Copyright Statement

The digital copy of this thesis is protected by the Copyright Act 1994 (New Zealand).

This thesis may be consulted by you, provided you comply with the provisions of the Act and the following conditions of use:

- Any use you make of these documents or images must be for research or private study purposes only, and you may not make them available to any other person.
- Authors control the copyright of their thesis. You will recognise the author's right to be identified as the author of this thesis, and due acknowledgement will be made to the author where appropriate.
- You will obtain the author's permission before publishing any material from their thesis.

To request permissions please use the Feedback form on our webpage.
http://researchspace.auckland.ac.nz/feedback

General copyright and disclaimer

In addition to the above conditions, authors give their consent for the digital copy of their work to be used subject to the conditions specified on the Library Thesis Consent Form.
The Effect of Oxygen Dosage Rate on the Chemical and Sensory Changes Occurring During Micro-oxygenation of New Zealand Red Wine

Stuart Dykes

This thesis is submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in Food Science, The University of Auckland, 2007

Academic Supervisor
Dr Paul A Kilmartin, Director of Wine Science, University of Auckland

Advisor
Dr Martin Pour Nikfardjam
LVWO Weinsberg
ABSTRACT

The technique of micro-oxygenation involves the deliberate addition of continuous, metered amounts of oxygen into a vessel of bulk wine during the maturation period (between the end of fermentation and bottling). The aim of the process is to improve the sensory properties of red wine, particularly the mouthfeel characteristics associated with the various polyphenol constituents. The success of the process appears to depend strongly on the ability to control the rate of oxygen dosage. The effect of dosage rate on the chemical and corresponding sensory changes of a red wine is the central theme of this thesis.

A method of dosing oxygen (at typical micro-oxygenation rates) into small volumes of wine (<100 litres) was developed using a dense polymer membrane diffuser. It was clearly demonstrated that wine could be reliably oxygenated at very low rates using a coiled length of FEP as the diffuser material. Oxygen dosage was regulated by adjusting the oxygen pressure inside the tube. The advantage with a dense polymer diffuser is that no bubbles are generated and the oxygenation efficiency is 100%. The diffuser was fully modeled and characterised for use in the laboratory scale trials detailed in Chapters Four and Six.

The small scale oxygenation equipment was used to conduct a fully replicated experiment to investigate the evolution of a Cabernet Sauvignon wine under four oxygenation treatments at dosage rates of 0, 10, 23 and 36 mg/L/mth. The total period of the trial was 105 days. HPLC analysis indicated that the rate change of low molecular weight polyphenols is directly related to the oxygen dosage rate. The concentration of the majority of the identifiable monomers, most notably the anthocyanins decreased throughout the course of the trial. The rate of decrease was directly related to oxygen dosage rate. Thiolysis results showed an increase in mDP for all treatments over the course of the trial until day 77 when they were observed to decrease for all treatments. The decrease in mDP coincided with an addition of SO$_2$ which was investigated in a subsequent trial. Spectrophotometric results indicated that the rate of formation of non-bleachable pigments was directly related to the rate of oxygen dosage with significant differences between the high rates (23 and 36 mg/L/mth) and the low rates (0 and 10 mg/L/mth). The trend for all treatments was for increased levels of stable pigments.
The sensory results show that the measured organoleptic temporal development exhibits a similar oscillatory behaviour compared to the anecdotally derived curve presented in figure 1-2. The distinction between the respective phases described in section 1.1.1 was, however, less clear. The most significant factor in the model weighting was mouthfeel and astringency which correlates with the observed changes occurring in the wine polyphenols during maturation.

Overall the laboratory scale trial showed that the chemical polyphenol development was directly related to the oxygen dosage rate. The sensory evolution also appeared to be accelerated with higher oxygen dosage rates, although the oscillatory nature of the sensory response given a single linear input indicates a complex underlying mechanism driving the changes.

The effect of SO₂ on the development of wine polyphenols with and without oxygen was also investigated. The presence of SO₂ was found to have a significant effect on both mDP and the concentration of non-bleachable pigments. mDP was observed to decrease over the six week trial period irrespective of whether oxygen had been added or not. The mDP for the treatments without SO₂ increased steadily over the course of the trial. Similarly the formation of non-bleachable pigments was suppressed and even retarded with SO₂ present whereas for the treatments without SO₂ a steady increase was observed. The implication of these results is that SO₂ may have a much larger effect on tannin development than oxygen.

The use of electrochemical micro-oxidation (or ELMOX) was examined ostensibly to determine proof of concept and also compare the performance of glassy carbon and titanium as electrode materials against traditional micro-oxygenation. Notable transformations occurred with titanium showing higher levels of ethanal than the other treatments both chemically and by sensory measure. A greater rate of stable pigment formation was also observed for the titanium compared to the other treatments. The respective dosage rates for the glassy carbon ELMOX and traditional micro-oxygenation treatments were too low to be able to discriminate any significant differences compared to the control wine.
Gross oxygen consumption kinetics was measured using a device developed at the University of Auckland. Autocatalytic behaviour was observed in wines containing, even small quantities of yeast lees. Tightly filtered wines however exhibited first order kinetic behaviour. The result indicates that any residual yeast lees remaining in the wine dominate the consumption of dissolved oxygen effectively decreasing the net availability of oxygen for wine polyphenol reactions.

The computational fluid dynamics (CFD) analysis of the micro-oxygenation system showed that for a typical micro-oxygenation dosage rate there is very little mixing occurring in the tank. Localised flow loops form around the bubble plume and there is only slight lateral dispersion of the bubble column limiting the transport of the dissolved oxygen from the central plume. Despite some limitations in the method the ability to be able to visualise the physical system provides a powerful tool to help in analyzing and subsequently optimising the process.
In memory of
Ian Dykes
1941-2001
ACKNOWLEDGEMENTS

The work described in this thesis would not have been possible without the considerable help from a large number of people.

First and foremost I would like to acknowledge the support of my academic supervisor Dr Paul Kilmartin. Over the period of my research Paul has been fully supportive of my ideas and work giving me technical, logistical and financial assistance when required. Paul has also been a great mentor over the period of my post-graduate studies and someone I am honored and grateful to have as a supervisor.

I was fortunate enough to be awarded an AGMARDT scholarship, and this assisted me greatly in being able to carry out this research. Moreover, the support of the AGMARDT administration team was excellent throughout. Thanks also go to Dr Martin Pour Nikfardjam who was active in getting this project off the ground and for Lincoln University for providing much needed initial support.

Paul Mooney, the winemaker at Mission Estate Wines in the Hawke’s Bay was heavily involved in my own and other research projects at the University of Auckland Wine Science Programme. Paul was a tremendous help through the course of my research either providing wine samples or as a source of insight and pragmatic comment on my work.

I am also extremely grateful to the University of Auckland Statistics department who gave me wise counsel in terms of experimental design and subsequent data analysis. I would like to acknowledge particularly the assistance of Associate Professor Chris Triggs and Dr Marti Anderson for providing me with many useful tools and ideas to examine and better interpret my data. Thanks also for the final chapter go to Professor Peter Hunter who gave me much guidance in terms of converting the equations into working code.

Jan Robinson, the University of Auckland Wine Science Programme Laboratory Manager, gave me significant support throughout the experimental phase of the study, particularly the winemaking and associated tasks over the harvest period.
Acknowledgement should also be made of the sensory panelists who made themselves available for often tedious training and data collection sessions purely on a voluntary basis. Their enthusiasm and dedication was tremendous and is very much appreciated.

Finally I would like to thank my long suffering family for the continued love and support in this endeavor. Particular thanks go to my wife who always managed to put things in perspective when I was not able to. I am also very much looking forward to getting to know my children a little better from now on.
# TABLE OF CONTENTS

## CHAPTER ONE  Introduction & Literature Review  1

1.1  Introduction  1

1.1.1  Micro-oxygenation - Definition  1

1.1.2  Rate Dependence of Micro-oxygenation  5

1.2  Research Objectives  7

1.3  Review of Oxygen Mass Transfer and Mass Transport Processes  8

1.3.1  Oxygen Delivery Methods  8

1.3.2  Micro-bullage  9

1.3.3  Dense Polymer Membrane Oxygenation.  15

1.4  The Role of Oxygen in Winemaking  17

1.4.1  Molecular Oxygen as the Primary Oxidant  17

1.4.2  The Production and Role of Ethanal  22

1.4.3  Direct Condensation of Flavanols and Anthocyanins  23

1.4.4  The Effect of Oxidation on Colour Stability  24

1.4.5  The effect of Oxygen on the Evolution of Sensory Properties  29

## CHAPTER TWO  Preliminary Commercial Trial and Method Development  31

2.1  Introduction  31

2.2  Trial Setup  31

2.2.1  Grapes  31

2.2.2  Wine Processing  31

2.2.3  Wine Sampling  33

2.3  Method Development  34

2.3.1  Monomeric Polyphenols  34

2.3.2  Chemometric Analysis of RP-HPLC data  36

2.3.3  Peak Alignment Pre-Processing  37

2.3.4  Sample Fractionation Procedure  40

2.3.5  Thiolysis  41

2.3.6  Optimising Hydrolysis Conditions  44

2.3.7  Thiolysis Procedure  46
CHAPTER THREE  

Development of a Method for Oxygenation of Small Volumes of Wine  

3.1 Introduction  

3.2 Trials using Micro-Bullage in Large Aspect Ratio Tanks  

3.3 Polymer Membrane Oxygenation  

3.3.1 PMO Mass Transfer Model  

3.3.2 Sealed-End Oxygen Partial Pressure Model  

3.4 Methods and Materials  

3.4.1 Membrane Material Selection  

3.4.2 Hollow Fibre Contactors  

3.4.3 Test Vessel  

3.5 Procedure  

3.6 Results  

3.6.1 Oxygen Pressure  

3.6.2 Mixing  

3.6.3 Diffuser Loop Diameter  

3.6.4 Effect of Ethanol  

3.6.5 Effect of Temperature  

3.6.6 Membrane fouling  

3.7 Discussion  

3.7.1 Oxygen Partial Pressure  

3.7.2 Mixing  

3.7.3 Ethanol  

3.7.4 Temperature
3.7.5 Development of Oxygen Dosage Rate Curve.

3.8 Conclusion

CHAPTER FOUR Cabernet Sauvignon: Laboratory Scale Trials

4.1 Introduction

4.2 Trial Setup

4.2.1 Grapes

4.2.2 Wine Processing

4.2.3 Oxygenation Treatment Setup

4.2.4 Wine Sampling

4.2.5 Malolactic Fermentation

4.2.6 Sulfur Dioxide Treatment

4.3 Chemical Analysis

4.3.1 Low Molecular Weight Polyphenol Analysis

4.3.2 Thiols

4.3.3 Spectrophotometric Analysis

4.3.4 Polyphenol Measurement by Folin-Ciocalteau Method

4.3.5 Sensory Analysis

4.3.6 Data Analysis

4.4 Results

4.4.1 Sensory Results

4.4.2 Principal Response Curves

4.4.3 Folin Ciocalteau

4.4.4 Colour Measurements

4.4.5 Low Molecular Weight HPLC Analysis

4.4.6 Thiols

4.5 Summary

4.6 Discussion

4.6.1 Sensory Evaluation

4.6.2 LMW Polyphenol Analysis

4.6.3 Thiols
CHAPTER FIVE  

Effect of Sulfur Dioxide and Oxygen on the Tannin Development in Red Wine  

5.1  Introduction  

5.2  Materials and Methods  

5.2.1  Grapes  

5.2.2  Wine Processing  

5.2.3  Experimental Treatment Setup  

5.3  Chemical Analyses  

5.3.1  Colour measurement  

5.3.2  Thiolysis  

5.3.3  SO₂ Measurements  

5.3.4  Data Analysis  

5.4  Results & Discussion  

5.4.1  Colourmetric Results  

5.4.2  Thiolysis Results  

5.4.3  SO₂ Concentrations  

5.4.3  Discussion  

CHAPTER SIX  

Electrochemical Micro-oxidation of Red Wine  

6.1  Introduction  

6.2  Trial Setup  

6.2.1  Grapes  

6.2.2  Wine Processing  

6.2.3  Experimental Design  

6.2.4  Wine Sampling  

6.2.5  Sulfur Dioxide Treatment  

6.3  Chemical Analysis  

6.3.1  Low Molecular Weight Polyphenol Analysis  

6.3.2  Thiolysis  

6.3.3  Colour measurement  

6.3.4  Sensory Analysis  

6.3.5  Ethanal Analysis  

6.3.6  Data Analysis
6.4 Results and Discussion

6.4.1 Data Quality Control
6.4.2 Low Molecular Weight Polyphenols
6.4.3 Thiolysis
6.4.4 Spectrophotometric Colour Analysis
6.4.5 Sensory Analysis
6.4.6 Ethanal Analysis
6.4.7 General Discussion

CHAPTER SEVEN Modeling of Oxygen Mass Transfer with Chemical Reaction

7.1 Introduction
7.2 Model Development
  7.2.1 Discrete phase governing equations
  7.2.2 Continuous phase governing equations
  7.2.3 Mass transport governing equations
7.3 Numerical Implementation
  7.3.1 Time Discretisation
  7.3.2 Bubble Dynamics
  7.3.3 Hydrodynamics
  7.3.4 Dissolved oxygen transport and chemical equation
  7.3.5 Bubble-liquid coupling
  7.3.6 Boundary conditions
  7.3.7 Computational Flow
7.4 Experimental Determination of Oxygen Consumption Kinetics
  7.4.1 Experimental Setup
  7.4.2 Experimental Design
7.5 Results & Discussion
  7.5.1 Determination of Oxygen Consumption Kinetics
  7.5.2 Results from Numerical Modeling

CHAPTER EIGHT Conclusion

8.1 Conclusion
8.2 Potential Future Research
APPENDICES

Appendix I  HPLC Standard Curves – Low Molecular Weight Polyphenols  237
Appendix II  HPLC Standard Curves – Thiolyis  237
Appendix III  Thiolyis Molar Yield Curves  238
Appendix IV  Sensory Analysis - Standard Data Collection Form  240
Appendix V  Forms Required for Ethical Approval to Run a Sensory Panel  245
Appendix VI  Experimental Oxygenation Tank Drawings  248
Appendix VII  Discretisation and Solution Procedure for Volume Average Navier Stokes Equation  256
Appendix VIII  Detailed Drawing of Oxygenation Cell  264

REFERENCES  272
**LIST OF FIGURES**

| Figure 1-1 | Analysis of overhead associated with barrel maturation compared to micro-oxygenation. Assumptions are: the barrels are amortised over 3 years, depreciation calculated on both the micro-oxygenation unit and the stainless steel tanks. Oak adjuncts are calculated using oak staves at 20% new wood equivalent. Labour overheads include all barrel operations including storage and laboratory monitoring. |
| Figure 1-2 | Different phases of red wine under micro-oxygenation treatment, adapted from (Lemaire 1995) |
| Figure 1-3 | The three processes of micro-oxygenation |
| Figure 1-4 | a) Schematic representation of micro-bullage oxygen delivery system. b) close up photograph of a diffuser in water |
| Figure 1-5 | Schematic representation of mass transfer across the bubble interface. |
| Figure 1-6 | Measured values of mass transfer coefficient ($K_L$) versus bubble diameter ($d_b$) for $O_2$ dissolution into pure water, from (Motarjemi and Jameson 1978) |
| Figure 1-7 | Bubble diameter at the diffuser as a function of pore size for a 12% v/v ethanol solution, adapted from (Gaddis and Vogelpohl 1986) |
| Figure 1-8 | Terminal rise velocity as a function of bubble diameter based on model developed by (Jamialahmadi, Branch et al. 1994) |
| Figure 1-9 | Proportion of $O_2$ transferred from bubbles containing pure $O_2$ only released at different depths from the surface of the liquid (pure water). The curves are for different initial bubble diameters (mm) from (Motarjemi and Jameson 1978). |
| Figure 1-10 | Bubble terminal velocity and bubble diameter as a function of ethanol concentration, from (Jamialahmadi and Mueller-Steinhagen 1992) |
| Figure 1-11 | Molecular orbital energy diagram for ground state ($\Sigma_g$) dioxygen |
| Figure 1-12 | Autoxidation of catechol with electron donation from the phenolate ion |
| Figure 1-13 | Summary of overall chemical oxidation process in wines from (Danilewicz 2003) |
| Figure 1-14 | Proposed mechanism for aldehyde induced condensation of Tannin-Tannin and Tannin-Anthocyanin reactions, adapted from (Cheynier 2000) |
| Figure 1-15 | Proposed mechanism for the formation of vinyl linked pigments, from (Mateus et al. 2002) |
Figure 1-16  Proposed reaction mechanisms for the T-A type direct condensation of anthocyanins and proanthocyanidins, from (Cheynier, Remy et al. 2000)  

Figure 1-17  Proposed reaction mechanisms for the A-T type direct condensation of anthocyanins and proanthocyanidins, from (Cheynier, Remy et al. 2000)  

Figure 2-1  Micro-oxygenation setup at Mission Estate Winery  

Figure 2-2  HPLC traces for a Cabernet Sauvignon wine. Main peak are identified as: 1 = gallic acid, 2 = t-caftaric acid, 3 = s-glutathionylcaftaric acid (GRP), 4 = cis-caftaric acid, 5 = t-caftaric acid, 6 & 7 = unknown flavanols possibly B3 and B1 respectively, 8 = catechin, 9 = caffeic acid, 10 = delphinidin-3-glucoside, 11 = epicatechin, 12 = petunidin-3-glucoside, 13 = peonidin-3-glucoside, 14 = malvidin-3-glucoside, 15 = unidentified (61.7 mins), 16 = p-coumaric acid, 17 = quercetin-3-glucoside, 18 & 19 = unknown flavonols, 20 = quercetin.  

Figure 2-3  Process flow for time-wise alignment of chromatographic peaks  

Figure 2-4  Nine unaligned chromatograms of red wine at 520nm using gradient elution described in table 2-1  

Figure 2-5  The same nine chromatograms as in figure 2-4 after alignment  

Figure 2-6  Data Matrix structure for PCA analysis of RP-HPLC results  

Figure 2-7  Hydrolytic cleavage of a proanthycanidin  

Figure 2-8  Nucleophilic addition of toluene-α-thiol to the extension unit of a condensed tannin after hydrolysis  

Figure 2-9  Hydrolysis optimisation trial  

Figure 2-10  280 nm HPLC chromatogram trace of thiolysed polymeric fraction of Cabernet Sauvignon wine sample. Identified peaks are: 1 = catechin, 2 = epicatechin, 3 = epicatechin-3-O-gallate, 4 = epigallocatechin-4-benzylthioether, 5 & 6 = catechin-4-benzylthioether, 7 = epicatechin-4-benzylthioether, 8 = epicatechin-3-O-gallate-4-benzylthioether.  

Figure 2-11  The two stereoisomers of catechin-4-thioether as observed in figure 2-10  

Figure 2-12 a)  Mean sensory scores for each attribute and sampling date. Error bars represent 95% confidence intervals  

Figure 2-12 b)  Mean sensory scores for each attribute and sampling date. Error bars represent 95% confidence intervals  

Figure 2-13 a)  Separation of treatment means 14 days after the start of treatment. Ellipses represent 95% confidence regions of group means. Individual panelist scores are overlaid onto the projection
Figure 2-13 b) Separation of treatment means 28 days after the start of treatment. Ellipses represent 95% confidence regions of the group means. Individual panelist scores are overlaid onto the projection.

Figure 2-13 c) Separation of treatment means 42 days after the start of treatment. Ellipses represent 95% confidence regions of group means. Individual panelist scores are overlaid onto the projection.

Figure 2-13 d) Separation of treatment means 56 days after the start of treatment. Ellipses represent 95% confidence regions of group means. Individual panelist scores are overlaid onto the projection.

Figure 2-14 PCA projection of the LMW Polyphenol HPLC data.

Figure 2-15 PCA loading plots for the LMW polyphenol HPLC data.

Figure 2-16 Schematic representation of coding for loadings plot interpretation.

Figure 2-17 a) Evolution of individual phenolic compounds over the course of the trial. Concentrations of B4 and unidentified compounds at 61.8 and 88.4 minutes are expressed as catechin equivalents.

Figure 2-17 b) Evolution of individual phenolic compounds over the course of the trial. Concentrations of t-caftaric acids and the two isomers of coutaric acid are expressed as caffeic and coumaric acid equivalents respectively. The concentration of the unidentified compound at 83.8 minutes is expressed as quercetin equivalents.

Figure 2-17 c) Evolution of individual phenolic compounds over the course of the trial. All compounds are expressed in terms of chromatogram peak area (mAu•s).

Figure 2-17 d) Evolution of individual phenolic compounds over the course of the trial. All compounds are expressed in terms of chromatogram peak area (mAu•s).

Figure 2-18 Evolution of mDP for treatment and control wines through the course of the trial. The labels above points represent the significance of the difference between the control and treatment wines and the sampling date as determined by ANOVA (P<0.05).

Figure 3-1 High aspect ratio tanks mounted in a large warehouse facility.

Figure 3-2 Schematic representation of the solute concentration gradients across a liquid-membrane-gas configuration.

Figure 3-3 Different types of polymer membranes.

Figure 3-4 Schematic representation of bi-directional solute fluxes in a sealed end membrane.

Figure 3-5 Photograph of the actual FEP diffuser used for the main trial.
Figure 3-6 Oxygen partial pressure as a function of distance from supply end to the opposite diameter for different feed pressures.

Figure 3-7 Dissolved Oxygen Meter Cradle; a) shows the component pieces, including the dissolved oxygen probe and b) is the components assembled and ready for installation into the tank.

Figure 3-8 Experimental setup shown as a schematic diagram and a photograph of the actual experimental apparatus

Figure 3-9 Typical dissolved oxygen accumulation for the following experimental parameters: bulk liquid = deionised water, gas = O₂ (food grade), O₂ feed pressure = 446.0 kPa, membrane specific area = 0.685 m⁻¹, stirring rate = 350 rpm, temperature = 15°C

Figure 3-10 Regression analysis used to calculate $K_L$ for the trial described in figure 3-9, $K_L = 0.323 \times 10^{-9}$ ms⁻¹.

Figure 3-11 Variation in local mass transfer coefficient with oxygen partial pressure (feed pressure corrected using the model derived in section 3.3.2). Experimental parameters: bulk liquid = deionised water, gas = O₂ (food grade), membrane specific area = 0.685 m⁻¹, stirring rate = 350 rpm, temperature = 20°C.

Figure 3-12 Variation in local mass transfer coefficient with mixing. Experimental parameters: bulk liquid = deionised water, gas = O₂ (food grade), O₂ pressure = 441 kPa, membrane specific area = 0.685 m⁻¹, temperature = 15°C.

Figure 3-13 Variation in local mass transfer coefficient with diffuser diameter. Experimental parameters: bulk liquid = deionised water, gas = O₂ (food grade), O₂ pressure = 441 kPa, mixing rate 350 rpm, temperature = 15°C.

Figure 3-14 Variation in local mass transfer coefficient with ethanol concentration. Experimental parameters: gas = O₂ (food grade), O₂ feed pressure = 441 kPa, mixing rate 350 rpm, membrane specific area = 0.685 m⁻¹, temperature = 15°C.

Figure 3-15 Mass transfer coefficient as a function of increasing surface tension (from decreasing ethanol concentration ). Experimental parameters as figure 3-14

Figure 3-16 Relationship between $K_L$ (10% ethanol) and $K$ (0% ethanol) for different oxygen feed pressures

Figure 3-17 Variation in local mass transfer coefficient with temperature. Experimental parameters: Bulk phase = de-ionised water, gas = O₂ (food grade), O₂ pressure = 441 kPa, mixing rate 350 rpm, membrane specific area = 0.685 m⁻¹, temperature = 15°C

Figure 3-18 Diffuser condition after 15 weeks in Cabernet Sauvignon wine
Figure 3-19  Tangential velocity profile assuming Rankine’s Combined vortex (adapted from Nagata (1975)). 99
Figure 3-20  Radial and axial velocity profiles for unbaffled stirred tank (adapted from Nagata (1975)) 100
Figure 3-21  $K_L$ as a function of theoretical tangential velocity 100
Figure 3-22  Dosage rate curve for FEP membrane diffuser 102
Figure 4-1  Summary of processing steps 107
Figure 4-2  Schematic representation of experimental arrangement 108
Figure 4-3  Sampling apparatus 110
Figure 4-4  Sampling procedure and process flow 110
Figure 4-5  Canonical variates analysis plots of sensory results for sampling dates at 7 and 21 days after the start of treatment. Bold symbols represent treatment mean scores. Other symbols are individual panelist data. The circles surrounding treatment means indicate 95% confidence regions for the respective treatment group means. 118
Figure 4-6  Canonical variates analysis plots of sensory results for sampling dates at 35 and 49 days after the start of treatment. Bold symbols represent treatment mean scores. Other symbols are individual panelist data. The circles surrounding treatment means indicate 95% confidence regions for the respective treatment group means. 119
Figure 4-7  Canonical variates analysis plots of sensory results for sampling dates at 63 and 77 days after the start of treatment. Bold symbols represent treatment mean scores. Other symbols are individual panelist data. The circles surrounding treatment means indicate 95% confidence regions for the respective treatment group means. 120
Figure 4-8  Canonical variates analysis plots of sensory results for sampling dates at 91 and 105 days after the start of treatment. Bold symbols represent treatment mean scores. Other symbols are individual panellist data. The circles surrounding treatment means indicate 95% confidence regions for the respective treatment group means. 121
Figure 4-9  Unconstrained PCA of the mean sensory scores for each treatment and sample date. Data labels indicate sample date and dosage rate as “days since start of treatment”/”oxygen dosage rate” e.g.7/23 = 7 days from start of treatment and 23 mg/L/mth dosage rate. The lines indicate the evolution of each treatment chronologically. 123
Figure 4-10  Principal Response curves for the three oxygenation treatments relative to the control wine. Sensory attribute weights are presented in the lineset plot to the right of the actual PRC plot. 124

Figure 4-11  Development of mean Folin-Ciocaltaeu polyphenol index for each treatment over the course of the trial. Error bars indicate standard deviation ($n=3$) 127

Figure 4-12  Development of mean visual intensity for each treatment over the course of the trial. Error bars indicate standard deviation ($n=3$) 129

Figure 4-13  Development of mean hue for each treatment over the course of the trial. Error bars indicate standard deviation ($n=3$) 130

Figure 4-14  PCA projection of UV-vis spectral data from all treatments and observation dates. Data labels indicate observation dates 133

Figure 4-15  Corresponding loadings plot vs wavelength for the PCA presented in figure 4-15 133

Figure 4-16  Results of bleached pigment assay for all treatments and observation dates. Data point represent mean values for each treatment and error bars indicate standard deviation ($n=3$) 134

Figure 4-17  PCA projection of HPLC chromatograms (280, 320, 365 and 520nm) 136

Figure 4-18  Loadings plots corresponding to the PCA scores projection shown in figure 4-18. The numbers adjacent to each peak correspond to the peak number from table 4-12 137

Figure 4-19  Evolution of individual phenolic compounds for each treatment through the course of the trial 139

Figure 4-20  Evolution of individual phenolic compounds for each treatment through the course of the trial 140

Figure 4-21  Evolution of individual phenolic compounds for each treatment through the course of the trial 141

Figure 4-22  Evolution of individual phenolic compounds for each treatment through the course of the trial. Error bars indicate standard deviation ($n=3$) 142

Figure 4-23  Evolution of individual phenolic compounds for each treatment through the course of the trial. Error bars indicate standard deviation ($n=3$) 143

Figure 4-24  spectral distribution of two unidentified flavanol at 61.8 and 69.6 minutes. While the peak absorbance is at 280 nm for both cases there is notable absorbance at 520-530 nm for both compounds. 144
Evolution of mDP for each treatment through the course of the trial. Error bars indicate standard deviation (n=3).

Principal Components Analysis of the relative proportions of proanthocyanidin subunits after thiolysis. Score data labels refer to the observation day. Abbreviated attribute labels refer to: cat = catechin, epi = epicatechin, ECG = epicatechin-3-O-gallate, EGC-TE = epigallocatechin-4-benzylthioether, cat-TE(i) = (2R,3R,4S)-catechin-benzylthioether, cat-TE(ii) = (2R,3R,4R)-catechin-benzylthioether, epi-TE = epicatechin-4-benzylthioether, ECG-TE = epicatechin-3-O-gallate-4-benzylthioether.

Correlation loadings plot of summary data matrix.

Summary PCA for all sensory and chemical attributes discussed above. The data labels for the scores refer to observation date/dosage rate. The abbreviated attributes refer to: bleach = 520nm absorbance after addition of SO₂ (figure 4-17), FP1 and FP2 = the unidentified flavanol compounds eluting at 61.8 and 69.6 mins respectively (table 4-12), AP1 and AP2 = unidentified pigmented compounds eluting at 75 and 86 mins respectively (figure 4-12), quer-3-glu = quercetin-3-glucoside, mv-3-glu = malvidin-3-glucoside, del-3-glu = delphinidin-3-glucoside, pet-3-glu = petunidin-3-glucoside, peo-3-glu = peonidin-3-glucoside, cy-3-glu = cyanidin-3-glucoside.

Proposed reaction mechanism for nucleophilic addition of bisulfite ion as a competing reaction for tannin-tannin condensation.

Summary of wine-making processing steps.

Results of the bleaching assay. Error bars indicate standard deviation (n=3).

mDP as measured by thyolysis. Error bars indicate standard deviation (n=3).

SO₂ concentrations (bound and free) for treatment with oxygen.

SO₂ concentrations (bound and free) for treatment without oxygen.

Schematic representation of experimental arrangement.

Modifications made for provision of a titanium electrode into the 15 litre research vessels.

Galvanostats used for the ELMOX trial described in this trial.

Scores plot from PCA analysis of HPLC chromatograms (280, 320, 365 and 520 nm). The data labels for each score refer to the “treatment”/“days since start of treatment”. The respective key is: control = C, Micro-
oxygenation = MOX, ELMOX-titanium = Ti and ELMOX-glassy carbon = GC.

Figure 6-5 Loading plot from PCA analysis of HPLC chromatograms (280, 320, 365 and 520 nm). The red and blue traces correspond to the PC1 and PC2 loadings respectively. The number adjacent to the peaks refer to the peaks listed in table 6-3

Figure 6-6 Evolution of individual phenolic compounds for each treatment through the course of the ELMOX trial (n = 3)

Figure 6-7 Evolution of individual phenolic compounds for each treatment through the course of the ELMOX trial (n = 3)

Figure 6-8 Evolution of individual phenolic compounds for each treatment through the course of the ELMOX trial (n = 3)

Figure 6-9 Evolution of individual phenolic compounds for each treatment through the course of the ELMOX trial (n = 3)

Figure 6-10 Evolution of mDP for each treatment through the course of the ELMOX trial. Error bars indicate standard deviation (n = 3)

Figure 6-11 Development of mean visual intensity for each treatment over the course of the ELMOX trial. Error bars indicate standard deviation (n=3)

Figure 6-12 Development of mean visual hue for each treatment over the course of the ELMOX trial. Error bars indicate standard deviation (n=3)

Figure 6-13 Results of the bleached pigment assay for all treatments and observation dates. Data point represent mean values for each treatment and error bars indicate standard deviation (n=3)

Figure 6-14 Canonical variates analysis plots of sensory results for sampling dates at 7 (a) and 14 (b) days after the start of treatment. Bold symbols represent treatment mean scores. Other symbols are individual panelist data. The circles surrounding treatment means indicate 95% confidence regions for the respective group means.

Figure 6-15 Canonical variates analysis plots of sensory results for sampling dates at 21 (a) and 35 (b) days after the start of treatment. Bold symbols represent treatment mean scores. Other symbols are individual panelist data. The circles surrounding treatment means indicate 95% confidence regions for the respective group means.

Figure 6-16 Principal Response curves for the three treatments relative to the control wine. Sensory attribute weights are presented in the linestack plot to the right of the actual PRC plot.
Figure 6-17  Ethanal concentration for all treatments and observation dates. Data point represent mean values for each treatment and error bars indicate standard deviation ($n=3$)

Figure 6-18  Ethanal treatment means from sensory data the x-axis labels indicate the observation date (e.g. d07 = 7 days since the start of the trial) followed by the treatment information. The error bars indicate 95% confidence intervals.

Figure 7-1  Schematic representation of the string coupling between factors affecting a bubble plume performance (adapted from Darmana 2005). $k_i$ = interphase mass transfer coefficient; $a$ = bubble-wine interfacial surface area and $d_b$ = bubble diameter.

Figure 7-2  Representation of interphase mass transfer of O$_2$ and CO$_2$ on an individual bubble level

Figure 7-3  Schematic representation of time marching approach

Figure 7-4  Schematic representation of liquid to bubble phase mapping method, the notation accompanies equation [7.23] adapted from (Darmana, Deen et al. 2005) where $S$ is the bubble position in space at time $t$

Figure 7-5  Schematic representation of the boundary conditions adapted from (Darmana, Deen et al. 2005)

Figure 7-6  Computational flow of bubble plume model

Figure 7-8  Photograph of Oxygenation cell described in section 7.4.1.

Figure 7-9  The oxygen consumption rate data for treatment T4c (table 7-2). The red circles represent the raw data. The blue line represents the fitted curve (using equation [7.25] and the dashed blue lines either side of the solid blue line represent the 95% confidence interval bands.

Figure 7-10  The oxygen consumption rate data for treatment T5 (table 7-3). The red circles represent the raw data. The blue line represents the fitted curve (using equation [7.28] and the dashed blue lines either side of the solid blue line represent the 95% confidence interval bands.

Figure 7-11  Oxygen consumption rate for Cabernet Sauvignon (T5 and T5b in table 7-3) including a repeated saturation (T5b)

Figure 7-12  Comparison of the oxygen consumption rates of the same wine after different filtration treatments (refer table 7-1)

Figure 7-13  Visualisation of CFD simulation 1 (refer table 7-4) with an oxygen dosage rate of 25 mg/L/mth and a bubble size of 670 $\mu$m at $t=120$ seconds; a) shows the bubble position, b) represents a diagonal slice of the wine phase
velocity field and c) represents the dissolved oxygen concentration in mg/L.

Figure 7-14 Visualisation of CFD simulation 4 (refer table 7-4) with an oxygen dosage rate of 200 mg/L/mth and a bubble size of 670 µm at t=120 seconds; a) shows the bubble position, b) represents a diagonal slice of the wine phase velocity field and c) represents the dissolved oxygen concentration in mg/L.

Figure 7-15 Visualisation of CFD simulation 4 (refer table 7-4) with an oxygen dosage rate of 1000 mg/L/mth and a bubble size of 670 µm at t=120 seconds; a) shows the bubble position, b) represents a diagonal slice of the wine phase velocity field and c) represents the dissolved oxygen concentration in mg/L.

Figure 7-16 Visualisation of CFD simulation 4 (refer table 7-4) with an oxygen dosage rate of 25 mg/L/mth and a bubble size of 310 µm at t=120 seconds; a) shows the bubble position, b) represents a diagonal slice of the wine phase velocity field and c) represents the dissolved oxygen concentration in mg/L.

Figure 7-17 Visualisation of CFD simulation 4 (refer table 7-4) with an oxygen dosage rate of 25 mg/L/mth and a bubble size of 1000 µm at t=120 seconds; a) shows the bubble position, b) represents a diagonal slice of the wine phase velocity field and c) represents the dissolved oxygen concentration in mg/L.

Figure 7-18 Oxygen mass fraction remaining in the bubble as a function of fluid column height above the diffuser
LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-1</td>
<td>Summary of published Micro-oxygenation dosage rates</td>
<td>6</td>
</tr>
<tr>
<td>2-1</td>
<td>Gradient elution profile for evaluation of monomeric polyphenols where: A = H2O, B = H2O +5% CH3COOH and C= CH3CN.</td>
<td>36</td>
</tr>
<tr>
<td>2-2</td>
<td>Summary of thiolysis conditions in recently published studies</td>
<td>44</td>
</tr>
<tr>
<td>2-3</td>
<td>Gradient elution profile for thiolysis run</td>
<td>46</td>
</tr>
<tr>
<td>2-4</td>
<td>Summary of proanthocyanidin constitutive units used to calculate from degree of polymerization</td>
<td>48</td>
</tr>
<tr>
<td>2-5</td>
<td>Summary of sensory attributes</td>
<td>51</td>
</tr>
<tr>
<td>2-6</td>
<td>Univariate Sensory Analysis Results</td>
<td>53</td>
</tr>
<tr>
<td>2-7</td>
<td>Table of results from a single factor MANOVA</td>
<td>54</td>
</tr>
<tr>
<td>2-8</td>
<td>Major peaks from PCA loadings plot Figure 2-15</td>
<td>63</td>
</tr>
<tr>
<td>2-9</td>
<td>Molar proportion of individual extension and terminal units after thiolysis. The significance of the difference between the treatment and control wines is indicated.</td>
<td>72</td>
</tr>
<tr>
<td>3-1</td>
<td>Limitations of commercial trials</td>
<td>74</td>
</tr>
<tr>
<td>3-2</td>
<td>Comparison of corrected oxygen partial pressure vs feed pressures</td>
<td>85</td>
</tr>
<tr>
<td>3-3</td>
<td>Gas permeability values of fluoropolymers based on 100µm film thickness at 23°C using ASTM D1434 for gases and DIN53122 for water vapour. Adapted from (Seiler 1997)</td>
<td>86</td>
</tr>
<tr>
<td>3-4</td>
<td>Experimental design matrix</td>
<td>89</td>
</tr>
<tr>
<td>4-1</td>
<td>Oenological parameter of the Cabernet Sauvignon must</td>
<td>106</td>
</tr>
<tr>
<td>4-2</td>
<td>Dosage Rate Summary</td>
<td>108</td>
</tr>
<tr>
<td>4-7</td>
<td>Summary of sensory attributes</td>
<td>116</td>
</tr>
<tr>
<td>4-8</td>
<td>Results of single factor MANOVA for each sample date</td>
<td>122</td>
</tr>
<tr>
<td>4-9</td>
<td>mean (n=3)ab mean Folin-Ciocalteau polyphenol index (mg/L gallic acid equivalent) for all treatments and observation dates.</td>
<td>128</td>
</tr>
<tr>
<td>4-10</td>
<td>mean (n=3)ab raw visual intensity (420+520+620nm) for all treatments and observation dates.</td>
<td>129</td>
</tr>
<tr>
<td>4-11</td>
<td>mean (n=3)ab raw hue (420/520nm) for all treatments and observation dates.</td>
<td>130</td>
</tr>
<tr>
<td>4-12</td>
<td>mean (n=3)ab absorbance at 520nm after bleaching with bisulphite for all treatments and observation dates.</td>
<td>134</td>
</tr>
<tr>
<td>4-13</td>
<td>Major peaks from PCA loadings plot Figure 4-19</td>
<td>138</td>
</tr>
<tr>
<td>4-14</td>
<td>mean (n=3)ab degree of polymerisation after thiolysis and quantification with HPLC.</td>
<td>146</td>
</tr>
<tr>
<td>Table 5-1</td>
<td>Oenological parameters of the Cabernet Sauvignon must</td>
<td>155</td>
</tr>
<tr>
<td>Table 5-2</td>
<td>Experimental Design</td>
<td>156</td>
</tr>
<tr>
<td>Table 5-3</td>
<td>Mean (n=3)ab results of bleaching assay for all treatments and observation dates.</td>
<td>158</td>
</tr>
<tr>
<td>Table 5-4</td>
<td>Mean (n=3)ab results of mDP for all treatments and observation dates.</td>
<td>160</td>
</tr>
<tr>
<td>Table 6-1</td>
<td>Summary of oenological parameters of the wine used for the ELMOX trial</td>
<td>165</td>
</tr>
<tr>
<td>Table 6-2</td>
<td>Summary of respective treatments</td>
<td>165</td>
</tr>
<tr>
<td>Table 6-3</td>
<td>Major peaks identified from PCA loading plot figure 6-5</td>
<td>176</td>
</tr>
<tr>
<td>Table 6-4</td>
<td>Mean (n=3)ab degree of polymerisation after thiolysis and quantification with HPLC.</td>
<td>183</td>
</tr>
<tr>
<td>Table 6-5</td>
<td>Mean (n=3)ab intensity (420+520+620 nm) for all treatments and observation dates</td>
<td>184</td>
</tr>
<tr>
<td>Table 6-6</td>
<td>Mean (n=3)ab hue (420/520 nm) for all treatments and observation dates</td>
<td>185</td>
</tr>
<tr>
<td>Table 6-7</td>
<td>Mean (n=3)ab absorbance at 520 nm after bleaching with bisulfite for all treatments and observation dates.</td>
<td>186</td>
</tr>
<tr>
<td>Table 6-8</td>
<td>Results of single factor MANOVA for each sample date</td>
<td>188</td>
</tr>
<tr>
<td>Table 6-9</td>
<td>Mean (n=3)ab ethanal concentrations for all treatments and observation dates.</td>
<td>193</td>
</tr>
<tr>
<td>Table 7-1</td>
<td>Experimental treatments for oxygen consumption measurements described in section 7.4.1</td>
<td>214</td>
</tr>
<tr>
<td>Table 7-2</td>
<td>Curve fitting results for oxygen consumption rate curves exhibiting first order behaviour</td>
<td>215</td>
</tr>
<tr>
<td>Table 7-3</td>
<td>Curve fitting results for oxygen consumption rate curves exhibiting autocatalytic behaviour</td>
<td>217</td>
</tr>
<tr>
<td>Table 7-4</td>
<td>Summary of the CFD simulation parameters</td>
<td>220</td>
</tr>
<tr>
<td>Table 7-5</td>
<td>Model parameters and values</td>
<td>220</td>
</tr>
<tr>
<td>Table A-1</td>
<td>Standard curves for commercially available phenolic compounds. Used in Chapters Two, Four and Six</td>
<td>240</td>
</tr>
<tr>
<td>Table A-2</td>
<td>Standard curves for commercially available phenolic compounds. Used in Chapters Two, Four, Five and Six</td>
<td>240</td>
</tr>
</tbody>
</table>
# NOMENCLATURE

**CHAPTERS ONE through SIX**

- \( a \) = specific area (contact area divided by the volume of fluid being aerated) \((1/m)\)
- ANOVA = analysis of variance
- \( A - T \) = anthocyanin – tannin condensation product
- \( b \) = attribute weighting factor
- \( c \) = response pattern
- \( C \) = concentration (mol/L)
- CFD = computational fluid dynamics
- COW = correlation optimised warping
- CRZ = cylindrical rotating zone
- CVA = canonical variates analysis
- \( d \) = bubble diameter
- DO = dissolved oxygen
- \( E^\circ \) = standard electrode potential (V)
- ELMOX = electrochemical micro-oxidation
- ESI = electrospray ionisation
- FEP = fluorinated ethylene-propylene co-polymer
- FP1 = unidentified flavanol at 61.8 minutes
- FP2 = unidentified flavanol at 69.6 minutes
- FVZ = free vortex zone
- \( H \) = Henry’s law coefficient (Pa·m³/mol)
- HPLC-DAD = high performance liquid chromatography – diode array detector
- \( I \) = electrical current (A)
- \( j \) = mass flux (kg/s)
- \( k \) = individual phase mass transfer coefficient – ms⁻¹
- \( K_L \) = overall mass transfer coefficient (m/s)
- \( K_a \) = acid dissociation constant
- \( l_e \) = effective membrane thickness (m)
- LC-MS = liquid chromatography mass spectrometry
- LMW = low molecular weight
- MANOVA = multivariate analysis of variance
- MLF = malo-lactic fermentation
- MOX = micro-oxygenation
- \( n_e \) = number of electrons
- \( P \) = pressure
- \( p \) = permeability (mol/m·s·Pa)
- PET = polyethylene terephthalate
- PMO = polymer membrane oxygenation
- PCA = principal components analysis
- PC1 = principal component 1
- PC2 = principal component 2
- PRC = principal response curve
- \( Q \) = charge (coulomb)
- \( r \) = radius
R = ideal gas constant - J·mol⁻¹
RDA = redundancy analysis
ROTE = roll on tamper evident
RP-HPLC = reversed phase high performance liquid chromatography
s = fibre length (m)
S = membrane partition coefficient
t = time
T = temperature (K)
T = treatment effect
T - A = tannin – anthocyanin condensation product
TOF = time of flight
UV-VIS = ultra violet - visible
v = velocity (m/s)
v/v = volume per volume
w/v = weight per volume

Greek Symbols
ε = error term in principal response model
φ = diameter (m)
Γ = diffusion coefficient (m²/s)
λ = wavelength (nm)
Λ = Wilks lambda statistic
μ = viscosity (kg/ms)
ω = rotational velocity (rad/s)
ρ = density (kg/m³)
σ = surface tension (N/m)
σ² = variance
³Σₖ = triplet spin state
χ² = chi-squared test statistic

Subscripts
c = cylinder
d = treatment index
e = effective
ext = external
g = gas phase
G = gas phase
GO = bulk gas phase
GI = interfacial gas phase
int = internal
k = attribute index
l = liquid phase
L = liquid phase
LI = interfacial liquid phase
LO = bulk liquid phase
\( m \) = membrane
\( M \) = membrane
\( MG \) = membrane-gas interface
\( ML \) = membrane-liquid interface
\( N \) = nitrogen
\( O \) = oxygen
\( t \) = time index
\( T \) = total
\( w \) = wine

**CHAPTER SEVEN**

\( a \) = bubble wine interfacial area (m²)
\([A]\) = concentration of reactant A (kg/m³)
\([B]\) = concentration of product B (kg/m³)
\( C \) = bubble force coefficient (dimensionless)
\( c \) = solute concentration (kg/m³)
\( \text{CFD} \) = computational fluid dynamics
\( d \) = bubble diameter (m)
\( e \) = exponential operator
\( \text{E-E} \) = Euler-Euler two phase model
\( \text{E-L} \) = Euler Lagrange two phase model
\( \text{F} \) = force (N)
\( g \) = acceleration vector due to gravity (m/s²)
\( \text{GAE} \) = gallic acid equivalent
\( H \) = Henry’s law coefficient (Pa·m³/mol)
\( I \) = identity tensor
\( j \) = mass flux
\( k \) = reaction rate constant
\( K \) = interphase mass transfer coefficient (m/s)
\( m \) = mass (kg)
\( \text{NTU} \) = nephelometric turbidity units
\( P \) = pressure (Pa)
\( r \) = radius (m)
\( R^2 \) = quality control parameter from regression
\( \text{Re} \) = Reynolds number
\( \text{RMSE} \) = root mean squared error
\( S \) = source and sink terms for the transport equation
\( Sc \) = Schmidt number, \( Sc = \frac{\mu}{\rho \Gamma} \)
\( Sh \) = Sherwood number, \( Sh = \frac{k_d}{\Gamma} \)
\( \text{SSE} \) = sum of square due to error
\( t \) = time (s)
\( u \) = liquid phase velocity vector (m/s)
\( v \) = bubble phase velocity vector (m/s)
\( V \) = volume (m³)

**Greek Symbols**
\( \alpha = \) coefficient in the autocatalytic rate equation
\( \beta = \) coefficient in the autocatalytic rate equation
\( \delta t = \) time increment
\( \varepsilon = \) volume fraction (dimensionless)
\( \xi = \) volume fraction of bubble included in a cell (dimensionless)
\( \phi = \) interphase coupling term (N/m³)
\( \Gamma = \) diffusion coefficient (m²/s)
\( \lambda = \) interpolation factor (dimensionless)
\( \mu = \) viscosity (kg/ms)
\( \omega = \) Lagrangian quantity
\( \Omega = \) Eulerian quantity
\( \rho = \) density (kg/m³)
\( \tau = \) viscous stress tensor (N/m³)
\( \nabla = \) gradient operator (1/m)

**Subscripts**

\( b = \) bubble
\( bub = \) time increment for bubble phase momentum calculation
\( b \rightarrow l = \) bubble to liquid
\( CO_2 = \) carbon dioxide
\( Drag = \) drag force
\( D = \) sink
\( equivalent = \) equivalent volume
\( flow = \) time increment for liquid phase momentum calculation
\( g = \) gas phase
\( L = \) source
\( l = \) liquid phase
\( l \rightarrow b = \) liquid to bubble
\( Lift = \) lift force
\( P = \) pressure force
\( O_2 = \) oxygen
\( VM = \) virtual mass force
\( 0 = \) initial (i.e. at time = 0)

**Superscripts**

\( * = \) interfacial property
\( \kappa = \) iteration counter
CHAPTER ONE
Introduction & Literature Review

1.1 Introduction

The influence of oxygen on the organoleptic development of red wines during maturation has been recognised for more than 130 years. The now widely used quote ‘C’est l’oxygène qui fait le vin, c’est par son influence qu’il vieillit.’ (oxygen makes the wine, which ages under its influence) (Pasteur 1873) has, in the last decade, found a fresh resonance with both winemakers and oenologists. Two globally significant developments have been largely responsible for the renewed interest; the proliferation of a process known as “micro-oxygenation” and the rapid adoption (particularly in Australasia) of roll-on tamper-evident (ROTE) or screw caps as an increasingly important alternative for the traditional cork closures. The resulting enthusiasm from the wine industry has, understandably, inspired considerable research effort at an applied and a more fundamental level. A complete understanding of the role of oxygen in wine development either pre- or post bottling, at present however, remains some way off.

The scope of this thesis is concerned only with micro-oxygenation and more specifically the effect of oxygen dosage rate on the chemical and corresponding sensory evolution of red wine.

1.1.1 Micro-oxygenation - Definition

Micro-oxygenation can be defined generally as the deliberate treatment of wine with continuous and metered doses of oxygen at various times between the end of primary fermentation and bottling. This, somewhat broad definition is given in much of the micro-
oxygenation equipment supplier literature and it encompasses all of the key aspects of the process. Terms such as “macro-” and “meso-oxygenation” are sometimes used to describe what is essentially a variation of the same treatment (Paul 2002). Micro-oxygenation was formally developed in France in the mid 1990s in an attempt to replicate barrel conditions for wine matured in large stainless steel and cement vessels (Ducournau and Laplace 1995; Lemaire 1995). Since that time the leading supplier of micro-oxygenation equipment has indicated that almost 10,000 units have been sold, and an estimated 2 billion litres have undergone micro-oxygenation treatment (Renard 2006).

Wooden barrels (barriques) are a traditional way of storing wine during maturation (the period of development between fermentation and bottling). Barriques, however, confer many other benefits to the wine over-and-above merely storage; most significant is the addition of complimentary flavours and textures being derived from chemicals partitioned into the wine from the wood (Singleton 1994; Vivas 1996). Despite the many advantages of using barriques they represent a significant cost overhead to a winery. Not only is the direct cost of a barrique relatively high for a vessel with a useful life of just three years (USD600-1,000 per unit); the associated labour required to maintain a barrel (filling, emptying, racking, topping, monitoring, cleaning and storing) is also considerable. Wood can harbour harmful bacteria and yeast which can contribute to irreparable wine spoilage (e.g. actobacter and brettanomyces) if cellar hygiene practices are not maintained. Each barrel (typically 225 litres) requires the same level of analysis as a single stainless steel vessel, hence laboratory costs can also be very high for wineries with large inventories of cooperage.

For medium to large scale wineries it is often significantly more cost effective to mature medium and low quality red wines in large tanks (5,000 – 1,000,000 litres) made from an inert material such as stainless steel. Moreover large vessel maturation in combination with oak adjuncts such as staves and chips has become increasingly popular as a means of producing wines with oak flavour characteristics without the expense of barriques. The addition of controlled doses of oxygen to the tanks of wine was thus a further attempt to mirror the effects of oxygen transfer experienced through permeable wooden barrel staves.
A crude cost comparison analysis is presented in figure 1-1 which gives an indication of the savings that can be realised by using micro-oxygenation over traditional barrel maturation. The NZD 0.92/litre reduction in the production overhead of a wine is significant when blends of 1,000,000 litres are considered.

Figure 1-1  Analysis of overhead associated with barrel maturation compared to micro-oxygenation. Assumptions are: the barrels are amortised over 3 years, depreciation calculated on both the micro-oxygenation unit and the stainless steel tanks. Oak adjuncts are calculated using oak staves at 20% new wood equivalent. Labour overheads include all barrel operations including storage and laboratory monitoring.

The principal aim of micro-oxygenation is to improve the overall organoleptic profile of the wine under treatment. Specific claims that are made of micro-oxygenation by equipment suppliers and consultants are (Paul 2002):

- Improving the wine “structure” – softening of the mouthfeel
- Colour stability
- Amelioration of “green” herbaceous aromas
- Removal of reductive characters
Experience suggests that the sensory development of the wine under micro-oxygenation treatment is not linear but undergoes oscillatory behaviour over time. This behaviour has been partitioned into definable stages which are best represented graphically and are shown in figure 1-2.

**Structuring Phase**

This phase generally occurs before SO$_2$ additions and is characterised by a building of the structure (defined generally as the combined effects of astringency, bitterness and palate weight – also termed “tannic force”) and a decrease in the fermentation and varietal aromas. The wine appears to be deteriorating during this phase and developing in the opposite direction to what is desired! The structuring phase can last anywhere from three days to six weeks. Dosage rates tend to be relatively high during this period, typically between 20-90 mg of O$_2$/ litre of wine / month.

![Figure 1-2](image.png)

**Figure 1-2** Different phases of red wine under micro-oxygenation treatment, adapted from (Lemaire 1995)

The end of the structuring phase generally coincides with the completion of malo-lactic fermentation (MLF) and, it is argued more importantly, the addition of sulfur dioxide. Table 1-1 summarises a number of previously published micro-oxygenation studies. A distinction
is made between pre- and post-MLF which likely correspond to structuring and harmonisation respectively.

**Harmonisation Phase**

The harmonisation stage commences when the structure of the wine has reached its peak intensity (figure 1-2). From this point structure softens and there is a marked increase in varietal aromas and overall aromatic complexity. This progression continues until a structural minimum is achieved at which point micro-oxygenation treatment should be ceased. Dosage rates are typically much lower than for the Structuring phase (1-10 mg/L/mth). The harmonisation phase can last from 3 weeks to six months depending on the type of wine.

**Over-oxygenation Phase**

Once the structural minimum is reached further treatment leads to the over-oxygenation stage characterised by an increase in astringency (often described as “dry” tannins) and an increase in oxidative aromas. Constant monitoring by the winemaker is imperative to ensure that the treatment is not carried over into the over-oxygenation stage.

### 1.1.2 Rate Dependence of Micro-oxygenation

An (apparently) important stipulation of micro-oxygenation is that the oxygen is introduced at a rate equal to or less than the oxygen uptake rate of the wine (Paul 2002b), therefore, no resulting buildup of dissolved oxygen is observed. Anecdotal evidence suggests that an excess of oxygen for any length of time is detrimental to the sensory properties and future development potential of the wine (Parish, Wollan et al. 2000; Cheynier, Atanasova et al. 2002; Paul 2002), thus the success of micro-oxygenation depends significantly on controlling the rate of oxygen exposure. Typical dosage rates are relatively small ranging from 2 to 90 mg of O₂ / litre of wine / month depending on the type of wine and the stage of maturation. A range of actual dosage rates quoted in published trials are summarized in Table 1-1. Note the order of magnitude difference between the dosage rates applied pre- and post-MLF.

An interesting corollary to the rate dependence of this process is the implication of an optimum dosage rate. Too little oxygen and no change in the wine is observed. Too much oxygen and the wine begins to deteriorate. What determines this optimum rate and,
importantly, can it be determined before treatment is commenced? This question forms the central theme of the research presented in this thesis.

<table>
<thead>
<tr>
<th>Table 1-1</th>
<th>Summary of published Micro-oxygenation dosage rates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grape Variety</td>
<td>Dosage Rate (mg/L/mth)</td>
</tr>
<tr>
<td><strong>Post MLF and SO₂ addition</strong></td>
<td></td>
</tr>
<tr>
<td>Cabernet Sauvignon</td>
<td>5-10</td>
</tr>
<tr>
<td>Cabernet Sauvignon</td>
<td>1-5</td>
</tr>
<tr>
<td>Shiraz</td>
<td>1-3</td>
</tr>
<tr>
<td>Shiraz</td>
<td>0.5-2</td>
</tr>
<tr>
<td>Cabernet Sauvignon</td>
<td>2-5</td>
</tr>
<tr>
<td>Cabernet Sauvignon</td>
<td>2</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Pre MLF and SO₂ addition</strong></td>
<td></td>
</tr>
<tr>
<td>Cabernet Sauvignon</td>
<td>60</td>
</tr>
<tr>
<td>Cabernet Sauvignon</td>
<td>60-90</td>
</tr>
<tr>
<td>Merlot</td>
<td>30-50</td>
</tr>
<tr>
<td>Cabernet Sauvignon</td>
<td>20-60</td>
</tr>
<tr>
<td>Merlot</td>
<td>30-60</td>
</tr>
<tr>
<td>Shiraz</td>
<td>20-60</td>
</tr>
<tr>
<td>Cabernet Sauvignon</td>
<td>20-60</td>
</tr>
<tr>
<td>Cabernet Sauvignon</td>
<td>20-60</td>
</tr>
<tr>
<td>Malbec</td>
<td>30-60</td>
</tr>
<tr>
<td>Cabernet Sauvignon</td>
<td>20-40</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Wine still on skins</strong></td>
<td></td>
</tr>
<tr>
<td>Cabernet Sauvignon</td>
<td>20-40</td>
</tr>
</tbody>
</table>
1.2 Research Objectives

As previously stated, the aim of the research work contained within this thesis is to gain insight into the dependence of oxygen dosage rate on the sensory and chemical transformations of red wine under micro-oxygenation treatment. The implication of the existence of an optimum oxygen dosage rate is intriguing in that it is not clearly understood what factors determine the rate. It is also recognised that controlled oxygenation is a physicochemical process and any consideration of dosage rate must include physical as well as chemical aspects (i.e. the interplay between spatial distributions and reaction kinetics, which is examined in Chapter 7) This is believed to be particularly important when using micro-oxygenation with large volumes of wine. From a chemistry perspective focus is given to the evolution of the wine polyphenols as these are the major oxidisable substrates and are known to contribute significantly to wine mouthfeel and colour. In terms of the physics, a comprehensive review of the various transport processes is given below combined with modeling in Chapter 7.

The thesis can be broadly categorised into three sections. The first section aims to investigate the effect of oxygen dosage rate on red wine under very controlled conditions. Analyses are made of both sensory and chemical transformations over time. Contained within this is a significant amount of method development and preliminary trial work performed on commercial treatments.

Section two investigates the use of electrochemical micro-oxidation of wine (ELMOX). This is a novel application that was developed at the University of Auckland and the results of the inaugural trial are given in Chapter Six.

The final section aims to investigate the spatial effects of the traditional micro-oxygenation treatments using multiphase computational fluid dynamics to model the oxygen transport and the effect of different physical configurations on the dosage rate and spatial gradients observed in the treatment vessel.
1.3 Review of Oxygen Mass Transfer and Mass Transport Processes

Micro-oxygenation is a physicochemical process, which can be categorised into three subprocesses as illustrated in figure 1-3. The first is concerned with delivery and dissolution of oxygen into the wine, the second with the transport of dissolved oxygen through the volume of wine contained within the maturation vessel. The final sub-process is concerned with the chemical reactions occurring between the oxygen and oxidisable substrates of the wine. All three sub-processes play a key role in the success of the treatment and will be reviewed at length below.

**Figure 1-3**  The three processes of micro-oxygenation

1.3.1 Oxygen Delivery Methods

What separates micro-oxygenation from traditional techniques such as aerative racking or air/oxygen sparging is that the dosage rate is controlled and metered (note definition above). Two methods have been developed to achieve this end, both of which are described below and are discussed in detail in Chapter Three:

Oxygen must be in the liquid phase for reactions with wine to occur. The equilibrium solubility of oxygen (from air) in wine (12% v/v EtOH) at standard temperature and pressure (25°C, 1 atm) is approximately 11 mg/L. In practice the bulk phase (wine) will not
approach this value, however it is important in determining the interphase mass transfer rates described below.

Two methods of oxygen delivery are currently in commercial use: the traditional micro-bullage or bubble-column oxygenation and more recently introduced technique using dense polymer membranes allowing oxygenation without bubbles.

### 1.3.2 Micro-bullage

Micro-bullage is, currently, the most widely employed technique for commercial micro-oxygenation and fully packaged units are available from several suppliers. Typically bubbles are sparged from a micro-porous diffuser that is suspended in the centre and in close proximity to the bottom of a wine storage vessel (refer figure 1-4). Oxygen is dissolved from the bubbles into the wine as they rise through the plume. The interphase transfer of oxygen is determined principally by the bubble size distribution or the interfacial area density (Mewes and Wiemann 2003).

The mass transfer of oxygen from the dispersed (gas) phase, into the continuous (wine) phase is best explained on the individual bubble level. The oxygen flux is described by the mass transfer equation

\[
\frac{dC_{O_2,w}}{dt} = -K_L a (C_{O_2,w}^* - C_{O_2,w})
\]  

[1.1]

where \( C_{O_2,w} \) is the bulk concentration of dissolved oxygen in the wine, \( K_L \) is the mass transfer coefficient and \( a \) is the contacting surface area between the bubble and the wine phase. The asterisk superscript on the first concentration term inside the parentheses refers to the concentration of dissolved oxygen on the liquid side of the interface (refer to figure 1.5). The driving force for the flow of solute is the concentration gradient shown schematically in figure 1-5. It is assumed that the concentration of dissolved oxygen in the bulk wine is maintained at zero \( (C_{O_2,w} = 0) \). The equilibrium concentration at the liquid side of the interface can be calculated using Henry’s law as

\[
C_{O_2,w}^* = \frac{P_{O_2}}{H_{O_2,w}}
\]  

[1.2]
Where \( P \) is the partial pressure on the gas side of the interface and \( H \) is Henry’s law coefficient.

1.3.2.1 Bubble Size

A key parameter in the mass transfer performance of a bubble plume is the bubble diameter. The rate of oxygen dissolution from the bubble into the wine can be influenced by the mass transfer coefficient, \( K_L \), and the specific interfacial area, \( a \) (defined as the ratio of total interfacial area divided by the volume of wine), as shown in equation [1.1]. The relationship between interfacial area and bubble size is obvious; the smaller the bubbles the greater the value of \( a \) for a given volume of liquid phase. \( K_L \) is also strongly related to bubble size as shown by figure 1-6 (Motarjemi and Jameson 1978).

**Figure 1-4**  a) Schematic representation of micro-bullage oxygen delivery system. b) close up photograph of a diffuser in water

**Figure 1-5**  Schematic representation of mass transfer across the bubble interface.
Below 150µm bubbles behave as solid spheres (i.e. no internal circulation of the gas phase within the bubble) and \( K_L \) shows a weak dependence on bubble diameter. Values of \( K_L \) between 2mm and 5mm can be explained using the penetration theory (Higbie 1935) where the internal atmosphere is allowed to circulate leading to a larger mass transfer coefficient. Between 150µm and 2mm there is a corresponding increase in \( K_L \). Motarjemi and Jameson (1978) postulate that this is a transition between the states described above. Bubbles above 5mm are characterised by highly deformable interfacial surfaces and as a result \( K_L \) is difficult to predict.

1.3.2.2 Determining Bubble size

In the bubbly flow regime the bubble size is generally a strong function of the orifice (diffuser pore) diameter and the surface tension (\( \sigma \)) of the solution and a weak function of the superficial gas velocity (Jamialahmadi and Mueller-Steinhagen 1992). A model was proposed by (Gaddis and Vogelpohl 1986) which shows good agreement with experimental results and is shown as a function of orifice diameter in figure 1-7. Most micro-oxygenation diffusers are typically of the order of 1-10 µm giving a range of bubble diameters (at the diffuser) of between 310 and 670 µm. Obviously bubble diameter will change as the bubble rises through the plume with the combined effects of hydrostatic pressure, desorption of oxygen and absorption of \( \text{CO}_2 \), water and ethanol vapour.
1.3.2.3 Terminal Rise Velocity

A bubble released from a diffuser pore will initially accelerate upwards through a column of liquid until the drag force on the surface of the bubble equals the buoyancy force, at which point the bubble is said to have reached terminal rise velocity. The terminal rise velocity is important to the overall aeration efficiency as this determines the amount of time the bubble has in the column before being expelled at the free surface. Unsurprisingly the rise velocity is strongly related to bubble size and liquid surface tension. Figure 1-8 shows the relationship using a model developed by (Jamialahmadi, Branch et al. 1994).

![Figure 1-7](image)

**Figure 1-7** Bubble diameter at the diffuser as a function of pore size for a 12% v/v ethanol solution, adapted from (Gaddis and Vogelpohl 1986)
1.3.2.4 Optimum Bubble Size

One way of defining the oxygenation efficiency of a bubble plume (with diffuser at a given depth) is to determine the initial bubble diameter such that when it reaches the surface it has lost a specified fraction of the oxygen to the wine (e.g. 99%). If the bubbles are too large there will be insufficient time for the oxygen transfer to occur. Conversely if the bubbles are too small the oxygen will be dissolved close to the diffuser thus reducing the spatial distribution of oxygen.

(Moterjemi and Jameson 1978) used this approach to develop a series of curves shown in figure 1-9 which were verified experimentally. These curves correct for changes in bubble diameter with depth and resulting changes in terminal rise velocity. For an initial bubble size of 0.3 mm all of the oxygen is dissolved into the liquid in approximately 0.65 m. It should also be noted that these curves were developed for oxygen and pure water and are likely to be optimistic for wine. The effect of ethanol on $K_L$ and bubble size is discussed below.
1.3.2.5 Surfactant Effects of Ethanol

Ethanol behaves as a surface-active agent in aqueous solutions, which has a significant effect on the overall transport properties (Kulkarni, Shah et al. 1987; Jamialahmadi and Mueller-Steinhagen 1992). There is a strong tendency for ethanol to accumulate at interfaces (i.e. bubble surfaces) (Adamson 1982) resulting in a decrease in liquid surface tension with increasing ethanol concentrations. These surface tension gradients at the bubble-liquid interfaces cause tangential stresses along the bubble surface suppressing motion and internal circulation within the bubble. The net effect is to retard $K_L$ relative to those for pure water given in figures 1-6 and 1-8. Smaller bubbles are affected to a greater extent due to the larger relative interfacial surface area.

The terminal rise velocity model shown in figure 1-8 accounts for the decreased surface tension due to ethanol and is in very good agreement with experimental results (Jamialahmadi and Mueller-Steinhagen 1992).

Bubble terminal velocity and mean bubble diameter as a function of ethanol mole fraction (12% v/v EtOH ~ 5% mole fraction) are shown in figure 1-10. It is notable that over the typical range of wine ethanol concentrations (3.6 – 5.6%) there is considerable variation in bubble diameter and thus $K_L$. 

---

**Figure 1-9** Proportion of $O_2$ transferred from bubbles containing pure $O_2$ only released at different depths from the surface of the liquid (pure water). The curves are for different initial bubble diameters (mm) from (Motarjemi and Jameson 1978).
In addition to decreasing bubble diameter, increasing concentrations of ethanol in aqueous solutions tend to decrease the incidence of coalescence for smaller bubbles and break-up for larger bubbles thus mitigating the associated decrease in $K_L$ described above. The decrease in surface tension and associated reduction in size makes the bubbles more stable (Jamialahmadi and Mueller-Steinhagen 1992).

1.3.3 Dense Polymer Membrane Oxygenation.

A significant limitation of gaseous oxygen diffusion is that a minimum height of wine above the diffuser is required for complete oxygen dissolution to occur. The use of oxygen-permeable dense-polymer tubing for the oxygenation of wine is a recent innovation with the commercial introduction of a system for barrels in 2003 (Kelly and Wollan 2003); similar claims of have been made by the suppliers of dense polymer (polyethylene) tanks (Flecknoe-Brown 2005) although, hitherto, neither system has been fully characterised in terms of oxygen transmission rates. The main advantage of this technique is that oxygenation occurs without bubbles being formed thus avoiding the need for tall storage vessels. Moreover the absence of bubbles ensures that desirable volatiles are not stripped from wine.


\[\text{Figure 1-10 Bubble terminal velocity and bubble diameter as a function of ethanol concentration, from (Jamialahmadi and Mueller-Steinhagen 1992)}\]
of a tube, is placed in the liquid and oxygen (or air) is passed through at a set pressure. Oxygen is absorbed into the polymer on the gas side and transported by diffusion to the liquid side where desorption of the oxygen into the liquid occurs. Significant limitations exist with this technique which are discussed in detail in Chapter Three.
1.4 The Role of Oxygen in Winemaking

Since the time of Pasteur, considerable research effort has gone into attempting to understand the chemistry of oxygen and wine. The increasing and wide spread use of micro-oxygenation since 1996 has encouraged oenologists to refocus on the role of oxygen in winemaking. Comprehensive reviews on oxygen in winemaking and specifically the interactions between polyphenols and oxygen have been recently published (Singleton 1987; Cheynier, Remy et al. 2000; Singleton 2000; Danilewicz 2003; Waterhouse and Laurie 2006). For the sake of completeness and context, the key points of these reviews will be summarised below.

It has been recognised for some time that the wine polyphenols are the primary substrate for oxidation (Singleton 1987) and the classical mechanisms of oxidative coupling reactions are well established (McDonald and Hamilton 1973). Current research focuses on the nature of the tannin-anthocyanin interactions and the corresponding effects on the organoleptic properties of the wine (Vidal, Cheynier et al. 2002; Vidal, Courcoux et al. 2003; Vidal, Francis et al. 2003).

While there has been considerable recent effort to understand the chemistry of oxygen and polyphenols, there has not, hitherto, been a commensurate effort to understand the importance of oxygen availability (i.e. a stoichiometric excess or deficit) on either reaction mechanism(s) or the sensory transformations (beyond winemaking observations). Some pioneering work was carried out by Jean Ribereau Gayon to measure the effect of oxygen uptake rate in red wines in France (Riberau-Gayon 1933). More recent work using model solutions has given some insight into kinetics of complex oxygen-polyphenol reactions (Tulythan, Boulton et al. 1989; Gilliers and Singleton 1991), however, these were done at either elevated pH or temperature and may not be indicative of the mechanisms observed under normal wine conditions.

1.4.1 Molecular Oxygen as the Primary Oxidant

The main oxidant of interest is molecular oxygen ($O_2$), which is reduced in a stepwise manner to water. Dioxygen has a rather complicated chemistry and in order to properly consider the oxidation of wine, an understanding of the principal mechanisms is essential.
The predominant naturally occurring (ground-state) molecule combines a high oxidising potential \( (E^o=1.229V) \) with a significant kinetic barrier to oxidation reactions, which will be discussed below (Ingraham 1985).

Ground state oxygen exists as a triplet; i.e. it contains two electrons with parallel spins in the \( 2p \) antibonding orbital \( (2\pi^*) \). The molecular orbital energy level diagram is given in figure 1-11. Reaction of triplet dioxygen \( (^3\Sigma_g) \) with singlet compounds is a spin forbidden process. The majority of organic compounds exist as singlets and do not have stable triplet forms required to react with \(^3\Sigma_g\text{O}_2\).

The kinetic barrier to oxidation by the \(^3\Sigma_g\) dioxygen, mentioned above is the result of two phenomena: firstly the triplet nature of \(^3\Sigma_g\) and secondly that ground state oxygen is difficult to reduce by one electron. Despite the relatively high redox potential of \(^3\Sigma_g\) it is difficult to add the first electron to form the superoxide anion \( (\text{O}_2^{-} + e^- \rightarrow \text{O}_2^- \ E^o= -0.16V) \). After one electron is added the bond length between the oxygen atoms increases, meaning the \( \pi \)-orbitals behave like lone pairs (Ingraham 1985).

Oxygen can also be activated to two singlet forms; the most active \(^1\Sigma_g\) (a single electron in each \( 2\pi^* \) orbital with paired spins) quickly decays to \(^1\Delta_g\) (both electrons in one \( 2\pi^* \) orbital with paired spins) which has a half life of 2 \( \mu \)sec deemed sufficient for many reactions (Ingraham 1985). Singlet oxygen can be produced by the action of light on photosynthesising dyes and certain chemical or enzymatic reactions. It is believed to be involved in the increased oxidation rate observed when wine is exposed to ultraviolet radiation, but is not thought to play a part in the “dark” autoxidation reactions.

\(^3\Sigma_g\text{O}_2\) can also be activated by complexing with a transition metal. The resulting complex is a singlet instead of a triplet. The metal adds electrons to the dioxygen and the antibonding \( \pi \) orbital behaves more like an electron pair thus reducing the barrier to electron acceptance (Ingraham 1985). This is believed to be the dominant mechanism in the coupled oxidation of polyphenols.
Introduction and Literature Review

One possible route for $^3\Sigma_g^-$ activation is believed to be phenolate ions. It has been observed, in numerous studies (Ingraham 1985; Singleton 2000), that in the presence of certain anions $^3\Sigma_g^-$ can accept one electron and be reduced to the superoxide free radical anion ($O_2^\cdot$). The phenolate ion would be consequently oxidised to its semi-quinone radical. A second reaction would presumably be required to remove the second electron and produce the quinone. The conjugate acid of the superoxide radical is the perhydroxyl radical (equation [1.3]). In protic aqueous solutions superoxide radicals have a strong tendency ($K_a$ at pH $7 = 4 \times 10^{30}$) to disproportionate to produce $H_2O_2$ and $O_2$ (which is recycled) according to reaction [1.4] and [1.5] (Ingraham 1985; Singleton 1987).

\[
O_2^\cdot + H^\cdot \rightleftharpoons HO_2^\cdot \quad \text{pK} = 4.69 \quad [1.3]
\]

\[
2HO_2^\cdot \rightleftharpoons H_2O_2 + O_2 \quad [1.4]
\]

\[
H_2O + HO_2^\cdot + O_2^\cdot \longrightarrow H_2O_2 + O_2 + OH^- \quad [1.5]
\]

Figure 1-11  Molecular orbital energy diagram for ground state ($^3\Sigma_g^-$) dioxygen

![Molecular orbital energy diagram for ground state ($^3\Sigma_g^-$) dioxygen](image)
The superoxide anion can also generate another very reactive species, the hydroxyl radical (OH•) through a reaction with H₂O₂ as given in equation [1.6] (Heikkila and Cohen 1973)

\[
O_2^\cdot + H_2O_2 \rightarrow OH^\cdot + OH^\cdot + O_2
\]  

[1.6]

Given that the proposed autoxidation reactions lead to the formation of both O₂• and H₂O₂, it seems likely that OH• is also generated.

Based on studies performed on catechol (1,2-dihydroxy benzene) a reaction scheme is proposed in figure 1-12 (Nanni et al. 1980; Heikkila and Cohen 1973; Singleton 1987). Reaction [1.7] and [1.11] account for the consumption of two atoms of oxygen per molecule of catechol and the corresponding formation of one molecule each of quinone and H₂O₂. The reaction rate also depends on the initial concentration of phenolate ions which is pH dependent; the rapid consumption of oxygen in alkaline conditions is thus explained. The reactions at wine pH (3-4) would be expected to give the same products but at a much slower rate (Singleton 1987).

The low concentration of phenolate ions would limit the reaction initially (reaction [1.7]). The superoxide reacts with phenol producing more phenolate ion (reaction [1.8]). Reactions [1.10] and [1.11] represent a chain sequence and could propagate as long as there remained semi-quinones available for reaction. Condensation reactions (section 1.4.2 and 1.4.3) and reaction [1.12] are competing mechanisms that limit and eventually terminate the reaction sequence.

As oxygen reacts with the phenolate ion (reaction [1.7]) the proximity of the newly formed superoxide to the adjacent hydroxyl group may allow almost simultaneous take up of the second electron and proton forming the quinone and H₂O₂ (reaction [1.12]). The net effect of this reaction scheme is that two superoxide radicals are produced, two atoms of oxygen are expended and one molecule each of quinone and H₂O₂ are produced.
Although this reaction seems feasible at pH 7 and above, is unlikely to go to completion at wine pH (Danilewicz 2003). Another more thermodynamically favourable reaction pathway involves iron as a catalyst to overcome the high activation energy barrier of the first electron transfer discussed above. Transition metals which can exist in various spin states are able to relieve the spin restrictions of ground state dioxygen thereby increasing the rates of polyphenolic oxidation. This is achieved by the formation of metal-phenol complexes.
With dioxygen present in solution the Fe$^{2+}$ ion is reoxidised back to Fe$^{3+}$ and the oxidation of orthodihydroxyphenol groups is allowed to continue provided that O$_2$ remains in solution as an oxidant. This reaction is strongly facilitated by the presence of organic acids (tartaric and malic) which form complexes with the iron (Danilewicz 2003) which tends to reduce the reduction potential of the Fe$^{2+}$/Fe$^{3+}$ couple and ensure that Fe$^{2+}$ is reoxidised quickly. A summary of the overall oxidation process is given in figure 1-13. Iron mediated oxidation of catechol is very pH dependent; at pH 6-7 Fe$^{3+}$ is no longer susceptible to oxidation by catechol (Jovanovic, Simic et al. 1998) and has been found to stop for pH > 5 (Danilewicz 2003).

One recent study clearly demonstrated the effectiveness of particularly copper (Cu$^{2+}$) and iron (Fe$^{3+}$) as oxidation catalysts of procyanidins compared to enzymes (Vivas de Gaulejac et al., 2001). The experiments were carried out on model solutions at wine pH (3.2), thus being indicative of mechanisms observed in wines.

![Figure 1-13](image-url)  
Figure 1-13  Summary of overall chemical oxidation process in wines from (Danilewicz 2003)

1.4.2 The Production and Role of Ethanal

It has been demonstrated for some time that coupled oxidation of polyphenols leads to the formation of ethanal (Wildenradt and Singleton 1974) due principally to the production of hydrogen peroxide as one of the products of the reoxidation of iron (figure 1-13). The significance of ethanal in the context of micro-oxygenation however is that it has been shown to play a role in the both tannin-tannin and tannin-anthocyanin condensation reactions (Timberlake and Bridle 1976; Baranowski and Nagel 1983; Dallas, Ricardo-da-Silva et al. 1996; Fulcrand, Doco et al. 1996; Francia-Aricha, Guerra et al. 1997; Saucier, Bourgeois et al. 1997; Saucier, Little et al. 1997b; Fulcrand, Carime et al. 1998; Es-Safi, Fulcrand et al. 1999; Es-Safi, Fulcrand et al. 1999b; Cheynier, Remy et al. 2000; Mateus, Pasual-Teresa et al.)
2002; Vivar-Quintana, Santos-Buelga et al. 2002; Atanasova, Fulcrand et al. 2002b; Mateus, Silva et al. 2002b; Salas, Fulcrand et al. 2003). The proposed reaction mechanisms are presented in figure 1-14.

Ethanal induced polymerisation reactions are important as they are believed to lead to more stable pigments. Notably the production of vinyl linked pigments (Mateus, Pasual-Teresa et al. 2002; Mateus, Silva et al. 2002b) which have been elucidated in port and model wines. The proposed mechanism, described in figure 1-15, depends on the ethanal addition outlined in figure 1-14.

Despite the considerable research that has gone into exposing the various compounds associated with ethanal induced polymerisation the quantitative significance of this mechanism is not fully established. Two recent studies performed on actual wines (as opposed to model solutions) failed to detect any products resulting from ethanal induced polymerisation as predicted (Atanasova, Fulcrand et al. 2002; Hayasaka and Kennedy 2003).

1.4.3 Direct Condensation of Flavanols and Anthocyanins

Recently developed analytical techniques have enabled the study of red wine tannin using various forms of mass spectrometry. An excellent summary of the techniques and the elucidated phenolic structures has just been published (Monagas, Bartolome et al. 2005) and the reader is referred to this review for further insight.

These direct condensation reactions are essentially addition reactions and can occur without the presence of oxygen. The products are generally defined as T-A (tannin-anthocyanin) or A-T (anthocyanin-tannin) types depending on which position the anthocyanin forms a bond with the tannin. T-A condensation is characterised by the nucleophilic addition of an anthocyanin (in the hemiketal form at the C6 or C8 position) to the C4 position of a flavanyl-4-carbocation formed by acid hydrolysis of the interflavanic bond of a condensed tannin molecule. This mechanism is described in figure 1-16. In contrast the A-T condensation product is characterised by the nucleophilic attack of the tannin (in either C6 or C8 position) by the electrophilic C4 position of the anthocyanin in the cation form. It is postulated that the A-T dimer either proceeds to a xanthylum salt, a colourless bicyclic form of the dimer (Remy-Tanneau, Le Guerneve et al. 2003) or undergoes an oxidation step to recover the
flavylium form of the anthocyanin moiety. The respective A-T mechanisms are also described in figure 1-17.

The role of oxygen in this type of reaction is limited to the recovery of the flavylium form of the anthocyanin in both the cycloadDITION pathway and the direct A⁺-T condensation reactions. Unsurprisingly the relative abundance of each of the species is dependant on the wine pH (Salas, Fulcrand et al. 2003) with the T-A reaction dominating at low pH and the A-T more prevalent at higher pH.

1.4.4 The Effect of Oxidation on Colour Stability

Two of the principal benefits of micro-oxygenation (refer to section 1.1.1) are to “soften” tannins (i.e. reduce astringency or “tannic-force”) and stabilise colour. Both of these result directly from a chemical modification of the polyphenols in the wine. These will be the main focus of the research contained within this thesis.

Colour loss in red wines is generally attributed to a degradation of anthocyanins into non-pigmented compounds (e.g. bisulfite bleaching (reversible), A-T cycloaddition type condensation reactions and other less well understood phenomenon). It is preferential for red wines that certain condensation reactions occur early to promote more stable pigmented tannins (such as those described above). It has been observed for some time that more stable wine pigments develop over time both during maturation and bottle aging (Somers 1971; Riberau-Gayon and Glories 1986)

Numerous pigments have been characterised in wines and model solutions by researchers in recent times. These are summarised in the review cited above (Monagas, Bartolome et al. 2005). The relative importance of the respective compounds however is unclear and the exact structure of many of the pigmented polymers is yet to be resolved. The role of oxygen in the stabilisation of colour is somewhat indirect via the formation of stable pigments either through ethanal linked condensation or direct condensation. What is unknown is the ideal dosage rate to accelerate these colour stabilising pathways without doing irreparable damage to the wine (Cano-Lopez, Pardo-Minguez et al. 2006; Llaudy, Canals et al. 2006).
Figure 1-14  Proposed mechanism for aldehyde induced condensation of Tannin-Tannin and Tannin-Anthocyanin reactions, adapted from (Cheynier 2000)
Figure 1-15  Proposed mechanism for the formation of vinyl linked pigments, from (Mateus et al. 2002)
Figure 1-16  Proposed reaction mechanisms for the T-A type direct condensation of anthocyanins and proanthocyanidins, from (Cheynier, Remy et al. 2000)
Figure 1-17  Proposed reaction mechanisms for the A-T type direct condensation of anthocyanins and proanthocyanidins, from (Cheynier, Remy et al. 2000)
1.4.5 The effect of Oxygen on the Evolution of Sensory Properties

It has been recognised for some time that polyphenols are one of the principal contributors to astringency in natural products (Haslam 1998). Astringency on the palate is a tactile sensation that has been generally defined as drying, puckering, rough and constricting (Haslam 1998; Vidal, Francis et al. 2003). The sensation is thought to be caused by selective cross linking by the polyphenol substrates with the proline rich proteins in the saliva decreasing the effective lubrication on the inside of the mouth. Astringency in its various forms (descriptors such as: “fine”, “coarse”, “chalky” and “pucker” have been applied to wine (Vidal, Cheynier et al. 2002)) is a significant sensory component of red wines and is generally considered undesirable if present with any significant intensity – particularly “coarse” or “rough” astringency (Peynaud 1996).

Recent work has shown that astringency appears to be a function mainly of tannin length (Vidal, Francis et al. 2003). Moreover a direct correlation was observed between degree of galloylation and the coarseness of the tannins, whereas trihydroxylation of the B-ring was found to decrease the amount of coarseness. Anthocyanins and the pigmented polymers described above were found not to contribute significantly to astringency whereas both ethyl-linked flavanols and proanthocyanidins were directly correlated (Vidal, Francis et al. 2004). This finding is significant in that it may explain why the synthesis of pigmented polymers, formed in competition with the tannin adducts is important, not only for stabilising colour but also in reducing the intensity of astringency perception. This result highlights the importance of anthocyanin to tannin ratio. Other studies correlate astringency to total phenolics and (to a lesser extent) copigmented anthocyanins (Cliff, Brau et al. 2002).

Bitterness, a taste linked to the sensory receptors on the tongue, has also been directly linked to the presence of polyphenols (Fischer and Noble 1994). Monomeric and oligomeric flavanols are typically attributed to having the most intense perception of bitterness compared with longer chain proanthocyanidins (Haslam 1998).

With both astringency and bitterness however other wine components show significant influence on perceived intensities. Notably increasing ethanol concentration appears to increase bitterness perception (Fischer and Noble 1994; Vidal, Courcoux et al. 2003) and
astringency intensity decreased with increasing concentrations of polysaccharides (Vidal, Courcoux et al. 2003). These potentially significant interactions make the effect of different treatments on the sensory properties of wine problematic from an experimental design point of view.

In the context of better understanding the micro-oxygenation treatment, the importance of understanding and quantifying the sensory changes that occur cannot be overstated. The single overriding goal of micro-oxygenation is to improve the organoleptic properties of a wine and any changes to wine chemistry should be considered relative to sensory transformations.
CHAPTER TWO
Preliminary Commercial Trial and Method Development

2.1 Introduction

As part of preliminary investigations into the effects of controlled oxygenation of wine during maturation, a suite of analyses (described below) was performed periodically on a commercial wine undergoing micro-oxygenation (MOX) treatment compared to the same wine stored in a separate vessel without oxygen. The principal aim of the experiment was to validate the anecdotally derived organoleptic development (figure 1-2) using formal sensory analysis and to attempt to correlate those sensory transformations with chemical changes, specifically changes to the polyphenolic profile. A secondary aim was to develop the chemical and sensory protocols to apply to subsequent, more controlled trials. These methods are fully detailed in this chapter.

2.2 Trial Setup

2.2.1 Grapes

Cabernet Sauvignon is a red Vitis Vinifera variety typically used in micro-oxygenation treatments. The grapes were machine-harvested at commercial maturity between the 18th and 26th April 2003 from three vineyard blocks (Ellingham Heavy, Ellingham Light and Cotton) in the Hawkes Bay, New Zealand.

2.2.2 Wine Processing

The grapes were all crushed, destemmed and fermented on skins in stainless steel tanks. The wines were inoculated immediately (i.e. no significant pre-fermentation maceration was
undertaken) with various commercial yeast strains and the ferments ran to dryness within 10 days. Additional sugar was added in the form of crystal sucrose (Chelsea, New Zealand) to bring the effective concentration of soluble solids (°brix) up to 22. Cap management consisted of twice daily pump-overs where nominally 50% of the liquid volume in each of the fermentation vessels was displaced.

After 18 days of post fermentation maceration the wine was removed from the pomace, initially by draining the free-run fraction then by pressing using a side membrane type press (Diemme AR150, maximum membrane pressure 1 bar).

On 15th May 2003 the three components were mixed to create a single blend to undergo micro-oxygenation (approximately 45,000 litres). Malo-lactic fermentation in all cases was observed to have commenced spontaneously and was tracked through to completion before the wines were blended. Once MLF had been completed all wines were dosed with SO₂ to a concentration of 30 mg/L.

Oxygenation was commenced on 17th May 2003 at a rate of 4 mg/L/mth. The micro-oxygenation apparatus is shown in figure 2-1. This system was developed locally and the regulation is based on precision flowmeters (Matheson) as shown in figure 2-1a). The oxygen is diffused into the wine using sintered stainless steel diffusers with a mean pore diameter of approximately 10 μm. In addition to the oxygenation treatment oak chips (Boise, France) were added at a concentration of 2 g of oak per litre of wine to the 45,000 litre blend (hereinafter referred to as the treatment wine). Prior to the commencement of the treatment, 100 litres was decanted off into two 50 litre vessels to be used as a control (hereinafter referred to as the control wine). No oak additions were made to the control wine.
2.2.3 Wine Sampling

For this trial the wines were sampled every 14 days into 750 ml wine bottles. The bottles were initially filled with CO$_2$ to displace the air and, in the case of the treatment wine, filled from the sample valve at a height of approximately 1.6 m from the base of the tank. The control wine was filled by syphoning from the top of the 50 litre storage vessel through a 12 mm polypropylene tube. Two bottles of each of the treatment and control wines were collected, dispatched to the University of Auckland wine science lab and stored at 4°C until sensory and chemical analysis could be performed.

For logistical reasons all sampled wines were consolidated under storage and both the sensory and chemical analyses were performed simultaneously over a two day period. The trial spanned ten weeks (i.e. four treatment samples, four control samples and one baseline sample). The analyses were conducted on the 22$^{nd}$ and 23$^{rd}$ July 2003.
2.3 Method Development

2.3.1 Monomeric Polyphenols

Analysis of the monomeric (also dimeric and trimeric) polyphenols was undertaken using a reverse phase (RP) HPLC-DAD method developed at the University of Auckland (Zou 2001). Four wavelengths ($\lambda = 280$, 320, 365 and 520 nm) were analysed to give a comprehensive profile of the monomeric and oligomeric polyphenol fraction.

The samples were run through an Agilent 1100 HPLC (diode array detector – G1315B, column heater G1316A, Auto-sampler – G1313A, quaternary pump – G1311A) using a Luna C18(2) 250mm x 4.6mm x 5µm column (Phenomenex); the acquisition software was Chemstation for LC 3D Rev A.10.02 (Agilent Technologies). The gradient elution profile for the method is given in table 2-1 the respective solvents correspond to: A = H$_2$O, B = H$_2$O + 5% CH$_3$COOH (Scharlau), C = CH$_3$CN (Unichrom). All solvents were HPLC grade and membrane filtered to 0.22 µm prior to use.

The wine aliquots were prepared, using freshly sampled wine, by filtering through a 0.45 µm syringe filter (Sartorious Minsart RC-4) into 1.5 ml amber HPLC (Agilent) vials and sealed with screw caps and PTFE septa (Agilent). The samples were run immediately following preparation.

An example of the HPLC traces at the different wavelengths is given in figure 2-2. The previously identified peaks (Zou 2001) are indicated on each of the traces.
Figure 2-2  HPLC traces for a Cabernet Sauvignon wine. Main peak are identified as: 1=gallic acid, 2 = t-caftaric acid, 3 = s-gluthionylcaftaric acid (GRP), 4 = cis-coumaric acid, 5 = t-coumaric acid, 6 & 7 = unknown flavanols possibly B, and B, respectively, 8 = catechin, 9 = caffeic acid, 10 = delphinidin-3-glucoside, 11 = epicatechin, 12 = petunidin-3-glucoside, 13 = peonidin-3-glucoside, 14 = malvidin-3-glucoside, 15 = unidentified (61.7 mins), 16 = p-coumaric acid, 17 = quercetin-3-glucoside, 18 & 19 = unknown flavonols, 20 = quercetin.
Table 2-1 Gradient elution profile for evaluation of monomeric polyphenols where: 
\[ A = \text{H}_2\text{O}, \quad B = \text{H}_2\text{O} + 5\% \text{CH}_3\text{COOH} \quad \text{and} \quad C = \text{CH}_3\text{CN}. \]

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>A%</th>
<th>B%</th>
<th>C%</th>
<th>Flowrate (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>45</td>
<td>55</td>
<td>0</td>
<td>0.8</td>
</tr>
<tr>
<td>10.0</td>
<td>25</td>
<td>75</td>
<td>0</td>
<td>0.8</td>
</tr>
<tr>
<td>20.0</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>0.8</td>
</tr>
<tr>
<td>30.0</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>0.8</td>
</tr>
<tr>
<td>50.0</td>
<td>0</td>
<td>90</td>
<td>10</td>
<td>0.8</td>
</tr>
<tr>
<td>70.0</td>
<td>0</td>
<td>85</td>
<td>15</td>
<td>0.8</td>
</tr>
<tr>
<td>80.0</td>
<td>0</td>
<td>80</td>
<td>20</td>
<td>0.8</td>
</tr>
<tr>
<td>95.0</td>
<td>0</td>
<td>60</td>
<td>40</td>
<td>0.8</td>
</tr>
<tr>
<td>105.0</td>
<td>0</td>
<td>55</td>
<td>45</td>
<td>0.8</td>
</tr>
<tr>
<td>115.0</td>
<td>45</td>
<td>55</td>
<td>0</td>
<td>0.8</td>
</tr>
<tr>
<td>125.0</td>
<td>45</td>
<td>50</td>
<td>0</td>
<td>0.8</td>
</tr>
</tbody>
</table>

2.3.2 Chemometric Analysis of RP-HPLC data

A method was required to systematically analyse the many HPLC chromatograms in order to identify patterns of degradation and accumulation between and within each treatment over the course of the trial. The use of multivariate statistical techniques such as principal components analysis (PCA) has become increasingly popular for the purposes of exploratory analysis of spectrometric and chromatographic data sets such as those acquired for this study (Esbensen 2002). The advantage of PCA in this application was that the entire data set can be analysed. The traditional method of integrating discrete peaks can cause significant errors due to co-elution and variation in baseline absorbance. These effects are mitigated and even eliminated using the PCA approach.

PCA was applied to each set of chromatograms (a baseline sample (i.e. a sample that was collected before the oxygenation treatment was begun) plus two treatments x four sampling dates) and each wavelength (280, 320, 365 and 520 nm). The aim of this analysis was, in the first instance, to gain a geometric intuition of the “distance” between the respective treatments and sampling times (scores). Another useful output of the analysis allows the identification the chromatographic peaks largely responsible for those differences in the respective wines (loadings). A detailed explanation of PCA, in the context described above, is given in several recent texts (Esbensen 2002; Brereton 2003).
2.3.3 Peak Alignment Pre-Processing

A particular problem with HPLC chromatograms is the relatively large shifts in time that can occur between respective chromatographic peaks from run to run. This is due, mainly, to small but significant variations in temperature and solvent concentrations. The effect, when applied to the PCA analysis described above is to reduce the overall resolution of the resulting analysis and thus the ability to detect small changes in the chromatographic profile. To overcome this problem some initial pre-processing of the data was required.

The pre-processing essentially involved mutually aligning the peaks along a common time axis. Numerous methods have been developed to achieve this with varying degrees of success (Wang and Isenhour 1987; Vest Nielsen, Cartensen et al. 1998; Fraga, Prazen et al. 2001; Byland, Danielsson et al. 2002; Pravdova, Walczak et al. 2002; Torgrip, Aberg et al. 2003; Idborg, Per-Olof et al. 2004). The most appropriate technique of those considered, for this application, was correlation optimised warping (COW) which is a piecewise warping method (Vest Nielsen, Cartensen et al. 1998). Alignment is achieved by linear stretching and compressing of sequential data segments of predefined length. The optimal alignment is determined by correlation of the individual data segments with a reference chromatogram. The algorithm detailed by Vest Nielson (1998) by was converted into code using Matlab® (v7.0.1 R14). All chromatograms were aligned relative to the baseline sample. The general process of alignment is given in figure 2-3. An example of the chromatograms before and after alignment processing is given in figures 2-4 and 2-5.

Each individual chromatogram signal of around 18,000 data points (for a particular sample and wavelength) was exported from the LC acquisition software as a comma separated text file (.csv). Each file (corresponding to one chromatogram was imported directly into Matlab® as a column vector containing absorbance value only (no corresponding retention time information was necessary for the alignment). After alignment the chromatograms were collected into a single matrix and plotted against the time axis for the reference chromatogram (baseline sample).
Chapter Two

Figure 2-3  Process flow for time-wise alignment of chromatographic peaks

Figure 2-4  Nine unaligned chromatograms of red wine at 520nm using gradient elution described in table 2-1
After a visual inspection, to check that all peaks were mutually aligned, the matrix of processed chromatograms was exported for principal components analysis. The PCA was performed using The Unscrambler® v9.1. As the PCA model is only able to process two dimensional data sets the respective chromatograms of different wavelengths of interest are appended sequentially (in time) in the manner shown in figure 2-6.

![Chromatograms](image)

**Figure 2-5** The same nine chromatograms as in figure 2-4 after alignment

In addition to the PCA approach described above, the traditional peak area integration was also performed and the evolution of individual phenolic compounds was tracked for each treatment. Where possible phenolic standards (Sigma Aldrich) were used to generate standard curves; these are given in appendix I. Where standard curves have been used concentrations are given in mg/L, otherwise raw peak area is stated.
2.3.4 Sample Fractionation Procedure

The oligomeric and polymeric phenol fractions were separated with Sephadex LH-20 gel (Amersham Biosciences) using a method adapted from (Kantz and Singleton 1990; Kantz and Singleton 1991). In order to batch process samples, 16 glass columns (internal diameter = 5 mm x 200 mm) were fabricated and connected to a 16-port vacuum manifold (Alphatech) with silicone tubing (internal diameter = 1.57 mm). The Sephadex was swelled and packed in the columns according to the manufacturer’s recommendation.

The columns were equilibrated using acidified water (0.2% acetic acid) in the manner described by Kantz (1990). Initially, the ethanol was removed from the 5 ml wine samples by rotary evaporation. The remaining volume of each sample was then loaded directly on to the column by gravity only (i.e. no vacuum pressure was applied to the manifold). After the samples were loaded, the monomeric polyphenol fraction was washed with approximately 40 ml (or 10 column volumes) of 60:40 methanol:water. Finally the polymeric fraction was eluted with 20 ml (5 column volumes) of 1:1 acetone:water solution. All solvents contained 0.2% acetic acid to minimise dissociation of the phenolic acids. For the washing and elution steps, again, only gravity was used to drive the flow. Even small vacuum pressures were found to cause channelling and the process was far less efficient than using only gravity.

Initially, to confirm the entire monomeric fraction was being washed from the column in the volumes stated above, a UV (280nm) detector was connected, in-line, to monitor absorbance at the output of the column. In all trials UV absorbance had returned to baseline values after 5 column volumes. Further corroboration was gained by running the polymeric fraction through RP-HPLC (described in section 2.3.1). Verification of complete elution of the
polymeric fraction was more complicated as the acetone in the elution solvent interfered in the UV region. The output fractions (0-20 ml, 20-30 ml, 30-40 ml) were collected and each analysed using Folin Ciocalteau procedure which established that all of the polymeric material had been eluted using less than the 20ml of solvent used for the method.

The monomeric polyphenol fraction was discarded and the polymeric material collected and taken to near-dryness by rotary evaporation (water bath maintained at 30°C). The near-dry sample was made up to 5 ml with methanol, sealed in a 5 ml polycarbonate vial and stored at -10°C until required for thiolysis.

2.3.5 Thiolysis

Numerous techniques have been developed to analyse proanthocyanidins, these have been the subject of excellent recent reviews (Lazarus, Adamson et al. 1999; de Beer, Harbertson et al. 2004; Monagas, Bartolome et al. 2005). One technique which has been utilised perhaps more than any other is thiolysis, principally, it seems, because the results are able to be quantified. In the context of the studies described in this thesis, the ability to compare results is of considerable importance to assess whether a treatment is effective or not.

As stated above, thiolysis is a technique used for the analysis of condensed tannins. The general principle is to break the proanthocyanidin down in to its constituent units (flavanols). All of the subunits, with the exception of the one located at the terminal position of the tannin (terminal unit), will form a flavanyl-4-carbocation following hydrolysis and these units are generally termed extension units. The electrophilic site on the carbocation (usually in the number 4 position of the C ring) is rapidly quenched by the addition of an aggressive nucleophile (in this case toluene-α-thiol, however phloroglucinol is also used in this capacity). The terminal units will not undergo the same addition reaction as the extension units and remain as flavanol monomers. The mechanism is described in figures 2-7 and 2-8. By quantifying the molar concentrations of extension units and terminal units (using HPLC), and assuming the proanthocyanidins have been entirely broken down, an estimate of the mean tannin length (or mean degree of polymerisation (mDP)) can be made using the following formula:
Chapter Two

\[ \sum [\text{Extension units}] + \sum [\text{Terminal subunits}] \]
\[ \sum [\text{Terminal subunits}] \]

[2.1]

Using this technique insight can also be gained into the component makeup of the condensed tannin, particularly the degree of galloylation and the degree of trihydroxylation of the “B” ring of the flavanol monomer, which have been observed to affect sensory properties in wine. (Section 1.4.5).

While Thiolysis gives indicative results it should be pointed out that the mDP has some significant limitations and care should be taken not be over-interpret the results. The main issue relates to the relative low and variable molar yield of the thiolysis products obtained from the proanthocyanadin extract. This low conversion rates have been clearly demonstrated in two studies (Kennedy, Matthews et al. 2000; Kennedy and Jones 2001) where on a mass basis, yields (i.e. the proportion of proanthocyanadin fraction undergoing thiolytic cleavage) vary from as little as 56% up to 80.1%. This may be as a result of the overall reliability of the method but may also be due to an increase in the oxidatively cross-linked polymers as discussed in section 1.4.4 and Figure 1-17. Any interpretation of changes in mDP (either between or within treatments) or the relative proportion of constituent units should thus be done with respect to the ratio given in [2.1] and the relative yield of thiolyzed material from a given mass of proanthocyanadin extract.
Figure 2-7  Hydrolytic cleavage of a proanthocyanidin
2.3.6 Optimising Hydrolysis Conditions

The numerous studies conducted using thiolysis methods have stated a broad range of hydrolysis conditions summarised in tables 2-2. An observation is that the environment was different in each case and the hydrolysis conditions must be optimised accordingly. The aim of the hydrolysis step, in the thiolysis process, is to cleave only the interflavan bonds and leave the proanthocyanidin constitutive units intact. If the conditions are too weak the proanthocyanidins will not be completely broken down; too much time and/or temperature the individual flavonoid units will begin to degrade. In reality a set of conditions where both can be achieved is unlikely, however conditions can be enhanced to give the best compromise.

Table 2-2 Summary of thiolysis conditions in recently published studies

<table>
<thead>
<tr>
<th>Reference</th>
<th>Acid</th>
<th>Acid Conc</th>
<th>Hydrolysis Time (mins)</th>
<th>Hydrolysis Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Rigaud, Perez-Ilzarbe et al. 1991)</td>
<td>H$_2$SO$_4$</td>
<td>2%</td>
<td>15, 30, 45, 60, 90</td>
<td>60</td>
</tr>
<tr>
<td>(Prieur, Rigaud et al. 1994)</td>
<td>HCl</td>
<td>0.2 M</td>
<td>2</td>
<td>90</td>
</tr>
<tr>
<td>(Sun, Conceicao et al. 1998)</td>
<td>HCl</td>
<td>0.2 M</td>
<td>2</td>
<td>90</td>
</tr>
<tr>
<td>(Atanasova, Fulcrand et al. 2002)</td>
<td>HCl</td>
<td>0.2 M</td>
<td>300</td>
<td>60</td>
</tr>
<tr>
<td>(Monagas, Carmen et al. 2003)</td>
<td>HCl</td>
<td>0.2 M</td>
<td>7</td>
<td>55</td>
</tr>
</tbody>
</table>
A set of experiments were run to establish the best hydrolysis conditions for the red wine under treatment. The thiolysis procedure is described in section 2.3.7. For all trials a standard thiolysing reagent was used and hydrolysis conditions were varied by changing the length of time the sample was held at the hydrolysis temperature (60ºC). Thiolysis was performed on the same wine using increasingly longer periods at : 1, 2, 5, 10, 18, 24 and 37 hours.

The results of the optimisation experiments are shown in figure 2-9. Catechin and epicatechin terminal unit concentrations peaked around 2.5 hours, similarly for catechin-4-benzylthioether (an extension unit). Epicatechin-4-benzylthioether, epicatechin-3-O-gallate and epicatechin-3-O-gallate-4-benzylthioether concentrations peaked sometime later at around 10 hours. The differences in the peak concentrations are believed to be due to the relative positions of the constitutive units within the proanthocyanidin polymer. Based on the above data, five hours was determined to be the best hydrolysis conditions to be used for the micro-oxygenation trial analyses.
2.3.7 Thiolysis Procedure

The polymeric wine fraction was isolated using Sephadex LH-20 as described in section 2.3.4. The thiolysis reagent, for all trials, consisted of 0.2 M HCl, toluene-\(\alpha\)-thiol (5%) made up in methanol. 400 µl of thiolysis reagent was added to an equal volume of polymeric wine fraction. The sample was then sealed in a 2 ml amber glass ampoule and placed in a heating block at 60°C for five hours. The ampoules were stored at 4°C immediately after removal from the heating block. Prior to running on the HPLC, the samples were filtered (0.45 µm Minisart RC-4 syringe filters) into 1.5 ml HPLC vials and sealed using a PTFE screwcap/septum configuration (Agilent Technologies).

The HPLC and column configuration used for evaluating the thiolysed samples was identical to that described in section 2.3.1. The gradient elution profile is given in table 2-3. Solvents A, B and C correlate to H\(_2\)O, H\(_2\)O + 5% CH\(_3\)COOH (Scharlau) and CH\(_3\)CN (Unichrom) respectively. The injection volume used was 20 µl and the column temperature was set at 30°C.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>A%</th>
<th>B%</th>
<th>C%</th>
<th>Flowrate (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>48</td>
<td>48</td>
<td>4</td>
<td>1.0</td>
</tr>
<tr>
<td>0.0</td>
<td>48</td>
<td>48</td>
<td>4</td>
<td>1.0</td>
</tr>
<tr>
<td>0.0</td>
<td>48</td>
<td>48</td>
<td>4</td>
<td>1.0</td>
</tr>
<tr>
<td>0.0</td>
<td>48</td>
<td>48</td>
<td>4</td>
<td>1.0</td>
</tr>
<tr>
<td>0.0</td>
<td>48</td>
<td>48</td>
<td>4</td>
<td>1.0</td>
</tr>
</tbody>
</table>

A typical HPLC chromatogram is presented in figure 2-11. The identified peaks are also indicated on the chromatogram. This particular example is a young wine made from Cabernet Sauvignon grapes, prior to any oxygenation treatment.
Peaks for catechin, epicatechin and epicatechin-3-O-gallate were verified from literature (Prieur, Rigaud et al. 1994), by standards and by LC-MS. Likewise, the thioether peaks (4, 5, 6, 7 and 8 from figure 2-10) were verified from literature (Prieur, Rigaud et al. 1994) and from LC-MS run at the University of Auckland. The two catechin-4-benzylthioether peaks (5 and 6 in figure 2-10) correspond to two diastereoisomers as shown in figure 2-11. Table 2-4 summarises the peaks used to calculate the degree of polymerisation outlined in section 2.3.5.
Table 2-4  Summary of proanthocyanidin constitutive units used to calculate from degree of polymerisation

<table>
<thead>
<tr>
<th>Phenolic Unit</th>
<th>Peak</th>
<th>Retention Time / mins</th>
<th>Verification</th>
<th>m/z * [M+H]+</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Terminal Units</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>catechin</td>
<td>1</td>
<td>12.1</td>
<td>Standard, LC-MS</td>
<td>290.1</td>
<td>(Prieur, Rigaud et al. 1994)</td>
</tr>
<tr>
<td>epicatechin</td>
<td>2</td>
<td>15.1</td>
<td>Standard, LC-MS</td>
<td>290.1</td>
<td>(Prieur, Rigaud et al. 1994)</td>
</tr>
<tr>
<td>epicatechin-3-O-gallate</td>
<td>3</td>
<td>20.1</td>
<td>Standard, LC-MS</td>
<td>442.1</td>
<td>(Prieur, Rigaud et al. 1994)</td>
</tr>
<tr>
<td><strong>Extension Units</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>epigallocatechin-4-benzylthioether</td>
<td>4</td>
<td>30.4</td>
<td>LC-MS</td>
<td>428.1</td>
<td></td>
</tr>
<tr>
<td>catechin-4-benzylthioether</td>
<td>5&amp;6</td>
<td>34.4 &amp; 34.7</td>
<td>LC-MS</td>
<td>412.1</td>
<td>(Prieur, Rigaud et al. 1994)</td>
</tr>
<tr>
<td>epicatechin-4-benzylthioether</td>
<td>7</td>
<td>35.3</td>
<td>LC-MS</td>
<td>412.1</td>
<td>(Prieur, Rigaud et al. 1994)</td>
</tr>
<tr>
<td>epicatechin-3-O-gallate-4-benzylthioether</td>
<td>8</td>
<td>38.0</td>
<td>LC-MS</td>
<td>564.1</td>
<td>(Prieur, Rigaud et al. 1994)</td>
</tr>
</tbody>
</table>

* determined using LC-MS with MS in positive ion mode using ESI source and TOF detector

Figure 2-11  The two stereoisomers of catechin-4-thioether as observed in figure 2-10

Standard curves for the thioether derivatives were generated by collecting relevant fractions from 40 thiolysis runs removing any organic solvent by rotary evaporation and freeze drying to remove the water. The mass of the dry residue was recorded then the stock solution was prepared by dissolving the fraction using 1 ml of methanol as the solvent. Serial dilutions
were then made and the samples run on the HPLC using the method described above. The resulting standard curves are given in Appendix II (Tables A-2 and A-3).

2.3.8 Sensory Analysis
Quantitative descriptive analysis of a range of sensory attributes was carried out concurrently with chemical analysis at each sampling date. A trained panel of 12 volunteer members was asked to discriminate the wines based on several organoleptic parameters listed in table 2-5. The panellists were selected from people who consume wine on a regular basis. They were initially screened on their ability to perceive certain basic sensory attributes (refer to table 2-5). After selection, training was conducted twice weekly for four months prior to actual data collection. The training was established based on the advice of the Hort Research Food Sensory Group (Lund 2003)

Training consisted initially of triangle testing of individual sensory standards in water (three sets per session on any attribute listed in table 2-5). The aim of this training phase was to improve the cognitive ability and confidence of the panelists to recognise the sensory character of each attribute. The triangle testing of standards was followed by triangle testing of wines (Mission Estate Wines Cabernet Sauvignon 2001) spiked with one or more of the standards. During these sessions the panellists were asked to develop an appropriate terminology for the attributes (refer to table 2-5).

After the panelists became comfortable in being able to detect the spiked wines in a triangle test context they were then asked to rank each attribute on a 100 mm horizontal line (the left and right edge of the line denoting the absence and the extreme presence of the attribute respectively – refer to appendix IV for an example of the training form) (Lund 2003). The panelist was generally presented with two identical base wines, one being spiked with standard(s). In this test the panelist was asked to recognise the attribute difference of each wine in both absolute and relative terms. Standard concentrations were also varied to determine the response of the tasters. Numerous tests were run and the data analysed to calculate the mean scores for the base wines and for the effect of spiking the standards. The base wine mean scores for each attribute were then indicated on the 100 mm horizontal line with vertical checks (refer to appendix IV). Subsequent training could then be “calibrated”
based on the historical training data (i.e. the individual panelist was forced to adjust their respective scores based on the group’s mean score).

The data collections were held in two sessions over a two day period (22
\textsuperscript{nd} and 23
\textsuperscript{nd} July 2003). Each session was commenced at 10 am. No more than 30 minutes before the beginning of the tasting, 30 ml of each wine was decanted from the 750 ml sample bottle into standard INAO tasting glasses. The glasses were sealed with parafilm, and each glass then labelled with a three digit randomly generated numerical code corresponding to the treatment and sample date.

During the data collection each panelist was presented with four wines (one of each treatment). The presentation order was randomised. The panelists were asked to score the wines independently (i.e. no comparison) on the aforementioned attributes. Printed on the score sheets were the base wine calibration checks described above (refer to Appendix IV).

The sessions were conducted under controlled lighting in individual booths. Panelists were requested not to drink or eat for two hours before each collection session. Panelists were given water and slices of bread (baguette) to aid palate freshness during tasting.

\textbf{2.3.9 Ethics Approval}

As part of the University of Auckland research policies any trial conducted on humans must comply with the ethics guidelines set down by the University. A comprehensive submission was prepared and presented to the committee. The ethics approval was granted and the sensory trial were allowed to proceed. The submission is given in Appendix V.
<table>
<thead>
<tr>
<th>Attribute</th>
<th>Description (given to panellists)</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Visual</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intensity</td>
<td>The concentration of colour as perceived when the glass is held at approximately 30° to the horizontal against a white card.</td>
<td>Mission Estate Cabernet 2001</td>
</tr>
<tr>
<td>Hue</td>
<td>The degree of browning in the wine when the glass is held at approximately 30° to the horizontal against a white card</td>
<td>Mission Estate Cabernet 2001</td>
</tr>
<tr>
<td><strong>Aromatic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetative</td>
<td>Herbaceous characters, e.g. capiscum, freshly cut grass</td>
<td>iso-butyl methoxypyrazine (0.5-40 µg/L in water)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cis-3-hexen-1-ol (0.2-1 mg/L in water)</td>
</tr>
<tr>
<td>Fruity</td>
<td>Berry characters, black currant, strawberry, raspberry</td>
<td>Barkers blackcurrant concentrate diluted in water to 50%</td>
</tr>
<tr>
<td>Aromatic</td>
<td>The intensity of the wine aroma when first sniffed.</td>
<td>Mission Estate Cabernet 2001</td>
</tr>
<tr>
<td>intensity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aromatic</td>
<td>The number of aromatic components in the wine.</td>
<td>Mission Estate Cabernet 2001</td>
</tr>
<tr>
<td>complexity</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sapid</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acidity</td>
<td>Sour/tart character on the tongue</td>
<td>Tartaric Acid (4 g/L in water)</td>
</tr>
<tr>
<td>Bitterness</td>
<td>Perceived on tongue</td>
<td>Caffeine (0.4 g/L in water)</td>
</tr>
<tr>
<td><strong>Tactile/Mouthfeel</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Astringency</td>
<td>Drying/puckering in cheeks</td>
<td>OceanSpray Cranberry juice</td>
</tr>
<tr>
<td>Tannic force</td>
<td>Mouthfeel is a measure of how the wine “fills” the palate. A wine with a weak mouthfeel will tend to only be perceived on the centre-line of the tongue. If the wine is felt strongly on the inside of the cheeks and the tongue, then it can be considered full or big.</td>
<td>Mission Estate Cabernet 2001</td>
</tr>
<tr>
<td>Length</td>
<td>The amount of time the wine is able to be perceived on the palate.</td>
<td>Mission Estate Cabernet 2001</td>
</tr>
<tr>
<td>Overall</td>
<td>A general liking of the wine relative to the standard wine.</td>
<td>Mission Estate Cabernet 2001</td>
</tr>
<tr>
<td>impression</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2.3.10 Data Analysis

Statistical analysis was performed using Microsoft Excel 2003, The Unscrambler v9.1, Matlab v7.0.1 R14 and R v2.4.0.
2.4 Results and Discussion

2.4.1 Sensory Analysis

The sensory results did not discriminate any significant differences between the treatment and the control for any of the sampling dates analysed either by univariate or multivariate test. The mean scores for each of the surveyed attributes with corresponding 95% confidence interval bands are given in figures 2-13 a) and 2-13 b).

Three factor univariate analysis of variance (ANOVA) and single factor multivariate analysis of variance (MANOVA) results are presented in Tables 2-6 and 2-7. A graphical representation of the multivariate dataset is given in figure 2-14 a) – d).

Table 2-6 Univariate Sensory Analysis Results

<table>
<thead>
<tr>
<th>Attribute</th>
<th>F-Statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual</td>
<td></td>
</tr>
<tr>
<td>Intensity</td>
<td>0.00 0.37</td>
</tr>
<tr>
<td>Hue</td>
<td>0.97 0.71</td>
</tr>
<tr>
<td>Aromatic</td>
<td></td>
</tr>
<tr>
<td>Vegetativeness</td>
<td>1.68 0.07</td>
</tr>
<tr>
<td>Fruitiness</td>
<td>0.17 0.35</td>
</tr>
<tr>
<td>Aromatic Intensity</td>
<td>0.00 1.34</td>
</tr>
<tr>
<td>Aromatic Complexity</td>
<td>0.03 1.69</td>
</tr>
<tr>
<td>Sapid/Mouthfeel</td>
<td></td>
</tr>
<tr>
<td>Astringency</td>
<td>0.06 0.09</td>
</tr>
<tr>
<td>Bitterness</td>
<td>0.00 1.64</td>
</tr>
<tr>
<td>Acidity</td>
<td>2.05 2.40</td>
</tr>
<tr>
<td>Tannic Force</td>
<td>0.26 0.46</td>
</tr>
<tr>
<td>Length</td>
<td>5.31* 1.46</td>
</tr>
<tr>
<td>Overall Impression</td>
<td>0.77 0.41</td>
</tr>
</tbody>
</table>

* P < 0.05
** P < 0.01
***P < 0.001

The output of 3-way ANOVAs run on each of the attributes is given in Table 2-6. The results presented in Table 2-6 indicate that panelists are the most significant source of variation in this data set (P<0.001). Only the “length” attribute (P<0.05) showed a significant difference between the control and treatment. Judge variation is commonly observed (Girard, Yuksel et al. 2001) and is due mainly to physiological and scoring
differences. Due to logistical and time constraints replicates were not built into the design, hence interpretation of the panel x wine interaction was not possible for this trial.

Table 2-7 Table of results from a single factor MANOVA

<table>
<thead>
<tr>
<th>Days from start of Treatment</th>
<th>Mahalanobis distance between group means</th>
<th>Wilks $\Lambda$</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>3.181</td>
<td>0.5355</td>
<td>9.9941</td>
</tr>
<tr>
<td>28</td>
<td>1.085</td>
<td>0.7717</td>
<td>4.1461</td>
</tr>
<tr>
<td>42</td>
<td>0.738</td>
<td>0.8324</td>
<td>2.9354</td>
</tr>
<tr>
<td>56</td>
<td>1.355</td>
<td>0.7302</td>
<td>5.0314</td>
</tr>
</tbody>
</table>

Table 2-7 shows that the greatest statistical distance between any two group means occurs at 14 days from the start of treatment. No significant differences, however, were observed in the multivariate data. The canonical variates analysis (CVA) plots (figure 2-13 a – d) highlight the insignificant separation of the group means. The circles represent 95% confidence regions for each of the respective treatments means (i.e. the probability that the population group mean lie within that region) and are based on a $\chi^2$ test (Krzanowski 2000). Overlayed are the individual panellist scores transformed into canonical space.

Analysis of the sensory results and the overall training and data collection process allowed the refinement of the sensory panel for the following year. It was decided to reduce the number of attributes analysed; particularly those attributes that could not be easily matched to a standard (e.g. visual attributes, aromatic intensity and complexity and overall impression). More focus on “calibration” of judges to normalise the scores between panellists was to be given. Replicate trials would also be performed as part of the training regime to assess panellist x wine interactions and thus the quality of the mean scores.
Figure 2-12 a) Mean sensory scores for each attribute and sampling date. Error bars represent 95% confidence intervals.
**Chapter Two**

**Figure 2-12 b)** Mean sensory scores for each attribute and sampling date. Error bars represent 95% confidence intervals.
One issue that arose was the appropriateness of some of the standards used. The astringency standard in particular was found to be inadequate and not representative of wine-specific astringency. The sweetness of the cranberry juice was also found to interfere with the overall astringency perception. An alternative standard was sourced for future trials.

**Figure 2-13 a)** Separation of treatment means 14 days after the start of treatment. Ellipses represent 95% confidence regions of group means. Individual panelist scores are overlaid onto the projection.
Figure 2-13 b) Separation of treatment means 28 days after the start of treatment. Ellipses represent 95% confidence regions of the group means. Individual panelist scores are overlaid onto the projection.

Figure 2-13 c) Separation of treatment means 42 days after the start of treatment. Ellipses represent 95% confidence regions of group means. Individual panelist scores are overlaid onto the projection.
Figure 2-13 d) Separation of treatment means 56 days after the start of treatment. Ellipses represent 95% confidence regions of group means. Individual panelist scores are overlaid onto the projection.
### 2.4.2 Low Molecular Weight Polyphenol HPLC Results

All the samples were analysed using the low molecular weight (LMW) polyphenol HPLC analysis described in section 2.3.1. The resulting chromatograms were then processed using the peak alignment and principal components analysis techniques outlined in sections 2.3.2 and 2.3.3. This set of analyses gives an initial insight into the evolution and variation of the LMW polyphenols throughout the trial.

![PCA projection of the LMW Polyphenol HPLC data.](image)

**Figure 2-14** PCA projection of the LMW Polyphenol HPLC data.

The output of the PCA is shown in figures 2-14 and 2-15. Figure 2-14 shows the traditional PCA projection (PC1 vs PC2), and figure 2-15 is a series of line plots of the PCA loadings versus time; the interpretation of this plot will be discussed below. The majority of the model variance (80%) was explained by the first two principal components (PCs). With the exception of the treatment wine at 14 days the variation occurs mainly along the PC1 axis (from right to left as the axis is drawn).
Figure 2-15  PCA loading plots for the LMW polyphenol HPLC data

With a typical PCA, scores and loadings can be plotted on the same axes in the form of a biplot. With large datasets it is preferable to plot the loadings separately as a line plot versus the respective variables (in this case time). Each loading is overlayed on the same plot.
allowing for mutual comparison. The simplest way of interpreting this plot is to consider the four quadrants of the PCA projection (figure 2-16). The right hand quadrants are positive in PC1 and positive and negative in PC2 for the upper and lower quadrants respectively. Similarly the left hand quadrants are negative in PC1 and positive and negative in PC2 as shown in figure 2-16.

Figure 2-16  Schematic representation of coding for loadings plot interpretation

In general, if the loading curves track along the zero point of the vertical axis they contribute nothing to the variance model. Deviation from zero implies that there is some difference between treatments and/or sample dates. A clear example is the 520 nm (bottom) trace in Figure 2-15. The baseline trace for both PC1 and PC2 track along the zero loading line. The main peaks for each loading trace occur at: 48.0, 51.5, 53.4, 57.7, 62.6, 69.0, 75.6, 85.9 and 87.3 minutes. The sense of these peaks is consistent with the PC1 and PC2 loadings being in the positive and negative directions respectively. This implies that the loadings for the compounds (anthocyanins) is in the bottom right hand quadrant (+,-) of the PCA projection (Figure 2-14). The maximum levels (or biggest difference) of anthocyanins are when PC1 is positive and PC2 is negative (e.g. Baseline, C_{14days}, C_{28days}, C_{56days}) and lowest when PC1 and PC2 are most negative and positive respectively (e.g. C_{42days}, T_{42days}, T_{56days}). Another general feature is the unresolved “hump” which represents oligomeric/polymeric material (identified in section 2.3.1) this appears as a (+,+). loading in the top trace of Figure 2-16. This result, rather surprisingly as it suggests that the greatest amount (or largest difference) of oligomeric and polymeric material is in the Baseline, C_{28days} and C_{56days} samples. Table 2-8 lists the major peaks, retention time and where possible the corresponding compound.
Table 2-8 Major peaks from PCA loadings plot Figure 2-15

<table>
<thead>
<tr>
<th>Peak</th>
<th>Trace</th>
<th>Retention Time (min)</th>
<th>Loading Sense</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>280nm</td>
<td>9.3</td>
<td>(+,-)</td>
<td>Gallic Acid</td>
</tr>
<tr>
<td>2</td>
<td>320nm</td>
<td>23.2</td>
<td>(-,-)</td>
<td>t-caftaric acid</td>
</tr>
<tr>
<td>3</td>
<td>320nm</td>
<td>34.7</td>
<td>(+,+))</td>
<td>cis-coutaric acid</td>
</tr>
<tr>
<td>4</td>
<td>320nm</td>
<td>36.4</td>
<td>(+,+))</td>
<td>t-coutaric acid</td>
</tr>
<tr>
<td>5</td>
<td>280nm</td>
<td>37.2</td>
<td>(+,-)</td>
<td>catechin</td>
</tr>
<tr>
<td>6</td>
<td>280nm</td>
<td>47.0</td>
<td>(+,+)</td>
<td>B₄</td>
</tr>
<tr>
<td>7</td>
<td>520nm</td>
<td>48.0</td>
<td>(+,-)</td>
<td>delphinidin-3-glucoside</td>
</tr>
<tr>
<td>8</td>
<td>520nm</td>
<td>53.4</td>
<td>(+,-)</td>
<td>petunidin-3-glucoside</td>
</tr>
<tr>
<td>9</td>
<td>280nm</td>
<td>53.7</td>
<td>(+,-)</td>
<td>epicatechin</td>
</tr>
<tr>
<td>10</td>
<td>520nm</td>
<td>56.6</td>
<td>(+,+))</td>
<td>peonidin-3-glucoside</td>
</tr>
<tr>
<td>11</td>
<td>520nm</td>
<td>57.7</td>
<td>(+,+))</td>
<td>malvidin-3-glucoside</td>
</tr>
<tr>
<td>12</td>
<td>280nm</td>
<td>61.8</td>
<td>(+,-)</td>
<td>unidentified</td>
</tr>
<tr>
<td>13</td>
<td>520nm</td>
<td>68.9</td>
<td>(+,-)</td>
<td>unidentified</td>
</tr>
<tr>
<td>14</td>
<td>520nm</td>
<td>75.4</td>
<td>(+,-)</td>
<td>unidentified</td>
</tr>
<tr>
<td>15</td>
<td>365nm</td>
<td>83.8</td>
<td>(0,+)</td>
<td>unidentified</td>
</tr>
<tr>
<td>16</td>
<td>365nm</td>
<td>84.9</td>
<td>(+,+))</td>
<td>unidentified</td>
</tr>
<tr>
<td>17</td>
<td>520nm</td>
<td>85.8</td>
<td>(+,-)</td>
<td>unidentified</td>
</tr>
<tr>
<td>18</td>
<td>365nm</td>
<td>85.7</td>
<td>(+,+))</td>
<td>myricetin</td>
</tr>
<tr>
<td>18</td>
<td>520nm</td>
<td>87.3</td>
<td>(+,+))</td>
<td>unidentified</td>
</tr>
<tr>
<td>19</td>
<td>280nm</td>
<td>88.3</td>
<td>(+,+))</td>
<td>unidentified</td>
</tr>
<tr>
<td>20</td>
<td>365nm</td>
<td>93.9</td>
<td>(+,+))</td>
<td>quercetin</td>
</tr>
</tbody>
</table>

The peaks of all the compounds identified in table 2-8 are integrated and plotted, for each treatment in figures 2-17 a) – d).
Figure 2-17 a) evolution of individual phenolic compounds over the course of the trial. Concentrations of B4 and unidentified compounds at 61.8 and 88.4 minutes are expressed as catechin equivalents.
Figure 2-17 b) evolution of individual phenolic compounds over the course of the trial. Concentrations of t-caftaric acids and the two isomers of coutaric acid are expressed as caffeic and coumaric acid equivalents respectively. The concentration of the unidentified compound at 83.8 minutes is expressed as quercetin equivalents.
Figure 2-17 c) evolution of individual phenolic compounds over the course of the trial. All compounds are expressed in terms of chromatogram peak area (mAu*s)
Figure 2-17 d) evolution of individual phenolic compounds over the course of the trial. All compounds are expressed in terms of chromatogram peak area (mAU*s).

Gallic acid, catechin, B4 and the unidentified flavanol at 61.8 mins show very consistent behaviour over the course of the trial for both the treatment and control wine (figure 2-17 a)). Epicatechin and the unidentified flavanol at 88.4 mins show a more complicated evolution which may be influenced by simultaneous condensation and hydrolysis reactions that are proceeding in the wine possibly releasing monomeric and oligomeric flavanols into the wine. The unidentified peak at 88.4 mins is particularly interesting as the behaviour of the control and treatment is very different indicating some effect of the treatment.

The three hydroxycinnamates (t-caftaric acid, cis-coumaric acid and t-coumaric acid) show remarkably similar patterns of degradation (figure 2-17 b)). The treatment shows a sharp decrease in concentration after 14 days compared to control. The concentration then falls until the final sample date (56 days) when both show a sudden increase. This behaviour may be to do with these compounds being hydrolysed to their non-esterified forms (e.g. coumaric and coumaric acids) however there is no corresponding increase in the concentration of these products. They may also be involved in some direct condensation reactions with other quinones as has been proposed in recent reviews (Monagas, Bartolome et al. 2005).

The flavonol kinetics are similarly complicated (quercetin, and the unidentified peaks at 83.8 and 85.7 minutes in figure 2-17 c)) with decreases, followed by increases in detected concentrations. There appears to be a phase shift between the control and treatment; with
the treatment wine “leading” the control wine by approximately 28 days. Further data points would be required to confirm this however the different behaviour of the control and treatment wines is interesting.

The degradation of anthocyanins for both control and treatment wines was consistent for the period of the trial. All peaks exhibited a steady decline consistent with numerous published studies (Baranowski and Nagel 1983; Bakker 1986; Riberau-Gayon and Glories 1986; Bakker, Picinelli et al. 1993; Dallas, Ricardo-da-Silva et al. 1995).

It should also be noted that there were no replicate treatments for this trial therefore the variance between either observation dates or treatments could not be assessed.
2.4.3 Thiolysis Results

The thiolysis results are presented in figure 2-18 and table 2-9. All samples were performed in triplicate (including the fractionation step described in section 2.3.4).

![Figure 2-18](image)

**Figure 2-18** Evolution of mDP for treatment and control wines through the course of the trial. The labels above points represent the significance of the difference between the control and treatment wines and the sampling date as determined by ANOVA (P<0.05).

No significant differences were observed between the treatment and control wines at any of the sampling dates. An initial decrease in the mDP (significant for the control wine) after 14 days of oxygenation was followed by a significant rise in mDP for both wines at the 28 and 42 day sampling points. The final sampling date again showed a significant decrease in mDP for both treatment and control wines with a larger decrease detected in the control wine.

An analysis of the composition of the constitutive units revealed the absence of any prodelphinidins as terminal units, with the terminal positions being taken up entirely by procyanidin flavanols. Epigallocatechin, however, was detected as a significant component of the extension unit fraction. Moreover, pigmented compounds were detected (at 520 nm) but in such small quantities they were unable to be resolved for quantification and hence were not included in the mDP calculation.
Table 2-9 details the relative proportions of the respective terminal and extension subunits. Interestingly from the 14 day point, the treatment wine showed proportionately less epicatechin-3-O-gallate both as a terminal and an extension subunit (although from day 28 the relative concentration was relatively stable). No discrete peaks of gallic acid were detected in any of the HPLC analyses of the thiolysed samples (peak retention time at 5.9 minutes determined by analytical standard) indicating that the hydrolysis of the gallic acid moiety may occur in the wine or due to loss or conversion in the thiolysis analysis.

Small, but significant increases can be seen in the relative amounts of catechin and epicatechin terminal units in the final sample (56 days). Also of note is the significantly higher proportion of epigallocatechin-4-benzylthioether in the treatment wine compared to the control in the 28 and 42 day sample dates.

As discussed in section 1.4.5 the degree of galloylation tends to increase the “coarseness” of the astringency perception, conversely trihydroxylation of the “B” ring has the opposite effect. While significant differences in the organoleptic assessment of astringency or “tannic force” were unable to be resolved in this trial, the subtle changes in the tannin profile observed in the thiolysis results may contribute to the positive changes in sensory properties of a wine under oxygenation treatment.

Finally, the above interpretation comes with a caveat based on the limitation of the thiolysis procedure as outlined in section 2.3.5. As the total mass of proanthocyanidin extract was not measured the percentage yield as described in (Kennedy, Matthews et al. 2000) could not be calculated. The absolute molar yield however has been calculated and is given in figure A-1 (Appendix III). Note the relatively large variation in both within and between treatment yields over the course of the trial.

2.5 Discussion
While the trial allowed some method development the overall design meant that the data gathered was of limited use. A significant issue proved to be the different way the control and treatment wines were handled. Specifically: the different sized vessels – and the effects
of ullage on the control wine particularly, the oak treatment given to the treatment but not the control wine and the lack of temperature control – again a more significant effect would be seen on the control wine. Moreover, it proved, despite the best efforts, difficult to keep the control wine free of oxygen during sampling. Given the relatively small volume, the impact of this was significant.

A further limitation with commercial trials is the impracticality of incorporating replicate treatments into the design. Given the relatively large intrinsic variability in the various stages of the micro-oxygenation, replication of treatment is required to ensure the statistical validity of the data.

In the normal course of treatment a winemaker would routinely sample the wine directly from the treatment vessel in order to make decision on the continuation of the treatment or the appropriateness of the dosage rate. The wine is generally sampled fresh and not stored in bottles for a period of time prior to analysis (as was the case in this trial). In order to better replicate the actual conditions experienced by a winemaker during maturation, an experimental setup that allowed the sampling of wines just prior to analysis (sensory and chemical) was required.

A further implication of the above study is that the sensory and chemical changes manifest in a wine undergoing micro-oxygenation are very subtle and require a higher level of analysis to resolve. Some potential ways this could be achieved are through: better experimental design (e.g. replication), more accurate treatment methods (e.g. oxygen dosage), fresher samples and better analytical techniques.
### Table 2-9  
Molar proportion of individual extension and terminal units after thiolysis. The significance of the difference between the treatment and control wines is indicated.

<table>
<thead>
<tr>
<th></th>
<th>14 days</th>
<th>28 days</th>
<th>42 days</th>
<th>56 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treatment</td>
<td>Control</td>
<td>Treatment</td>
</tr>
<tr>
<td>Catechin</td>
<td>6.22%</td>
<td>6.00%</td>
<td>ns</td>
<td>6.04%</td>
</tr>
<tr>
<td>Epicaechin</td>
<td>4.11%</td>
<td>3.80%</td>
<td>**</td>
<td>3.94%</td>
</tr>
<tr>
<td>Epicatechins-3-O-gallate</td>
<td>2.06%</td>
<td>2.16%</td>
<td>ns</td>
<td>2.14%</td>
</tr>
<tr>
<td>Epigallocatechin-4-benzylthioether</td>
<td>8.25%</td>
<td>8.45%</td>
<td>ns</td>
<td>8.29%</td>
</tr>
<tr>
<td>(2R,3R,4R)-catechin-4-benzylthioether</td>
<td>5.29%</td>
<td>4.45%</td>
<td>ns</td>
<td>4.57%</td>
</tr>
<tr>
<td>(2R,3R,4R)-catechin-4-benzylthioether</td>
<td>1.53%</td>
<td>1.77%</td>
<td>ns</td>
<td>1.37%</td>
</tr>
<tr>
<td>Epicatechins-4-benzylthioether</td>
<td>53.97%</td>
<td>53.54%</td>
<td>ns</td>
<td>53.99%</td>
</tr>
<tr>
<td>Epicatechins-3-O-gallate-4-benzylthioether</td>
<td>18.58%</td>
<td>19.83%</td>
<td>ns</td>
<td>19.66%</td>
</tr>
</tbody>
</table>

ns = not significant  
* = p < 0.05  
** = p < 0.01  
*** = p < 0.001
CHAPTER THREE

Development of a Method for Oxygenation of Small Volumes of Wine

3.1 Introduction

The major limitations of studying a commercial micro-oxygenation treatment were detailed in Chapter Two, and are summarised in Table 3-1. These limitations represent potentially significant effects on the treatment response, some of which have been investigated (McCord 2003). Moreover there is little practical scope for replication in a commercial context further reducing the certainty of results.

In order to study the effects of controlled oxygenation in a scientifically rigorous and practical way, a method of accurately dosing small volumes of wine (<100 litres) was required.

As discussed in the preceding chapters, controlled oxygenation has traditionally been achieved using a technique known as micro-bullage (very small bubbles). This involves a gas regulation system connected by a conduit (usually an oxygen impermeable polymer tube) to a micro-porous diffuser suspended in the wine tank. The diffuser is generally located in the centre of the tank close to the bottom, as shown in figure 1-3. The system is designed to operate above the bubble-point pressure of the diffuser. The bubbles, formed at the
diffuser, rise through buoyancy in a plume through a column of wine (diameter of the
column is determined by dispersion factors). The oxygen in the bubble dissolves into the
wine by interphase mass transfer. The system should be designed such that 100% of the
oxygen within the bubble is transferred into the wine before the bubble reaches the free
surface (liquid-gas interface) at the top of the wine volume.

Table 3-1  Limitations of commercial trials

<table>
<thead>
<tr>
<th>Vessel size</th>
<th>Volume and aspect ratio. Smaller volume used for control compared to the oxygenated wine; aspect ratio is important in influencing the oxygen mass transport in the tank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ullage</td>
<td>Variations in surface area of wine in contact with the atmosphere and the respective surface area to volume ratio.</td>
</tr>
<tr>
<td>Oak treatments</td>
<td>Slats/chips used in treatment not in control</td>
</tr>
<tr>
<td>Temperature</td>
<td>Storage vessels are generally not temperature controlled and there may be significant temperature differences within and between tanks</td>
</tr>
</tbody>
</table>

In the trial described in Chapter Two periodic measure of the storage vessel headspace oxygen concentration were made using a dissolved oxygen meter (Orbisphere 3650). In all cases no oxygen was detected (< 1 ppb). The presence of bubbles, however, on the free surface of the wine was clearly observed throughout the trial. Bubble volume is maintained, despite the loss of oxygen, by the influx of other gases present in the wine solution, most notably CO₂, N₂, water and ethanol vapour. A comprehensive review of the interphase mass transfer of bubbles in a column of fluid is given in Section 1.3.

An obvious limitation with *micro-bullage* is that for a given diffuser configuration a minimum tank height is required to dissolve all the oxygen into the wine (for the reasons stated above). The implication is that as the volume of wine decreases the tank aspect ratio (ratio of height to diameter) must increase in order to maintain the minimum fluid column height; the limiting case being a tube of slightly larger diameter than the diffuser as shown in figure 3-1.
3.2 Trials using Micro-Bullage in Large Aspect Ratio Tanks

An initial experiment was undertaken using a commercially available micro-oxygenation system (Oenodev Compact VisiO2). The trial was conducted using 6 m lengths of 75 mm diameter 304 stainless steel schedule tubing (diffuser diameter 50 mm) giving a useable capacity of approximately 25 litres (figure 3-1). Several problems were encountered with this configuration. Positioning of the tanks and providing access for sampling initially posed some logistical difficulties. Temperature regulation also proved problematic due to the vertical dimension of the vessel; gradients of 1.5°C were observed from the bottom to the top of the vessel during the trials.

Moreover it was discovered that the commercial regulation systems were not well suited to small volumes of wine. Diffuser head pressure was found to be noticeably saw-toothed in character leading to significant fluctuations in oxygen dosage rate with time. It should be noted however, that this effect was limited to small volumes of wine and was not observed when used in lots greater than 1000 litres.

As a result of these trials, it was concluded that commercial micro-oxygenation dosage systems were not suitable for small volumes of wines required for replicated laboratory trials.
Figure 3-1   High aspect ratio tanks mounted in a large warehouse facility
3.3 Polymer Membrane Oxygenation

An alternative to micro-bullage is oxygenation by a gas-permeable membrane. This technique utilises a dense or micro-porous polymer typically in the form of a tube with the liquid and gas phase present on either side. Solute (oxygen, in this case) flow is driven by the concentration gradient across the membrane as shown in figure 3-2. The primary advantage of polymer membrane oxygenation (PMO) is that the solute is dissolved directly into the wine without the formation of bubbles; tank geometry, therefore, becomes less important in achieving 100% oxygenation efficiency.

With a dense polymer membrane, oxygen is absorbed by the polymer on the gas side and is transported by diffusion to the liquid side where it is then desorbed directly into the liquid. For a micro-porous membrane, the gas pressure is maintained below the bubble-point. In the case of a hydrophobic micro-porous membrane, bubbles actually form at the membrane pores but surface tension keeps them attached. Conversely the liquid phase penetrates the pores of hydrophilic micro-porous polymers, as a consequence no bubbles are formed and the interphase mass transport mechanisms are different from the hydrophobic case as will be explained below. The three types of polymer membranes are illustrated in figure 3-3.

**Figure 3-2** Schematic representation of the solute concentration gradients across a liquid-membrane-gas configuration.
As described above the selective mass transfer properties of the membrane are important in determining the system transport characteristics. The overall mass transport, however, depends not only on the membrane properties but also on the interfacial characteristics between the membrane and the contacting phases (Yasuda and Lin 2003). Preliminary PMO research has noted that oxygen transfer rates of wet films (gas-membrane-liquid) were reduced by factors of between 5 and 10 when compared with the same membrane in a dry (gas-membrane-gas) configuration (Schaffer, Ludzack et al. 1960; Robb 1965; Yasuda and Lamaze 1972; Yang and Cussler 1986; Cote, Bersillon et al. 1989). The difference in the mass transfer rates was attributed, in all cases to the effect of the liquid side boundary layer.

The boundary layer effects can best be explained by considering the rate at which the solute is being “carried away” by the contacting phase through convective transport mechanisms. The greater the liquid bulk phase velocity (generally expressed as the Reynolds ($Re$) or Peclet ($Pe$) number) the more influence the convective transport has on the overall solute mass transfer at the membrane/liquid interface (generally expressed as the Sherwood number ($Sh$)) and the smaller the boundary layer. In the limiting case where the fluid around the diffuser is stagnant (i.e. zero flow) the only solute transport mechanism is diffusion (typically several orders of magnitude slower than advective transport) and a very large liquid side boundary layer would exist. The boundary layers are represented schematically in figure 3-2.

Note the relatively large boundary layer on the liquid side compared to that of the gas-side. For typical configurations the gas-side mass transfer resistance (see below for definition) is four to six orders of magnitude less than the liquid side due, principally to the boundary layer effects (Cote, Bersillon et al. 1989; Ahmed and Semmens 1992).
Figure 3-3 Different types of polymer membranes

3.3.1 PMO Mass Transfer Model

In bubble oxygenation, the mass transfer characteristics are typically expressed as the product of the mass transfer coefficient and the specific area ($K_{L}a$). The two variables are combined in this way as the bubble surface area is generally unknown. In the case of PMO, $a$ is simply taken as the surface area of membrane in contact with the liquid and thus the overall mass transfer coefficient ($K_L$) can be determined independently.

The following derivation is adapted from a model proposed by (Cote, Bersillon et al. 1989).

The solute flux ($j$) across a wet membrane can be given as (refer to figure 3-2):

$$j = k_G (C_{GO} - C_{GI}) = k_M (C_{MG} - C_{ML}) = k_L (C_{LI} - C_{LO})$$  \[3.1\]

The equilibrium partition coefficients (used to account for the different solute solubilities in the respective phases) are defined as:

$$S_{MG} = \frac{(C_m)_E}{(C_G)_E}$$  \[3.2\]
\[ S_{ML} = \frac{(C_m)_E}{(C_L)_E} \tag{3.3} \]

for the gas and liquid interfaces respectively. Combining equations [3.1], [3.2] and [3.3] and rearranging gives the flux equation:

\[
j = \frac{1}{k_G} + \frac{1}{S_{MG}k_M} + \frac{1}{(S_{MG}/S_{ML})k_L} \left( C_{GO} - \frac{C_{LO}}{S_{MG}/S_{ML}} \right) \tag{3.4} \]

Equation [3.4] is analogous to Ohms law with the flux term being equivalent to electrical current, the concentration difference to voltage and the \(1/k\) terms representing resistance. In this instance the resistance terms are additive indicating a resistance-in-series model. The resistance terms can be simplified by assuming the gas film resistance \((1/k_G)\) is very small compared to the membrane \((1/S_{MC}k_M)\) and liquid film resistances \((1/(S_{MC}/S_{ML})k_L)\) (Cote, Bersillon et al. 1989). By applying Henry’s law \((P=HC_L)\) and the ideal gas law \((P=C_GRT)\) equation [3.4] can be more conveniently written as:

\[
j = \frac{1}{S_{MC}^I k_M} + \frac{H}{k_L} \left( P - HC_{LO} \right) \tag{3.5} \]

Where \(S_{MC}^I = S_{MC}/RT\). The mass transfer coefficient of a membrane is given by [3.6] (Cussler 1984)*:

\[
k_M = \frac{\Gamma}{l} \tag{3.6} \]

Where \(\Gamma\) is the diffusion coefficient of oxygen in the membrane and \(l\) is the membrane thickness. The product \(S_{MC}^I\Gamma\) is termed the permeability \(\mu\) in the case of a gas-membrane-gas configuration (Cussler 1984). In the case of a hollow tube (fibre) the flux equation derived in cylindrical coordinates uses an equivalent thickness \((l^*)\) (Yamane, Matsuda et al. 1981) given as:

\[
l^* = r_{\text{out}} \ln \left( \frac{r_{\text{out}}}{r_{\text{int}}} \right) \tag{3.7} \]

* Table 9.3-2, page 230
where \( r_{ext} \) and \( r_{int} \) are the external and internal radii of the tubular membrane respectively. Substituting [3.6] and [3.7] into [3.5] and rearranging gives:

\[
\frac{1}{K_L} = \frac{l_e}{pH} + \frac{1}{k_L}
\]

Which gives an overall mass transfer resistance as the sum of the membrane resistance \((l_e/PH)\) and the liquid film resistance \((1/k_L)\) or:

\[
\frac{1}{K_L} = \frac{l_e}{pH} + \frac{1}{k_L}
\]

The motive force for the flux is the concentration difference between the oxygen side (determined by the oxygen partial pressure) and the liquid side dissolved oxygen concentration.

**Determination of the mass transfer coefficient**

The overall mass transfer coefficient \((K_L)\) can be determined from measurements of dissolved oxygen concentration in the liquid phase as a function of time (assuming no chemical reaction). A mass balance on a fully mixed volume of wine leads to:

\[
\frac{dC_L}{dt} = K_L a \left( \frac{P}{H} - C_L \right)
\]

with initial conditions of \(C_L(0) = 0\)

The above can be integrated to give:

\[
C_L(t) = \frac{P}{H} \left(1 - e^{-K_L a t}\right)
\]

Equation [3.11] can be used to evaluate the mass transfer resistance \(K_L\).

### 3.3.2 Sealed-End Oxygen Partial Pressure Model

The above model assumes constant oxygen partial pressure along the length of the membrane fibre. In the case where oxygen (or air) is being continuously pumped through
the fibre this assumption is justified as the gas is being continuously renewed. For a sealed-end configuration, however, account must be taken of the solute transport in both directions.

The bulk phase (water) used in the characterisation trials (described below) was chemically deoxygenated prior to each experiment (section 3.5); however nitrogen remained in solution at saturation levels. Because the membrane is permeable to N$_2$ as well as O$_2$ and concentration gradients exist for both solutes, it is reasonable to assume that transport will occur across the membrane in both directions i.e. a net flux of oxygen from within the fibre into the bulk phase and a net flux of nitrogen from the liquid into the fibre. The effect of contamination from a second gas will be to reduce the oxygen partial pressure and thus the oxygen flux through the membrane.

A model, adapted from (Weiss, Oakley et al. 1996) was used to quantify the effect of a membrane permeable gas in the bulk phase. The following assumptions are made to allow model development:

1. The nitrogen entering the fibre is carried away from the feed end by the velocity of the oxygen entering the tube.
2. Total gas pressure (feed pressure = $P_T$) remains constant along the length of the fibre (i.e. no pressure losses). This assumption is justified given the small length of the fibre and low oxygen flux rates.
3. Oxygen and nitrogen diffusivities are the same and vary only with temperature and pressure

4. Oxygen and nitrogen are the only gases present and behave as ideal gases.

Assumptions 2 and 4 give:

\[ \frac{dP_t}{ds} = \frac{dP_o}{ds} + \frac{dP_n}{ds} = 0 \]  

[3.12]

Equating the diffusive and convective transport of oxygen within the fibre to the oxygen flux out gives:

\[ \frac{1}{RT} \left( \Gamma \frac{d^2 P_o}{ds^2} - P_o \frac{dv}{ds} - v \frac{dP_o}{ds} \right) = K_t a \left( P_o - C_o H_o \right) \]  

[3.13]

Finally by performing a mass balance on a differential element inside the lumen gives:

\[ \frac{dv}{ds} = - \frac{1}{P_t} \left( \frac{RTK_o a}{H_o} \left( P_o - C_o H_o \right) + \frac{RTK_n a}{H_n} \left( P_n - C_n H_n \right) \right) \]  

[3.14]

Boundary conditions are

1. \( P_o(0) = P_f \)
2. \( P_o(0) = 0 \)
3. \( v(L) = 0 \)
4. \( P_n'(L) = 0 \)

Oxygen partial pressure at the fibre end is equal to the feed pressure and by assumption 1 the nitrogen partial pressure is equal to zero. Gas velocity and flux at the sealed end are assumed to be zero giving boundary conditions 3 and 4.

Solution

To obtain a solution this problem the MATLAB (v 7.0.1 R14) boundary value solver was used (bvp4c). The diffuser used for the trials was circular in shape with the oxygen being fed to each end of the tube via a manifold (refer to figure 3-5). For the purposes of the model, symmetry is assumed and the oxygen partial pressure profile is calculated for half the length of the diffuser.

The following parameters were used for the calculation: \( C_o = 0.51 \times 10^3 \text{ mol/L} \), \( C_n = 0 \text{ mol/L} \), \( H_o = 7.38 \times 10^4 \text{ Pa m}^3/\text{mol} \), \( H_n = 1.564 \times 10^5 \text{ Pa m}^3/\text{mol} \).
Chapter Three

\[ RT = 2.479 \times 10^3 \text{ J/mol} \] (Atkins and de Paula 2002), \[ \Gamma = 17.75 \times 10^{-6} \text{ m}^2\text{s}^{-1} \] (Cusseler 1984), \[ K = 3.27 \times 10^{-8} \text{ ms}^{-1} \] and \[ a = 1500 \text{ m}^{-1} \left(4d_6/d_i^2\right) \]

The oxygen partial pressure profiles for different feed pressures, according to the above model are shown in figure 3-5. The most notable feature is that the effect of the contaminating nitrogen gas on O\textsubscript{2} partial pressure is more pronounced at lower feed pressures. Mean oxygen partial pressures were calculated by numerically integrating the profiles in figure 3-6\footnote{\[ \frac{1}{L} \int_L f(s)ds \]}. Mean oxygen partial pressures as percentage of corresponding feed pressure are also given in table 3-2
Table 3-2  Comparison of corrected oxygen partial pressure vs feed pressures

<table>
<thead>
<tr>
<th>Feed Pressure (kPa)</th>
<th>Average Corrected Oxygen Partial Pressure (kPa)</th>
<th>Average Corrected O₂ Partial Pressure as a percentage of Feed Pressure (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>104</td>
<td>52</td>
<td>50</td>
</tr>
<tr>
<td>236</td>
<td>171</td>
<td>72</td>
</tr>
<tr>
<td>340</td>
<td>271</td>
<td>79</td>
</tr>
<tr>
<td>430</td>
<td>359</td>
<td>83</td>
</tr>
<tr>
<td>521</td>
<td>448</td>
<td>86</td>
</tr>
<tr>
<td>625</td>
<td>551</td>
<td>88</td>
</tr>
</tbody>
</table>

Figure 3-6  Oxygen partial pressure as a function of distance from supply end to the opposite diameter for different feed pressures.
3.4 Methods and Materials

3.4.1 Membrane Material Selection

Several membrane materials were considered however fluorinated ethylene-propylene copolymer (FEP) was chosen because of its low permeability to water (vapour) and relatively good permeability to oxygen (refer to table 3.3). Moreover, the polymer is mechanically strong and the surface is smooth reducing the likelihood of fouling as experienced with polymers such as dimethyl silicone rubber.

Table 3-3 Gas permeability values of fluoropolymers based on 100μm film thickness at 23°C using ASTM D1434 for gases and DIN53122 for water vapour. Adapted from (Seiler 1997)

<table>
<thead>
<tr>
<th></th>
<th>PTFE</th>
<th>PFA</th>
<th>FEP</th>
<th>ETFE</th>
<th>PCTFE</th>
<th>ECTFE</th>
<th>PVDF</th>
<th>PVF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water Vapour (g/m²d.bar)</td>
<td>5</td>
<td>8</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Air (cm³/m²d.bar)</td>
<td>2000</td>
<td>1150</td>
<td>600</td>
<td>175</td>
<td>N/A</td>
<td>40</td>
<td>7</td>
<td>50</td>
</tr>
<tr>
<td>Oxygen (cm³/m²d.bar)</td>
<td>1500</td>
<td>N/A</td>
<td>2900</td>
<td>350</td>
<td>60</td>
<td>100</td>
<td>20</td>
<td>12</td>
</tr>
<tr>
<td>Nitrogen (cm³/m²d.bar)</td>
<td>500</td>
<td>N/A</td>
<td>1200</td>
<td>120</td>
<td>10</td>
<td>40</td>
<td>30</td>
<td>1</td>
</tr>
<tr>
<td>Carbon dioxide (cm³/m²d.bar)</td>
<td>15000</td>
<td>7000</td>
<td>4700</td>
<td>1300</td>
<td>150</td>
<td>400</td>
<td>100</td>
<td>60</td>
</tr>
</tbody>
</table>

The tubing used for the membrane was of a 6 mm outside diameter with a 1 mm wall thickness and a dry (gas-membrane-gas) permeability of $1.906 \times 10^{-15}$ mol/m·s·Pa (from the tubing supplier).

3.4.2 Hollow Fibre Contactors

The circular diffuser was constructed of a single tube as shown in figure 3-5. A diameter was initially, and somewhat arbitrarily, chosen as 0.188 m (effective contact area $10.27 \times 10^{-3}$ m²) in a sealed-end (no gas flow) configuration (figure 3-5). The dead-end arrangement was chosen for two reasons, firstly because of the safety issues involved with purging pure oxygen into the atmosphere and secondly to limit the performance of the membrane to achieve the desired oxygen dosage rates as described in earlier work (Cote, Bersillon et al. 1989; Ahmed and Semmens 1992; Ahmed and Semmens 1992b).
3.4.3 Test Vessel

The experiments were conducted in a purpose-built 15 litre vessel fabricated from 316 stainless steel with a diameter of 285 mm (refer to appendix VI for detailed engineering drawings). The dissolved oxygen meter (Orbisphere 3650) was mounted in a specially designed cradle (figure 3-7) and suspended from the top of the tank with the probe located approximately 5 cm below the surface of the liquid. The headspace of approximately 180 ml was sparged daily with argon gas delivered through a non-return valve in the lid of the tank.

The full test setup is shown in figure 3-8 and consisted of the test vessel and diffuser described above connected to a pure oxygen (BOC food grade) supply. Feed pressure was regulated through a two stage primary regulator (BOC-8000) and a single stage high purity line regulator (Victor SGL 500) as shown in figure 3-8. The primary regulator was maintained at 700 kPa output and Feed pressure to the diffuser was set by adjusting the high precision regulator to the required pressure. Accuracy of the regulator was not verified, precision was estimated to be ±10 kPa.

![Figure 3-7](image)

**Figure 3-7** Dissolved Oxygen Meter Cradle; a) shows the component pieces, including the dissolved oxygen probe and b) is the components assembled and ready for installation into the tank.
Figure 3-8  Experimental setup shown as a schematic diagram and a photograph of the actual experimental apparatus

3.5  Procedure

Each trial was conducted in the manner described below. The tank was filled with 15 litres of de-ionised water, sealed and the headspace sparged with argon for approximately 60 seconds. The initial dissolved oxygen (DO) concentration was then recorded. The water was deoxygenated by adding a 10% stoichiometric excess of sodium sulphite (Na$_2$SO$_3$) and 3-5 mg of Cobalt Chloride (as CoCl$_2$·6H$_2$O). The tank was then resealed and the DO noted again to ensure no oxygen remained in solution. Oxygen was generally consumed by the Na$_2$SO$_3$ in the order of minutes, however, in some cases two to three hours was required to achieve a completely de-oxygenated tank.

Once the initial DO in the water had been removed, pure oxygen (BOC - food grade) at a prescribed feed pressure was applied to the membrane. The DO was then recorded every
hour for the duration of the experiment. The trial was ceased when sufficient data points had been collected to allow a reliable analysis (Typically 200-300 hours).

The water in the tank was mixed using a 50 x 5 mm magnetic stirrer bar at various angular velocities. The experiments were conducted in a temperature controlled room maintained at 20°C (unless otherwise stated). The solution temperature was verified using the integrated temperature sensor on the DO meter. A thermally insulating pad was placed between the magnetic stirrer and the tank to avoid heating of the water from the magnetic stirrer plate.

The aim of the experiments was to characterise the membrane in terms of oxygen partial pressure, mixing rate, diffuser configuration, temperature and ethanol concentration. Each experiment was performed in triplicate and the results represent the mean of the three trials with corresponding standard deviations shown as error bars. Table 3-4 describes the experimental design employed.

<table>
<thead>
<tr>
<th>Expt No.</th>
<th>Oxygen partial pressure (kPa)</th>
<th>Temperature (°C)</th>
<th>Mixing Rate (rpm)</th>
<th>Diffuser diameter (m)</th>
<th>Ethanol Concentration (% v/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>205</td>
<td>20</td>
<td>350</td>
<td>0.188</td>
<td>0.0</td>
</tr>
<tr>
<td>2</td>
<td>337</td>
<td>20</td>
<td>350</td>
<td>0.188</td>
<td>0.0</td>
</tr>
<tr>
<td>3</td>
<td>441</td>
<td>20</td>
<td>350</td>
<td>0.188</td>
<td>0.0</td>
</tr>
<tr>
<td>4</td>
<td>532</td>
<td>20</td>
<td>350</td>
<td>0.188</td>
<td>0.0</td>
</tr>
<tr>
<td>5</td>
<td>622</td>
<td>20</td>
<td>350</td>
<td>0.188</td>
<td>0.0</td>
</tr>
<tr>
<td>6</td>
<td>725</td>
<td>20</td>
<td>350</td>
<td>0.188</td>
<td>0.0</td>
</tr>
<tr>
<td>7</td>
<td>441</td>
<td>9</td>
<td>350</td>
<td>0.188</td>
<td>0.0</td>
</tr>
<tr>
<td>8</td>
<td>441</td>
<td>30</td>
<td>350</td>
<td>0.188</td>
<td>0.0</td>
</tr>
<tr>
<td>9</td>
<td>441</td>
<td>350</td>
<td>200</td>
<td>0.188</td>
<td>0.0</td>
</tr>
<tr>
<td>10</td>
<td>441</td>
<td>20</td>
<td>600</td>
<td>0.188</td>
<td>0.0</td>
</tr>
<tr>
<td>11</td>
<td>441</td>
<td>20</td>
<td>350</td>
<td>0.076</td>
<td>0.0</td>
</tr>
<tr>
<td>12</td>
<td>441</td>
<td>20</td>
<td>350</td>
<td>0.138</td>
<td>0.0</td>
</tr>
<tr>
<td>13</td>
<td>441</td>
<td>20</td>
<td>350</td>
<td>0.202</td>
<td>0.0</td>
</tr>
<tr>
<td>14</td>
<td>441</td>
<td>20</td>
<td>350</td>
<td>0.188</td>
<td>2.5</td>
</tr>
<tr>
<td>15</td>
<td>441</td>
<td>20</td>
<td>350</td>
<td>0.188</td>
<td>5.0</td>
</tr>
<tr>
<td>16</td>
<td>441</td>
<td>20</td>
<td>350</td>
<td>0.188</td>
<td>10.0</td>
</tr>
<tr>
<td>17</td>
<td>441</td>
<td>20</td>
<td>350</td>
<td>0.188</td>
<td>15.0</td>
</tr>
<tr>
<td>18</td>
<td>441</td>
<td>20</td>
<td>350</td>
<td>0.076</td>
<td>10.0</td>
</tr>
<tr>
<td>19</td>
<td>441</td>
<td>20</td>
<td>350</td>
<td>0.138</td>
<td>10.0</td>
</tr>
<tr>
<td>20</td>
<td>441</td>
<td>20</td>
<td>350</td>
<td>0.202</td>
<td>10.0</td>
</tr>
</tbody>
</table>

* All experiments performed in triplicate
3.6 Results

Figure 3-9 shows a typical response of the FEP membrane diffuser in pure water. Note the transient period in the first 60 hours, followed by more steady-state behavior. $K_L$ was calculated using equation [3.11] and the slope of the line was derived by regression analysis. The result for figure 3-9 is shown in figure 3-10.

![Figure 3-9](image)

**Figure 3-9** Typical dissolved oxygen accumulation for the following experimental parameters: bulk liquid = deionised water, gas = O$_2$ (food grade), O$_2$ feed pressure = 446.0 kPa, membrane specific area = 0.685 m$^{-1}$, stirring rate = 350 rpm, temperature = 15ºC

![Figure 3-10](image)

**Figure 3-10** Regression analysis used to calculate $K_L$ for the trial described in figure 3-9. $K_L = 0.323 \times 10^{-9}$ ms$^{-1}$.
3.6.1 Oxygen Pressure

Figure 3-11 illustrates the local mass transfer coefficient behaviour with oxygen partial pressure. $K_L$ appears to be relatively independent of oxygen partial pressure as predicted by the model derived in section 3.3.1 and 3.3.2. An average mass transfer coefficient of $3.32\pm0.16 \times 10^{-8}$ m/s was calculated and is shown as a horizontal line through the data in figure 3-11.

![Figure 3-11](image)

Figure 3-11 Variation in local mass transfer coefficient with oxygen partial pressure (feed pressure corrected using the model derived in section 3.3.2). Experimental parameters: bulk liquid = deionised water, gas = O$_2$ (food grade), membrane specific area = 0.685 m$^{-2}$, stirring rate = 350 rpm, temperature =20ºC.

Equation [3.9] can be used to quantify the relative contribution of the membrane and the liquid boundary layer on the overall mass transfer performance of the system. $k_M (=PH/\ell)$ was calculated as $8.21 \times 10^{-8}$ m/s using the following parameters: Henry’s law constant = $73.8 \times 10^3$ Pa m$^3$/mol (Atkins and de Paula 2002), $\ell$ from equation (7) = $1.216 \times 10^{-3}$ m and $P = 1.906 \times 10^{-15}$ mol/m·s·Pa (from tubing supplier). As $K_L$ and $k_M$ are known $k_L$ can be calculated using equation [3.9] and expressed as a proportion of $K_L$ and is shown as triangles in figure 3-11 a mean value of $71\pm6\%$ is superimposed as a line over the individual points.
3.6.2 Mixing

The effect of mixing on $K_L$ was determined by varying the angular velocity of the magnetic stirrer bar. The results of this series of experiments are summarized in figure 3-12. A small increase in $K_L$ was observed when the mixing speed was increased from 200 to 350 rpm, however no significant change was observed from 350 to 600 rpm. It was not possible to measure $K_L$ with zero mixing as accurate operation of the DO meter required a small liquid velocity across the sensor membrane to operate accurately. Spatial effects also become important with little or no active mixing.

![Figure 3-12](image)

Figure 3-12  Variation in local mass transfer coefficient with mixing. Experimental parameters: bulk liquid = deionised water, gas = $O_2$ (food grade), $O_2$ pressure = 441 kPa, membrane specific area = 0.685 m$^{-1}$, temperature =15$^\circ$C.

3.6.3 Diffuser Loop Diameter

A further trial was run to observe the behavior of various diffuser lengths. Four different loop diameters were trialed (refer to table 3-3), each was mounted concentrically and 30mm above the bottom surface of the tank. As observed in figure 3-13 the variation in $K_L$ with diffuser loop diameter was considerably more dramatic than that observed by figure 3-12. Note the relatively small decrease in $K_L$ of 11% for the two largest membranes ($\Phi = 0.188$...
and 0.203 m) compared to the smaller diameter diffusers ($\Phi = 0.076$ and 0.138 m showed decreases of 52 and 77% respectively).

![Figure 3-13](image)

**Figure 3-13** Variation in local mass transfer coefficient with diffuser diameter. Experimental parameters: bulk liquid = deionised water, gas = O$_2$ (food grade), O$_2$ pressure = 441 kPa, mixing rate 350 rpm, temperature = 15°C.

### 3.6.4 Effect of Ethanol

Still table wine contains ethanol, typically, at concentrations between 9 and 14% v/v (8-13% mass fraction and 3.3-5.4% mole fraction). Ethanol, in water, acts as a surfactant and even at low concentrations can dramatically reduce the surface tension. For the values stated above (20°C), the surface tension ranges from 51.4 to 44.8 x 10$^{-3}$ N/m (Vazquez, Alvarez et al. 1995), or a 7.8% difference. The decrease from pure water to 12% v/v ethanol is an even more dramatic at 34% (72.75 to 47.06 x 10$^{-3}$ N/m).

To investigate the effect of ethanol on the mass transfer performance a series of trials were run using various bulk phase ethanol:water concentrations ranging from 0 to 15% v/v. Mass transfer performance is shown in figure 3-14. The increase in $K_L$ with higher ethanol concentration is believed to be primarily attributable to the surfactant effect on the
membrane as discussed above. The positive effect on the performance of $K$ has also been observed by other researchers (Cote, Bersillon et al. 1989)

Figure 3-14 also shows decreasing influence of the liquid side mass transfer resistance ($1/k_L$) with increasing ethanol concentration. For a typical wine, at a mixing rate of 350 rpm the influence of $1/k_L$ is reduced to 3.15%. Figure 3-15 depicts the same data as presented in figure 3-14 as a function of surface tension rather than ethanol concentration.

As such a large difference in $K_L$ was observed for 0% to 15 % ethanol a further two trials were run to better understand the relationship between oxygen partial pressure and Ethanol. The results of these trials (shown in figure 3-16) show a consistent ratio of approximately 3 (i.e. $K_L$ is three times greater at 10% than 0% Ethanol) between the ethanol concentrations for all pressures.

![Figure 3-14](image)

**Figure 3-14**  Variation in local mass transfer coefficient with ethanol concentration. Experimental parameters: gas = $O_2$ (food grade), $O_2$ feed pressure = 441 kPa, mixing rate 350 rpm, membrane specific area = 0.685 $m^{-1}$, temperature $=15^\circ C$
Figure 3-15  Mass transfer coefficient as a function of increasing surface tension (from decreasing ethanol concentration). Experimental parameters as figure 3-14

Figure 3-16  Relationship between $K_L$ (10% ethanol) and $K$ (0% ethanol) for different oxygen feed pressures
3.6.5 Effect of Temperature

Figure 3-17 shows no significant variation in $K_L$ for the range of temperatures expected in a micro-oxygenation treatment. Reference literature suggests that the permeability of FEP to oxygen varies considerably over this range (Scheirs 2000), however it should be recognised that the overall mass transfer resistance is dominated by the liquid-side boundary layer for the trial configuration, thus apparently reducing the overall sensitivity of the system to temperature variations.

![Figure 3-17](image)

**Figure 3-17**  Variation in local mass transfer coefficient with temperature. Experimental parameters: Bulk phase = de-ionised water, gas = $O_2$ (food grade), $O_2$ pressure = 441 kPa, mixing rate 350 rpm, membrane specific area = 0.685 m$^{-1}$, temperature =15°C.

3.6.6 Membrane fouling

A potentially significant problem with using membranes for oxygenation is the effect of fouling (solid particles adhering to the membrane and reducing mass transfer performance). A trial using FEP membranes in exactly the configuration described above was run on red wine for a duration of 15 weeks. Despite the fact that the wine had been filtered prior to the trial, precipitation of polyphenolic material is common for red wines during maturation. Figure 3-18 illustrates the condition of two (typical) membranes immediately following the experiment. There was relatively little visible fouling on any of the membranes.
In order to quantify the effect of any contamination, repeat trials (as described in section 3-5) were run on three membranes in order to compare the mass transfer coefficients before and after exposure to red wine. In all cases the variation in $K_L$ was less than 2% which is within the standard deviation of the original trial replicates.

![Diffuser condition after 15 weeks in Cabernet Sauvignon wine](image)

**Figure 3-18** Diffuser condition after 15 weeks in Cabernet Sauvignon wine

### 3.7 Discussion

#### 3.7.1 Oxygen Partial Pressure

There is good agreement with the model derived in section 3.3.1 and 3.3.2 and the behaviour of $K_L$ with oxygen partial pressure. The effect of a second, contaminating gas on the oxygen partial pressure was observed and the model derived in 3.3.2 appeared to account for the reduction in transport performance. In the case of a wine, particularly after fermentation, the solution is likely to be saturated with dissolved CO$_2$. The same model can be used to correct for the oxygen partial pressure using CO$_2$ as the contaminating gas.

#### 3.7.2 Mixing

The initial trials to determine the effect of mixing rate produced surprisingly little variation in $K_L$ (figure 3-12). The strong dependence of $K_L$ on the liquid phase boundary layer, as shown
Chapter Three

in figure 3-11, suggested that the variation should have been more significant. The observed changes in $K_i$ can be explained through an understanding of the flow fields induced by the rotating magnetic bar-stirrer.

The hydrodynamics of stirred tanks is given a detailed treatment in the reference literature (Nagata 1975) and the salient points will be summarized below. Liquid flow in an unbaffled, stirred tank can be characterised by defining two distinct flow regions: the cylindrical rotating zone (CRZ) and the free vortex zone (FVZ). The CRZ represents a cylinder of liquid directly above the stirrer with radius $r_c$ that rotates at constant angular velocity (the same rate as the stirrer) without a velocity gradient. Beyond the CRZ there exists a free vortex where the tangential velocity ($v$) is inversely proportional to the radius of the tank i.e.:

$$ r \leq r_c; \quad v = r \omega $$

$$ r \geq r_c; \quad v = \frac{\omega r_c^2}{r} $$

where $\omega$ is the angular velocity. The model is known as the Rankine's combined vortex. The tangential velocity field is depicted in figure 3-19. These theoretical profiles have been verified experimentally (Plion, Costes et al. 1985) (the discontinuity in velocity at $r_c$ notwithstanding). The radial and axial velocities are more difficult to predict using analytical models, however comparable experimental results exist (Nagata 1975) that allow a qualitative comparison of the relative contribution of each of the flow components. The axial and radial flow profiles are presented in figure 3-20.

The major influence of the radial velocity component is at the stirrer. The liquid is driven outward from the stirrer to the tank wall. The flow is localized, however, at the bottom of the tank. The axial velocity profile is characterised by up-flows directly above the stirrer and at the tank wall and down-flows in the annulus between (as depicted in figure 3-18)
The experiments performed by Nagata (1975) show that for the Reynolds numbers* above $10^3$ the flow is completely turbulent and the tangential component dominates (5 to 10 times greater than the largest axial or radial velocities). Reynolds numbers calculated for the membrane oxygenation trials described in section 3.5 are of the order of $1 \times 10^6$. Previous studies have shown that overall mass transfer is more effective when the liquid is flowing perpendicular (cross-flow) rather than parallel to the membrane axis (Yang and Cussler 1986). The difference can be a great as 150 for typical flows velocities observed around the membrane. For this reason the axial velocities, in particular cannot be ignored.

The original diffuser (figure 3-5) was mounted concentrically in the tank 30 mm from the bottom. The horizontal distance of the membrane from the tank wall was approximately

* Reynolds number defined, in this case, as $d'n\rho/\mu$ where $d$ = diameter of the stirrer and $n$ = rotation speed in r.p.s.
Based on figure 3-12 the local velocity field around the diffuser appears to have low sensitivity to changes in stirring rate.

**Figure 3-20** Radial and axial velocity profiles for unbaffled stirred tank (adapted from Nagata (1975))

**Figure 3-21** $K_L$ as a function of theoretical tangential velocity
The effect of reducing the diffuser diameter is to substantially increase the tangential velocity (up to $r_c$ - figure 3-19). Axial and radial velocities may vary, but the results of figure 3-13 suggest that the tangential velocity appears to have the major effect on $K_L$. This is further illustrated by Figure 3-21 which shows $K_L$ as a function of theoretical tangential velocity.

From a design perspective however the original configuration ($\Phi_{\text{diffuser}} = 0.188$ m) offers the advantage of low sensitivity to stirring rate. This is useful for replicated trials where each tank will be run by an independent stirring mechanism.

### 3.7.3 Ethanol

The significant effect of ethanol on $K_L$ is demonstrated clearly in figure 3-14 and 3-15. Moreover the relationship between ethanol concentration, oxygen partial pressure and $K_L$ was found to be predictable (figure 3-16). For design purposes the values of $K$ in figure 3.10 will be multiplied by three to account for the ethanol concentration.

### 3.7.4 Temperature

No correction will be made for temperature based on the results of the results described in figure 3-17.

### 3.7.5 Development of Oxygen Dosage Rate Curve.

For future trials the aim is to vary oxygen dosage rate by adjusting oxygen partial pressure inside the diffuser and maintaining all other variables constant. In the case of the test tanks (15 litres) it is desirable to have a dosage rate curve to allow selection of the required oxygen delivery rate. Figure 3-22 presents the relationship between the oxygen feed pressure and the dosage rate in mg/L/mth.

One limitation with the membrane configuration as tested is that the lowest achievable dosage rate is 10 mg/L/mth for wine. This is slightly higher than the commercially recommended levels. The response of the diffuser at lower or higher pressures could be extrapolated from the curve shown in figure 3-22 however this has not been verified experimentally.
Figure 3-22  Dosage rate curve for FEP membrane diffuser
3.8 Conclusion

Two methods of delivering controlled amounts of oxygen into small volumes of wine were examined; micro-bullage in high aspect ratio stainless steel tubes and dense polymer membrane oxygenation. Micro-bullage, in this case, was found to be unsuitable due to the inability to regulate the oxygen dosage, significant temperature gradients along the tube and the overall logistical difficulties that were encountered.

Dense polymer membrane oxygenation was found to be appropriate for small volumes of wine using FEP fluoropolymer tubing as the diffuser. Reliable and accurate oxygenation could be achieved over the range of dosage rates required for typical treatment of red wine. Significant increases in overall mass transfer performance were observed with increasing ethanol concentration attributed principally to the surfactant effect of the ethanol.

While little variation in mass transfer coefficient was observed with mixing rate for a 0.188 m diameter diffuser, altering the diffuser diameter lead to large changes in $K_L$. A strong correlation of $K_i$ with tangential velocity field was noted (figure 3-21), indicating a strong influence of the bulk-phase flow field. Moreover the dominance of the liquid phase boundary layer on $K_L$ is further illustrated in figure 3-11 where $1/k_L$ as a proportion of $1/K_L$ was found to be 71%. The effect of the liquid boundary layer resistance is significantly mitigated, however, by the surfactant effect of the ethanol. At concentrations typical in wine (12% v/v) $1/k_L$ as a proportion of $1/K_L$ was reduced to 3.2%.
4.1 Introduction

The principal objective of the experiments described in this thesis is to better understand the effects of oxygen dosage rate on the chemical and corresponding sensory changes in red wine. Hitherto experimental studies on the effects of oxygen in wine have generally been conducted on model wines at either elevated temperature or pH (Singleton 1987; Tulythan, Boulton et al. 1989; Cilliers and Singleton 1991) or using commercial treatments where important between treatment effects (oak adjuncts, suspended yeast lees, vessel geometries, ullage and temperature) were uncontrolled and hence there effects could not be determined (McCord 2002). Two published studies have investigated particular chemical transformations occurring in MOX wines (Atanasova, Fulcrand et al. 2002; Cano-Lopez, Pardo-Minguez et al. 2006) however no study has looked at both chemical and sensory transformations concurrently.

The aim of this particular trial was to quantify chemical and organoleptic transformations occurring in a red wine exposed to oxygen at different dosage rates, while controlling for all of the potentially important variables listed above.

To achieve this, special treatment vessels were designed and fabricated (figure 3-8) utilising oxygen-permeable dense-polymer membrane as the oxygenation mechanism. The diffuser configuration used for this trial was fully characterised in chapter three and the dosage rates are taken direct from figure 3-22.
4.2 Trial Setup

4.2.1 Grapes

Cabernet Sauvignon is a red *Vitis Vinifera* variety typically used in micro-oxygenation treatments. The grapes were hand-harvested at commercial maturity on the 1st May 2004 from a vineyard in West Auckland, New Zealand.

4.2.2 Wine Processing

The processing steps are summarised in figure 4-1. A pectolytic enzyme (VR-C) was added to the pomace at crushing to aid with colour extraction. 15 mg/L sulfur dioxide (as 10% w/v solution of potassium metabisulfite) was also added, principally to suppress polyphenol oxidase and wild yeast activity. The oenological details of the must are given in table 4-1.

| Table 4-1 Oenological parameter of the Cabernet Sauvignon must |
|--------------------------|---------|
| Parameter               | Value   |
| Brix (juice) (%SS)      | 21.5    |
| pH                      | 3.5     |
| TA (g/L)                | 8.8     |
| Malic Acid (g/L)*       | 2.8     |

After a period of pre-fermentation maceration (72 hours at 12°C) the temperature was increased to 15°C and a commercial strain of *Saccharomyces cerevisiae* (Lalvin D254) was added at the (supplier’s) recommended concentration of 250 mg/L. The fermentation proceeded normally to dryness with 200 mg/L diammonium phosphate (DAP) and 20 g/L sugar (as sucrose) being added two and three days after inoculation respectively. Cap management involved thorough plunging every 12 hours from the time of crushing until the end of fermentation and then every 24 hours until the grapes were pressed.

* Measured using an enzyme assay (Boeringher Mannhiem)
It was desirable, for this particular trial, to have material that was rich in tannins hence the wine was left to macerate for 15 days following the completion of fermentation. Informal tastings were conducted every two days to judge the extent of astringency and bitterness on the palate. The wine was pressed-off skins, transferred to a 500 litre holding tank and maintained at 12°C.

### 4.2.3 Oxygenation Treatment Setup

The trial was conducted using 12 x 15 litre tanks identical to that described in chapter three. The experimental design consisted of four treatments x three replicates; each treatment involved applying a different oxygen dosage rate as shown in figure 4-2.

The aim of the experiment was to evaluate the chemical and sensory characteristics of a wine treated at different rates of continuous oxygen dosage. Respective rates were chosen to represent the typical range employed for commercial micro-oxygenation treatments. The treatment details are given in table 4-2.
Table 4-2  Dosage Rate Summary

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dosage Rate (mg/L/mth)</th>
<th>Diffuser feed pressure (kPa absolute)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Treatment 1</td>
<td>10</td>
<td>200</td>
</tr>
<tr>
<td>Treatment 2</td>
<td>23</td>
<td>400</td>
</tr>
<tr>
<td>Treatment 3</td>
<td>36</td>
<td>600</td>
</tr>
</tbody>
</table>

Prior to filling of the treatment vessels, the wine was racked and filtered, initially using a plate-and-frame (20 cm x 20 cm) filter using #30 filter pads (3M Cuno) to remove the gross and medium particulate matter. Subsequently the wine was passed through a 0.45 μm membrane filter to remove remaining yeast lees and other fine material. Each of the 12 x 15 litres experimental tanks was then immediately filled with the filtered wine. All tanks were purged with argon immediately prior to filling and a blanket of argon was maintained over the free liquid surface during filling. The tanks were sealed after filling and the headspace volume (approximately 180 cm³) was displaced with argon through a non-return valve configuration on the lid as shown in figure 4-3 a).
48 hours after the filling operation (allowing for temperature equilibration) controlled oxygenation was commenced by applying feed pressure to the oxygen diffusers at rates shown in figure 4-2 and table 4-2. The feed pressure was applied continuously for the duration of the trial.

The temperature was maintained at 15°C using a temperature controlled rooms at the University of Auckland winery. The headspace was systematically displaced with argon every three days to minimise the extent of oxygen contact from the atmosphere.

### 4.2.4 Wine Sampling

Sampling of the wine for analysis (chemical and sensory) began one week after commencement of oxygenation and then every two weeks following, for the duration of the trial (15 weeks). 250 ml of each treatment replicate was required for analysis. As the aim of the experiment was to measure the effect of controlled amounts of oxygen on wine, sampling without oxygen contamination from the atmosphere was critical. To achieve this, a sampling apparatus was designed and fabricated and is shown in figure 4-3 b) and c). The corresponding sampling procedure is described in figure 4-4. The aliquot of sampled wine was immediately replaced with bulk wine after each sampling (the same wine as used in the trial but stored at 10°C without oxygen). This was necessary as the cumulative effect of sampling 250 ml x eight observations (2 litres) would significantly impact the oxygen dosage rate (i.e. by changing the specific area \(a\) – refer to section 3.3.1) and the potential for oxygen contamination due to an increased headspace volume. As 250 ml represents only 1.7% of the total tank volume, the overall effect on the respective treatment was considered to be insignificant.
Chapter Four

Figure 4-3  Sampling apparatus

Figure 4-4  Sampling procedure and process flow
4.2.5 Malolactic Fermentation

52 days after the commencement of the oxygenation treatment the temperature in the controlled environment was raised to 18°C and a commercial strain of *oenococcus oeni* (Enoferm Alpha) was added to each treatment at the recommended dosage rate of 10 mg/L. The levels of L-malic acid were monitored initially by enzymatic analysis (Boeringher Mannhheim) followed by paper chromatography (Iland 2000) 7 and 14 days after inoculation. After 14 days, the paper chromatography indicated that malo-lactic fermentation had been completed in all 12 treatment replicates. This result was confirmed with a final series of analyses again using the commercial L-malic enzyme assay described above.

4.2.6 Sulfur Dioxide Treatment

At the completion of malo-lactic fermentation 30 mg/L sulfur dioxide (as 10% w/v solution of potassium metabisulfite in water) was added to each of the treatment and control wines. The addition was made 84 days after the commencement of the trial. The aim of the sulfur dioxide (SO2) was to further replicate the typical treatment of a commercial wine and to gain some insight into the effects of SO2 on the development of the polyphenol and sensory characteristics. SO2 levels (free and bound) were measured by the aspiration method at the remaining two observation dates (91 and 105 days).

4.3 Chemical Analysis

4.3.1 Low Molecular Weight Polyphenol Analysis

The low molecular weight HPLC analysis and subsequent chemometric treatment is fully described in Chapter Two (section 2.3.1 through 2.3.3). Identical equipment, methods and materials (with the exception of the samples) were used for the trial described above as for that described in Chapter Two. Standard curves for the commercial available polyphenolic compounds were run prior to the commencement of the trial and are presented in appendix 1.
4.3.2 **Thiolysis**

Thiolysis and associated procedures (sample fractionation) are fully described in Chapter Two (sections 2.3.4 through 2.3.7). Thiolysis was run on all samples at each of the observation dates.

4.3.3 **Spectrophotometric Analysis**

Colour was assessed using a spectrophotometer (Varian Cary 50) with a 2 mm path-length fibre-optic probe. Wines were filtered (0.45 µm RC syringe filter, Sartorius) before measurement. Full spectral measurements (340-800 nm) as well as discrete wavelengths (420, 520 and 620 nm) were recorded for each sample. As all the samples were at identical pH no adjustment was made for these readings.

In addition to the raw spectrophotometric data outlined above a bleaching assay was also performed. This procedure involved adding a stoichiometric excess of bisulfite (30 µl of 25% (w/v) potassium metabisulfite) leaving for 45 minutes and measuring the absorbance at 520 nm. The full method details are described in (Ilard 2000). The idea is that the loss of red colour is inversely related to the extent of formation of stable pigments (condensed tannins, pyranoanthocyanins, vitisins etc.) and hence overall colour stability.

4.3.4 **Polyphenol Measurement by Folin-Ciocalteau Method**

The Folin Ciocalteau method was used as a measure of total polyphenols to compliment the HPLC analyses described in 4.3.1. The method was adapted from (Slinkard and Singleton 1977) and is described below.

Gallic acid was used as the standard for this particular assay and stock solutions of 0, 50, 100, 500 and 1000 mg/L were prepared. 20 µl of each stock solution were pipetted into UV-Vis plastic cuvette. 1.58 ml of de-ionised water was then added to each cuvette followed by 100 µl of Folin-Ciocalteau reagent (Sigma Aldrich). The cuvettes were covered with parafilm, mixed thoroughly (by hand) and left for 5 minutes. 300 µl of previously prepared 200 g/l sodium carbonate solution was then added to each cuvette and left at 20°C (in the incubator) for 2 hours. The absorbance of each cuvette was then measured at 765 nm.
Each sample was prepared in the manner described above except the stock solution was replaced with wines diluted 1:1 with de-ionised water prior to analysis. All analyses were performed in triplicate and the results are expressed in concentration as gallic acid equivalents (GAE).

4.3.3 Sensory Analysis

Quantitative descriptive analysis of sensory attributes was carried out concurrently with chemical analysis at each sampling date. A trained panel of (nominally) 12 volunteer members was asked to discriminate the wines based on several organoleptic parameters listed in table 4-7. The panelists were selected from people who consume wine on a regular basis. The majority of the panelists participated in the trials the previous year (described in chapter Two), new members, however, were initially screened on their ability to perceive certain basic sensory attributes (refer to table 4-7). After selection, training was conducted twice weekly for four months prior to actual data collection.

As with the previous year preliminary training consisted of conducting triangle testing of individual sensory standards in water (three sets per session on any attribute listed in table 4-7). The aim of this training phase was to improve the cognitive ability and confidence of the panelist to recognise the sensory character of each attribute. The triangle testing of standards was followed by triangle testing of wines (Mission Estate Cabernet Sauvignon 2003) spiked with one or more of the standards. During these sessions the panelist were asked to develop an appropriate terminology for the attributes (refer 4-7). Note that this terminology is subtly different from the panel of the previous year.

After the panelists became comfortable being able to detect the spiked wines within a triangle test context they were then asked to rank each attribute on a 100 mm horizontal line (the left and right edge of the line denoting the absence and the extreme presence of the attribute respectively – refer to appendix IV for an example of the training form). The panelist was generally presented with two identical base wines, one only being spiked with standard(s). In this training phase the panelist was asked to recognise the attribute difference of each wine in both absolute and relative terms. Standard concentrations were also varied to determine the response of the tasters and develop response curves (perception vs
concentration) for each of the attributes. Numerous tests were run and the data analysed to calculate the mean scores for the base wines and for the effect of spiking the standards. The base wine mean scores for each attribute were then indicated on the 100 mm horizontal line with vertical checks (refer to appendix IV). Subsequent training could then be “calibrated” based on the historical training data (i.e. individual panelists were required to adjust their respective scores based on the group’s mean score).

A number of changes were made from the sensory trials conducted the previous year (Chapter Two). The total number of attributes requiring discrimination was reduced from 12 to 8. Visual attributes were eliminated as it was felt these could be easily measured. Aromatic intensity and complexity, length and overall impression were also eliminated as these were felt to be too esoteric to get meaningful results. In place of length and overall impression the attribute of “balance” was included with the corresponding definition given in table 4-7. The “tannic force” attribute was changed to “mouthfeel” on the recommendation of the panelists. Aldehyde was included as this is an important sensory property associated with MOX wines.

Further research on appropriate standards to better reflect the wine sensory attributes was conducted. As a result the bitterness standard was changed from caffeine to quinine sulfate and the astringency standard was changed from Ocean Spray cranberry juice to aluminium sulfate. The respective concentrations in wine are given in table 4-7. The calibration points for the standard wine are also given in table 4-7. This represents the mean sensory response (over multiple trials) of the base wine (Mission Estate Cabernet 2003) to each sensory attribute.

Sensory data collections were held at 10am on the day of sampling (approximately 2 hours after the wine had been sampled). No more than 30 minutes before the commencement of tasting 30 ml of each wine was decanted from the 250 ml Schott bottle (used to sample the wine) into standard INAO tasting glasses. The glasses were sealed with parafilm, and each glass then labeled with a three digit randomly generated numerical code corresponding to the treatment and replicate.
During the data collection each panelist was presented with four wines (1 of each treatment). The presentation order was randomised except that each panelist received a different treatment replicate each data collection session (i.e. the same replicate would be repeated every fourth session). The panelists were asked to score the wines independently (i.e. no comparison) on the aforementioned attributes. Printed on the score sheets were the base wine calibration checks described above.

The sessions were conducted under controlled lighting in individual booths. Panelists were requested not to drink or eat for two hours before each collection session. Panelists were given water and slices of bread (baguette) to aid palate freshness during tasting.

Training was conducted weekly throughout the data collection period using the aforementioned techniques.

4.3.6 Data Analysis
Statistical analysis was performed using Microsoft Excel 2003, The Unscrambler v9.1, Matlab v7.0.1 R14 and R v 2.4.0.
<table>
<thead>
<tr>
<th>Attribute</th>
<th>Description (developed by panellists)</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aromatic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetative</td>
<td>herbaceous characters, e.g. capsicum freshly cut grass</td>
<td>Isobutyl methoxypyrazine (0.05 µg/L)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gis-hexenol (0.2 mg/L)</td>
</tr>
<tr>
<td>Fruity</td>
<td>Berry characters, black currant, strawberry, raspberry</td>
<td>Barkers blackcurrant concentrate</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>Rotting fruit, sweet-sickly fruit</td>
<td>CH₃CHO (50 mg/L in water)</td>
</tr>
<tr>
<td><strong>Sapid</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acidity</td>
<td>Sour/tart character on tongue</td>
<td>Tartaric Acid (3.0 g/L in water)</td>
</tr>
<tr>
<td>Bitterness</td>
<td>Perceived on tongue</td>
<td>Quinine Sulfate (0.5 mg/L in water)</td>
</tr>
<tr>
<td><strong>Tactile/Mouthfeel</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Astringency</td>
<td>Drying/puckering in cheeks</td>
<td>Al₂(SO₄)₃ (2 g/L in water)</td>
</tr>
<tr>
<td>Balance</td>
<td>Balance is a measure of a wine’s harmony – i.e. the way in which all attributes come together to make a complete wine. If you perceive one or more attribute(s) over the other(s) then the wine is poorly balanced</td>
<td>Mission Estate Cabernet 2003</td>
</tr>
<tr>
<td>Mouthfeel</td>
<td>Mouthfeel is a measure of how the wine “fills” the palate. A wine with a weak mouthfeel will tend to only be perceived on the centre-line of the tongue. If the wine is felt strongly on the inside of the cheeks and the tongue, then it can be considered full or big</td>
<td>Mission Estate Cabernet 2003</td>
</tr>
</tbody>
</table>
4.4. Results

4.4.1 Sensory Results

The results of the sensory trials are presented in figures 4-5 through 4-8 and in table 4-8. Figures 4-5 through 4-8 show plots of the two first canonical variables (linear combination of original variables that maximise the separation between treatment means) for each sampling date. The treatment means are presented as bold symbols surrounded by circles of the same colour. The circles represent 95% confidence regions for each of the respective treatments means (i.e. the probability that the population group mean lie within that region) and are based on a $\chi^2$ test (Krzanowski 2000). Overlayed are the individual panellist scores transformed into canonical space.

Table 4-8 presents the results of a single factor multivariate analysis of variance (MANOVA) for each of the sampling dates. The Wilks Lambda test statistic ($\Lambda$) is calculated for every possible dimension of the data (i.e. number of treatments – 1) the corresponding probability estimation is shown in the adjacent column (based on the $\Lambda$ value). The overall significant (Probability<0.05) dimensionality of the treatments is presented in the right-hand column (i.e. if the means are the same then $d=0$ or separated along a line ($d=1$), plane ($d=2$) or 3 dimensional space ($d=3$)).
Chapter Four

Figure 4-5  Canonical variates analysis plots of sensory results for sampling dates at 7 and 21 days after the start of treatment. Bold symbols represent treatment mean scores. Other symbols are individual panelist data. The circles surrounding treatment means indicate 95% confidence regions for the respective treatment group means.
Figure 4-6  Canonical variates analysis plots of sensory results for sampling dates at 35 and 49 days after the start of treatment. Bold symbols represent treatment mean scores. Other symbols are individual panelist data. The circles surrounding treatment means indicate 95% confidence regions for the respective treatment group means.
Figure 4-7  Canonical variates analysis plots of sensory results for sampling dates at 63 and 77 days after the start of treatment. Bold symbols represent treatment mean scores. Other symbols are individual panelist data. The circles surrounding treatment means indicate 95% confidence regions for the respective treatment group means.
Figure 4-8  Canonical variates analysis plots of sensory results for sampling dates at 91 and 105 days after the start of treatment. Bold symbols represent treatment mean scores. Other symbols are individual panellist data. The circles surrounding treatment means indicate 95% confidence regions for the respective treatment group means.
Table 4-8  Results of single factor MANOVA for each sample date

<table>
<thead>
<tr>
<th>Days From Start of Treatment</th>
<th>No. of Panellists</th>
<th>Wilks Lambda (Λ)</th>
<th>Estimated Probability</th>
<th>Dimensionality (based on P&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>12</td>
<td>0.2340</td>
<td>0.0000</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.4680</td>
<td>0.0019</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.7401</td>
<td>0.0352</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>12</td>
<td>0.2036</td>
<td>0.0000</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.4648</td>
<td>0.0049</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.8027</td>
<td>0.1729</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>12</td>
<td>0.3239</td>
<td>0.0003</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5351</td>
<td>0.0062</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.7782</td>
<td>0.0558</td>
<td></td>
</tr>
<tr>
<td>49</td>
<td>11</td>
<td>0.2291</td>
<td>0.0040</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5515</td>
<td>0.0782</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.7864</td>
<td>0.1798</td>
<td></td>
</tr>
<tr>
<td>63</td>
<td>8</td>
<td>0.3676</td>
<td>0.4047</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.6657</td>
<td>0.7495</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.8963</td>
<td>0.8410</td>
<td></td>
</tr>
<tr>
<td>77</td>
<td>10</td>
<td>0.3506</td>
<td>0.0748</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5826</td>
<td>0.2148</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.7854</td>
<td>0.2402</td>
<td></td>
</tr>
<tr>
<td>91</td>
<td>8</td>
<td>0.1300</td>
<td>0.0011</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.4435</td>
<td>0.1202</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.7538</td>
<td>0.3147</td>
<td></td>
</tr>
<tr>
<td>105</td>
<td>10</td>
<td>0.3150</td>
<td>0.0337</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5123</td>
<td>0.0772</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.7442</td>
<td>0.1356</td>
<td></td>
</tr>
</tbody>
</table>

Figure 4-6 to 4-9 and table 4-8 show a complex evolution of the relative sensory profiles of each of the treatments through the course of the trial. All sampling dates showed significant differences in treatment means except for those at 63 and 77 days. An analysis of the CVA plots (figure 4-8 a) and b)) and table 4-8 indicate that this was because the wines were perceived as being similar by the panellists rather than an overly large variance in the data.
Figure 4-9  Unconstrained PCA of the mean sensory scores for each treatment and sample date. Data labels indicate sample date and dosage rate as “days since start of treatment”/”oxygen dosage rate” e.g. 7/23 = 7 days from start of treatment and 23 mg/L/mth dosage rate. The lines indicate the evolution of each treatment chronologically.
Figure 4-10  Principal Response curves for the three oxygenation treatments relative to the control wine. Sensory attribute weights are presented in the linestack plot to the right of the actual PRC plot.
An unconstrained principal components analysis (PCA) was performed on the mean sensory scores for each of the treatment and observation dates. The typical ordination output for this type of analysis is an x-y plot scaled to include both the scores (sample data) as points and loadings (variables) as vectors in principal component space. Such a biplot is given in figure 4-9. Connecting lines are drawn between each sample score to indicate the chronological sequence of observation dates for each treatment. The plot is very cluttered and difficult to interpret fully, particularly the differences between the respective treatments and the control wine. Moreover time is not expressed in a single direction leading to very jagged trajectories.

### 4.4.2 Principal Response Curves

A method, known as principal response curves (PRC), has recently been developed to mitigate these issues by focussing on the sensory differences between the treatments and the controls at each corresponding observation dates. The model is briefly outlined below, however it is fully developed and discussed by (Van Den Brink and Ter Braak 1999).

Let, for sensory attribute $k$ (e.g. astringency) $T_{dk}$ be the treatment effect of treatment $d$ at sample date $t$ ($k=1,...,7$; $d = 0,...,3; t=7,21,35,49,63,77,91$ and 105 days). Thus the response pattern of interest for each sensory attribute is a set of $4 \times 7$ treatment effects ($T_{dk}$) for a given $k$. By further modelling the response pattern $T_{dk}$ for each attribute as a multiple of some weighting factor ($b_k$) of a basic response pattern ($c_{dt}$) [i.e. $T_{dk} = b_k c_{dt}$] the statistical model of the sensory attribute data becomes:

$$y_{d(j)tk} = \bar{y}_{0tk} + b_k c_{dt} + \epsilon_{d(j)tk}$$  \[4.1\]

Where $y_{d(j)tk}$ is the sensory score of attribute $k$ in replicate $j$ of treatment $d$ at time $t$; $\bar{y}_{0tk}$ is the mean score of attribute $k$ at sample date $t$ in the control ($d=0)$ and $\epsilon_{d(j)tk}$ is an error term with mean $= 0$ and variance $= \sigma^2_k$. It should also be noted that $c_{dt} = 0$ for every sample date because, by definition, $T_{dk} = 0$ for every $t$ and $k$ (recall we are concerned with the differences between the treatment and the control rather than the absolute treatment scores). Estimates for $\epsilon_{d}$ are determined by redundancy analysis (RDA) which is a constrained form of PCA (Legendre and Legendre 1998). The desired estimates of the coefficients ($c_{dt}$) are the canonical coefficients of the RDA. These coefficients are then plotted against sampling date.
for each treatment. The resulting curves are interpreted as the principal response curves. These are generally accompanied by the attribute weights ($b_i$) which allow interpretation of the individual attribute contribution. The higher the weight the more the response pattern of the attribute is likely to follow the PRC. Attributes with high negative weights follow the opposite pattern, and attributes with zero weights show either no response or a response that is unrelated to the PRC.

The PRC for the sensory data set described above is presented in figure 4-10. The most obvious feature of the PRCs for each of the treatments is the relative similarity in their patterns. 36 mg/L/mth treatment shows an initial decrease and the coefficients become negative at 21 days (recall the effect is measured relative to the control). An increase in the response is then seen and the treatment coefficient becomes positive and a peak is seen at 63 days. A further cycle (decrease followed by increase) can be seen in the remaining 42 days of the trial. Similar patterns are observed for the 23 and 10 mg/L/mth treatments; however the pattern appears to be shifted in time approximately two weeks (i.e. limited by the resolution of the sampling dates). Response peaks occur at 91, 77 and 63 days for the 10, 23 and 36 mg/L/mth treatments respectively. This indicates that the sensory evolution follows the same pattern irrespective of the oxygen dosage rate and the rate of oxygen dosage determines the rate of sensory development.

Another notable feature of the response curves is the magnitude of the response of the 36 mg/L/mth treatment at the 63 day sampling date (positive) and the 23 mg/L/mth treatment at the 35 and 105 day sampling dates (negative).

An examination of the attribute weights shows that mouthfeel, astringency, aldehyde (aroma) and bitterness all contribute positively toward the model. Acidity vegetativeness and fruitiness contribute almost nothing to the treatment response and balance weights negatively. This is very similar to the attribute vectors displayed on the PCA biplot in figure 4-10 (unsurprisingly as they were derived from the same data).

The PRC plot allows a much clearer interpretation of the entire dataset and particularly the evolving sensory behaviour of each treatment (relative to the control wine). One of the main
objectives of this study was to ratify the anecdotally derived sensory development plot presented in figure 1-2. While the behaviour is oscillatory as in figure 1-2 the much discussed structuring and harmonisation phases are less obvious.

### 4.4.3 Folin Ciocalteau

The Folin Ciocalteau results are summarised in figure 4-11 and table 4-9. While there were significant differences between observation dates there was only one significant within treatment difference on any of the sampling days (between the 10 and 23 mg/L/mth treatment on days 77). The characteristic development for all treatments begins with a rise in the Folin index at day 21 (significant for the 23 mg/L/mth treatments) followed by a small decrease at day 35 (significant from 10 and 23 mg/L/mth treatments). A steady increase in mean Folin index ensues for the remainder of the trial and is consistent (and significant) for all treatments.

![Figure 4-11](image-url)  
Figure 4-11 Development of mean Folin-Ciocalteau polyphenol index for each treatment over the course of the trial. Error bars indicate standard deviation ($n=3$)
Table 4-9  mean (n=3)ab mean Folin-Ciocalteau polyphenol index (mg/L gallic acid equivalent) for all treatments and observation dates.

<table>
<thead>
<tr>
<th>Days from start of Treatment</th>
<th>0 mg/L/mth</th>
<th>10 mg/L/mth</th>
<th>23 mg/L/mth</th>
<th>36 mg/L/mth</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>565 (±45)bc</td>
<td>611 (±6)ab</td>
<td>631 (±48)ab</td>
<td>580 (±29)ab</td>
</tr>
<tr>
<td>21</td>
<td>705 (±34)bc</td>
<td>721 (±43)bc</td>
<td>744 (±31)c</td>
<td>703 (±35)bc</td>
</tr>
<tr>
<td>35</td>
<td>443 (±30)ab</td>
<td>694 (±29)a</td>
<td>679 (±39)a</td>
<td>508 (±44)ab</td>
</tr>
<tr>
<td>49</td>
<td>563 (±47)ab</td>
<td>581 (±51)ab</td>
<td>520 (±27)a</td>
<td>526 (±45)a</td>
</tr>
<tr>
<td>63</td>
<td>587 (±43)b</td>
<td>611 (±26)bc</td>
<td>790 (±60)bc</td>
<td>532 (±23)ab</td>
</tr>
<tr>
<td>77</td>
<td>722 (±67)bc</td>
<td>707 (±64)ab</td>
<td>820 (±31)c</td>
<td>744 (±84)bc</td>
</tr>
<tr>
<td>91</td>
<td>593 (±45)bc</td>
<td>702 (±63)bc</td>
<td>705 (±41)bc</td>
<td>788 (±57)c</td>
</tr>
<tr>
<td>105</td>
<td>775 (±57)c</td>
<td>773 (±52)c</td>
<td>797 (±55)c</td>
<td>736 (±57)c</td>
</tr>
</tbody>
</table>

a Standard deviations are given in parentheses after each value
b The bold-type letters indicate whether each treatment is significantly different from another based on a single factor ANOVA and multiple comparison using Fishers LSD0.05. Different letters indicate significant differences.

### 4.4.4 Colour Measurements

One of the assertions made by MOX practitioners is that the treatment improves colour intensity and colour stability. In order to test these claims the colour was measured at each observation date. The aim was to quantify the raw colour intensity (as observed by a winemaker) and the colour stability (assessed using the bleaching test described in section 4.3.3).

The raw colour of the wine was measured using traditional measures of intensity and hue. These results are presented in figures 4-12 and 4-13 and table 4-10 and 4-11. Full spectral measurements were also acquired and these were analysed using PCA in a similar way to that described in section 2.3.2. The results of the PCA are displayed in figures 4-14 and 4-15 for the scores and loadings plot respectively.
**Figure 4-12**  Development of mean visual intensity for each treatment over the course of the trial. Error bars indicate standard deviation (n=3)

**Table 4-10**  mean (n=3) raw visual intensity (420+520+620nm) for all treatments and observation dates.

<table>
<thead>
<tr>
<th>Days from start of Treatment</th>
<th>0 mg/L/mth</th>
<th>10 mg/L/mth</th>
<th>23 mg/L/mth</th>
<th>36 mg/L/mth</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>10.76 (±0.17) de</td>
<td>11.45 (±0.68) de</td>
<td>12.17 (±1.35) c</td>
<td>10.57 (±0.67) de</td>
</tr>
<tr>
<td>21</td>
<td>10.52 (±0.82) d</td>
<td>11.51 (±0.68) e</td>
<td>11.22 (±0.57) de</td>
<td>11.13 (±1.13) de</td>
</tr>
<tr>
<td>35</td>
<td>9.34 (±0.01) c</td>
<td>8.78 (±0.15) bc</td>
<td>8.92 (±0.14) bc</td>
<td>8.98 (±0.09) c</td>
</tr>
<tr>
<td>49</td>
<td>8.90 (±0.14) bc</td>
<td>8.91 (±0.23) bc</td>
<td>9.29 (±0.42) c</td>
<td>9.18 (±0.07) c</td>
</tr>
<tr>
<td>63</td>
<td>8.56 (±0.04) bc</td>
<td>8.95 (±0.21) bc</td>
<td>9.37 (±0.52) c</td>
<td>9.27 (±0.07) c</td>
</tr>
<tr>
<td>77</td>
<td>8.63 (±0.05) bc</td>
<td>9.06 (±0.21) c</td>
<td>9.44 (±0.37) c</td>
<td>9.45 (±0.07) c</td>
</tr>
<tr>
<td>91</td>
<td>6.98 (±1.17) a</td>
<td>7.99 (±0.48) b</td>
<td>8.68 (±0.92) bc</td>
<td>8.54 (±0.36) bc</td>
</tr>
<tr>
<td>105</td>
<td>6.75 (±0.81) a</td>
<td>7.70 (±0.66) ab</td>
<td>9.02 (±1.09) c</td>
<td>8.59 (±0.36) bc</td>
</tr>
</tbody>
</table>

* Standard deviations are given in parentheses after each value

b The bold-type letters indicate whether each treatment is significantly different from another based on a single factor ANOVA and multiple comparison using Fishers LSD$_{0.05}$. Different letters indicate significant differences.
Figure 4-13  Development of mean hue for each treatment over the course of the trial. Error bars indicate standard deviation \((n=3)\)

Table 4-11  mean \((n=3)^{ab}\) raw hue (420/520nm) for all treatments and observation dates.

<table>
<thead>
<tr>
<th>Days from start of Treatment</th>
<th>0 mg/L/mth</th>
<th>10 mg/L/mth</th>
<th>23 mg/L/mth</th>
<th>36 mg/L/mth</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>0.545 (±0.003) a</td>
<td>0.567 (±0.016) ab</td>
<td>0.591 (±0.025) b</td>
<td>0.551 (±0.017) a</td>
</tr>
<tr>
<td>21</td>
<td>0.545 (±0.022) a</td>
<td>0.612 (±0.036) bc</td>
<td>0.632 (±0.029) cd</td>
<td>0.636 (±0.029) cd</td>
</tr>
<tr>
<td>35</td>
<td>0.545 (±0.007) a</td>
<td>0.611 (±0.006) bc</td>
<td>0.606 (±0.015) bc</td>
<td>0.628 (±0.008) c</td>
</tr>
<tr>
<td>49</td>
<td>0.575 (±0.003) ab</td>
<td>0.623 (±0.006) bc</td>
<td>0.643 (±0.021) cd</td>
<td>0.647 (±0.005) cd</td>
</tr>
<tr>
<td>63</td>
<td>0.602 (±0.004) bc</td>
<td>0.640 (±0.004) cd</td>
<td>0.657 (±0.020) cd</td>
<td>0.658 (±0.007) cd</td>
</tr>
<tr>
<td>77</td>
<td>0.625 (±0.004) bc</td>
<td>0.664 (±0.005) d</td>
<td>0.675 (±0.013) de</td>
<td>0.683 (±0.010) de</td>
</tr>
<tr>
<td>91</td>
<td>0.732 (±0.064) e</td>
<td>0.677 (±0.012) d</td>
<td>0.686 (±0.017) d</td>
<td>0.694 (±0.010) d</td>
</tr>
<tr>
<td>105</td>
<td>0.666 (±0.047) de</td>
<td>0.699 (±0.020) e</td>
<td>0.686 (±0.010) de</td>
<td>0.709 (±0.008) e</td>
</tr>
</tbody>
</table>

\(a\) Standard deviations are given in parentheses after each value

\(b\) The bold-type letters indicate whether each treatment is significantly different from another based on a single factor ANOVA and multiple comparison using Fishers LSD\(_{0.05}\). Different letters indicate significant differences.
Figure 4-12 and table 4-10 show that the absolute visual intensity for all treatments decreases over the course of the trial. All treatments are characterised by a decline in the first five weeks followed by a relatively stable period until the final two observation dates (91 and 105 days) where another decrease in visual intensity is observed (note that SO$_2$ was added at day 84).

While all treatments show a decline in intensity the decrease was more pronounced for the control, particularly after the addition of SO$_2$ where the control was significantly lower than the 23 and 36 mg/L/mth treatments. Interestingly the 10 mg/L/mth treatment appears to track between the control and two higher dosage rate treatments.

Hue is the ratio of 420 nm to 520 nm absorbance and is an indication of the relative amount of brown to red pigments in the wine. Hue was observed to increase for all treatments over the course of the trial, as expected the most notable feature of figure 4-13 being that the control tracks consistently and significantly lower than the other three treatments until the 91 day observation date where it measures significantly higher. This sudden increase in hue correlates with the addition of SO$_2$ as discussed above the commensurate loss of red pigment through bleaching.

In recent studies on micro-oxygenation (Atanasova, Fulcrand et al. 2002) observed a similar chromatic evolution to that given above (i.e the absolute value of colour intensity decreases but less so for the MOX treatment, no significant result was observed for the hue). In contrast (Cano-Lopez, Pardo-Minguez et al. 2006) saw both an absolute and relative rise in colour intensity, similarly no significant increase in hue was detected. Both studies dealt with wines at very low dosage rates (5 ml/L/mth). Moreover, no indication is given regarding the SO$_2$ levels in either wine, which could have a major impact on the colour intensity.

Figures 4-14 and 4-15 show the results of a PCA on the raw spectral data. Figure 4-14 tracks the individual treatments through the course of the trial (the data labels indicate the observation date). 93% of the variance is explained by PC1 which shows a general evolution from right to left over time. One notable feature is the similar trajectories of the three treatment wines and the different behaviour of the control wine. For a complete
interpretation of the analysis the scores plot must be viewed in conjunction with the loading
plot (figure 4-15). The loadings plot indicates that the wines that plot in the (+,+ ) quadrant
(refer to section 2.4.2 for an explanation of the nomenclature) are highest in red pigments
(control wine at 7 and 21 days, 10 mg/L/mth wine at 7 and 21 days and the 36 mg/L/mth
wine at 7 days. Similarly the wines that exhibit the greater brown pigment (420nm) load in
the (+,-) quadrant (23 mg/L/mth treatment wine at 7 and 21 days and 36 mg/L/mth
treatment wine at 21 days). All treatments with the exception of the control show little
evolution from 35 to the 77 days (all activity is centred around the (0,0) point of the PCA
projection). All treatments (except the 23 mg/L/mth) show a shift from right to left after 77
days; this correlates with the addition of SO$_2$ as discussed above. The 23 mg/L/mth
treatment stays at the centre of the projection until the end of the trial and appears to be
unaffected by the SO$_2$ addition.

A common method of assessing the colour stability of red wines is to measure the resistance
to bisulphite bleaching (Iland 2000). The test involves dosing a wine with a stoichiometric
excess of bisulphite to bleach the remaining anthocyanins. The smaller the relative decrease
in red colour indicates a greater degree of formation of stable pigmented compounds (e.g. T-
A$^+$ adducts or similar as discussed in section 1.4.3). The results of the bleaching assay are
presented in figure 4-16 and table 4-12.
Figure 4-14  PCA projection of UV-vis spectral data from all treatments and observation dates. Data labels indicate observation dates

Figure 4-15  Corresponding loadings plot vs wavelength for the PCA presented in figure 4-15
Figure 4-16  Results of bleached pigment assay for all treatments and observation dates. Data point represent mean values for each treatment and error bars indicate standard deviation (n=3)

Table 4-12  mean (n=3)\textsuperscript{ab} absorbance at 520nm after bleaching with bisulphite for all treatments and observation dates.

<table>
<thead>
<tr>
<th>Days from start of Treatment</th>
<th>0 mg/L/mth</th>
<th>10 mg/L/mth</th>
<th>23 mg/L/mth</th>
<th>36 mg/L/mth</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>1.02 (±0.13) ab</td>
<td>0.93 (±0.07) a</td>
<td>0.99 (±0.06) ab</td>
<td>0.96 (±0.09) ab</td>
</tr>
<tr>
<td>21</td>
<td>1.09 (±0.03) b</td>
<td>1.08 (±0.06) bc</td>
<td>1.24 (±0.15) bc</td>
<td>1.26 (±0.04) bc</td>
</tr>
<tr>
<td>35</td>
<td>1.23 (±0.08) bc</td>
<td>1.27 (±0.04) bc</td>
<td>1.67 (±0.15) d</td>
<td>1.60 (±0.14) cd</td>
</tr>
<tr>
<td>49</td>
<td>1.35 (±0.01) c</td>
<td>1.40 (±0.05) cd</td>
<td>1.57 (±0.16) cd</td>
<td>1.49 (±0.05) cd</td>
</tr>
<tr>
<td>63</td>
<td>1.28 (±0.07) bc</td>
<td>1.23 (±0.09) bc</td>
<td>1.37 (±0.05) bc</td>
<td>1.39 (±0.06) cd</td>
</tr>
<tr>
<td>77</td>
<td>1.40 (±0.02) cd</td>
<td>1.40 (±0.10) cd</td>
<td>1.57 (±0.08) d</td>
<td>1.62 (±0.19) d</td>
</tr>
<tr>
<td>91</td>
<td>1.52 (±0.04) cd</td>
<td>1.54 (±0.06) cd</td>
<td>1.66 (±0.16) d</td>
<td>1.63 (±0.06) d</td>
</tr>
<tr>
<td>105</td>
<td>1.44 (±0.06) cd</td>
<td>1.43 (±0.05) cd</td>
<td>1.62 (±0.04) d</td>
<td>1.56 (±0.06) d</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Standard deviations are given in parentheses after each value
\textsuperscript{b} The bold-type letters indicate whether each treatment is significantly different from another based on a single factor ANOVA and multiple comparison using Fishers LSD\textsubscript{0.05}. Different letters indicate significant differences.
The most notable feature of figure 4-17 is the close correlation between the control and the 10 mg/L/mth treatment pair and the 23 and 36 mg/L/mth treatment pair. The sets of treatment pairs are significantly different from each other, however, only on observation day 35. This result indicates that the micro-oxygenated treatments produce more SO$_2$ resistant pigments as expected.

### 4.4.5 Low Molecular Weight HPLC Analysis

Figure 4-17 is the projection (PC1 and PC2) of a principal components analysis performed on four HPLC chromatograms of interest (280, 320, 365 and 520nm). The procedure is described in detail in sections 2.3.1 through 2.3.3. 84% of the model variance can be explained by principal component 1 and only 3% by principal component 2. The projection presents each treatment as a chronological trace. Each trace tracks from right to left. The notable feature is that all of the wines appear to follow a similar trajectory, the difference being the rate at which the development progresses.

The slowest wine is, unsurprisingly, the control which lags the 10 mg/L/mth treatment by up to six weeks. Similarly the 10 and 23 mg/L/mth treatments track an almost identical trajectory with the 10 mg/L/mth following the 23 mg/L/mth wine by two to six weeks. Although the 23 and 36 mg/L/mth treatments follow slightly different paths the end result is very similar for both wines. Another interesting feature of the plot is the rate of evolution (i.e. the point-to-point distance between observation days). The greatest distances occur, for all treatments between weeks 3 and 7. The points are considerably more compressed from weeks 9 to 15 indicating the rate of phenolic development is slower between these periods.
Figure 4-17   PCA projection of HPLC chromatograms (280, 320, 365 and 520nm)

In order to fully interpret figure 4-18 the loadings plot is required which is presented in figure 4-18. The major peaks are identified and listed in table 4-13 along with their corresponding loading sense (refer to section 2.4.2). Each of the peaks represents a compound(s) that has changed during the course of the trial and subsequently has driven the changes observed in figure 4-17. The relative behaviour of each of these compounds is presented in figures 4-19 through 4-23.
Figure 4-18  Loadings plots corresponding to the PCA scores projection shown in figure 4-18. The numbers adjacent to each peak correspond to the peak number from table 4-12.
Table 4-13  Major peaks from PCA loadings plot Figure 4-19

<table>
<thead>
<tr>
<th>Peak</th>
<th>Trace</th>
<th>Retention Time (min)</th>
<th>Loading Sense</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>280 nm</td>
<td>9.3</td>
<td>(+,+),</td>
<td>gallic acid</td>
</tr>
<tr>
<td>2</td>
<td>320 nm</td>
<td>23.2</td>
<td>(+,+),</td>
<td>t-caftaric acid</td>
</tr>
<tr>
<td>3</td>
<td>320 nm</td>
<td>34.7</td>
<td>(+,+),</td>
<td>cis-coutaric acid</td>
</tr>
<tr>
<td>4</td>
<td>320 nm</td>
<td>36.4</td>
<td>(+,+),</td>
<td>t-coutaric acid</td>
</tr>
<tr>
<td>5</td>
<td>280 nm</td>
<td>37.2</td>
<td>(+,-)</td>
<td>catechin</td>
</tr>
<tr>
<td>6</td>
<td>280 nm</td>
<td>46.3</td>
<td>(+,-)</td>
<td>caffeic acid</td>
</tr>
<tr>
<td>7</td>
<td>520 nm</td>
<td>48.0</td>
<td>(+,-)</td>
<td>delphinidin-3-glucoside</td>
</tr>
<tr>
<td>8</td>
<td>520 nm</td>
<td>51.6</td>
<td>(+,-)</td>
<td>cyanidin-3-glucoside</td>
</tr>
<tr>
<td>9</td>
<td>520 nm</td>
<td>53.4</td>
<td>(+,-)</td>
<td>petunidin-3-glucoside</td>
</tr>
<tr>
<td>10</td>
<td>280 nm</td>
<td>54.8</td>
<td>(+,-)</td>
<td>epicatechin</td>
</tr>
<tr>
<td>11</td>
<td>520 nm</td>
<td>56.6</td>
<td>(+,-)</td>
<td>peonidin-3-glucoside</td>
</tr>
<tr>
<td>12</td>
<td>520 nm</td>
<td>57.7</td>
<td>(+,-)</td>
<td>malvidin-3-glucoside</td>
</tr>
<tr>
<td>13</td>
<td>320 nm</td>
<td>60.4</td>
<td>(-,+),</td>
<td>p-coumaric acid</td>
</tr>
<tr>
<td>14</td>
<td>280 nm</td>
<td>61.8</td>
<td>(+,-)</td>
<td>unidentified</td>
</tr>
<tr>
<td>15</td>
<td>280 nm</td>
<td>69.6</td>
<td>(-,+),</td>
<td>unidentified</td>
</tr>
<tr>
<td>16</td>
<td>520 nm</td>
<td>75.7</td>
<td>(+,-)</td>
<td>unidentified</td>
</tr>
<tr>
<td>17</td>
<td>365 nm</td>
<td>76.3</td>
<td>(0,+),</td>
<td>quercetin-3-glucoside</td>
</tr>
<tr>
<td>18</td>
<td>520 nm</td>
<td>86.0</td>
<td>(+,-)</td>
<td>unidentified</td>
</tr>
<tr>
<td>19</td>
<td>320 nm</td>
<td>88.3</td>
<td>(+,+),</td>
<td>t-resveratrol</td>
</tr>
<tr>
<td>20</td>
<td>365 nm</td>
<td>93.9</td>
<td>(-,-)</td>
<td>quercetin</td>
</tr>
<tr>
<td>21</td>
<td>All</td>
<td>75-100</td>
<td>(-,0)</td>
<td>Unresolved polymeric material</td>
</tr>
</tbody>
</table>

A notable feature of figures 4-19 – 4-23 is the transformation of the predominant hydroxycinamate forms (caftaric acid, cis-coutaric and t-coutaric acid, figure 4-19 a)-c) respectively) to their corresponding non-esterified form (i.e. caffeic and p-coumaric acids, figure 4-20 b) and figure 4-22 a) respectively). The rate of transformation is the slowest for the control wine in all cases. While the changes are more rapid for the treatments with oxygen the kinetics appear to be independent of the oxygen dosage rate. One possible reason is the presence of residual pectolytic enzyme (section 4.2.2) which has a known hydrolytic side activity. The presence of oxygen may facilitate the hydrolysis reaction. It should also be noted that by the end of the trial the complete transformation had also occurred in the control treatment also.
Figure 4-19  Evolution of individual phenolic compounds for each treatment through the course of the trial
Figure 4-20  Evolution of individual phenolic compounds for each treatment through the course of the trial
Figure 4-21  Evolution of individual phenolic compounds for each treatment through the course of the trial
Figure 4-22  Evolution of individual phenolic compounds for each treatment through the course of the trial. Error bars indicate standard deviation ($n=3$)
Figure 4-23  Evolution of individual phenolic compounds for each treatment through the course of the trial. Error bars indicate standard deviation (n=3)
The anthocyanins showed remarkably consistent patterns of degradation (figures 4-20 c) and d), 4-21 a), c) and d), 4-22 d) and 4-23 b)) characterised by a rapid decrease from observation days 7 to 25 followed by a much slower rate of decrease until the end of the trial.

Two unidentified flavanols (figures 4-22 b) and c)) show interesting behaviour and it is likely based on their respective kinetic behaviour that the compound eluting at 61.8 mins (figure 4-22 b)) transforms into the compound eluting observed at 69.6 minutes (figure 4-22 c)). Moreover the significant transformation only seems to occur for the 23 mg/L/mth treatment. This phenomenon was observed for all replicates indicating that the chemical change is related to the dosage rate rather than as a result of the experimental setup. The compounds are yet to be identified, their spectral compositions are given below

![Spectral distribution of two unidentified flavanol at 61.8 and 69.6 minutes.](image)

A decrease in t-resveratrol levels were also observed for all treatments. However, like the hydroxycinnimates, the rate of degradation was markedly lower for the control wine compared to the treatments. The oxygenation treatments show very similar rates of decrease, indicating, like the hydroxycinnamates the kinetics are independent of oxygen dosage rate.

### 4.4.6 Thiolysis

The thiolysis results are presented in figure 4-25, 4-26 and table 4-14. Although statistically significant differences exist between treatments at all observation days except 21 there was a
consistent trend of increasing mDP until day 77. Following day 77, there was a significant decrease in mean tannin length until the end of the trial. As indicated in figure 4-25 the decrease in mDP correlates with the addition of SO$_2$ to each of the treatments.

![Figure 4-25 Evolution of mDP for each treatment through the course of the trial. Error bars indicate standard deviation (n=3)](image)

Moreover the decrease is greatest for the control, 10 and 23 mg/L/mth treatment. While a drop in mDP is also observed in the 36 mg/L/mth wine the effect is significantly less than for the other treatments. Whether the decrease in mDP is as a result of the SO$_2$ additions or not, one interpretation is that spontaneous cleavage of the interflavan bonds occurs and tannins are being broken up and reformed in solution. This effect has also been observed in recent studies (Vidal, Cartalade et al. 2002). One possible reason for reduction in mDP is the nucleophilic action of the bisulfite ion complexing with the newly formed favanyl-4-carbocation as has been shown by (Foo, McGraw et al. 1983). The effect of SO$_2$ on the development of proanthocyanidins was investigated further and the results given in Chapter Five.

Another possible reason for the decrease in mDP is an increase in the oxidatively linked flavan-3-ol polymers (bicyclic A-type oligomers and polymers) as described in section 1.4.4
and Figure 1-17. These additional bonds are known to be resistant to hydrolytic cleavage (Remy-Tanneau, Le Guerneve et al. 2003) and do not contribute to the pool of thiolyzed constituents thus leading to errors in the calculated mean degree of polymerisation. This phenomenon was highlighted in sections 2.3.5 and 2.4.6. The effect of this was not quantified as the mass of proanthocyanidin extract was not measured and thus the proportional yield of this extract undergoing thiolyis could not be calculated. The absolute molar yield of thiolyis products however is given in Appendix III (Figure A-2).

Table 4-14 mean \((n=3)^{ab}\) degree of polymerisation after thiolyis and quantification with HPLC.

<table>
<thead>
<tr>
<th>Days From Start of Treatment</th>
<th>0 mg/L/mth</th>
<th>10 mg/L/mth</th>
<th>23 mg/L/mth</th>
<th>36 mg/L/mth</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>11.98 (±0.69) (\textbf{bc})</td>
<td>11.43 (±0.24) (\textbf{ab})</td>
<td>10.85 (±0.13) (\textbf{a})</td>
<td>11.58 (±0.19) (\textbf{b})</td>
</tr>
<tr>
<td>21</td>
<td>12.85 (±0.41) (\textbf{e})</td>
<td>13.03 (±0.08) (\textbf{e})</td>
<td>12.51 (±0.15) (\textbf{c})</td>
<td>12.73 (±0.28) (\textbf{c})</td>
</tr>
<tr>
<td>35</td>
<td>16.27 (±0.14) (\textbf{fg})</td>
<td>16.05 (±0.05) (\textbf{fg})</td>
<td>15.09 (±0.07) (\textbf{e})</td>
<td>15.29 (±0.21) (\textbf{e})</td>
</tr>
<tr>
<td>49</td>
<td>17.03 (±0.35) (\textbf{gh})</td>
<td>17.31 (±0.99) (\textbf{h})</td>
<td>16.37 (±0.11) (\textbf{fg})</td>
<td>16.55 (±0.22) (\textbf{fg})</td>
</tr>
<tr>
<td>63</td>
<td>16.79 (±0.24) (\textbf{gh})</td>
<td>16.45 (±0.79) (\textbf{fg})</td>
<td>15.97 (±0.18) (\textbf{f})</td>
<td>15.20 (±0.86) (\textbf{e})</td>
</tr>
<tr>
<td>77</td>
<td>18.82 (±0.09) (\textbf{j})</td>
<td>18.07 (±0.20) (\textbf{i})</td>
<td>18.00 (±0.32) (\textbf{i})</td>
<td>17.79 (±0.26) (\textbf{hi})</td>
</tr>
<tr>
<td>91</td>
<td>16.58 (±0.02) (\textbf{g})</td>
<td>14.77 (±0.30) (\textbf{e})</td>
<td>17.53 (±0.14) (\textbf{hi})</td>
<td>18.22 (±0.13) (\textbf{i})</td>
</tr>
<tr>
<td>105</td>
<td>13.81 (±0.08) (\textbf{d})</td>
<td>14.07 (±0.05) (\textbf{d})</td>
<td>14.08 (±0.10) (\textbf{d})</td>
<td>16.15 (±0.45) (\textbf{fg})</td>
</tr>
</tbody>
</table>

\(^a\) Standard deviations are given in parentheses after each value

\(^b\) The bold-type letters indicate whether each treatment is significantly different from another based on a single factor ANOVA and multiple comparison using Fishers LSD\(_{0.05}\). Different letters indicate significant differences.

An analysis of the proportional changes in the constitutive subunits was also carried out. The result is given, in the form of a PCA biplot, in figure 4-26. The change in relative amounts of each of the extension and terminal sub-units is reasonably consistent for all four treatments. What is interesting about figure 4-26 is the decrease in the relative amount of epicatechin-3-O-gallate as an extension unit following observation day 77. This result correlates with the increase in free gallic acid as detected by LMW HPLC and presented in figure 4-19 a).
Figure 4-26  Principal Components Analysis of the relative proportions of proanthocyanidin subunits after thiolysis. Score data labels refer to the observation day. Abbreviated attribute labels refer to: cat = catechin, epi = epicatechin, ECG = epicatechin-3-O-gallate, EGC-TE = epigallocatechin-4-benzylthioether, cat-TE(i) = (2R,3R,4S)-catechin-benzylthioether, cat-TE(ii) = (2R,3R,4R)-catechin-benzylthioether, epi-TE = epicatechin-4-benzylthioether, ECG-TE = epicatechin-3-O-gallate-4-benzylthioether.
4.5 Summary

The considerable suite of analyses that were performed on the treatment wines throughout the course of the study, have in themselves given some insight into the rather complex mechanisms that are associated with MOX. In an attempt to summarise the various data that have been individually analysed above, multivariate statistical methods are again used to explore the interrelationships between the attributes and any underlying structure that may not be apparent through independent analysis of each variable.

The entire set of outputs discussed above were collected in a single matrix and a PCA was performed (using the correlation matrix for eigenvalue and eigenvector extraction). Initially the correlation loadings plot was inspected to ascertain which variables contributed significantly to the variance model. This diagnostic plot is shown, for interest, in figure 4-28.

Correlation loadings are computed for each variable for the displayed principal components. Figure 4-27 contains two ellipses; the outer and inner ellipse indicate 100% and 50% of explained variance respectively. This plot is useful because the relative importance of individual variables can be visualised more clearly in the correlation loading plot rather than the standard loadings plot. The important variables (i.e. those that load between the two ellipses) are chosen for the final PCA. Sensory variables mouthfeel and astringency were also included as they are the most dominant of the sensory attribute observed in the trial. Unknown flavanols Peaks 14 and 15 in table 4-12 are labeled as FP1 and FP2 respectively in this analysis.

The next stage was to perform a PCA with all of the important variables (those within the correlation ellipses and the other exceptions mentioned above). The result of the PCA is given as a biplot in figure 4-28. The most obvious feature of this projection is that all treatments track from left to right following the temporal progression of the trial. The extent of the progression is greatest for the 23 and 36 mg/L/mth treatments. Interestingly chemical and physical attributes load mainly on PC1 which explains 72% of the variance in the model. Sensory attributes (mouthfeel and astringency) load positively along PC2 and appear to be independent of much of the observed chemical transformations, with the exception of mDP and the two unidentified flavanol peaks at 61.8 and 69.6 minutes from the LMW HPLC analyses (table 4-12). mDP has previously been directly correlated with
astringency (Vidal, Francis et al. 2004), and this summary supports this finding. Note that the 36 mg/L/mth treatment tails up (positively along PC2) in the last two observation dates while the 23 mg/L/mth tails down (negatively along PC2). Given that PC2 can be interpreted as the sensory axis what this indicates is that the 23 mg/L/mth treatment is becoming more balanced and softer while the opposite is occurring to the 36 mg/L/mth treatment.

Figure 4-27  Correlation loadings plot of summary data matrix

Section 1.1.2 discussed the alleged importance of dosage rate on the success of the MOX treatment. An optimum dosage rate was defined as being one whereby the wine was sufficiently improved in an appropriate period of treatment without irreparable damage being caused. From the trial work detailed in this chapter it is apparent that the optimum dosage rate may be around 23 mg/L/mth. A definite sensory improvement was observed compared to any of the other treatment. The sensory properties of the 36 mg/L/mth treatment appeared to be deteriorating by the end of the trial, perhaps having been over-oxygenated.
The above claim is made with several qualifications. The lower oxygen treatment may have reached the same end with further exposure to oxygen. Certainly the various analyses done on the four treatments suggest that the effect of a higher oxygen dosage is to accelerate the process. SO₂ treatment (both the quantum and timing of the dosage) also appears critical to the development of the tannins and therefore the sensory properties. The noted reduction in applied oxygen dosage rate from pre- to post- MLF appears, in much of the literature to be associated with the malo-lactic bacteria (Paul 2002; Cano-Lopez, Pardo-Minguez et al. 2006). The above results, however, suggest it is more to do with the addition of SO₂ (generally made immediately following the end of MLF). Based on the findings of this trial the role and importance of SO₂ in MOX will be further investigated in Chapter Five.

The trial work described above is clearly limited by the inability to clearly resolve tannin structures. The role of the tannins in MOX (and wines in general) requires work at a more fundamental level both in terms of elucidating the chemical structures and determining the importance of different compounds in the sensory properties of the wine.

4.6 Discussion

The trial detailed in Chapter four involved a number of different analytical techniques all aimed at providing an insight into the effect of micro-oxygenation on Cabernet Sauvignon. There are a number of interesting results that require some critical discussion.

4.6.1 Sensory Evaluation

While the sensory trial provided some significant results in terms of the within and between treatment effects, a major limitation was the inability to resolve more subtle organoleptic changes brought about by modifications to the wine polyphenols. It was felt, given the experience of the panel and the fact that they were only volunteers that more abstract sensory properties such as those proposed in (Gawel, Oberholster et al. 2000) would be difficult to train given the logistical constraints (lack of suitable standards etc.).

It is recommended that in future studies on micro-oxygenation the sensory focus should be on the more subtle mouthfeel properties such as described in the mouth-feel wheel (Gawel, Oberholster et al. 2000). Sensory terms such as: ‘surface smoothness’, ‘complexity’, ‘particulate’ and ‘drying’ appear to be more relevant and perhaps easier to discriminate than
the more general sensory terms (such as bitterness and astringency) adopted for this trial (standards may be a challenge however). It is also recommended that the number of treatments be reduced to allow for a full factorial design so treatment interactions can be examined.

### 4.6.2 LMW Polyphenol Analysis

The low molecular weight polyphenol analysis approach worked very well with a large set of HPLC data being able to be visualised in two plots (Figures 4-18 and 4-19). Moreover the relative development of the LMW polyphenols could be visualised using principal components analysis to reduce the data set down to two dimensions.

The behaviour of the LMW polyphenols was predictable with the exception of the conversion of \( t \)-caftaric and \( p \)-coumaric acids to \( t \)-caffeic and \( p \)-coumaric acid respectively. The kinetics of the conversion appeared to be related to the presence of oxygen as indicated in figures 4-20 b), 4-20 c), 4-20 d) and 4-21 b). The conversion may be due to the presence of pectolytic enzymes.

### 4.6.3 Thiolysis

The thiolysis results were interesting in that they did not show any significant difference between treatments for any of the observation dates. There appeared to be an effect (albeit between observation dates) after the addition of 30 mg/L of \( \text{SO}_2 \) at day 84 from the start of treatment. While the effect of \( \text{SO}_2 \) appears to be reasonably clear, the resulting thiolysis response is not. One interpretation is that the bisulfite ion acts as a nucleophile competing for the electrophilic positions created by the hydrolysis of the interflavin bonds of the condensed tannins. The take-up of these electrophillic sites by the bisulfite ions appears to effectively retard or suppress the further development of condensed tannins.

Another interpretation is that the pool of thiolysable proanthocyanidins is decreasing due to the increase in the oxidatively linked (A-type) oligomers and polymers. Thiolysis yields have been calculated on grapes in a number of studies (Kennedy, Matthews et al. 2000; Kennedy and Jones 2001) and have been shown to be variable and decreasing as the berry matures. There appears to have been little work done on wine and it would be a very useful study to
look at thiolysis yields in maturing wines to investigate whether, by inference, there is an increase in the non-thiolysable fraction over time and the effect of oxygen on this aspect of development.
Figure 4-28 Summary PCA for all sensory and chemical attributes discussed above. The data labels for the scores refer to observation date/dosage rate. The abbreviated attributes refer to: bleach = 520nm absorbance after addition of SO₂ (figure 4-17), FP1 and FP2 = the unidentified flavanol compounds eluting at 61.8 and 69.6 mins respectively (table 4-12), API and AP2 = unidentified pigmented compounds eluting at 75 and 86 mins respectively (figure 4-12), quer-3-glu = queretin-3-glucoside, mv-3-glu = malvidin-3-glucoside, del-3-glu = delphinidin-3-glucoside, pet-3-glu = petunidin-3-glucoside, peo-3-glu = peonidin-3-glucoside, cy-3-glu = cyanidin-3-glucoside
Chapter Four
 CHAPTER FIVE
Effect of Sulfur Dioxide and Oxygen on the Tannin Development in Red Wine

5.1 Introduction

One of the most interesting results of the trial described in Chapter four was the observed decline in tannin length (as measured by mDP) following the addition of sulfur dioxide (figure 4-25). The decrease in mDP was observed for all four treatments most dramatically in the control (no oxygen), and also the low and medium oxygen treatments (10 and 23 mg/L/mth). The 36 mg/L/mth treatment also showed similar trends but the decrease was less marked at the conclusion of the trial.

As briefly discussed in section 4.4.6 the decrease in the mean tannin length was correlated to the addition of SO$_2$. The effect was observed irrespective of oxygen dosage rate of the treatment.

It has been argued that proanthocyanidins, in wine, exist in an equilibrium and continuous hydrolysis as well as condensation of interflavanic bonds occurs (Vidal, Cartalade et al. 2002). Over time (months) the net tannin length tends to increase until the tannins become insoluble in the wine medium and precipitate out (Somers 1971; Haslam 1998).

One possible explanation for the decrease in mDP relates to the strong nucleophilic action of the bisulfite ion competing for the electrophilic site on the carbocations formed from the hydrolysis of the interflavanic bonds. The effect of this competition will be to retard the tannin and hence the sensory development in the wine. The proposed reaction mechanism is shown in figure 5-1. The reaction described in figure 5-1 has been shown to occur in wine-
like conditions (Vidal, Cartalade et al. 2002) albeit indirectly and for pH and temperature conditions much higher than would be experienced in wine (Foo, McGraw et al. 1983).

The tannin length (as measured by thiolyis) has been shown to be highly correlated with astringency (Vidal, Francis et al. 2004), and this was also observed in the trial described in the previous chapter (figure 4-29). The aim of the experiments detailed in this chapter was to better understand the effects of sulfur dioxide (SO$_2$) and oxygen on the tannin development of a red wine.

![Proposed reaction mechanism for nucleophilic addition of bisulfite ion as a competing reaction for tannin-tannin condensation.](image)

**Figure 5-1** Proposed reaction mechanism for nucleophilic addition of bisulfite ion as a competing reaction for tannin-tannin condensation.

### 5.2 Materials and Methods

#### 5.2.1 Grapes
Cabernet Franc is a red *Vitis Vinifera* variety used typically as a component in Bordeaux style red wines. The grapes were hand-harvested at commercial maturity on the 16th April 2005 from a vineyard in West Auckland, New Zealand.

#### 5.2.2 Wine Processing
The processing steps are summarised in figure 5-2. 15 mg/L sulfur dioxide (as potassium metabisulfite) was also added, principally to suppress polyphenol oxidase and wild yeast activity. The oenological details of the pomace are given in table 5-1.
Table 5-1  Oenological parameters of the Cabernet Sauvignon must

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brix (juice) (%SS)</td>
<td>21.5</td>
</tr>
<tr>
<td>pH</td>
<td>3.54</td>
</tr>
<tr>
<td>TA (g/L)</td>
<td>8.06</td>
</tr>
</tbody>
</table>

After a short period of pre-fermentation maceration (72 hours at 12°C) the temperature was increased to 15°C and a commercial strain of *Saccharomyces cerevisiae* (Lalvin D254) was added at a concentration of 300 mg/L. The fermentation proceeded normally to dryness with 200 mg/L diammonium phosphate (DAP) being added on days two and three respectively. Cap management involved thorough plunging every 12 hours from the time of crushing until the end of ferment, the every 24 hours until the grapes were pressed.

Figure 5-2  Summary of wine-making processing steps

After pressing, the wine was left to settle in a 500 litre stainless steel tanks for three days. The settled wine was racked directly into a 225 litre French oak barrique. During the racking process sufficient wine for the trial (approximately 15 litres) was diverted into a 15 L capacity holding tank, sealed and held at 10°C.
5.2.3 Experimental Treatment Setup

The experimental design consisted of four treatments x three replicates of each treatment. The treatments are detailed in table 5-2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Oxygen</th>
<th>SO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No</td>
<td>100 mg/L</td>
</tr>
<tr>
<td>2</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>Yes</td>
<td>100 mg/L</td>
</tr>
<tr>
<td>4</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

The oxygen treatment consisted of sparging with pure oxygen (BOC gases) once per week. Sparging was continued until approximately one saturation had been achieved (9 mg/L). Dissolved oxygen concentration was monitored during sparging with a dissolved oxygen meter (Orbishere 3650) and sparging was stopped once 9 mg/L saturation was reached. 100 mg/L SO₂ was added at the start of the trial to treatments 2 and 4 (as 10% w/v potassium metabisulfite).

The 12 treatment vessels used were one litre polyethylene terephthalate (PET) enclosed containers with sealing lids. PET was chosen because of the very low permeability to oxygen (Pauly 1999). The wines were stored at 15°C in a temperature controlled facility at the University of Auckland winery for the duration of the trial.

Samples were taken once per week (on days 7, 14, 21, 35, 42). In order to prevent oxygen contamination from the atmosphere, flowing argon gas was used to inert the headspace volume while samples were being drawn. Samples were stored without light except for those occasions when sampling was being undertaken.

5.3 Chemical Analyses

5.3.1 Colour measurement

Colour measurements were conducted using a spectrophotometer (Varian Cary 50) using a 2 mm path-length fibre-optic probe. As the primary objective of the experiment was to gain an insight into the development of wine tannins, the only appropriate colourmetric analysis is
the bleaching assay described in section 4.4.4. This was performed immediately following each observation (sampling).

5.3.2 Thiolysis
Thiolysis was performed exactly as described in section 2.3.4 through 2.3.7. Like the colourmetric analysis, the thiolysis procedure was performed immediately following the acquisition of the sample.

5.3.3 SO₂ Measurements
SO₂ (free and bound) were measured using the aspiration method described in (Ilard 2000).

5.3.4 Data Analysis
Statistical analysis was performed using Microsoft Excel 2003, The Unscrambler v9.1, Matlab v7.0.1 R14 and R v2.4.0.
5.4 Results & Discussion

5.4.1 Colourmetric Results

The results of the bleaching assay are presented in figure 5-3. The corresponding statistical results are included in table 5-3.

![Figure 5-3](image-url)

**Figure 5-3** Results of the bleaching assay. Error bars indicate standard deviation ($n=3$)

**Table 5-3** Mean ($n=3$)$^{ab}$ results of bleaching assay for all treatments and observation dates.

<table>
<thead>
<tr>
<th>Experimental Treatment</th>
<th>Days From Start of Treatment</th>
<th>No SO$_2$, No O$_2$</th>
<th>SO$_2$ (100ppm), No O$_2$</th>
<th>No SO$_2$, O$_2$</th>
<th>SO$_2$ (100ppm), O$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>0.683 (±0.000) $^c$</td>
<td>0.683 (±0.000) $^c$</td>
<td>0.683 (±0.000) $^c$</td>
<td>0.683 (±0.000) $^c$</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>0.879 (±0.001) $^g$</td>
<td>0.872 (±0.007) $^c$</td>
<td>0.804 (±0.047) $^g$</td>
<td>0.912 (±0.111) $^fg$</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>0.970 (±0.016) $^g$</td>
<td>0.671 (±0.014) $^c$</td>
<td>0.943 (±0.008) $^g$</td>
<td>0.940 (±0.020) $^fg$</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>1.153 (±0.004) $^i$</td>
<td>0.627 (±0.004) $^b$</td>
<td>1.148 (±0.006) $^i$</td>
<td>0.922 (±0.004) $^f$</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>1.104 (±0.005) $^h$</td>
<td>0.596 (±0.016) $^{ab}$</td>
<td>1.114 (±0.009) $^h$</td>
<td>0.823 (±0.010) $^e$</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>1.232 (±0.036) $^j$</td>
<td>0.577 (±0.005) $^a$</td>
<td>1.225 (±0.020) $^j$</td>
<td>0.748 (±0.013) $^d$</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>1.433 (±0.014) $^k$</td>
<td>0.609 (±0.010) $^b$</td>
<td>1.466 (±0.010) $^l$</td>
<td>0.796 (±0.020) $^e$</td>
</tr>
</tbody>
</table>

$^a$ Standard deviations are given in parentheses after each value

$^b$ The bold-type letters indicate whether each treatment is significantly different from another based on a single factor ANOVA and multiple comparison using Fishers LSD$_{0.05}$. Different letters indicate significant differences.

The two obvious and notable features of figure 5-3 are the close correlation of the two treatments without SO$_2$ and their trend to increasing 520 nm absorbance throughout the
Effect of SO\textsubscript{2} and oxygen on the tannin development in a red wine

course of the trial. Wines without SO\textsubscript{2} appear to have a higher propensity to form non-bleachable pigments. The other striking feature is the behaviour of the treatments where SO\textsubscript{2} has been added. There was a significant difference between the two SO\textsubscript{2} treatments from day 7. Both show small within treatment variation over time with significantly higher 520nm absorbance observed in the “no oxygen” treatment compared to the oxygen+SO\textsubscript{2} treatment.

A possible reason for this is that oxygen will cause the formation of hydrogen peroxide (section 1.4) which tends to react with the quantitatively significant ethanol producing ethanal (acetaldehyde). Ethanal forms a relatively stable complex with SO\textsubscript{2} and hence competes with the flavanyl-4-carbocations. It is not surprising therefore for the treatments with SO\textsubscript{2} that more polymeric pigment has been able to form in the presence of oxygen compared to anaerobic conditions.

5.4.2 Thiolysis Results

The evolution of mean degree of polymerisation is presented in figure 5-4 with corresponding statistical summaries given in table 5-4. Figure 5-4 is similar in character to figure 5-3 in that there is a significant separation between the treatments with and without SO\textsubscript{2} irrespective of whether oxygen exposure occurs. For the two SO\textsubscript{2} treatments a significant decrease in mDP is observed from day 7 to 21. The same decrease is not manifest in the treatments without SO\textsubscript{2}. From day 28 a significant increase in mDP is observed in the treatments without SO\textsubscript{2}.

Clearly tannin development during maturation, as measured by thiolysis and the bleaching assay, is strongly influenced by the SO\textsubscript{2} treatment. In the case of the aforementioned trial the effect of SO\textsubscript{2} is more significant than that of oxygen. While oxygen appears to have a notable influence on the formation of polymeric pigments (as measured by the bleaching assay) the same effect was not observed with the mDP results.
Figure 5-4  mDP as measured by thyolysis. Error bars indicate standard deviation ($n=3$)

Table 5-4  Mean ($n=3$)\(^{a,b}\) results of mDP for all treatments and observation dates.

<table>
<thead>
<tr>
<th>Days From Start of Treatment</th>
<th>Experimental Treatment</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No SO(_2), No O(_2)</td>
<td>SO(_2) (100ppm), O(_2)</td>
<td>No SO(_2), O(_2)</td>
<td>SO(_2) (100ppm), No O(_2)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>8.73 (±0.12) (c)</td>
<td>8.73 (±0.12) (c)</td>
<td>8.73 (±0.12) (c)</td>
<td>8.73 (±0.12) (c)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>8.76 (±0.13) (c)</td>
<td>9.12 (±0.15) (c)</td>
<td>8.99 (±0.38) (c)</td>
<td>9.09 (±0.34) (c)</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>8.96 (±0.15) (c)</td>
<td>8.57 (±0.07) (bc)</td>
<td>9.00 (±0.21) (bc)</td>
<td>8.59 (±0.00) (c)</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>8.76 (±0.06) (c)</td>
<td>7.92 (±0.22) (b)</td>
<td>8.56 (±0.17) (c)</td>
<td>7.53 (±0.05) (ab)</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>8.60 (±0.35) (c)</td>
<td>7.18 (±0.18) (a)</td>
<td>8.63 (±0.23) (bc)</td>
<td>7.47 (±0.17) (ab)</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>10.20 (±0.41) (de)</td>
<td>7.74 (±0.20) (ab)</td>
<td>10.01 (±0.25) (d)</td>
<td>7.46 (±0.08) (ab)</td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>10.51 (±0.09) (de)</td>
<td>7.54 (±0.25) (ab)</td>
<td>10.65 (±0.05) (e)</td>
<td>7.46 (±0.07) (ab)</td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\) Standard deviations are given in parentheses after each value

\(^{b}\) The bold-type letters indicate whether each treatment is significantly different from another based on a single factor ANOVA and multiple comparison using Fishers LSD\(_{0.05}\). Different letters indicate significant differences.
5.4.3 \textit{SO}_2 \textit{Concentrations}

SO$_2$ concentrations are given for the oxygen and non-oxygen treatment in figure 5-5 and 5-6 respectively. A steady loss of free SO$_2$ is seen where oxygen was introduced, as would be expected. A smaller decrease in free SO$_2$ was also observed in the treatment without oxygen, indicating that some oxygen ingress is likely to have occurred during sampling despite the precautions taken.

5.4.3 \textit{Discussion}

The results presented in this trial support the hypothesis that the addition of SO$_2$ into a wine affects the development of tannins. Moreover the results observed also reflect those seen in section 4.4.6.

The significant decrease in tannin length, observed for the SO$_2$ treated wines infers firstly, that spontaneous hydrolytic cleavage of inflavanic proanthocyanidin bonds occurs in wine-like solutions as has already been observed in a recent study (Vidal, Cartalade et al. 2002). The second major inference of this study is that the presence of SO$_2$ (and specifically the bisulfite ion) the condensation reactions appear to be suppressed.

Based on the previous discussion of the limitations of thiolysis (section 2.3.5), it cannot be discounted that the reason for the observed decrease in mDP may be due to a reduced pool of proanthocyanidins being available for hydrolytic cleavage; due principally to an increase in oxidative cross linking described in section 1.4.4 and Figure 1-17. The proportional thiolysis yields were not however calculated (as discussed in sections 2.4.3 and 4.4.6) therefore the effect of a decreased thiolysable proanthocyanidin pool could not be quantified.

The implication of these results on micro-oxygenation is extremely significant. The observations show that the effect of SO$_2$ on the development of condensed tannins is at least as important, if not more important than the effect of oxygen during the treatment phase. Both the dosage rate and timing appear critical in managing the process. The anecdotal observation that much of the organoleptic changes occur pre-MLF is perhaps more related to the timing of the SO$_2$ addition than any micro-biological effect.
Subsequent to these findings a further replicated trial was undertaken. The trial was conducted using the 15 litre research vessels described in Chapter Three and the methodologies developed in this thesis. This study again confirmed the major influence that SO$_2$ concentration has on phenolic development during micro-oxygenation (Tao, Dykes et al. 2007; Tao, Dykes et al. 2007).

**Figure 5-5**  SO$_2$ concentrations (bound and free) for treatment with oxygen

**Figure 5-6**  SO$_2$ concentrations (bound and free) for treatment without oxygen
CHAPTER SIX

Electrochemical Micro-oxidation of Red Wine

6.1 Introduction

Hitherto consideration has only been given to dioxygen (O₂) as the primary oxidant driving the chemical and corresponding sensory changes that occur in red wine during maturation. Although the chemistry of dioxygen is a complicated process (as discussed in Chapter One), reduction and oxidation, at basic level, is simply the transfer of electrons. Oxidation of polyphenols and other wine constituents, in principle, can be achieved by the use of several methods, an obvious one being to apply an electrical potential across two appropriate conducting surfaces contacting the wine.

Electrochemical micro-oxidation (ELMOX) of wine is not a completely novel idea and a patent was lodged in 2002 (Guglielmi and Simoncelli 2002), concerning the application of continuous currents across (at least) one titanium electrode placed in a wine. Currents of between 0.1 and 100 µA/L were trialed, in which experiments yielded “preferable” sensory results at currents between 1 and 10 µA/L.

The use of electrochemical methods for wine analysis has been a key area of research at the University of Auckland and significant advances have been made in use of cyclic voltammetry to measure the antioxidant properties of solutions containing polyphenols and more specifically of actual wines (Kilmartin 2001; Zou 2001; Kilmartin, Zou et al. 2001a). Similarly, considerable research work has been carried out on the electrochemical behaviour of different electrode materials in solutions containing polyphenols (Kilmartin and Zou 2001b).
A potential advantage of using ELMOX over traditional controlled oxygenation methods is the precise control of the oxidation process by maintaining a predetermined electrical potential across the anode and cathode. This is particularly useful when very small dosage rates are required (e.g. for a low phenol wine such as Pinot noir or Chardonnay). Traditional micro-bullage also has the disadvantage that when the optimum level of oxygenation is reached (most often determined by sensory evaluation) there is some intrinsic overshoot because of the residual dissolved oxygen remaining in the treatment vessel after the dosage has been stopped. With ELMOX, however, once the current is switched-off the oxidation process is terminated with very little over-shoot.

The aim of the experiments described in this Chapter is to provide an initial comparison of the ELMOX method against traditional micro-oxygenation in terms of the sensory and chemical changes observed over a six week period. Based on the results of a previous electrode characterisation study (Fell 2005) it was decided to attempt the initial ELMOX trials using both glassy-carbon and titanium anode materials.

6.2 Trial Setup

6.2.1 Grapes

Cabernet Sauvignon is a red, polyphenol-rich *Vitis Vinifera* variety typically used in micro-oxygenation treatments. The grapes were hand-harvested at commercial maturity on the 30th April and 1st May 2005 from a vineyard in West Auckland, New Zealand.

6.2.2 Wine Processing

The grapes were destemmed, crushed and transferred (manually) to two 500 litre “open-top” fermentation vessels. SO₂ (as 10% (w/v) potassium metabisulfite) was added to the must at a rate 25 mg/kg immediately following crushing. No more SO₂ was added until after the trial had concluded. 48 hours after crushing, the must was inoculated with *Saccharomyces cerevisiae* (Lalvin D254) at the supplier recommended concentration of 250 mg/L. A pectolytic enzyme (Rohapect VR-C) was also added at a rate of 30 mg/L to aid with colour extraction and to help clarification after fermentation.
The fermentation proceeded normally to dryness, requiring six days. Cap management consisted of manually plunging the cap every 12 hours during fermentation and for one day following. The wine was pressed-off skins on 11th May 2005, given a coarse filtration through a plate-and-frame type filter arrangement (#30 filter pads – Cuno 3M) to remove any particulate matter and the majority of the yeast lees. Final oenological details of the Cabernet Sauvignon wine are given in table 6-1.

### Table 6-1 Summary of oenological parameters of the wine used for the ELMOX trial

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol Concentration (% v/v)</td>
<td>14%</td>
</tr>
<tr>
<td>pH</td>
<td>3.67</td>
</tr>
<tr>
<td>TA (g/L)</td>
<td>7.95</td>
</tr>
<tr>
<td>Residual sugar (g/L)</td>
<td>1.7</td>
</tr>
</tbody>
</table>

Following filtration the wine was transferred to 12 x 15 litre containers described in detail in Chapters Three and Four. A slight modification was made to six of these vessels to enable the provision of an anode for the two ELMOX treatments. The wines were left in a temperature controlled environment at 15°C for one week to equilibrate. Headspace volumes were sparged with argon twice weekly to avoid contamination of the treatments with headspace oxygen. Following the week of equilibration the trial was commenced.

#### 6.2.3 Experimental Design

The experimental design is presented schematically in figure 6-1. The respective treatments are summarized in table 6-2

### Table 6-2 Summary of respective treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Oxidation Method</th>
<th>Delivery Method</th>
<th>Delivery Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>N/A</td>
<td>N/A</td>
<td>0</td>
</tr>
<tr>
<td>MOX</td>
<td>Micro-oxygenation</td>
<td>dense polymer membrane</td>
<td>1 mL/L/mth</td>
</tr>
<tr>
<td>ELMOX-Ti</td>
<td>ELMOX</td>
<td>titanium anode</td>
<td>76.8 µA</td>
</tr>
<tr>
<td>ELMOX-C</td>
<td>ELMOX</td>
<td>glassy carbon anode</td>
<td>76.8 µA</td>
</tr>
</tbody>
</table>

Previous studies have shown that wine polyphenols are oxidized, using glassy carbon electrodes, at potentials less than 1.0 V (Kilmartin 2001). At higher potentials ethanol itself is oxidized, likely resulting in the formation of ethanal. Should an oxidation regime be
sought in which minimal ethanal is produced, then the electrical current should be kept low enough to maintain an electrode potential less than 1.0 V. One of the major advantages of ELMOX over traditional micro-oxygenation is that the potential can be accurately regulated therefore controlling the level of ethanal produced.

Figure 6-1  Schematic representation of experimental arrangement

The current levels for the ELMOX circuits were established after the electrodes were characterised in red wine (Fell 2005). Oxygen equivalents for ELMOX can be calculated theoretically using charge conservation laws and assuming that the full oxidation of a polyphenol is a four electron process (refer below to section 6.2.3.1). In the case of the 15 litre vessels used for this trial 7.7 mL/L/mth of oxygen is equivalent to 768 µA of electrode current (Fell 2005). It was determined that currents above 76.8 µA (i.e. 0.77 ml/L/mth oxygen equivalents) for the glassy carbon electrode resulted in potentials greater than 1.0 V. Lower currents were not investigated as achieving equivalent oxygen dosage rates was not practical given the setup.
Similar characterisation of the titanium electrodes registered steady-state electrode potentials around 2.5 V for currents of 38.4 µA (0.38 mL/L/mth oxygen equivalents). The oxidative processes occurring at the titanium electrode surface, however are not fully understood, so despite the large electrode potentials observed during characterisation the current was set at 0.77 mL/L/mth oxygen equivalents (i.e. the same as for the glassy carbon treatment).

6.2.3.1 Characterisation of Glassy Carbon Electrodes.

0.77 mL of O\textsubscript{2} \((V_{O_2} = 0.77 \times 10^{-3} \text{ L})\) represents 1.1 mg of O\textsubscript{2} at 0 °C (based upon the density of O\textsubscript{2} gas of 1.43 g/L), or \(3.44 \times 10^{-5}\) moles \((n_{O_2})\). As 4 electrons are passed in the reduction of each molecule oxygen to water, this would be equivalent to \(1.38 \times 10^{-4}\) moles of electrons \((n_e)\).

\[
\text{O}_2 + 4\text{H}^+ + 4e^- \rightarrow 2\text{H}_2\text{O} \quad [6.1]
\]

The equivalent oxidation achieved by applying a current at an electrode and the number of moles of oxygen can be determined using the following conversions:

\[
Q = I \cdot t = n_e F = 4n_{O_2} F = 4\left(\frac{V_{O_2}}{22.4}\right)F \quad [6.2]
\]

Using the Faraday constant \((F)\) of 96,485 C mol\(^{-1}\), the amount of charge \((Q)\) passed here is 13.3 coulombs \((Q = I \cdot t)\), for 0.77 mL of O\textsubscript{2} in a litre of wine. To pass this level of charge through an electrode in a litre of wine over a period of 30 days (2,592,000 seconds) would require a current \((I)\) of 5.1 µA, which translates to 77 µA in 15 L of wine.

6.2.3.2 Trial Vessel Modifications

As mentioned above the vessels used for these experiments were the same as those used for the trial described in Chapter Four with some small modifications to the ELMOX treatments:

- The ports that provided the oxygen feed were blanked-off
- The 2.5 inch blanking caps located on the lids were replaced with modified blanking caps with provision for the anodes (refer to figure 6-2).
6.2.3.3 Electrode Preparation and Configuration

The electrodes that were used were in the form of cylindrical rods approximately 250 mm long and 6 mm in diameter. The glassy carbon electrodes (Tokai Electrode Manufacturing Co. Ltd. Tokyo, Japan) were purchased as 6 mm diameter rod stock and simply cut to length. The titanium electrode material (Advent Research Materials Ltd – Oxford, England) was purchased as 8 mm rod stock, machined to 6 mm diameter and cut to four 250 mm lengths. These were suspended approximately 100 mm into the wine using electrical cable glands (figure 6-2) which provided both electrical insulation and a reasonable hermetic seal. The 100 mm of electrode submerged in the wine provided a current density of approximately 4 $\mu$A/cm$^2$ (based on electrode surface area contacting the wine).

6.2.3.3 Constant Current Source

The constant current source for each of the ELMOX treatment electrodes was provided by two three-channel galvanostats (figure 6-3). Each channel could be independently set using a rotary potentiometer (figure 6-3). Electrical connection to the anode was made using a bulldog type clip (figure 6-2). A similar connection was made to the outside skirt of the tank (which acted as the cathode).

Figure 6-2  Modifications made for provision of a titanium electrode into the 15 litre research vessels
6.2.4 Wine Sampling

An initial baseline sample was taken immediately before the trial was commenced. Sampling of the wine for analysis (chemical and sensory) began one week after commencement of the treatments and then every week following, for the duration of the trial (6 weeks). 250 mL of each treatment replicate was required for analysis. As with the trial described in Chapter Four, sampling without oxygen contamination from the atmosphere was critical. As a result an identical sampling procedure as described in section 4.2.4 was implemented and applied at each of the sampling dates.

![Figure 6-3](image)

**Figure 6-3** Galvanostats used for the ELMOX trial described in this trial

6.2.5 Sulfur Dioxide Treatment

As mentioned above no SO$_2$ was added to the wine except for the small amount added to the must immediately after harvest.
6.3 Chemical Analysis

6.3.1 Low Molecular Weight Polyphenol Analysis
The low molecular weight HPLC analysis and subsequent chemometric treatment is fully described in Chapter Two (section 2.3.1 through 2.3.3). Identical equipment, methods and materials (with the exception of the samples) were used for the trial described above as for that described in Chapter Two.

Due to technical problems with the HPLC the treatments for the week four (28 days after the start of the trial) were not able to be analysed. The samples were frozen immediately, however after thawing and running at a later date the overall phenolic profile was found to be dramatically different to the treatments at sample dates either immediately before or after week four. It was decided, therefore, to exclude these samples from the analysis.

6.3.2 Thiolysis
Thiolysis and associated procedures (sample fractionation) are fully described in Chapter Two (sections 2.3.4 through 2.3.7). Thiolysis was run on all samples at each of the observation dates.

6.3.3 Colour measurement
Colour was assessed using a spectrophotometer (Varian Cary 50) with a 2 mm path-length fibre-optic probe. Wines were filtered (0.45 μm RC syringe filter, Sartorius) before measurement. As all the samples were at an identical pH no adjustment was made for these readings. Colour intensity (420+520+620 nm) and hue (420/520 nm) were calculated from the discrete wavelength measurements.

In addition to the raw spectrophotometric data outlined above a bleaching assay was also performed as described in section 4.3.3. The idea is that the loss of red colour is inversely related to the extent of formation of stable pigments (condensed tannins, pyranoanthocyanins, vitisins etc.) and hence overall colour stability.
6.3.4 Sensory Analysis

The aim of this trial was to conduct quantitative descriptive analysis of sensory attributes concurrently with chemical analysis at each sampling date. A trained panel was assembled consisting of several members of the same panel that conducted the sensory analysis of the trials described in Chapter Four with some additional members (all volunteers). The panel was asked to discriminate the wines based on several organoleptic parameters listed in table 4-7 (i.e. the attributes were identical to those used for the MOX trial described in Chapter Four). As was the case with the previous panels, intensive training was conducted twice weekly for four months prior to actual data collection.

Sensory data collections were held at 10 am on the day of sampling (approximately 2 hours after the wine had been sampled). No more than 30 minutes before the commencement of tasting 30 mL of each wine was decanted from the 250 mL Schott bottle (used to sample the wine) into standard INAO tasting glasses. The glasses were sealed with parafilm, and each glass was then labeled with a three digit randomly generated numerical code corresponding to the treatment and replicate.

During the data collection each panelist was presented with four wines (one of each treatment). The presentation order was randomised except that each panelist received a different treatment replicate each session (i.e. the same replicate would be repeated every fourth session). The panelists were asked to score the wines independently (i.e. no comparison) on the aforementioned attributes. Printed on the score sheets were the base wine calibration checks described above.

The sessions were conducted under controlled lighting in individual booths. Panelists were requested not to drink or eat for two hours before each collection session. Panelists were given water and slices of bread (baguette) to aid palate freshness during tasting.

Due to logistical difficulties sensory analysis was unable to be conducted on either week four or week six of the trial (28 and 42 days since start of treatments respectively). Training was conducted weekly throughout the data collection period using the aforementioned techniques.
6.3.5 Ethanal Analysis

The following method for the quantification of acetaldehyde in both free and bound forms was adapted from (Peinado, Moreno et al. 2004). A Shimadzu (Kyoto, Japan) GC-17A series gas chromatograph was used coupled to a flame ionisation detector running at 220 °C. The column used was a capillary DB Wax 12-7012 column (15 m x 0.25 mm x 0.25 μm) supplied by Alltech Associates Inc. (Deerfield, IL). The flow rate of the column carrier gas (N2) was delivered at 1.4 mL/min with a split ratio of 43:1 and an injector temperature of 220 °C. The initial loading temperature was 35 °C for 5 min, increasing by 4 °C/min to 55 °C, then ramped at 20 °C/min to 200 °C and held at 200 °C for 5 min, for a total run time of 22.3 min. 50 μL of the internal standard 4-methylpentan-2-ol (98%, Aldrich), prepared as 50 g/L in 40% hydroalcohol solution, was added to 5 mL samples of wine, or to standard solutions of acetaldehyde (>99%, Merck-Schuchardt, Germany). After homogenisation 1.0 μL aliquots were manually injected onto the GC column. The limit of detection for the GC method was found to be 0.2 mg/L and the limit of quantitation 0.5 mg/L.

6.3.6 Data Analysis

Statistical analysis was performed using Microsoft Excel 2003, The Unscrambler v9.1, Matlab v7.0.1 R14 and R v 2.4.0.
6.4 Results and Discussion

6.4.1 Data Quality Control

Preliminary analysis of the chromatographic data followed by subsequent analysis of all the data sets found that the “a” replicate of the glassy carbon ELMOX (ELMOX-C) treatment was substantially different to the other replicates (“b” and “c”). This was thought to be due to:

a) A malfunctioning galvanostat or a current source set too high
b) Fouling of the electrode increasing the circuit resistance and thus the electrode potential
c) A leak in the tank allowing the ingress of atmospheric oxygen.

Irrespective of the cause the data for this replicate were excluded from all analyses.

6.4.2 Low Molecular Weight Polyphenols

The results from the chemometric (principal components) analysis described in section 6.3.1 are presented in figures 6-4 and 6-5. Figure 6-5 is the projection (PC1 and PC2) of the principal components scores calculated from the LMWP HPLC chromatograms using the discrete wavelengths 280, 320, 365 and 520 nm (the procedure is described in detail in sections 2.3.1 through 2.3.3).

The overall behaviour of each of the treatments was very similar in terms of the HPLC results. The largest distance occurred at day 35 with the control wine relative to the other three treatments. The trajectories and rate–of–progress of the four treatments, otherwise are very similar.

In identical fashion to section 4.4.5, figure 6-5 is used to identify peaks that exhibit temporal and/or between treatment variation. The significant peaks have been marked-up on figure 6-5 and are listed in table 6-3.
Figure 6-4  Scores plot from PCA analysis of HPLC chromatograms (280, 320, 365 and 520 nm). The data labels for each score refer to the “treatment”/ “days since start of treatment”. The respective key is: control = C, Micro-oxygenation = MOX, ELMOX-titanium = Ti and ELMOX-glassy carbon = GC.
Figure 6-5  Loading plot from PCA analysis of HPLC chromatograms (280, 320, 365 and 520 nm). The red and blue traces correspond to the PC1 and PC2 loadings respectively. The number adjacent to the peaks refer to the peaks listed in table 6-3.
Table 6-3  Major peaks identified from PCA loading plot figure 6-5

<table>
<thead>
<tr>
<th>Peak</th>
<th>Trace</th>
<th>Retention Time (min)</th>
<th>Loading Sense</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>280 nm</td>
<td>9.3</td>
<td>(-,+)</td>
<td>gallic acid</td>
</tr>
<tr>
<td>2</td>
<td>320 nm</td>
<td>23.2</td>
<td>(+,-)</td>
<td>t-caftaric acid</td>
</tr>
<tr>
<td>3</td>
<td>320 nm</td>
<td>34.6</td>
<td>(+,-)</td>
<td>cis-coutaric acid</td>
</tr>
<tr>
<td>4</td>
<td>320 nm</td>
<td>36.4</td>
<td>(+,-)</td>
<td>t-coutaric acid</td>
</tr>
<tr>
<td>5</td>
<td>280 nm</td>
<td>37.2</td>
<td>(+,-)</td>
<td>catechin</td>
</tr>
<tr>
<td>6</td>
<td>520 nm</td>
<td>48.0</td>
<td>(+,+)</td>
<td>delphinidin-3-glucoside</td>
</tr>
<tr>
<td>7</td>
<td>520 nm</td>
<td>51.6</td>
<td>(+,+)</td>
<td>cyanidin-3-glucoside</td>
</tr>
<tr>
<td>8</td>
<td>520 nm</td>
<td>53.4</td>
<td>(+,+)</td>
<td>petunidin-3-glucoside</td>
</tr>
<tr>
<td>9</td>
<td>280 nm</td>
<td>54.8</td>
<td>(+,-)</td>
<td>epicatechin</td>
</tr>
<tr>
<td>10</td>
<td>520 nm</td>
<td>56.6</td>
<td>(+,+)</td>
<td>peonidin-3-glucoside</td>
</tr>
<tr>
<td>11</td>
<td>520 nm</td>
<td>57.7</td>
<td>(+,0)</td>
<td>malvidin-3-glucoside</td>
</tr>
<tr>
<td>12</td>
<td>280 nm</td>
<td>61.8</td>
<td>(0,+)</td>
<td>unidentified</td>
</tr>
<tr>
<td>13</td>
<td>520 nm</td>
<td>75.7</td>
<td>(+,+),</td>
<td>unidentified</td>
</tr>
<tr>
<td>14</td>
<td>365 nm</td>
<td>76.3</td>
<td>(+,-)</td>
<td>quercetin-3-glucoside</td>
</tr>
<tr>
<td>15</td>
<td>520 nm</td>
<td>86.0</td>
<td>(+,+),</td>
<td>unidentified</td>
</tr>
<tr>
<td>16</td>
<td>365 nm</td>
<td>93.9</td>
<td>(+,+),</td>
<td>quercetin</td>
</tr>
<tr>
<td>17</td>
<td>All</td>
<td>80-95</td>
<td>(-,-)</td>
<td>Unresolved polymeric</td>
</tr>
</tbody>
</table>

The degradation kinetics for each of the peaks listed in table 6-3 are presented in figures 6-6 through 6-9. The curves demonstrate that the hydroxyxycamic and benzoic acids (figure 6-6 a through d) were relatively stable throughout the course of the trial and, moreover there was insignificant between treatment variation for any of the observation dates. Likewise catechin (figure 6-7a) and the unidentified flavanol (6-8d) showed little temporal or between treatment variance for any of the observation dates. Epicatechin (figure 6-8a), however showed a marked decline in concentration for all treatments. This may be due to the elution time of epicatechin and the fact that the peak area may be contaminated with co-eluting anthocyanins.

All the anthocyanins show very similar kinetic behaviour; characterized by an initially rapid decrease in concentration over the first seven days followed by a more gradual decline. For all anthocyanins presented above (figure 6-7b through d, 6-8b and d and 6-9a and c) by weeks five and six the largest decrease in concentration was observed with ELMOX-Ti followed by the ELMOX-C and the MOX treatments. The highest level of anthocyanins
was observed in the control wines. The decrease in monomeric anthocyanins is expected during maturation and the difference between the treatments may be explained by the higher levels of ethanal, produced using titanium as the anode material, which when formed readily reacts with monomeric anthocyanins.

The flavonol concentrations were generally found to decrease for all treatments. The between treatment variation was less clear due to the high between replicate variance obtained, particularly with the quercetin peak (figure 6-9d). A degradation of flavonols is generally seen in a wine during maturation.
Figure 6-6  Evolution of individual phenolic compounds for each treatment through the course of the ELMOX trial ($n = 3$)
Electrochemical micro-oxidation of red wine

Figure 6-7   Evolution of individual phenolic compounds for each treatment through the course of the ELMOX trial ($n = 3$)
Chapter Six

Figure 6-8  Evolution of individual phenolic compounds for each treatment through the course of the ELMOX trial (n = 3)
Evolution of individual phenolic compounds for each treatment through the course of the ELMOX trial ($n = 3$)
6.4.2 Thiolysis

A summary of the thiolysis results are given in terms of mean degree of polymerisation in figure 6-10 and table 6-4.

The mDP results from figure 6-10 show there was little between treatment variation for any of the observation dates (table 6-4). There was a slight increase in mDP until 35 days after the start of the trial for all treatments. The 42 day point showed a small, but statistically significant decrease in mDP relative to the 35 day values.

mDP has been directly correlated to astringency in both wines and model solutions (Vidal et al. 2003). Moreover mDP can be a useful indicator of the evolution of tannin length. Unfortunately in the case of this trial the measurement is inadequate to resolve any small differences in the tannin length that may exist between the treatments.
Figure 6-10  Evolution of mDP for each treatment through the course of the ELMOX trial. Error bars indicate standard deviation (n = 3)

Table 6-4  Mean (n=3)ab degree of polymerisation after thiolysis and quantification with HPLC.

<table>
<thead>
<tr>
<th>Days Since Start of Treatment</th>
<th>Control</th>
<th>MOX</th>
<th>ELMOX-Ti</th>
<th>ELMOX-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>12.40(±0.00)abcd</td>
<td>12.40(±0.00)abcd</td>
<td>12.40(±0.00)abcd</td>
<td>12.40(±0.00)abcd</td>
</tr>
<tr>
<td>7</td>
<td>12.03(±0.81)bcd</td>
<td>11.50(±1.03)cd</td>
<td>12.16(±0.57)bcd</td>
<td>12.17(±0.62)bcd</td>
</tr>
<tr>
<td>14</td>
<td>11.62(±0.41)cd</td>
<td>10.99(±0.28)cd</td>
<td>11.35(±0.61)cd</td>
<td>11.60(±0.15)cd</td>
</tr>
<tr>
<td>21</td>
<td>13.40(±0.38)abc</td>
<td>12.50(±0.47)bcd</td>
<td>13.29(±0.49)abc</td>
<td>13.34(±0.59)abc</td>
</tr>
<tr>
<td>28</td>
<td>13.16(±0.59)abcd</td>
<td>12.26(±0.30)bcd</td>
<td>12.61(±0.60)bcd</td>
<td>13.58(±0.19)abc</td>
</tr>
<tr>
<td>35</td>
<td>14.44(±0.60)ab</td>
<td>13.60(±0.47)abc</td>
<td>13.45(±0.52)abc</td>
<td>14.37(±0.22)ab</td>
</tr>
<tr>
<td>42</td>
<td>12.35(±0.26)bcd</td>
<td>11.70(±0.59)cd</td>
<td>11.48(±0.47)cd</td>
<td>11.47(±0.68)cd</td>
</tr>
</tbody>
</table>

a Standard deviations are given in parentheses after each value

b The bold-type letters indicate whether each treatment is significantly different from another based on a single factor ANOVA and multiple comparison using Fishers LSD0.05. Different letters indicate significant differences
6.4.3 Spectrophotometric Colour Analysis

The visual intensity and hue of the respective treatments were measured using the identical procedure to that described in section 4.3.3. The results are presented in figure 6-11 and table 6-5 for visual intensity and figure 6-12 and table 6-6 for hue.

![Graph](image)

**Figure 6-11** Development of mean visual intensity for each treatment over the course of the ELMOX trial. Error bars indicate standard deviation \((n=3)\)

**Table 6-5** Mean \((n=3)ab\) intensity \((420+520+620\) nm) for all treatments and observation dates

<table>
<thead>
<tr>
<th>Days since start of treatment</th>
<th>Control</th>
<th>MOX</th>
<th>ELMOX-Ti</th>
<th>ELMOX-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7.55(±0.00)</td>
<td>7.55(±0.00)</td>
<td>7.55(±0.00)</td>
<td>7.55(±0.00)</td>
</tr>
<tr>
<td>7</td>
<td>12.45(±0.23)b</td>
<td>12.67(±0.05)abc</td>
<td>12.68(±0.10)abc</td>
<td>13.36(±0.20)b</td>
</tr>
<tr>
<td>14</td>
<td>12.35(±0.29)b</td>
<td>12.59(±0.14)b</td>
<td>12.54(±0.17)b</td>
<td>12.28(±0.06)bcd</td>
</tr>
<tr>
<td>21</td>
<td>12.23(±0.18)b</td>
<td>12.37(±0.09)b</td>
<td>12.75(±0.38)abc</td>
<td>12.71(±0.16)abcd</td>
</tr>
<tr>
<td>28</td>
<td>11.83(±0.22)cd</td>
<td>12.15(±0.06)abc</td>
<td>12.03(±0.25)bcd</td>
<td>12.82(±0.13)abc</td>
</tr>
<tr>
<td>35</td>
<td>11.94(±0.39)b</td>
<td>12.32(±0.07)bcd</td>
<td>12.23(±0.29)b</td>
<td>12.61(±0.05)abcd</td>
</tr>
<tr>
<td>42</td>
<td>12.01(±0.23)b</td>
<td>12.36(±0.03)b</td>
<td>12.38(±0.35)b</td>
<td>12.74(±0.05)abcd</td>
</tr>
</tbody>
</table>

\(^a\) Standard deviations are given in parentheses after each value

\(^b\) The bold-type letters indicate whether each treatment is significantly different from another based on a single factor ANOVA and multiple comparison using Fishers LSD\(_{0.05}\). Different letters indicate significant differences.
Figure 6-12  Development of mean visual hue for each treatment over the course of the ELMOX trial. Error bars indicate standard deviation (n=3)

Table 6-6  mean (n=3)\(^a\)\(^b\). hue (420/520 nm) for all treatments and observation dates

<table>
<thead>
<tr>
<th>Days since start of Treatment</th>
<th>Control</th>
<th>MOX</th>
<th>ELMOX-Ti</th>
<th>ELMOX-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.435(±0.000)</td>
<td>0.435(±0.000)</td>
<td>0.435(±0.000)</td>
<td>0.435(±0.000)</td>
</tr>
<tr>
<td>7</td>
<td>0.425(±0.004)(^{d,e})</td>
<td>0.430(±0.004)(^{d,e})</td>
<td>0.430(±0.003)(^{d,e})</td>
<td>0.438(±0.005)(^{d,e})</td>
</tr>
<tr>
<td>14</td>
<td>0.442(±0.007)(^{c,d,e})</td>
<td>0.451(±0.006)(^{c,d,e})</td>
<td>0.448(±0.004)(^{c,d,e})</td>
<td>0.435(±0.000)(^{c,d,e})</td>
</tr>
<tr>
<td>21</td>
<td>0.457(±0.009)(^{b,c,d,e})</td>
<td>0.465(±0.007)(^{b,c,d,e})</td>
<td>0.472(±0.003)(^{b,c,d,e})</td>
<td>0.460(±0.003)(^{b,c,d,e})</td>
</tr>
<tr>
<td>28</td>
<td>0.463(±0.010)(^{b,c,d})</td>
<td>0.475(±0.008)(^{b,c,d})</td>
<td>0.473(±0.003)(^{b,c,d})</td>
<td>0.472(±0.005)(^{b,c,d})</td>
</tr>
<tr>
<td>35</td>
<td>0.474(±0.003)(^{b,c,d})</td>
<td>0.483(±0.007)(^{b,c,d})</td>
<td>0.483(±0.007)(^{b,c,d})</td>
<td>0.473(±0.005)(^{b,c,d})</td>
</tr>
<tr>
<td>42</td>
<td>0.477(±0.008)(^{b,c,d})</td>
<td>0.491(±0.010)(^{b,c})</td>
<td>0.493(±0.009)(^{b,c})</td>
<td>0.486(±0.007)(^{b,c,d})</td>
</tr>
</tbody>
</table>

\(^a\) Standard deviations are given in parentheses after each value

\(^b\) The bold-type letters indicate whether each treatment is significantly different from another based on a single factor ANOVA and multiple comparison using Fishers LSD\(_{0.05}\). Different letters indicate significant differences
As a measure of colour stability resistance to bisulfite bleaching was determined (Iland 2000). The test involved dosing a wine with a stoichiometric excess of bisulfite to bleach the remaining monomeric anthocyanins. The relative decrease in red colour indicates the degree of formation of stable pigmented compounds (e.g. T-A\(^+\) adducts or similar as discussed in section 1.4.3). The results of the bleaching assay are presented in figure 6-13 and table 6-7.

![Figure 6-13](image_url)

**Figure 6-13**  Results of the bleached pigment assay for all treatments and observation dates. Data point represent mean values for each treatment and error bars indicate standard deviation (\(n=3\))

**Table 6-7**  mean (\(n=3\))\(^{ab}\) absorbance at 520 nm after bleaching with bisulfite for all treatments and observation dates.

<table>
<thead>
<tr>
<th>Day since start of treatment</th>
<th>Control</th>
<th>MOX</th>
<th>ELMOX-Ti</th>
<th>ELMOX-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.25(±0.00)</td>
<td>1.25(±0.00)</td>
<td>1.25(±0.00)</td>
<td>1.25(±0.00)</td>
</tr>
<tr>
<td>7</td>
<td>1.33(±0.01)e</td>
<td>1.34(±0.02)e</td>
<td>1.36(±0.03)e</td>
<td>1.49(±0.14)d</td>
</tr>
<tr>
<td>14</td>
<td>1.45(±0.02)e</td>
<td>1.55(±0.09)e</td>
<td>1.51(±0.04)e</td>
<td>1.36(±0.02)e</td>
</tr>
<tr>
<td>21</td>
<td>1.58(±0.08)d</td>
<td>1.70(±0.10)d</td>
<td>1.74(±0.04)d</td>
<td>1.72(±0.09)d</td>
</tr>
<tr>
<td>28</td>
<td>1.57(±0.12)d</td>
<td>1.76(±0.20)d</td>
<td>1.88(±0.07)d</td>
<td>1.77(±0.07)d</td>
</tr>
<tr>
<td>35</td>
<td>1.78(±0.11)d</td>
<td>2.04(±0.27)d</td>
<td>2.19(±0.18)b</td>
<td>1.76(±0.01)d</td>
</tr>
<tr>
<td>42</td>
<td>2.10(±0.18)b</td>
<td>2.38(±0.32)b</td>
<td>2.66(±0.24)a</td>
<td>2.14(±0.06)b</td>
</tr>
</tbody>
</table>

\(^a\) Standard deviations are given in parentheses after each value

\(^b\) The bold-type letters indicate whether each treatment is significantly different from another based on a single factor ANOVA and multiple comparison using Fishers LSD\(_{0.05}\). Different letters indicate significant differences
Figure 6-11 and 6-12 show relatively small between treatment variation except for a small divergence with the ELMOX-C intensity relative to the other treatments from day 21 until the end of the trial. The other notable feature of figure 6-11 is the big increase in colour intensity of all treatments from the initial baseline measurement to the seven day point. This is presumably a result of the equilibration and perhaps the residual dissolved oxygen in the wine after filtration.

The results of the bleaching assay show an increasing trend in absorbance for all wines indicating that SO\(_2\) resistant pigmented polymers are being formed. The rate of increase in these compounds was greatest for the ELMOX-Ti treatment followed by the traditional MOX treatment. The ELMOX-C and Control wines showed the lowest rate of increase. The relatively high rate of formation of SO\(_2\) resistant pigments by the ELMOX-Ti treatment presumably has to do with the high rate of formation of ethanal (refer to section 6.4.5) and the formation of ethyl-linked polymers which go on to form more stable pigmented polymers (section 1.4).

### 6.4.4 Sensory Analysis

The results of the sensory trials are presented in figures 6-14 and 6-15 and in table 6-8. Figures 6-14 and 6-15 show plots of the two first canonical variables (linear combination of original variables that maximise the separation between treatment means) for each sampling date. The treatment means are presented as bold symbols surrounded by circles of the same colour. The circles represent 95% confidence loci for each of the respective treatments and are based on a \(\chi^2\) test (Krzanowski 2000). Overlayed are the individual panelist scores transformed into canonical space.

Table 6-8 presents the results of a single factor multivariate analysis of variance (MANOVA) for each of the sampling dates. The Wilks Lambda test statistic (\(\Lambda\)) is calculated for every possible dimension of the data (i.e. number of treatments – 1) the corresponding probability estimation is shown in the adjacent column (based on the \(\Lambda\) value). The overall significant (P<0.05) dimensionality of the treatments is presented in the right-hand column (i.e. if the means are the same then d=0 or separated along a line (d=1), plane (d=2) or 3 dimensional space (d=3)).
Table 6-8  Results of single factor MANOVA for each sample date

<table>
<thead>
<tr>
<th>Days From Start of Treatment</th>
<th>No. of Panelists</th>
<th>Wilks Lambda (Λ)</th>
<th>Estimated Probability</th>
<th>Dimensionality (based on P&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>12</td>
<td>0.5777</td>
<td>0.5499</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.7971</td>
<td>0.8116</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.9714</td>
<td>0.9775</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>10</td>
<td>0.6726</td>
<td>0.9646</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.8492</td>
<td>0.9796</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.9609</td>
<td>0.9707</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>12</td>
<td>0.3428</td>
<td>0.0079</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.6214</td>
<td>0.1465</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.8996</td>
<td>0.6308</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>8</td>
<td>0.4531</td>
<td>0.7087</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.8705</td>
<td>0.9979</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.9719</td>
<td>0.9942</td>
<td></td>
</tr>
</tbody>
</table>

The only sample date that yielded a significant difference between treatments was 21 days after the start of the trial. Figure 6-15a shows no significant difference between the MOX and ELMOX-Ti treatments however the control and ELMOX-C treatments are significantly different from each other and the MOX and ELMOX-Ti treatments. Figure 6-15b (35 days after the start of the trial) also shows the ELMOX-Ti treatment separated from the other three, however the difference was not significant.

Figure 6-16 shows the principal response curve (PRC) for the sensory data described above. A full explanation of the application of the PRC is described in section 4.4.2.
Figure 6-14  Canonical variates analysis plots of sensory results for sampling dates at 7 (a) and 14 (b) days after the start of treatment. Bold symbols represent treatment mean scores. Other symbols are individual panelist data. The circles surrounding treatment means indicate 95% confidence regions for the respective group means.
Figure 6-15  Canonical variates analysis plots of sensory results for sampling dates at 21 (a) and 35 (b) days after the start of treatment. Bold symbols represent treatment mean scores. Other symbols are individual panelist data. The circles surrounding treatment means indicate 95% confidence regions for the respective group means.
The most notable feature of figure 6-16 is the behaviour of the ELMOX-Ti treatment. The principal response curve increases continually over the course of the trial. Examination of the attribute weights (the line-stack plot on the right side of the main plot) shows that aldehyde contributes highly to the model followed by astringency and mouthfeel. As discussed in section 6.2.3 the electrical potential realised at the titanium electrode with a current of 76.8 $\mu$A was greater than 1.0 V. Production of ethanal is therefore likely for this treatment which is reflected in the sensory result. Interestingly the weighting of astringency and bitterness does not correlate with the mDP results, however higher concentrations of ethanal presumably means that more aldehyde linked polymers were being formed which are not resolved using thiolysis.

Also of note in the PRC diagram is the similarity in response of the MOX and ELMOX-C treatments and in contrast to the ELMOX-Ti treatment, shows little divergence relative to the Control. The MOX and ELMOX-C treatment responses also track negatively compared to the Control responses; this is, perhaps indicative of the fact that there is little sensory difference between the three wines.
Figure 6-16   Principal Response curves for the three treatments relative to the control wine. Sensory attribute weights are presented in the linestack plot to the right of the actual PRC plot.
6.4.5 Ethanal Analysis

**Figure 6-17**  Ethanal concentration for all treatments and observation dates. Data point represent mean values for each treatment and error bars indicate standard deviation ($n=3$)

**Table 6-9**  mean ($n=3$) ethanal concentrations for all treatments and observation dates.

<table>
<thead>
<tr>
<th>Days since start of treatment</th>
<th>Control</th>
<th>MOX</th>
<th>ELMOX-Ti</th>
<th>ELMOX-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>9.27(±1.20)cde</td>
<td>11.87(±2.34)bcde</td>
<td>13.17(±0.97)bcde</td>
<td>12.65(±1.63)bcde</td>
</tr>
<tr>
<td>14</td>
<td>11.20(±2.59)cde</td>
<td>13.43(±3.89)bcde</td>
<td>15.27(±4.53)bcde</td>
<td>11.90(±2.55)bcde</td>
</tr>
<tr>
<td>21</td>
<td>7.57(±0.25)cde</td>
<td>10.97(±2.03)cde</td>
<td>16.17(±4.95)bcd</td>
<td>9.85(±1.20)cde</td>
</tr>
<tr>
<td>28</td>
<td>9.43(±4.32)cde</td>
<td>12.47(±2.49)bcde</td>
<td>19.80(±9.43)abc</td>
<td>9.60(±0.85)cde</td>
</tr>
<tr>
<td>35</td>
<td>15.83(±2.49)bcd</td>
<td>17.33(±8.14)abcd</td>
<td>22.70(±8.39)abc</td>
<td>12.55(±3.04)bcd</td>
</tr>
<tr>
<td>42</td>
<td>19.93(±1.50)abc</td>
<td>22.30(±3.05)abc</td>
<td>24.93(±10.31)ab</td>
<td>18.25(±9.83)abcd</td>
</tr>
</tbody>
</table>

- Standard deviations are given in parentheses after each value.
- The bold-type letters indicate whether each treatment is significantly different from another based on a single factor ANOVA and multiple comparison using Fishers LSD$_{0.05}$. Different letters indicate significant differences.
Due to the interesting sensory result presented in figure 6-16 a chemical analysis of the levels of ethanal was undertaken for each of the treatments as described in section 6.3.6. The results are given in figure 6-17 and Table 6-9. In general the evolution of ethanal is similar in behaviour to the sensory result shown in figure 6-16. The ELMOX-Ti curve tracks consistently above the other treatments (although the within treatment differences are not significant even in the final observation date). The analysis is also influenced by the high variance of the acetaldehyde chemical analysis data despite the removal of the outlier for the ELMOX-C treatment (refer to section 6.4.1 for discussion).

Note that figure 6-16 is not only influenced by ethanal but all of the other sensory attributes and that the principal response curves represent the contrasts between the respective treatment and the control. 6-18 shows the treatment means for the sensory data and despite the low resolution of both the chemical and sensory data there is an apparent correlation between the two sets of data.

![Figure 6-18](image)

**Figure 6-18** Ethanal treatment means from sensory data the x-axis labels indicate the observation date (e.g. d07 = 7 days since the start of the trial) followed by the treatment information. The error bars indicate 95% confidence intervals.
6.4.6 General Discussion

The preliminary ELMOX trial yielded some interesting results. The most dramatic transformations both chemically and organoleptically occurred in the ELMOX-Ti treatment. While the chemistry of the wine on the electrode surface is not fully understood, it is believed that the titanium material has a much higher propensity to oxidise ethanol to ethanal. This was certainly reflected in both the sensory results and the chemical analyses. Determination of ethanal concentrations showed higher levels in the ELMOX-Ti compared to the other treatments. Sensory results correlated with the chemical findings. Higher levels of SO\textsubscript{2} resistant pigments were also measured in the ELMOX-Ti treatment compared to the traditional micro-oxygenation or the ELMOX-C trials. This is indicative of a greater rate of stable pigment formation.

In retrospect the traditional micro-oxygenation dosage rate was very low perhaps explaining the small, almost undetectable changes in the MOX treatment wine. Similarly for the glassy carbon electrode, the current was set so the potential would not exceed 1.0 V and promote the formation of ethanal. Perhaps a different material needs to be investigated or the same material in a different configuration (i.e. greater contacting surface area).

A subsequent trial was run using 300 litre treatments and the aim was to compare the glassy carbon ELMOX against traditional MOX (Fell, Dykes et al. 2007). For this trial a higher current was used (6144 \mu A) which allowed a more realistic comparable MOX rate of 3.1 mg/L/mth to be used. Similar trends were seen in both the MOX and ELMOX treatment in terms of an increase in the SO\textsubscript{2} resistant pigments, however increased amounts of ethanal and bound SO\textsubscript{2} were observed in the ELMOX compared to the MOX treatment. As with the ELMOX-Ti treatment in this trial the high levels of ethanal are believed to be due to the high potential at the electrode.
Chapter Six
CHAPTER SEVEN

Modeling of Oxygen Mass Transfer with Chemical Reaction

7.1 Introduction

It was stated in Chapter One that the main aim of the work contained within this thesis was to better understand the dependence of oxygen dosage rate on the efficacy of micro-oxygenation. This final chapter is concerned with the integration of the physical and chemical aspects of the process in order to better understand the observed dependence on the oxygen delivery rate.

The traditional micro-bullage oxygenation process can be described, in process technology terms, as a bubble column reactor. Bubble columns are encountered in a wide range of applications such as: hydrocarbon synthesis, hydrogenation of unsaturated oil, oxidation of hydrocarbons, fermentation, ozonation of water and (biological) wastewater treatment. These processes all involve gas-liquid mass transfer many with accompanying chemical reactions. The advantages of a bubble column are the simplicity of operation (i.e no moving parts), low operating cost as well as good mass and heat transfer characteristics. Despite the widespread use of bubble columns and the significant amount of research already undertaken (Joshi, Vitankar et al. 2002; Sokolichin, Eigenberger et al. 2004) a detailed understanding of hydrodynamics, mass transfer, chemical reaction behaviour and their respective interactions is still yet to be realised.

An analysis of the mass transfer within a bubble plume indicates that the mass transfer process is affected by a number of different factors. The principal ones being the interfacial
surface area between the bubbles and the liquid phase and the contact time the bubbles are in the plume. As discussed in section 1.3 the total surface area of bubble-liquid interface is inversely related to bubble size. Similarly, for the range of bubble diameters important for micro-oxygenation (300-1000 µm) the rise velocity is directly related to bubble diameter. Bubble size, however is not the only factor that influences the bubble-column performance, plume structure, rate of chemical reaction and mass transfer characteristics. These factors are strongly coupled and the relationship between them can be illustrated in figure 7-1.

![Figure 7-1 Schematic representation of the string coupling between factors affecting a bubble plume performance (adapted from Darmana 2005). $k_i$ = interphase mass transfer coefficient; $a$ = bubble-wine interfacial surface area and $d_i$ = bubble diameter.](image)

The behavior of a bubble column is complex; one problem arises from the fact that the local properties in the two-phase gas-liquid flow are inherently difficult to measure. Any type of probe tends to be intrusive and disturb the flow and resulting behaviour of the column. As a result there has been a growing interest in the development of models which can accurately predict the detailed characteristics of bubble plume.

Due, in no small part, to the advances in computer hardware and numerical solution methods, computational fluid dynamics (CFD) has become an increasingly powerful tool for both scientists and engineers. Two approaches are widely used for describing hydrodynamics of bubble columns: the Euler–Euler (E–E) and Euler–Lagrange (E–L) methods. The E–E model employs the volume or ensemble averaged mass and momentum conservation equations to describe the time dependent motion of both phases. Essentially both phases are treated as interpenetrating continua. The number of bubbles present in a computational cell is represented by a volume fraction and the information on the bubble size distribution is often obtained by incorporating population balance equations, which take into account
break-up and coalescence of bubbles as well as growth or shrinkage of bubbles as a result of mass transfer.

The E–L model on the other hand adopts a continuum description for the liquid phase and tracks each individual bubble using Newtonian equations of motion (i.e. the mutual bubble–liquid and the mutual bubble–bubble or bubble–wall interaction) in a lagrangian reference frame (hence the name). This allows for a direct consideration of additional effects related to bubble–bubble and bubble–liquid interaction. Mass transfer with and without chemical reaction, bubble coalescence and re-dispersion can be incorporated directly if desired. Unlike the E–E model, the E–L model does not require additional models to predict the bubble size distribution since this is intrinsic to the solution.

One main limitation of the E–L model is the number of bubbles that can be treated since for every bubble a set of mass and momentum balance equations requires solving. This means that the model is very computationally expensive.

The operation of a bubble columns typically involves bubbles being continuously injected into, an initially, quiescent liquid. The bubbles are driven upwards by buoyancy forces and as they rise the liquid is entrained inducing a mean upward flow of the bubble-liquid mixture (this is defined as the plume). Dissolution of the solute gas into the plume occurs as the bubble rise. Similarly dissolved gases will simultaneously be partitioned into the bubble (e.g. In the case of a wine immediately after fermentation, CO$_2$ will readily evolve into the bubble as it rises in the fluid column).

The bubble plume structure has been extensively researched and reviewed (Joshi et al. 2002, Sokolichin 2004) and comparisons have been made regarding the respective merits of the E-E and E-L approaches. In all cases however, It is generally regarded that a three-dimensional model is required to capture the essential behaviour of a bubble column.

Hitherto the majority of numerical studies have focused on bubbles columns with large gas flow-rates. The high gas velocities mean the fluid flow is generally in the turbulent regime, and models are developed accordingly using either $k$-$\varepsilon$ (Sokolichin and Eigenberger 1999) or
large eddy simulation (LES) based approaches (Darmana, Deen et al. 2005). Published studies of bubble columns with small gas flow rates (i.e. at the rates used in a typical micro-oxygenation treatment) are less common with one very recent publication investigating the mass transfer efficiency of ozonation of water (Gong, Takagi et al. 2007).

The study described in this chapter considers the dissolution of oxygen in a rectangular tank using realistic micro-oxygenation dosage rates. The tank volume is small compared to many commercial applications however the main objective of this study is to develop a method to predict mass transfer performance and to investigate the effect of dosage rate on the dissolved oxygen distribution in the vessel.

7.2 Model Development

7.2.1 Discrete phase governing equations

This model assumes that the bubbles are spherical which is reasonable given their small diameter (Motarjemi and Jameson 1978). The forces acting on a bubble rising in an unsteady, non-uniform liquid flow-field is made up of separate and uncoupled contributions from: far-field pressure gradient, drag, virtual mass, lift and gravity i.e.:

\[ F_{\text{Total}} = F_p + F_{\text{Drag}} + F_{\text{VM}} + F_{\text{Lift}} \]  

(0.1)

The acceleration of the bubble can then be calculated simply from applying a force balance to the bubble:

\[ m_b \frac{dv}{dt} = F_{\text{Total}} - \left( \rho_b \frac{dV_b}{dt} \right) v \]

[7.2]

Where the second term in equation [7.2] takes account of the changes due to mass transfer (refer below to section 7.2.2). The forces acting on bubbles within a bubble plume have been reviewed extensively in numerous publications (Sokolichin, Eigenberger et al. 2004; Clift, Grace et al. 2005; Prosperetti and Tryggvason 2007). For the sake of completeness however a brief description of each of the forces and the governing equations is given below.
7.2.1.1 Gravity and far-field pressure force

This is a general term for the forces acting on the bubble due to the liquid phase pressure gradient. This force incorporates the Archimedes displacement force, inertial forces and viscous strain. The sum of the liquid-phase pressure gradient can be given as:

\[ \mathbf{F}_g + \mathbf{F}_p = \rho \, g \, V_b \mathbf{g} - V_b \nabla P \]  \[ \text{[7.3]} \]

7.2.1.2 Drag force

A bubble moving with a constant velocity through a uniform flow field experiences a drag force which consists of a form and a friction drag exerted by the liquid on the moving bubble (Clift, Grace et al. 2005). Experiment has shown that the drag force is proportional to the slip velocity between the phases as described in equation 7.4:

\[ \mathbf{F}_{\text{Drag}} = -\frac{1}{2} C_{\text{Drag}} \rho \pi r_b^2 |\mathbf{v} - \mathbf{u}| (\mathbf{v} - \mathbf{u}) \]  \[ \text{[7.4]} \]

The drag coefficient \( C_{\text{Drag}} \) depends on the flow regime. For rigid spheres the flow regime can be given as (Clift, Grace et al. 2005).

\[ C_{\text{Drag}} = \begin{cases} \frac{24}{\text{Re}_b} \left(1 + \frac{3}{16} \text{Re}_b\right) & \text{Re}_b \leq 0.01 \\ \frac{24}{\text{Re}_b} \left(1 + 0.1315 \text{Re}_b^{(0.82 - 0.05 \log_{10} \text{Re}_l)}\right) & 0.01 < \text{Re}_b \leq 20 \\ \frac{24}{\text{Re}_b} \left(1 + 0.1935 \text{Re}_b^{0.6305}\right) & 20 < \text{Re}_b \leq 260 \end{cases} \]  \[ \text{[7.5]} \]

Where

\[ \text{Re}_b = \frac{2r_b \rho_l |\mathbf{v} - \mathbf{u}|}{\mu_l} \]  \[ \text{[7.6]} \]

7.2.1.3 Lift force

Bubbles rising in a non-uniform liquid flow field experience a lift force due to vorticity or shear of the fluid phase. The lift force is derived as being dependent on the slip velocity between phases and the curl of the liquid velocity field. If the bubble velocity is greater than the liquid velocity the lift force tends toward the direction of lower liquid velocity. Conversely if the bubble moves with a lower velocity than the liquid the lift force is directed toward the region of higher velocity. Mathematically the lift force can be expressed as:
Chapter Seven

\[ F_{\text{Lift}} = -C_L \rho V_b \left[ (\mathbf{v} - \mathbf{u}) \times \nabla \times \mathbf{u} \right] \quad [7.7] \]

The lift force coefficient is typically taken as 0.5 (Clift, Grace et al. 2005).

### 7.2.1.4 The virtual mass force

The virtual mass force can be seen as a resistance to acceleration of the bubble. This resistance is due to an effective “added mass” of liquid that has to be accelerated when a bubble accelerates. This force is proportional to the difference in acceleration between the liquid and the bubble and can be expressed as:

\[ F_{VM} = -C_{VM} \rho V_b \left( \frac{dv}{dt} - \frac{Du}{Dt} \right) \quad [7.8] \]

Where the D/Dt operator denotes the substantial or material derivative as the virtual mass force is being evaluated in the Lagrangian reference frame. The virtual mass coefficient is taken as \( C_{VM} = 0.5 \) for this study (Clift, Grace et al. 2005).

### 7.2.1.5 Direct bubble-bubble interactions

Very sophisticated models have been developed to manage bubble-bubble and bubble-wall interactions (Darmana, Deen et al. 2006). These are extremely important in bubble plumes with high specific gas velocities. It has been claimed that for bubble volume fractions below \( 1 \times 10^{-3} \) the bubble-bubble interaction are less important to the accuracy of the model. Even with the maximum gas flow-rates simulations described in this Chapter the bubble volume fraction are marginally greater than \( 1 \times 10^{-4} \) at the diffuser hence bubble-bubble interactions are ignored in this model.

### 7.2.2 Continuous phase governing equations

The liquid (wine) phase hydrodynamics are described by the volume-averaged Navier-Stokes equations:

\[ \frac{\partial}{\partial t} (\varepsilon, \rho, \mathbf{u}) + \nabla \cdot (\varepsilon, \rho, \mathbf{u}) = (j_{b,ed} - j_{l,b}) \quad [7.9] \]

\[ \frac{\partial}{\partial t} (\varepsilon, \rho, \mathbf{u}) + \nabla \cdot (\varepsilon, \rho, \mathbf{u}) \mathbf{u} = -\varepsilon \nabla P - \varepsilon \tau + \varepsilon \rho g + \phi \quad [7.10] \]

The liquid is assumed to behave as a Newtonian fluid moreover the bulk viscosity term is taken as zero for all simulations. The viscous stress tensor can, therefore be given as:
\[ \tau_t = \mu \left[ (\nabla \mathbf{u}) + (\nabla \mathbf{u})^T - \frac{2}{3} I (\nabla \cdot \mathbf{u}) \right] \]  

[7.11]

Because of the very low gas velocities the flow is assumed to be laminar and the viscosity is taken as constant over the entire domain.

### 7.2.2 Mass transport governing equations

The transport of oxygen can be divided into two different stages. Firstly, the interphase mass transfer of gaseous oxygen from the bubble into the wine (and the movement of other dissolved solutes from the liquid phase into the bubble – e.g. CO\(_2\)). To better follow the model development figure 1-5 has been reproduced as figure 7-2.

For an incompressible bubble, the mass balance equation can be written as:

\[ \rho_b \frac{dV_b}{dt} = j_{b\rightarrow l} - j_{l\rightarrow b} \]  

[7.12]

The interphase mass transfer from a bubble into a liquid phase (and vice versa) is driven by the concentration gradient (as described in section 1.4). The rate of mass transfer of any particular species \( j \) can thus be described as:

\[ j_b = K_L a_b (c_i^* - c_l) \]  

[7.13]

Where \( a_b \) is the bubble surface area and \( K_L \) is the overall mass transfer coefficient as defined in section 1.4. The * superscript (applied to the first concentration term) denotes the concentration at the liquid interface. If the concentration of the solute is known in the bubble, the liquid-side interfacial (equilibrium) concentration can be determined using Henry’s law\(^\text{\footnote{This is slightly different from equation [1.2] where partial pressure is given instead of gas concentration. The units of the Henry’s law constant obviously reflect the difference.}}\) as:

\[ c_i^* = \frac{c_{iG}}{H} \]  

[7.14]

The mass transfer coefficient can be determined using a common relation developed for a bubble in a moving flow field (Bird, Stewart et al. 2002):

\[ Sh = 2 + 0.6425(ReSc)^{1/2} \]  

[7.15]
Where $Sh$ is the Sherwood number and $Sc$ the Schmidt number. From the discussions in Chapter Three the mass transfer within the bubble was ignored due to the very small diameter of the bubble and the very high mass transfer coefficient relative to $K_L$.

The second part of the mass transfer problem is the distribution of the dissolved oxygen throughout the volume wine. This can be described using the scalar transport equation (convection-diffusion equation) given as:

$$\frac{\partial}{\partial t} (\varepsilon_i c_{O_2}) + \nabla \cdot (\varepsilon_i c_{O_2} \mathbf{u}) = \nabla \cdot \left[ \Gamma \nabla (\varepsilon_i c_{O_2}) \right] + S_L + S_D \quad [7.16]$$

Where $c_{O_2}$ is obviously the concentration of dissolved oxygen, $\Gamma$ is the diffusion coefficient for oxygen in the wine system, $S_L$ is the source term for the mass dissolution from the oxygen in the bubbles (from equation [7.13]) and $S_D$ is the term to account for the consumption of oxygen in the wine by chemical reaction.

**Figure 7-2** Representation of interphase mass transfer of $O_2$ and $CO_2$ on an individual bubble level
7.3 Numerical Implementation

7.3.1 Time Discretisation

The model essentially solves the liquid and bubble phase independently with coupling terms in the respective governing equations. In order to manage the time dependent behaviour of each of the models the approach used by (Darmana, Deen et al. 2005) is adapted for this model. The approach involves using two time steps which will be denoted $\delta t_{\text{bub}}$ and $\delta t_{\text{flow}}$ for the bubble and liquid phase solution time steps respectively. Both are fixed time steps with $\delta t_{\text{bub}}$ being a smaller step than $\delta t_{\text{flow}}$. The bubble mass and momentum balances are resolved every $\delta t_{\text{bub}}$ seconds after $\delta t_{\text{flow}}$ seconds the hydrodynamic equations are then solved. Figure 7-3 illustrates the approach taken schematically.

![Schematic representation of time marching approach](image)

7.3.2 Bubble Dynamics

Combining the mass and momentum balance equations described in section 7.2.1 the resulting equation for the net forces on a bubble can be given as:

$$\rho_g V_b \frac{d\mathbf{u}}{dt} = \rho_l V_b \frac{D\mathbf{u}}{Dt} + \rho_l V_b C_{\text{YM}} \left( \frac{D\mathbf{u}}{Dt} - \frac{dv}{dt} \right) + (\rho_g - \rho_l) V_b \mathbf{g}$$

$$-\rho_l \frac{\pi r_b^2}{2} C_{\text{Drag}} |\mathbf{v} - \mathbf{u}| (\mathbf{v} - \mathbf{u})$$

$$-\rho V_b C_L \left( (\mathbf{v} - \mathbf{u}) \times (\nabla \times \mathbf{u}) \right) \tag{7.17}$$

The first term on the right hand side of equation [7.17] is due to the acceleration of the surrounding liquid. The second term is the due to the virtual mass force. The third term combines gravity and buoyancy forces. The forth and fifth terms are drag and lift force respectively. Note that the density of the liquid is three orders of magnitude greater than the
density of the gas in the bubble (i.e. \( \rho \gg \rho_g \)). If the terms containing gas density are ignored equation [7.16] can be rewritten in terms of bubble acceleration as:

\[
\frac{d\mathbf{v}}{dt} = 3 \frac{D\mathbf{u}}{Dt} - 2g - \frac{3C_D}{4r_b} |\mathbf{v} - \mathbf{u}| (\mathbf{v} - \mathbf{u}) - \left[ (\mathbf{v} - \mathbf{u}) \times \nabla \times \mathbf{u} \right]
\]

Equation 7.17 is implemented directly into the discrete phase model.

The bubble acceleration is integrated numerically using a first order explicit scheme. Using \( \xi \) as a general variable the integration formula can be expressed as:

\[
\xi^{\kappa+1} = \xi^\kappa + \left( \frac{d\xi}{dt} \right)^\kappa \delta t_{\text{bub}}
\]

Where \( \kappa \) is the time step. The force and mass balance on each bubble within the column are solved sequentially. Initially the mass transfer of O\(_2\) from and CO\(_2\) into the bubble is calculated using equations [7.13] through [7.15]. The new bubble volume and diameter are then calculated using equation [7.18]. After calculation of the mass transfer the acceleration is obtain using equation [7.17] the new bubble velocity can be obtained by integrating the acceleration using equation [7.18]. Similarly the new bubble position can be obtained in the same manner by integration of the velocity.

### 7.3.3 Hydrodynamics

A finite difference scheme was used for the discretisation of the liquid phase flow equations [7.9] and [7.10]. A three-dimensional Cartesian staggered grid was employed to ensure numerical stability. All terms of the momentum equation were solved explicitly except the pressure gradient terms which were solved implicitly. The solution methods used were alternately based on a pressure implicit with splitting of operators (PISO) and a semi-implicit method for pressure linked equations (SIMPLE) algorithms. Both methods were adapted from (Prosperetti and Tryggvason 2007). The convective fluxes were discretised using first order upwind differencing (Ferziger and Milovan 2002). The resulting set of linear equations were solved using an incomplete Choleski conjugate gradient (ICCG) solver obtained from (Ferziger and Milovan 2002). The discretisation and solution details are given in appendix VII.
The dissolved mass of the gas phase (right hand side of equation [7.9]) is neglected due to the large difference between the liquid and gas phase densities and thus contributes little to the mass continuity of the wine phase.

### 7.3.4 Dissolved oxygen transport and chemical equation

All terms of the transport equation [7.16] were discretised and solved implicitly, with the exception of the source term \( (S_L) \) which was solved explicitly. The sink (or consumption) term of equation [7.16] \( (S_D) \) is determined experimentally as discussed in section 7.4 below. Like the momentum equations the convective fluxes in the transport equation were discretised using a first order upwind difference scheme. Although this results in a small diffusive error the method ensures numerical stability. The resulting set of linear equation were solved using the ICCG solver described in section 7.3.3

### 7.3.5 Bubble-liquid coupling

As discussed above the coupling between the two phases is achieved through the momentum transfer term \( (\phi) \) in equation [7.10], the liquid phase volume fraction \( (\varepsilon_L) \) and the mass transfer terms on the right-hand side of equation [7.9]. The \( \phi \) term in equation [7-10] represents the force the bubble exerts on the liquid and is simply the reaction force of the liquid on the bubble i.e.

\[
\phi = -\sum (F_D + F_{VM} + F_{Lift})
\]

Where the drag, virtual mass and lift force are appropriately mapped onto the Eulerian reference frame as described in section 7.3.5.2 below.

One of the challenges of the E-L method is to map the properties of one phase onto the other phase given each phase is defined using a different reference frame (i.e. Lagrangian for the bubble-phase and Eulerian for the liquid phase). A simple mapping technique that translates bubble properties onto the Eulerian grid and conversely maps liquid-phase properties onto individual bubbles was developed by (Darmana, Deen et al. 2005) and is used in this model.
7.3.5.1 Porosity Mapping
The bubble volume fraction for each three-dimensional cell can be measured directly at each \( \delta t_{\text{flow}} \) time-step using equation [7.19].

\[
\xi_b = \frac{1}{V_{\text{cell}}} \sum_{i \in \text{cell}} \xi_{\text{cell}}^i V_b^i \quad [7.19]
\]

Where \( \xi_{\text{cell}}^i \) is the volume fraction of the \( i^{th} \) bubble in the cell under calculation. The \( \xi_{\text{cell}}^i \) factor is necessary as bubbles may straddle cell boundaries in which case they may be counted twice. The liquid volume fraction is calculated once the bubble volume fraction has been determined by the simple closure relation:

\[
\xi_l = 1 - \xi_b \quad [7.20]
\]

In the case described above where bubbles do straddle boundaries, again an approach used by (Darmana, Deen et al. 2005) is adopted for the numerical implementation. For computational simplicity for bubbles that overlap boundaries are treated as cubes with sides \( d_{\text{equivalent}} \) calculated to give an equal volume as the spherical bubble i.e.:

\[
d_{\text{equivalent}} = \sqrt[3]{\frac{\pi d_b^3}{6}} \quad [7.21]
\]

7.3.5.2 Bubble-liquid phase mapping
A straightforward volume averaging method, first used by (Delnoij, Kuipers et al. 1999) is adopted for this model to map Lagrangian quantities onto the Eulerian grid. The bubble quantity \( \omega \) is mapped onto the liquid property \( \Omega \) in a similar method to that used for calculating porosity. Mathematically the relationship between \( \omega \) and \( \Omega \) can be expressed as:

\[
\Omega = \frac{1}{V_{\text{cell}}} \sum_{i \in \text{cell}} \xi_{\text{cell}}^i V_b^i \omega \quad [7.22]
\]

Where the other variables are identical to equation [7.19].

7.3.5.2 Liquid-bubble phase mapping
Again the method developed by (Delnoij, Kuipers et al. 1999) to map eulerian quantities to individual bubbles is applied to the model presented in this chapter. The technique involves calculating interpolation factors and calculating the bubble property from the eight surrounding grid nodes (scalar control volume nodes are used). The idea is shown schematically in figure 7.4 and mathematically in equation [7.23]
Figure 7-4 Schematic representation of liquid to bubble phase mapping method, the notation accompanies equation [7.23] adapted from (Darmana, Deen et al. 2005) where $S$ is the bubble position in space at time $t$

The value of property $ω$ at point $S$ in figure 7-4, in terms of the properties at each of the grid nodes ($Ω$) can be given by the following relation:

$$
ω = \lambda_1 \lambda_2 \lambda_3 Ω_A + \lambda_3 \lambda_4 \lambda_6 Ω_B + \lambda_4 \lambda_5 \lambda_6 Ω_C \\
+ \lambda_4 \lambda_5 \lambda_6 Ω_D + \lambda_3 \lambda_4 \lambda_5 Ω_E + \lambda_4 \lambda_5 \lambda_6 Ω_F \\
+ \lambda_4 \lambda_5 \lambda_6 Ω_G + \lambda_4 \lambda_5 \lambda_6 Ω_H \\
[7.23]
$$

Where $λ$ are interpolation factors and $0 \leq λ_i \leq 1$ and $λ_1 + λ_3 + λ_4 + λ_5 + λ_6 = 1$.

7.3.6 Boundary conditions

The vessel used for all simulations in this study is a rectangular tank (0.75 m x 0.75 m base area x 1.5 m and 2.25 m tall). The oxygen is introduced into the vessel through nine pores in a 3 x 3 matrix configuration 20 mm x 20 mm (i.e. each adjacent orifice is a minimum of 10 mm apart). This is similar in configuration to a typical micro-oxygenation diffuser. The bubbles are released in a regular but non-sequential order to prevent pulsing behaviour. Bubble size and rate of bubble release can be varied for each simulation.

The column is modeled using the methods and approaches detailed in the preceding sections. The computational grid consisted of 30 x 30 x 60 and 90 cells depending on the simulation. The liquid phase time-step ($δt_{flow}$) was set at $5 \times 10^{-3}$ seconds. The bubble phase time step
was varied depending on the bubble release time \( \delta t_{\text{sub}} \) and ranged from \( 1 \times 10^{-4} \) to \( 1 \times 10^{-3} \) seconds.

The boundary conditions were relatively easy to implement and are described in figure 7-5. It should be noted that prescribed pressure boundary conditions are applied to horizontal slits (one cell in height x 10 cells in width) at the top of each side wall. These act as a sink and source as the liquid volume changes as the bubbles are introduced at column start-up and to account for any small change in volume during the running of the column. Neumann boundary conditions are used for the transport equation (even the top surface - which is assumed to be continually covered with inert gas).

When a bubble hits the top surface of the tank it is flagged for removal. While mass and momentum calculations are no longer performed the porosity is still calculated until the bubble is completely removed for the calculation domain.

Figure 7-5  Schematic representation of the boundary conditions adapted from (Darmana, Deen et al. 2005)
7.3.7 Computational Flow

The computational sequence is best described schematically using a process map and is presented in figure 7-6. After initialization the bubble mass and momentum balance equations are solved the bubble positions advanced until $\delta t_{\text{flow}}$ is reached at which point the Lagrangian properties are mapped onto the Eulerian grid and the liquid momentum and mass transport equations are solved. The Eulerian gradients are then calculated and then mapped onto each bubble using the methods outlined in section 7.3.5.2 and the process is continued until the pre-specified number of bubbles have been released into the column.

As mentioned above the method is very computationally expensive and one minute of simulation takes approximately 24 hours of computation time (using an AMD Athlon64 3800+ running under Linux Fedora Core FC-6). The code was developed and written by the author in Fortran-95 and compiled using the G95 Fortran compiler. Flow visualization was achieved using Matlab V7 R14.

![Figure 7-6](image)

**Figure 7-6** Computational flow of bubble plume model
7.4 Experimental Determination of Oxygen Consumption Kinetics

As part of the mass transfer modeling of the bubble column a quantitative indication of the oxygen consumption kinetics is required to input into the mass transport equation [7.16]. There have been a small number of studies which have looked specifically at the oxidation kinetic of wine and model wine solutions (Singleton 1987; Tulythan, Boulton et al. 1989; Cilliers and Singleton 1991). These experiments however were carried out at either elevated temperature or wine pH. As an input to equation [7.16] a representative consumption rate model was required. As this was not available in any published studies it had to be determined experimentally, importantly at wine-like conditions.

The actual predominant reaction mechanisms related to the consumption of dissolved oxygen are not well understood. Rather than attempt to formally develop hypothetical reaction rate constants, the approach was to measure the gross rate of consumption of oxygen in an actual wine at wine-like conditions. Once the dissolved oxygen was recorded as a function of time the degradation behaviour was then modeled using curve fitting software and the modeled then applied to the dissolved oxygen transport equation [7.16] as the $S_D$ term.

7.4.1 Experimental Setup

In order to measure the lumped parameter rate of oxygen consumption a device had to be fabricated that allowed:

- effective mixing
- a stable temperature
- the ability to dose with oxygen
- provision of a dissolved oxygen meter

A device was designed (refer to appendix VIII for detailed drawings) and fabricated from glass by the University of Auckland glass blowers and is presented in figure 7-8. Good mixing of the oxygenation cell was achieved by the use of a standard laboratory magnetic stirrer. Temperature was controlled at 15°C by use of a water-bath/pump arrangement flowing water around an annular volume between the outer shell and the internal cell (refer to appendix VIII). The dissolved oxygen meter (Orbisphere 3650) was attached with a 316
stainless steel threaded collar which was glued to the cell with epoxy cement (refer appendix VIII).

The cell was initially slowly filled to overflowing (approximately 180 ml) with red wine using a glass funnel through the filling port (refer figure 7-8). The wine was left for several minutes to equilibrate and become thermally stable. Oxygen was then injected through a 1.6 mm (internal diameter) needle approximately 200 mm attached to a 3.2 mm (internal diameter) flexible high-density nylon hose attached to a regulator and oxygen cylinder (BOC, “Food Fresh” grade).

Oxygen was very slowly sparged into the cell while observing the dissolved oxygen concentration from the meter. Once 10 mg/L was recorded (approximately one saturation) then the sparging was stopped and the needle removed. The cell was then sealed and covered with a black velvet cloth (to shield the wine from UV radiation). The dissolved oxygen readings were recorded every hour using a data logger (Orbisphere) attached to a personal computer.

![Figure 7-8](image)

**Figure 7-8** Photograph of Oxygenation cell described in section 7.4.1.
7.4.2 Experimental Design

The aim of this particular experimental work was to measure some typical oxygen consumption rates from New Zealand red wines at wine conditions. Three wines made from Cabernet Sauvignon grapes, one from Cabernet Franc and one wine made from Merlot grapes were evaluated using the method described in section 7.4.1. The wines and corresponding experimental treatments are listed in table 7-1. The sampled wines had not had any sulfur additions except for a small amount added at crushing (prior to fermentation).

Table 7-1  Experimental treatments for oxygen consumption measurements described in section 7.4.1

<table>
<thead>
<tr>
<th>Wine Treatment</th>
<th>Variety</th>
<th>Polyphenol Concentration mg/l (GAE)*</th>
<th>Filtration</th>
<th>Tubidity (NTU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>Cabernet Sauvignon</td>
<td>1672</td>
<td>None</td>
<td>110</td>
</tr>
<tr>
<td>1b</td>
<td>Cabernet Sauvignon</td>
<td>1672</td>
<td>GF/C</td>
<td>9.0</td>
</tr>
<tr>
<td>1c</td>
<td>Cabernet Sauvignon</td>
<td>1672</td>
<td>RC 0.45 μm membrane</td>
<td>0.2</td>
</tr>
<tr>
<td>2</td>
<td>Merlot</td>
<td>2304</td>
<td>RC 0.45 μm membrane</td>
<td>0.1</td>
</tr>
<tr>
<td>3</td>
<td>Cabernet Sauvignon</td>
<td>4722</td>
<td>RC 0.45 μm membrane</td>
<td>0.1</td>
</tr>
<tr>
<td>4a</td>
<td>Cabernet Franc</td>
<td>1511</td>
<td>None</td>
<td>108</td>
</tr>
<tr>
<td>4b</td>
<td>Cabernet Franc</td>
<td>1511</td>
<td>None</td>
<td>108</td>
</tr>
<tr>
<td>5</td>
<td>Cabernet Sauvignon</td>
<td>3865</td>
<td>None</td>
<td>115</td>
</tr>
</tbody>
</table>

* Polyphenol concentration measured by Folin Ciocalteau method described in section 4.3.4

The turbidity values listed in Table 7-1 also indicate whether the wine had undergone filtration prior to sampling. Two levels of filtration were used: glass-fibre (Whatman GF/C 1.2 μm particle retention) and regenerated cellulose membrane filter (0.45 μm pore size). The aim was to simulate an unfiltered wine, a coarsely filtered wine (or wine left to settle before racking) and a tightly filtered wine (i.e. majority of yeast removed). The type of filtration for each treatment is indicated in table 7-1.
7.5 Results & Discussion

7.5.1 Determination of Oxygen Consumption Kinetics

Two types of curves were observed for the rate of oxygen consumption. Examples of these are given in figures 7-9 and 7-10. The first curve follows an exponential behaviour and is characteristic of a first order reaction (i.e. the rate of consumption of a reactant is proportional to the concentration of reactant at any time \( t \)). The rate law for a general reaction \( A \rightarrow B \) can be given by:

\[
v = k[A]
\]

Or in the differential equation form:

\[
\frac{d[A]}{dt} = -k[A]
\]

Which after integration gives:

\[
[A] = [A_0]e^{-kt}
\]

Where \( A_0 \) is the initial concentration of reactant A.

The proportionality constant \((k)\) can be calculated directly from an exponential regression of the raw oxygen consumption rate curve (figure 7-9). Three wines were found to exhibit this first order behaviour and the rate constant and the regression quality parameters are presented in table 7-2.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>([A_0]^* )</th>
<th>( k^* )</th>
<th>SSE</th>
<th>( R^2 )</th>
<th>RMSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1c</td>
<td>15.07(±0.10)</td>
<td>-0.01703(±0.0002)</td>
<td>2.08</td>
<td>0.9976</td>
<td>0.1602</td>
</tr>
<tr>
<td>T2</td>
<td>10.88(±0.08)</td>
<td>-0.009146(±0.0001)</td>
<td>1.80</td>
<td>0.9964</td>
<td>0.1146</td>
</tr>
<tr>
<td>T3</td>
<td>7.325(±0.03)</td>
<td>-0.04246(±0.0006)</td>
<td>0.48</td>
<td>0.9980</td>
<td>0.0864</td>
</tr>
</tbody>
</table>

* \([A_0]^* \) and \( k^* \) are the constants defined in equation [7.25]

SSE, \( R^2 \), and RMSE are standard quality control parameters from the curve fitting method and refer to sum of square due to error, R-square and the root mean squared error respectively.

An example of the second type of rate curve observed in the trials is shown in figure 7-10. This type of curve is which is sigmoid in shape is characteristic of autocatalytic behaviour discussed in section 1.4 (Atkins and de Paula 2002). This type of reaction proceeds slowly at
the start because there is little autocatalytic product present. As the reaction continues the concentration of autocatalytic product increases, and therefore the rate of reaction increases accordingly. Finally the reaction rate slows when the supply of reactants is exhausted.

The rate law for a general reaction $A \rightarrow B$ can be given by:

$$v = k[A][B]$$

[7.26]

And the corresponding differential equation given as:

$$\frac{d[A]}{dt} = -k[A][B]$$

[7.27]

The solution to this equation for appropriate initial conditions can be given by the expression:

$$[A] = \frac{\alpha}{1 + \beta e^{\alpha t}}$$

[7.28]

Where

$$\alpha = [A_0] + [B_0]$$

[7.29]

And

$$\beta = \frac{[B_0]}{[A_0]}$$

[7.29]

Where $A_0$ and $B_0$ are the initial concentrations of reactant $A$ and product $B$ respectively. By modelling the experimental curves in this way the reaction rate constant can be determined but also the initial concentrations of product (catalyst) can also be calculated. This is particularly notable for treatment T4a where T4b represents the consumption rate of a wine after a repeated dose of oxygen (i.e. wine was saturated with oxygen immediately after the first saturation had been consumed (refer to figure 7-11)). The constant $\beta$ is 17.5 times greater than for treatment T4a. This indicates that there is significantly more catalytic product than the first saturation at the start trial. A summary of the curves exhibiting autocatalytic behaviour is given in table 7-3.
### Table 7-3  Curve fitting results for oxygen consumption rate curves exhibiting autocatalytic behaviour

<table>
<thead>
<tr>
<th>Treatment</th>
<th>α* (±0.15)</th>
<th>β* (±0.00282)</th>
<th>k (±0.00053)</th>
<th>SSE</th>
<th>R²</th>
<th>RMSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>8.39</td>
<td>0.00875</td>
<td>0.01433</td>
<td>16.07</td>
<td>0.9866</td>
<td>0.3530</td>
</tr>
<tr>
<td>T1b</td>
<td>8.77</td>
<td>0.06578</td>
<td>0.01357</td>
<td>3.64</td>
<td>0.9902</td>
<td>0.2781</td>
</tr>
<tr>
<td>T4a</td>
<td>10.81</td>
<td>0.05797</td>
<td>0.01460</td>
<td>0.65</td>
<td>0.9989</td>
<td>0.1241</td>
</tr>
<tr>
<td>T4b</td>
<td>21.61</td>
<td>1.01900</td>
<td>0.00612</td>
<td>0.08</td>
<td>0.9998</td>
<td>0.0447</td>
</tr>
<tr>
<td>T5</td>
<td>11.09</td>
<td>0.08028</td>
<td>0.01294</td>
<td>2.08</td>
<td>0.9936</td>
<td>0.2254</td>
</tr>
</tbody>
</table>

* α, β and k are the constants defined in equation [7.28]

SSE, R², and RMSE are standard quality control parameters from the curve fitting method and refer to sum of square due to error, R-square and the root mean squared error respectively.

The rate constants are very similar for all treatments with the exception of T5b which may be affected by the very high amounts of initial product ([B]) and the resulting effect on the constant α (recall from equation [7.28] the coefficient of the time constant is the product of α and k).

The character of the wine oxygen consumption curves (ie. First-order or autocatalytic) may be due to the presence of yeast lees. Note from table 7-1 that treatment 3 was repeated three times: initially unfiltered, then through glass-fibre and finally through tight (0.45 μm) membrane filtration. The oxygen consumption curves for each respective treatment are presented in figure 7-12). Note the change from autocatalytic to first order after the wine is membrane filtered. The oxygen scavenging properties of yeast lees have been investigated and a similar autocatalytic kinetic behaviour has also been observed in other studies (Salmon, Fornairon-Bonnefond et al. 2000). The result presented in figure 7-12 indicates that yeast lees act in competition with the wine polyphenols for the available dissolved oxygen and even in low concentrations appears to dominate the overall consumption kinetics. The oxygen scavenging effect of yeast lees has major implications in terms of the dosage rate dependence of a wine undergoing micro-oxygenation.
Figure 7-9  The oxygen consumption rate data for treatment T4c (table 7-2). The red circles represent the raw data. The blue line represents the fitted curve (using equation [7.25]) and the dashed blue lines either side of the solid blue line represent the 95% confidence interval bands.

Figure 7-10  The oxygen consumption rate data for treatment T5 (table 7-3). The red circles represent the raw data. The blue line represents the fitted curve (using equation [7.28]) and the dashed blue lines either side of the solid blue line represent the 95% confidence interval bands.
**Figure 7-11** Oxygen consumption rate for Cabernet Sauvignon (T5 and T5b in table 7-3) including a repeated saturation (T5b)

**Figure 7-12** Comparison of the oxygen consumption rates of the same wine after different filtration treatments (refer table 7-1)
7.5.2 Results from Numerical Modeling

Several simulations were performed to attempt to better understand the influence of dosage rate and bubble size on the oxygenation performance of a typical micro-bullage setup. The simulations runs are indicated in table 7-4. Full scale simulations were unable to be run due to the length of time taken for each simulation. The output of each simulation summarized and visualized in figure 7-13 through 7-17 is the snap-shot at 120 seconds after the bubble column start up. This is practical limit of the simulation given the computational speed of the computers available.

<table>
<thead>
<tr>
<th>Simulation Number</th>
<th>Dosage Rate (mg/L/mth)</th>
<th>Bubble Size at diffuser (µm)</th>
<th>Tank Volume (L)</th>
<th>Tank aspect ratio (height:sides)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>670</td>
<td>1270</td>
<td>3:1</td>
</tr>
<tr>
<td>2</td>
<td>200</td>
<td>670</td>
<td>1270</td>
<td>3:1</td>
</tr>
<tr>
<td>3</td>
<td>1000</td>
<td>670</td>
<td>1270</td>
<td>3:1</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>310</td>
<td>1270</td>
<td>3:1</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>1000</td>
<td>1270</td>
<td>3:1</td>
</tr>
</tbody>
</table>

The common model parameters are specified in table 7-5, including their sources.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wine phase density ($\rho_w$)</td>
<td>985</td>
<td>kg/m³</td>
<td>(Gebhart 1988)</td>
</tr>
<tr>
<td>Wine phase viscosity ($\mu_w$)</td>
<td>1.32</td>
<td>mPa·s</td>
<td>(Gebhart 1988)</td>
</tr>
<tr>
<td>Surface tension ($\sigma_f$)</td>
<td>0.049</td>
<td>N/m</td>
<td>(Vazquez, Alvarez et al. 1995)</td>
</tr>
<tr>
<td>Oxygen phase density ($\rho_g$)</td>
<td>1.309</td>
<td>kg/m³</td>
<td>Assuming ideal gas behaviour</td>
</tr>
<tr>
<td>Diffusion Coefficient for $O_2$ in water* ($\Gamma_{O_2}$)</td>
<td>$2.10 \times 10^{-9}$</td>
<td>m²/s</td>
<td>(Cussler 1984)</td>
</tr>
<tr>
<td>Diffusion Coefficient for $CO_2$ in water* ($\Gamma_{CO_2}$)</td>
<td>$1.92 \times 10^{-9}$</td>
<td>m²/s</td>
<td>(Cussler 1984)</td>
</tr>
<tr>
<td>Henry’s law coefficient for $O_2$ in water* ($H_{O_2}$)</td>
<td>$1.28 \times 10^{-5}$</td>
<td>mol/m³Pa</td>
<td>(Sander 1999)</td>
</tr>
<tr>
<td>Henry’s law coefficient for $CO_2$ in water* ($H_{CO_2}$)</td>
<td>$3.55 \times 10^{-4}$</td>
<td>mol/m³Pa</td>
<td>(Sander 1999)</td>
</tr>
</tbody>
</table>

* The value used are for a water liquid phase due to the unavailability of reliable values for wine (i.e. 10% v/v ethanol)
Figure 7-13 Visualisation of CFD simulation 1 (refer table 7-4) with an oxygen dosage rate of 25 mg/L/mth and a bubble size of 670 µm at t=120 seconds; a) shows the bubble position, b) represents a diagonal slice of the wine phase velocity field and c) represents the dissolved oxygen concentration in mg/L.
Figure 7-14  Visualisation of CFD simulation 4 (refer table 7-4) with an oxygen dosage rate of 200 mg/L/mth and a bubble size of 670 µm at t=120 seconds; a) shows the bubble position, b) represents a diagonal slice of the wine phase velocity field and c) represents the dissolved oxygen concentration in mg/L.
Visualisation of CFD simulation 4 (refer table 7-4) with an oxygen dosage rate of 1000 mg/L/mth and a bubble size of 670 µm at \( t = 120 \) seconds; a) shows the bubble position, b) represents a diagonal slice of the wine phase velocity field and c) represents the dissolved oxygen concentration in mg/L.

---

**Figure 7-15**
Figure 7-16  Visualisation of CFD simulation 4 (refer table 7-4) with an oxygen dosage rate of 25 mg/L/mth and a bubble size of 310 µm at t=120 seconds; a) shows the bubble position, b) represents a diagonal slice of the wine phase velocity field and c) represents the dissolved oxygen concentration in mg/L.
Figure 7-17  Visualisation of CFD simulation 4 (refer table 7-4) with an oxygen dosage rate of 25 mg/L/m²h and a bubble size of 1000 µm at t=120 seconds; a) shows the bubble position, b) represents a diagonal slice of the wine phase velocity field and c) represents the dissolved oxygen concentration in mg/L.
The rate constant for treatment T3 (refer table 7-1) was used as the sink term $S_D$ (refer equation [7.16] for all simulations. This was done firstly for ease of implementation and secondly because the practical simulation times were only 120 seconds. This was insufficient to examine the equilibrium behaviour of dissolved oxygen field.

Figure 7-13 through 7-17 summarises the bubble position, hydrodynamics and dissolved oxygen fields at various dosage rates and bubbles sizes given in table 7-4. The visualisations are all at 120 seconds from the bubble column start-up. The most striking feature of this set of plots is the lack of dispersion of the bubble plume particularly at the top of the column and consequently the poor distribution of dissolved oxygen around the tank, even with the 1000 mg/L/mth simulation. While dispersion does occur the scale is so small so as to appear unnoticeable in the plot scales above. With the exception of the 1000 μm bubble size simulation all the velocity fields exhibit an absence large flow loops and rather several local flow structures manifest around the plume. As stated above A direct consequence of the lack of large scale mixing is the shape of the dissolved oxygen field (figure 7-13 c)) which essential follows the bubble column up the tank. Very little lateral dispersion is observed, however this would be expected over time particularly around the top of the tank. Despite the low bubble density the larger bubble size has a considerable effect on the hydrodynamic behaviour of the liquid phase.

The one dimensional trajectory of the bubbles is largely a result of the very strong buoyancy force relative to the lateral (horizontal) forces on the bubble due to the liquid phase hydrodynamics. More significant lateral forces are observed at higher specific gas velocities and smaller vessels where the resulting down-flows are greater (Darmana, Deen et al. 2006). Buoyancy forces were typically three to five orders of magnitude greater than the lateral forces. Entrained liquid phase velocities are also small as a consequence of the bubble diameter and the local bubble density (gas phase volume fractions of $1 \times 10^{-4}$ are typical for dosage rates of 25 mg/l/mth). Larger bubbles particularly and, to a lesser extent, higher bubble densities induce larger liquid phase velocities and hence more mixing.

As a consequence of the dominating effect of the buoyancy forces the mixing of the dissolved oxygen through the vessel is very poor. For all simulated dosage rates a substantial
dissolved oxygen gradient exists around the bubble plume. Although the time scale of this simulation is small compared to a typical micro-oxygenation treatment the hydrodynamics are steady and the gradient would be expected to remain (although the absolute concentration would increase).

As discussed above one of the major limitation of the E-L method is computational cost calculating force and mass balances on large numbers of bubbles ($10^3-10^4$ bubbles in the the bubble column at any time in the simulations run in this trial, depending on the dosage rate). The practical effect of this restriction is to limit simulation times to minutes without parallelization and/or the use of very powerful computers. Micro-oxygenation simulation times ideally should be days or weeks to get a sense of the steady state dissolved oxygen gradients. The observations made over minutes of simulation can give good insight into the long term behaviour of this type of bubble column system.

These simulations however are useful in terms of determining the oxygenation efficiency for a given bubble size. Figure 7-18 plots the bubble oxygen mass fraction as a function of column height for three given bubble diameters (310, 670 and 1000 $\mu$m). This is a very useful plot in practical terms for deciding the suitability of a certain vessel for micro-oxygenation treatment. As can be observed from figure 7-18, clearly very small bubbles are required to efficiently oxygenate the fluid volume. Even with 310 $\mu$m bubbles 1.7% of the gaseous oxygen remains in the bubble. This is considerably more effective than using 1000 $\mu$m bubbles which would be expelled to the free surface with 17.1% of the bubble volume containing oxygen.

The use of CFD as a tool to make possible the integration of the chemical and physical aspects of the micro-oxygenation process is clearly an evolving and important area of research that can very nicely complements the chemical and sensory methods described previously in this thesis. A more efficient code using parallelization and solvers such as multigrid will allow large scale (space and time) simulations.
Figure 7-18 Oxygen mass fraction remaining in the bubble as a function of fluid column height above the diffuser.
CHAPTER EIGHT

Conclusion

8.1 Conclusion

The central aim of the work contained within this thesis was to better understand the importance of dosage rate on the chemical and sensory properties of red wine. The initial work involved using a commercially treated Cabernet Sauvignon wine. Neither the sensory nor chemical analyses were able to discriminate between the control and the treatment wines. This may have been due to the very low oxygen dosage rates used for the treatment (4 mg/L/mth); but more likely due to the experimental setup which involved unsuitable storage vessel for the control wine which probably allowed the ingress of air and thus contamination. Moreover the wines were store for weeks in bottle before being analysed which may also have led to the normalisation between wines.

The benefit of the preliminary commercial trial was however to enable the development of the sensory and chemical methods used in the subsequent trials. The commercial trial also highlighted the requirement for an appropriate method for dosing oxygen (at typical micro-oxygenation rates) into small volumes of wine (<100 litres). After considerable research the method of dense polymer membrane oxygenation was investigated and trialed in purpose-built 15 litre research tanks. It was demonstrated that wine could be reliably oxygenated at very low rates using an FEP tubular diffuser. The advantage with a dense polymer diffuser is that no bubbles are generated and the oxygenation efficiency is thus 100%. The diffuser was fully modeled and characterised for use in the trials detailed in Chapters Four and Six.

The next step was to use the small scale oxygenation equipment to conduct a fully replicated experiment to investigate the evolution of a Cabernet Sauvignon wine under different oxygen dosage rates. The experimental design consisted of four oxygenation treatments at 0, 10, 23 and 36 mg/L/mth. The total period of the trial was 105 days. A full suite of sensory and
chemical analyses were conducted to observe what changes occur in a wine undergoing oxygenation at the impact of oxygen dosage rate on those changes.

HPLC analysis indicated that the rate change of low molecular weight polyphenols is directly related to the oxygen dosage rate (figure 4-18). The concentration of most of the identifiable monomers, most notably the anthocyanins decreased throughout the course of the trial. While the kinetic behaviour was similar the rate of decrease was greater with the higher oxygen dosage rates.

Thiolysis results showed a positive increase in mDP for all treatments over the course of the trial up to day 77 when a decrease was observed for all treatments. The decrease in mDP coincided with an addition of SO₂ which was thought to be suppressing (or in this case retarding) the tannin development by nucleophilic addition to the flavanyl-4-carbocation of a hydrolysed tannin. This observation was further investigated in a later trial.

The spectrophotometric results indicated that the rate of formation of stable (non-bleachable) pigments was directly related to the rate of oxygen dosage with significant differences between the high rates (23 and 36 mg/L/mth) and the low rates (0 and 10 mg/L/mth). The trend for all treatments was for increased levels of stable pigments over the course of the trial. This result correlated with the thiolysis results and is what would be expected for a wine as it evolves.

The sensory results, as interpreted from the principal response curve (figure 4-11), show that the measured sensory development exhibits the same oscillatory behaviour relative to the control wine) as that shown in figure 1-2, albeit with a less clear distinction between the structuring, harmonisation and over-oxygenation phases defined in section 1.1.1. The most significant factor in the model weighting is mouthfeel followed by astringency which correlates with the changes occurring in the wine chemistry also.

Overall the laboratory scale trial using Cabernet Sauvignon presented some very interesting results. The chemical analyses indicated that the polyphenol development was directly related to the oxygen dosage rate. The sensory evolution also appeared to be accelerated.
Conclusion

with a higher oxygen dosage rate, although the oscillatory nature of the sensory response given a single linear input indicates a complex underlying mechanism driving the changes. The correlation between the chemical and sensory results did not however yield any direct relationship, with the exception of mDP which has been previously correlated with astringency. The trial was hence limited by the inability to resolve the actual tannin structures and their corresponding transformations.

A small trial was run to measure the effect of SO$_2$ on the development of wine polyphenols with and without oxygen. The presence of SO$_2$ had a significant effect on both mDP and the concentration of stable (non-bleachable) pigments. mDP was observed to decrease over a six week period irrespective of whether oxygen had been added or not. The mDP for the treatments without SO$_2$ increased steadily over the course of the trial. Similarly the formation of non-bleachable pigments was suppressed and even retarded with SO$_2$ present whereas for the treatments without SO$_2$ a steady increase was observed. The implication of these results is that SO$_2$ has a much larger effect on tannin development than oxygen and the timing of the SO$_2$ addition is critical in the overall success of the treatment. A further similar trial has been run supporting the finding of this study (Tao, Dykes et al. 2007).

The application of alternative oxidants (to oxygen) was investigated and specifically the novel use of electrochemical micro-oxidation (or ELMOX). An initial trial was run principally to investigate the proof of concept. The trial also sought to compare the performance of glassy carbon and titanium as electrode materials against traditional micro-oxygenation. The most dramatic transformations occurred with the titanium treatments showing higher levels of ethanal than the other treatments both chemically and organoleptically. A greater rate of stable pigment formation was also observed for the titanium compared to the other treatments.

One of the major potential benefits with the ELMOX method is that by controlling electrical potential in the wine certain polyphenols can be selectively oxidised. Moreover titanium appears to have a propensity to oxidise ethanol to ethanal which, again, if controlled could be a positive thing for the development of tannins given the discussion in section 1.4.
result of this trial were sufficiently convincing to conduct another larger scale replicated trial in 300 litre tanks which has recently been accepted for publication (Fell, Dykes et al. 2007).

Gross oxygen consumption kinetics were measured using a device developed at the University of Auckland. Autocatalytic behaviour was observed in wines containing, even small quantities, of yeast lees. Tightly filtered wines however exhibited first order kinetic behaviour presumably related to the wine chemistry in the absence of yeast lees. This results is important as it implies that any residual yeast lees remaining in the wine dominates the consumption of dissolved oxygen and hence the actual dissolved oxygen available for polyphenol reaction is less than the dosage rate.

The CFD analysis of the micro-oxygenation system showed that for typical dosage rates there is very little mixing of the tank. Localised flow loops form around the bubble plume and there is little lateral dispersion of the bubble column. The lack of mixing means that in the wine volumes around the bubble plume localised oxidation is most likely occurring. In large tanks however there is likely to be significant volumes of wine that are not exposed to any concentration of dissolved oxygen. At typical micro-oxygenation dosage rates significant dissolved oxygen gradients are observed with high concentrations around the around the central plume.

One of the main limitations of E-L CFD analysis is that realistic simulation time scales are of the order of minutes whereas the appropriate time scales for micro-oxygenation are days and week. Despite this the ability to be able to visualise the dissolved oxygen and velocity fields provides a powerful insight into the micro-oxygenation physical system.

8.2 Potential Future Research

There are a number of areas of the preceding thesis that deserve further research and development. These are discussed briefly below, the complete understanding of micro-oxygenation, however, will largely be determined by the ability to better resolve wine tannins. Hitherto there is no assay available that will give a good measure of tannins in wine.
Conclusion

Significant potential for development exists in developing the CFD model developed in the last chapter of this thesis. The model that has been developed gives enormous scope for refinement in both the application of the physics and also the efficiency of the solution which present a major limitation at present. Perhaps the implementation of an Euler-Euler two-fluid model should be investigated to better deal with the long time scales appropriate for simulating a micro-oxygenation system.

There is also scope to better understand the oxidation chemistry in terms of the overall reaction mechanism and the corresponding kinetic models. The CFD model can then be used to track not only the temporal and spatial reactant field but also that of product (e.g. quinones). The integration of the physical and chemical models may have significant application in better understanding and optimising the micro-oxygenation process.

The dense polymer membrane oxygenation also requires some further development to extend the range of dosage rates. Different material should be characterised to allow the use of this type of accurate oxygenation in vessels larger than 15 litres.

The ELMOX concept is particularly exciting given the potential control one could have over the process compared to traditional micro-oxygenation. As discussed above a further trial has been conducted and the results published. The next step should be to investigate different electrode material and electrode configurations combined with some intensive sensory and chemical analysis.

The effect of SO$_2$ on tannin development and particularly as it applies to micro-oxygenation is another interesting area that should be further investigated. As mentioned above a further trial has been conducted and the results accepted for publication. The dramatic effect of SO$_2$ on the tannins and presumably the development of the wine during maturation has serious implications for micro-oxygenation practitioners.

Finally, in a broader context, micro-oxygenation is a tool that holds tremendous promise for not only for wines made from tannic red varieties rich in polyphenols, but potentially all wines that are matured in oak barrels; this includes Pinot noir and Chardonnay. To do this
successfully the chemistry of process must be well understood and there must be very good control over the process. With this in place micro-oxygenation is part of a package of tools (including oak adjuncts) that lead to the obsolescence of barrels and all their associated issues; to this end, further research in micro-oxygenation needs to focus on the influence of micro-oxygenation with oak products. Another aspect that has not been investigated is the long term effects of micro-oxygenation on the organoleptic development of wine once it has been bottled.
APPENDICES

Appendix I  HPLC Standard Curves – Low Molecular Weight Polyphenols

Table A-1  Standard curves for commercially available phenolic compounds. Used in Chapters Two, Four and Six

<table>
<thead>
<tr>
<th>Standard</th>
<th>Slope (mg/L/mAU·s)</th>
<th>Intercept (mg/L)</th>
<th>R²</th>
<th>SSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>catechin</td>
<td>0.0476</td>
<td>0.3802</td>
<td>1.000</td>
<td>1.2491</td>
</tr>
<tr>
<td>epicatechin</td>
<td>0.0438</td>
<td>1.5245</td>
<td>0.999</td>
<td>3.7807</td>
</tr>
<tr>
<td>epicatechin-3-O-gallate</td>
<td>0.0200</td>
<td>-0.9380</td>
<td>0.999</td>
<td>2.0132</td>
</tr>
<tr>
<td>gallic acid</td>
<td>0.0148</td>
<td>-1.1639</td>
<td>0.999</td>
<td>3.7211</td>
</tr>
<tr>
<td>quercetin</td>
<td>0.0093</td>
<td>0.2496</td>
<td>1.000</td>
<td>0.3258</td>
</tr>
<tr>
<td>caffecic acid</td>
<td>0.0077</td>
<td>-0.0593</td>
<td>0.999</td>
<td>1.9076</td>
</tr>
<tr>
<td>coumaric acid</td>
<td>0.0058</td>
<td>-2.4920</td>
<td>0.997</td>
<td>8.9960</td>
</tr>
</tbody>
</table>

Appendix II  HPLC Standard Curves – Thiolyis

Table A-2  Standard curves for commercially available phenolic compounds. Used in Chapters Two, Four, Five and Six

<table>
<thead>
<tr>
<th>Standard</th>
<th>Mass basis</th>
<th>Molar basis</th>
<th>R²</th>
<th>SSE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slope (mg/L/mAU·s)</td>
<td>Intercept (mg/L)</td>
<td>Slope (mmol/L/mAU·s)</td>
<td>Intercept (mmol/L)</td>
</tr>
<tr>
<td>catechin</td>
<td>0.1125</td>
<td>3.034</td>
<td>0.387×10⁻³</td>
<td>0.0105</td>
</tr>
<tr>
<td>epicatechin</td>
<td>0.0527</td>
<td>3.167</td>
<td>0.182×10⁻³</td>
<td>0.0109</td>
</tr>
<tr>
<td>epicatechin-3-O-gallate</td>
<td>0.0251</td>
<td>-0.590</td>
<td>5.681×10⁻⁴</td>
<td>-0.0013</td>
</tr>
<tr>
<td>epigallocatechin</td>
<td>1.428</td>
<td>2.145</td>
<td>4.662×10⁻³</td>
<td>0.0070</td>
</tr>
<tr>
<td>gallic acid</td>
<td>0.0235</td>
<td>1.714</td>
<td>0.138×10⁻³</td>
<td>0.0198</td>
</tr>
</tbody>
</table>

Table A-3  Standard curves for thio-ether derivative (thiolyis products) used to calculate mDP as described in section 2.3.7

<table>
<thead>
<tr>
<th>Peak Sample Fraction</th>
<th>Mass basis</th>
<th>Molar basis</th>
<th>R²</th>
<th>SSE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slope (mg/L/mAU·s)</td>
<td>Intercept (mg/L)</td>
<td>Slope (mmol/L/mAU·s)</td>
<td>Intercept (mmol/L)</td>
</tr>
<tr>
<td>(2R,3R,4S) catechin-4-benzylthioether</td>
<td>1.003</td>
<td>-22.39</td>
<td>2.431×10⁻³</td>
<td>-0.054</td>
</tr>
<tr>
<td>(2R,3R,4R) catechin-4-benzylthioether</td>
<td>1.103</td>
<td>-5.67</td>
<td>1.044×10⁻³</td>
<td>0.178</td>
</tr>
<tr>
<td>epicatechin-4-benzylthioether</td>
<td>0.4307</td>
<td>73.34</td>
<td>1.044×10⁻³</td>
<td>0.178</td>
</tr>
<tr>
<td>epigallocatechin-4-benzylthioether</td>
<td>1.5813</td>
<td>-0.03</td>
<td>2.801×10⁻³</td>
<td>4.702×10⁻⁵</td>
</tr>
<tr>
<td>Epicallocatechin-3-O-gallate-4-benzylthioether</td>
<td>1.1394</td>
<td>0.88</td>
<td>2.018×10⁻³</td>
<td>1.560×10⁻³</td>
</tr>
</tbody>
</table>
Appendix III  Thiolysis Molar Yield Curves

**Figure A-1**  Molar concentrations of terminal and extension sub-units for thiolysis analysis described in Chapter Two. Error bars represent standard deviation ($n=3$)

**Figure A-2**  Molar concentrations of terminal and extension sub-units for thiolysis analysis described in Chapter Four. Error bars represent standard deviation ($n=3$)
Figure A-2  Molar concentrations of terminal and extension sub-units for thiolysis analysis described in Chapter Five. Error bars represent standard deviation (n=3)
Appendix IV  Sensory Analysis - Standard Data Collection Form

Example of difference testing training form:

**Training Session 15th April 2005**

Name:_________________________________________

This session continues the triangle test on wine standards

You are presented with four sets of three glasses; each set containing samples with a single wine standard in water. Two of the three samples in each set are at an identical concentration and the third is different (either a higher of lower attribute concentration than the other two).

*An olfactory examination is required for the first two suites, i.e. samples should be smelt only. A sapid examination is required for the last two suites, i.e. the samples should be tasted then expectorated.*

The samples should be appraised in the order presented.

All that is required is that the sample containing the different concentration be identified by writing the three digit code in the space provided below. Additionally, the panellist is required to indicate whether the different sample is of lower or higher concentration than the two matching samples in the particular suite. Finally please try and name the attribute

**e.g.**

<table>
<thead>
<tr>
<th>Set 1:</th>
</tr>
</thead>
<tbody>
<tr>
<td>275</td>
</tr>
<tr>
<td>654</td>
</tr>
<tr>
<td><strong>711</strong></td>
</tr>
</tbody>
</table>

Sample with different concentration: __________ lower☐ higher☐

Attribute name: __________  Bitterness
Set 1:

Sample with different concentration: ______________lower\(\checkmark\) higher\(\square\)

Attribute name:____________________

Set 2:

Sample with different concentration: ______________lower\(\checkmark\) higher\(\square\)

Attribute name:____________________

Set 3:

Sample with different concentration: ______________lower\(\checkmark\) higher\(\square\)

Attribute name:____________________

Set 4:

Sample with different concentration: ______________lower\(\square\) higher\(\square\)

Attribute name:____________________
Example of Data collection form:

**Data Collection 7th June '05**

Name : __________________

**Instructions:**
For each attribute check the line at the point on the horizontal line where you feel the wine rates for that particular attribute. The vertical mark on the horizontal score line represents the mean scores of your tests over the last month. Use it as a calibration for assessing the wines that are presented to you.

**OLFACTORY EXAMINATION**

1. **Vegetativeness**

Score the numbered wine on the line below.

- Absent : 0%                       Extreme 100%

2. **Fruitiness**

Score the numbered wine on the line below.

- Absent : 0%                       Extreme 100%

3. **Acetaldehyde**

Score the numbered wine on the line below.

- Absent : 0%                       Extreme 100%
SAPID EXAMINATION

4. Acidity

Score the numbered wine on the line below.

Absent : 0%                          Extreme 100%

5. Bitterness

Score the numbered wine on the line below.

Absent : 0%                          Extreme 100%

TACTILE EXAMINATION

6. Astringency

Score the numbered wine on the line below. Mark the check with the number of the wine.

Absent : 0%                          Extreme 100%

GENERAL EXAMINATION

7. Balance

Balance is a measure of a wine’s harmony - i.e. the way in which all attributes come together to make a complete wine. If you perceive one or more attribute(s) over the other(s) then the wine is poorly balanced.

Score the numbered wine on the line below.

Absent : 0%                          Extreme 100%
8. **Mouthfeel**

Mouthfeel is a measure of how the wine “fills” the palate. A wine with a weak mouthfeel will tend to only be perceived on the centre-line of the tongue. If the wine is felt strongly on the inside of the cheeks and the tongue, then it can be considered full or big.

Score the numbered wine on the line below.

Weak/thin : 0%  
Big/full 100%
Appendix V

Forms Required for Ethical Approval to Run a Sensory Panel

Participant Information Sheet:

Title: Sensory Evolution of Micro-oxygenated Cabernet Sauvignon

To: Prospective Participants

My name is Stuart Dykes I am a student at The University of Auckland enrolled for a PhD Degree in the Department of Chemistry I am conducting this research for the purpose of my thesis on “The Effect of Micro-oxygenation of the Polyphenols in Red Wine”.

You are invited to participate in a trained sensory panel as part of a PhD research study into the effects of controlled oxidation during the maturation of red wine. The technique, called micro-oxygenation, is being used extensively in the manufacture of commercial red wine throughout the world including many wineries in New Zealand.

Trained Sensory panel Overview:

The panellist will be asked to taste Cabernet Sauvignon wines from a commercial winery in New Zealand that are currently undergoing micro-oxygenation treatment. The aim of the trial is to quantitatively track the evolution of important wine sensory attributes such as bitterness, astringency and fruitiness. The trial duration will be over a period of approximately 12 weeks.

As the evolution of the wine is of interest in this study it is extremely important that the same panellist are present during the entire study.

Initial Screening

As the aim is to obtain quantitative sensory data initial screening will be undertaken to determine the sensory thresholds for certain relevant properties (e.g. bitterness, acidity, sweetness etc.). Obviously people differ in their sensitivity to certain tastes and aromas. If the prospective panellist is unable to successfully discriminate any of the attributes to these thresholds they may be asked to leave the panel.

Training

Considerable time and effort is spent in “training” before the actual tasting. Training involves the use of standards (e.g. canned blackcurrant juice to represent “fruity” aroma, caffeine to represent bitterness) to allow the panellists to identify the particular attribute of interest and calibrate themselves relative to a fixed standard. Training will be initially three sessions for...
approximately one hour in the first week, followed by one session per week thereafter on the
day prior to the data collection.

Data Collection

Data Collection sessions will be conducted once per week in specialised tasting booths. The
exercise should take the panellist no more than twenty minutes. He or she will be required to
assess four wines for the relevant attributes.

Additional Information

If the participant is an asthmatic or has an allergy to sulphur compounds (sulphituria), they
must inform the Principal Researcher before the commencement of the trial.

Participation is entirely voluntary and panellists are able to withdraw from the study at any
time without reason. Sensory information provided by a panellist may also be withdrawn at
any time upon request from the individual panellist.

Although the data collected will be analysed individually and collectively and possibly used in
publication, all links between the data and the participant's name will remain strictly
confidential.

Thank you very much for your time and help in making this study possible. If you have any
queries or wish to know more please phone me at home at the number given above or write to
me at:

Wine Science,
Department of Chemistry,
The University of Auckland
Private Bag 92019
Auckland.      Tel ..........................

My supervisor is:       Dr. Paul Kilmartin
Department of Chemistry
The University of Auckland
Private Bag 92019
Auckland.      Tel. 373-7999 extn. .......

The Head of Department is:       Professor Graeme Bowmaker
Department of Chemistry
The University of Auckland
Private Bag 92019
Auckland.      Tel. 3737-7999 extn ....

For any queries regarding ethical concerns please contact:

The Chair, The University of Auckland Human Subjects Ethics Committee,
The University of Auckland, Research Office - Office of the Vice Chancellor, Private Bag 92019,
Auckland. Tel. 373-7999 extn 87830

APPROVED BY THE UNIVERSITY OF AUCKLAND HUMAN SUBJECTS ETHICS
COMMITTEE on ............................ for a period of ............ years, from ..../..../....
Reference ........./........
CONSENT FORM

THIS CONSENT FORM WILL BE HELD FOR A PERIOD OF SIX YEARS

Title: Sensory Evolution of Micro-oxygenated Cabernet Sauvignon

Researcher: Stuart Dykes

I have been given and have understood an explanation of this research project. I have had an opportunity to ask questions and have them answered.

I understand that I may withdraw myself or any information traceable to me at any time up to 15th September 2003 without giving a reason.

• I agree to take part in this research.

• I do not suffer from suphituria or severe asthma

Signed:

Name: (please print clearly)

Date:

APPROVED BY THE UNIVERSITY OF AUCKLAND HUMAN SUBJECTS ETHICS COMMITTEE

on ........................ for a period of ........ years, from
.........../........../......... Reference ............../.........
Appendix VI

Experimental Oxygenation Tank Drawings

Wine Research Tank 15l
Tank Assembly Detail

Wine Science Programme

S. Dykes

DRAWN BY
S. Dykes

SHEET

SCALE 1:100

WRT-AD-TANK-1/2

REV

DRAWN BY
S. Dykes

SHEET

SCALE 1:100

WRT-AD-TANK-1/2

REV

All parts 316 Stainless steel (unless otherwise stated). All dimensions in mm.
All parts 316 Stainless steel (unless otherwise stated). All dimensions in mm.

Wine Science Programme

Wine Research Tank 15l
Sub-assembly Drawing - 6" RJT Blanking Plate

Drawn by:
S. Dykes

Designed by:
S. Dykes

REV

SCALE 1:50

SIZE

FROM NO.

DRAW NO.

WRT-AD-6RJTBP

APPROVED

REVISIONS

ZONE

REV

DESCRIPTION

DATE
6" RJT blanking plate from Stainless Alloys p/n SANB41524. Holes cut to drawing specifications.

All part 316 Stainless steel unless otherwise stated. All dimensions in mm.

Wine Research Tank 15l
Component Drawing - 6" RJT Blanking Plate

DRAWN BY: S. Dykes
DESIGNED BY: S. Dykes

SIZE: DRAW NO.: WRT-CD-6RJTBP
REV: 1
SHEET

SCALE: 1:50
skirt to be fabricated and attached to tank by four spot welds at 45 degrees intervals

Wine Science Programme
Component Drawing - Tank Skirt

Wine Research Tank 15l

All parts 316 Stainless steel (unless otherwise stated). All dimensions in mm

S. Dykes

S. Dykes
Tank to be fabricated and fitted with 6" ZRJT male weld port (p/n SANMP1524). Continuous weld with inside polish required.

Double ended 1/8" NPT nipples (p/n SS-2-CN) should be fitted as shown in drawing. 10mm holes are required and the nipples should be welded and polished on the interior of the tank.

All parts 316 Stainless steel (unless otherwise stated). All dimensions in mm.

Wine Science Programme

Wine Research Tank 15l
Sub-assembly Drawing - Tank

S. Dykes

Drawn by
S. Dykes

Described by
S. Dykes

SIZE
PSW No.
Dwg No.
WRT-SAD-TANK
REV

DESIGNED BY
S. Dykes

SCALE 1:100
SHEET
All parts 316 Stainless steel (unless otherwise stated). All dimensions in mm.

Wine Science Programme

Wine Research Tank 15l
Tank Assembly Detail

DRAWN BY:
S. Dykes

DESIGNED BY:
S. Dykes

SCALE: 1:40
Appendix VII  Discretisation and Solution Procedure for Volume Average Navier Stokes Equation

Below is the discretisation details of the Navier Stokes equations used for the Euler-Lagrange CFD modelling of a bubble column reactor described in Chapter Seven. Included are two solution procedures, One based on the SIMPLE and the other based on the PISO algorithm.

Starting from the volume averaged Navier Stokes equations:

\[ \frac{\partial}{\partial t}(\varepsilon_i \rho_i) + \nabla \cdot \varepsilon_i \rho_i \mathbf{u} = 0 \]  \hspace{2cm} [A.1]

\[ \frac{\partial}{\partial t}(\varepsilon_i \rho_i \mathbf{u}) + \nabla \cdot \varepsilon_i \rho_i \mathbf{u} \mathbf{u} = -\varepsilon_i \nabla P - \nabla \cdot \varepsilon_i \rho_i \mathbf{g} + \phi \]  \hspace{2cm} [A.2]

Using finite difference discretisation on a uniform Cartesian grid gives the following equations for momentum in each direction:

Momentum in the u-CV in three dimensions can be given as:

\[
\left(\varepsilon_i \rho_i\right)_{i+\frac{1}{2},j,k}^n = \frac{u_{i+1/2,j,k}^n - u_{i-1/2,j,k}^n}{\Delta t} + \left. \left( \frac{\delta (\varepsilon_i \rho_i \mathbf{u})}{\delta x} \right) \right|_{i+\frac{1}{2},j,k}^n + \left. \left( \frac{\delta (\varepsilon_i \rho_i \mathbf{w})}{\delta z} \right) \right|_{i+\frac{1}{2},j,k}^n - \varepsilon_i^n \frac{P_{i+1/2,j,k}^{n+1} - P_{i-1/2,j,k}^{n+1}}{\Delta x} + \left( \frac{\delta \tau_{xx}}{\delta x} \right)_{i+\frac{1}{2},j,k}^n + \phi_x
\]

Where the convective terms are evaluated using first order upwind difference scheme, e.g. for the x-direction

\[
\left. \left( \frac{\delta (\varepsilon_i \rho_i \mathbf{u})}{\delta x} \right) \right|_{i+\frac{1}{2},j,k}^n = \frac{1}{\Delta x} \begin{cases} 
(\varepsilon_i \rho_i \mathbf{u})_{i+1/2,j,k}^n - (\varepsilon_i \rho_i \mathbf{u})_{i-1/2,j,k}^n \ u_{i+1/2,j,k}^n - u_{i-1/2,j,k}^n \\
\text{if } u_{i+1/2,j,k}^n \geq 0 \\
(\varepsilon_i \rho_i \mathbf{u})_{i+3/2,j,k}^n - (\varepsilon_i \rho_i \mathbf{u})_{i+1/2,j,k}^n \ u_{i+3/2,j,k}^n - u_{i+1/2,j,k}^n \\
\text{if } u_{i+1/2,j,k}^n < 0
\end{cases}
\]

[A.4]

Similarly for the y-direction:
\[
\left\langle \frac{\delta (\varepsilon, \rho, v)^n}{\delta y} u^n \right\rangle_{i+1/2,j,k} = \frac{1}{\Delta y} \begin{cases} 
\left( \varepsilon, \rho, v \right)_{i+1/2,j,k}^n u^n_{i+1/2,j,k} - \left( \varepsilon, \rho, v \right)_{i-1/2,j,k}^n u^n_{i-1/2,j,k} & \text{if } \left( \varepsilon, \rho, v \right)_{i+1/2,j,k}^n \geq 0 \\
\left( \varepsilon, \rho, v \right)_{i+1/2,j,k}^n u^n_{i+1/2,j,k} - \left( \varepsilon, \rho, v \right)_{i-1/2,j,k}^n u^n_{i-1/2,j,k} & \text{if } \left( \varepsilon, \rho, v \right)_{i+1/2,j,k}^n < 0 
\end{cases} \quad [A.5]
\]

Where \( v \) is evaluated using the average of the four adjacent nodes i.e.:

\[
\left( \varepsilon, \rho, v \right)_{i+1/2,j,k}^n = \frac{1}{4} \left[ \left( \varepsilon, \rho, v \right)_{i+1,j+1/2,k} + \left( \varepsilon, \rho, v \right)_{i+1,j-1/2,k} + \left( \varepsilon, \rho, v \right)_{i,j+1/2,k} + \left( \varepsilon, \rho, v \right)_{i,j-1/2,k} \right] \quad [A.6]
\]

And finally the z-direction:

\[
\left\langle \frac{\delta (\varepsilon, \rho, w)^n}{\delta z} u^n \right\rangle_{i+1/2,j,k} = \frac{1}{\Delta z} \begin{cases} 
\left( \varepsilon, \rho, w \right)_{i+1/2,j,k}^n u^n_{i+1/2,j,k} - \left( \varepsilon, \rho, w \right)_{i-1/2,j,k}^n u^n_{i-1/2,j,k} & \text{if } \left( \varepsilon, \rho, w \right)_{i+1/2,j,k}^n \geq 0 \\
\left( \varepsilon, \rho, w \right)_{i+1/2,j,k}^n u^n_{i+1/2,j,k} - \left( \varepsilon, \rho, w \right)_{i-1/2,j,k}^n u^n_{i-1/2,j,k} & \text{if } \left( \varepsilon, \rho, w \right)_{i+1/2,j,k}^n < 0 
\end{cases} \quad [A.7]
\]

Where \( \varepsilon, \rho, w \) is evaluated the same way as in equation [A.6]

The viscous stress term in the x-direction is discretised as follows:

\[
\left( \frac{\delta \tau_x}{\delta x} \right)_{i+1/2,j,k} = \mu \left[ \varepsilon_{i+1/2,j,k}^n \frac{u^n_{i+3/2,j,k} - u^n_{i+1/2,j,k}}{\Delta x} - \varepsilon_{i-1/2,j,k}^n \frac{u^n_{i+1/2,j,k} - u^n_{i-1/2,j,k}}{\Delta x} \right] \quad [A.8]
\]

And the corresponding term for the y-direction

\[
\left( \frac{\delta \tau_y}{\delta y} \right)_{i+1/2,j,k} = \mu \left[ \varepsilon_{i+1/2,j,k}^n \frac{u^n_{i+3/2,j,k} - u^n_{i+1/2,j,k}}{\Delta y} - \varepsilon_{i-1/2,j,k}^n \frac{u^n_{i+1/2,j,k} - u^n_{i-1/2,j,k}}{\Delta y} \right] \quad [A.9]
\]

Similarly for the vertical z-direction

\[
\left( \frac{\delta \tau_z}{\delta z} \right)_{i+1/2,j,k} = \mu \left[ \varepsilon_{i+1/2,j,k}^n \frac{u^n_{i+3/2,j,k} - u^n_{i+1/2,j,k}}{\Delta z} - \varepsilon_{i-1/2,j,k}^n \frac{u^n_{i+1/2,j,k} - u^n_{i-1/2,j,k}}{\Delta z} \right] \quad [A.10]
\]

Collecting terms and solving for \( u \) gives:
Similarly the momentum equations can be written respectively in \( v \)-CV and \( w \)-CV as:

\[
\begin{align*}
\langle \varepsilon, \rho \rangle_{i,j,k}^{n} \, u_{i+1/2,j,k}^{n+1} &= \langle \varepsilon, \rho \rangle_{i,j,k}^{n} \, u_{i+1/2,j,k}^{n} - \Delta t \left( \delta \left( \varepsilon, \rho \right) u \right)_{i+1/2,j,k}^{n} - \Delta t \left( \delta \left( \varepsilon, \rho \right) w \right)_{j+1/2,i+1/2,k}^{n} \\
-\Delta t \left( \delta \frac{\partial \left( \varepsilon, \rho \right) w}{\partial z} \right)_{i+1/2,j,k}^{n} &= \Delta t \left( \frac{\partial \left( \varepsilon, \rho \right) w}{\partial x} \right)_{i,j+1/2,k}^{n} + \Delta t \left( \frac{\partial \tau_{xy}}{\partial z} \right)_{i+1/2,j,k}^{n} + \Delta t \phi_x
\end{align*}
\]

\[\text{[A.12]}\]

The conservative and diffusive terms in equations [A.12] and [A.13] are calculated in a similar fashion to those described in equations [A.4] through [A.10].

**Solution Methods**

Two methods are presented here. The first solution scheme is essentially the SIMPLE method described in detail in (Ferziger and Peric 2002). The second scheme is based on the PISO approach (Ferziger and Peric 2002). Both algorithms are adapted from (Prosperetti and Tryggvason 2007).
SIMPLE Scheme for multiphase flows

When the continuity equation is calculated the equation can be written in terms of a mass residual $D_{ijk}$ or:

$$
D_{ijk} = (\varepsilon_i \rho_{i,j,k}^*) - (\varepsilon_i \rho_{i,j,k}) + \frac{\Delta t}{\Delta x} \left[ (\varepsilon_i \rho_{i,j,k}^*) - (\varepsilon_i \rho_{i,j,k}) \right] + \frac{\Delta t}{\Delta y} \left[ (\varepsilon_i \rho_{i,j,k+1}^*) - (\varepsilon_i \rho_{i,j,k+1}) \right] + \frac{\Delta t}{\Delta z} \left[ (\varepsilon_i \rho_{i,j,k+1}^*) - (\varepsilon_i \rho_{i,j,k+1}) \right]
$$

[A.14]

From the momentum equations we have:

$$
(\varepsilon_i \rho_{i,j,k}^*) = A_i - v_i \left[ P_{i,j+1,k} + P_{i,j-1,k} \right]
$$

[A.15]

$$
(\varepsilon_i \rho_{i,j,k}^*) = B_i - v_j \left[ P_{i,j+1,k} + P_{i,j-1,k} \right]
$$

[A.16]

$$
(\varepsilon_i \rho_{i,j,k}^*) = C_i - v_k \left[ P_{i,j+1,k} + P_{i,j,k-1} \right]
$$

[A.17]

To write the mass residual $D_{ijk}$ in terms of the pressure we substitute equations [A.15] through [A.17] into the continuity equation [A.14]:

$$
D_{ijk} = (\varepsilon_i \rho_{i,j,k}^*) - (\varepsilon_i \rho_{i,j,k}) + \frac{\Delta t}{\Delta x} \left[ A_i - v_i \left[ P_{i,j+1,k} + P_{i,j-1,k} \right] \right] + \frac{\Delta t}{\Delta y} \left[ B_i - v_j \left[ P_{i,j+1,k} + P_{i,j-1,k} \right] \right] + \frac{\Delta t}{\Delta z} \left[ C_i - v_k \left[ P_{i,j+1,k} + P_{i,j,k-1} \right] \right]
$$

[A.18]

The idea is that pressures will be updated iteratively driving $D_{ijk}$ to zero. A Newton-Raphson argument is employed looking for pressure increments such that:

$$
D_{ijk} \left( P_{i,j,k}^* + \delta P_{i,j,k}^* \right) = 0
$$

[A.19]

where $\kappa$ is the iteration counter. Using a Taylor series expansion of [A.19] we get:
Appendices

\[
\frac{\partial D^x_{i,j,k}}{\partial P^x_{i+1,j,k}} \delta P_{i+1,j,k} + \frac{\partial D^x_{i,j,k}}{\partial P^x_{i-1,j,k}} \delta P_{i-1,j,k} + \frac{\partial D^x_{i,j,k}}{\partial P^x_{i,j+1,k}} \delta P_{i,j+1,k} + \frac{\partial D^x_{i,j,k}}{\partial P^x_{i,j-1,k}} \delta P_{i,j-1,k} + \frac{\partial D^x_{i,j,k}}{\partial P^x_{i,j,k+1}} \delta P_{i,j,k+1} + \frac{\partial D^x_{i,j,k}}{\partial P^x_{i,j,k-1}} \delta P_{i,j,k-1} + \frac{\partial D^x_{i,j,k}}{\partial P^x_{i,j,k}} \delta P_{i,j,k} = -D^x_{i,j,k}
\]

[A.20]

By calculating the derivatives analytically a set of linear equations can be developed of the form

\[
A_p \delta P_p + \sum_l A_l \delta P_l = -D_{i,j,k} \quad l = E, W, N, S, T, B
\]

[A.21]

where the coefficients are calculated from [A.18] and given as:

\[
A_E = \frac{\partial D^x_{i,j,k}}{\partial P^x_{i+1,j,k}} = -\frac{\Delta t}{\Delta x} v^l_{i+1,j,k/2}
\]

[A.22]

\[
A_W = \frac{\partial D^x_{i,j,k}}{\partial P^x_{i-1,j,k}} = -\frac{\Delta t}{\Delta x} v^l_{i-1,j,k/2}
\]

[A.23]

\[
A_N = \frac{\partial D^x_{i,j,k}}{\partial P^x_{i,j+1,k}} = -\frac{\Delta t}{\Delta x} v^l_{i,j+1/2,k}
\]

[A.24]

\[
A_S = \frac{\partial D^x_{i,j,k}}{\partial P^x_{i,j-1,k}} = -\frac{\Delta t}{\Delta x} v^l_{i,j-1/2,k}
\]

[A.25]

\[
A_T = \frac{\partial D^x_{i,j,k}}{\partial P^x_{i,j,k+1}} = -\frac{\Delta t}{\Delta x} v^k_{i,j,k+1/2}
\]

[A.26]

\[
A_B = \frac{\partial D^x_{i,j,k}}{\partial P^x_{i,j,k-1}} = -\frac{\Delta t}{\Delta x} v^k_{i,j,k-1/2}
\]

[A.27]

Where the subscripts E,W,N,S,T,B are defined in figure A.VI-1

Equation [A.21] can be easily solved numerically using a conjugate gradient solver or similar. After the pressure corrections have been calculated the pressure and velocities are updated. The velocities however may not satisfy the momentum equations in which case the velocities are recalculated with the corrected pressure iteratively until the equations are solved to a pre-specified precision.
Appendices

**PISO solver for multiphase flows**

The SIMPLE scheme described above can be slow to converge in some situations. It may be computationally cheaper to use a PISO solver. The approach is detailed below.

The discretised continuity equation [A.14] can be written as:

\[
\frac{\Delta t}{\Delta x} \left[ (e_i \rho_i)_i + (e_i \rho_i)_i - (e_i \rho_i)_i \right] + \Delta y \Delta z \left[ (e_i \rho_i u)_i - (e_i \rho_i u)_i \right] + \Delta x \Delta z \left[ (e_i \rho_i v)_i - (e_i \rho_i v)_i \right] + \Delta x \Delta y \left[ (e_i \rho_i w)_i - (e_i \rho_i w)_i \right] = 0
\]  
\[\text{[A.28]}\]

Writing the velocity corrections in terms of pressure corrections gives:

\[
u_i = \nu_i + \delta u_i = \nu_i + \nu_i (\delta P_i - \delta P_i) \]
\[\text{[A.29]}\]

\[
u_i = \nu_i + \delta u_i + \nu_i (\delta P_i - \delta P_i) \]
\[\text{[A.30]}\]

\[
u_i = \nu_i + \delta u_i + \nu_i (\delta P_i - \delta P_i) \]
\[\text{[A.31]}\]

Where \( \nu \) is given as:

\[
\nu = \frac{\Delta t}{\Delta x \rho}, \quad \nu = \frac{\Delta t}{\Delta y \rho} \quad \text{and} \quad \nu = \frac{\Delta t}{\Delta z \rho}
\]

for the x-, y- and z- components respectively.

Substituting into the above continuity equation gives:

Figure A.VI-1 Designation of Subscripts for cell sides i.e. E=east, W=west, N=north, S=south, T=top, B=bottom


\[ B_{i,j,k} + \Delta x \Delta y \left[ e_{i+\frac{1}{2},j,k}^{n+1} u_{i+\frac{1}{2},j,k}^{x} - e_{i-\frac{1}{2},j,k}^{n+1} u_{i-\frac{1}{2},j,k}^{x} \right] + \]

\[ \Delta x \Delta y \left[ e_{i,j+\frac{1}{2},k}^{n+1} v_{i,j+\frac{1}{2},k}^{x} - e_{i,j-\frac{1}{2},k}^{n+1} v_{i,j-\frac{1}{2},k}^{x} \right] + \Delta x \Delta y \left[ e_{i,j,k+\frac{1}{2}}^{n+1} w_{i,j,k+\frac{1}{2}}^{x} - e_{i,j,k-\frac{1}{2}}^{n+1} w_{i,j,k-\frac{1}{2}}^{x} \right] \]

\[ -\Delta y \Delta z \epsilon_{i+\frac{1}{2},j,k}^{j,j+1} \delta P_{i+1,j,k} + \Delta y \Delta z e_{i+\frac{1}{2},j,k}^{j,j+1} \delta P_{i,j,k} + \]

\[ +\Delta y \Delta z \epsilon_{i+\frac{1}{2},j+1,k}^{j,j+1} \delta P_{i,j+1,k} + \Delta y \Delta z \epsilon_{i+\frac{1}{2},j,k}^{j,j+1} \delta P_{i,j,k} \]

\[ +\Delta x \Delta z \epsilon_{i,j+\frac{1}{2},k}^{j,j+1} \delta P_{i,j+1,k} + \Delta x \Delta z \epsilon_{i,j,k+\frac{1}{2}}^{j,j+1} \delta P_{i,j,k} + \]

\[ -\Delta x \Delta z \epsilon_{i,j,k+\frac{1}{2}}^{j,j+1} \delta P_{i,j,k+1} + \Delta x \Delta y \epsilon_{i,j,k}^{j,j+1} \nu_{i,j,k}^{K} \delta P_{i,j,k} \]

\[ +\Delta x \Delta y \epsilon_{i,j,k}^{j,j+1} \nu_{i,j,k}^{K} \delta P_{i,j,k} - \Delta x \Delta y \epsilon_{i,j,k}^{j,j+1} \nu_{i,j,k}^{K} \delta P_{i,j,k} = 0 \]

Where \( B \) is given as:

\[ B_{i,j,k} = \frac{\Delta x \Delta y \Delta z}{\Delta t} \left[ e_{i,j,k}^{n+1} - e_{i,j,k}^{n} \right] \]

Collecting terms gives the set of linear equation to solve similar to equation \([A.21]\)

\[ A_{E} \delta P_{i+1,j,k} + A_{W} \delta P_{i-1,j,k} + A_{N} \delta P_{i,j+1,k} + A_{S} \delta P_{i,j-1,k} + A_{P} \delta P_{i,j,k+1} + A_{P} \delta P_{i,j,k-1} + A_{P} \delta P_{i,j,k} = Q_{i,j,k} \]

Where

\[ A_{E} = -\Delta y \Delta z e_{i+\frac{1}{2},j,k}^{j,j+1} \nu_{i+\frac{1}{2},j,k}^{j,j+1} \]

\[ A_{W} = -\Delta y \Delta z e_{i-\frac{1}{2},j,k}^{j,j+1} \nu_{i-\frac{1}{2},j,k}^{j,j+1} \]

\[ A_{N} = -\Delta x \Delta z e_{i,j+\frac{1}{2},k}^{j,j+1} \nu_{i,j+\frac{1}{2},k}^{j,j+1} \]

\[ A_{S} = -\Delta x \Delta z e_{i,j-\frac{1}{2},k}^{j,j+1} \nu_{i,j-\frac{1}{2},k}^{j,j+1} \]

\[ A_{P} = -\Delta x \Delta y e_{i,j,k+\frac{1}{2}}^{j,j+1} \nu_{i,j,k+\frac{1}{2}}^{K} \]

\[ A_{P} = -\Delta x \Delta y e_{i,j,k-\frac{1}{2}}^{j,j+1} \nu_{i,j,k-\frac{1}{2}}^{K} \]

And

\[ A_{P} = -(A_{E} + A_{W} + A_{N} + A_{S} + A_{P} + A_{P}) \]
The source term $Q$ is given as:

$$Q = B_{i,j,k} + \Delta y \Delta z \left[ \begin{array}{c} \epsilon^{n+1}_{i,j+\frac{1}{2},k} \delta^x_{i,j+\frac{1}{2},k} - \epsilon^{n+1}_{i,j-\frac{1}{2},k} \delta^x_{i,j-\frac{1}{2},k} \\ \epsilon^{n+1}_{i,j,k+\frac{1}{2}} \delta^y_{i,j,k+\frac{1}{2}} - \epsilon^{n+1}_{i,j,k-\frac{1}{2}} \delta^y_{i,j,k-\frac{1}{2}} \end{array} \right] +$$

$$\Delta x \Delta z \left[ \begin{array}{c} \epsilon^{n+1}_{i+\frac{1}{2},j,k} \delta^x_{i+\frac{1}{2},j,k} - \epsilon^{n+1}_{i-\frac{1}{2},j,k} \delta^x_{i-\frac{1}{2},j,k} \\ \epsilon^{n+1}_{i,j,k+\frac{1}{2}} \delta^y_{i,j,k+\frac{1}{2}} - \epsilon^{n+1}_{i,j,k-\frac{1}{2}} \delta^y_{i,j,k-\frac{1}{2}} \end{array} \right] +$$

$$\Delta x \Delta y \left[ \begin{array}{c} \epsilon^{n+1}_{i+\frac{1}{2},j,k+\frac{1}{2}} \delta^x_{i+\frac{1}{2},j,k+\frac{1}{2}} - \epsilon^{n+1}_{i-\frac{1}{2},j,k+\frac{1}{2}} \delta^x_{i-\frac{1}{2},j,k+\frac{1}{2}} \\ \epsilon^{n+1}_{i,j+\frac{1}{2},k+\frac{1}{2}} \delta^y_{i,j+\frac{1}{2},k+\frac{1}{2}} - \epsilon^{n+1}_{i,j-\frac{1}{2},k+\frac{1}{2}} \delta^y_{i,j-\frac{1}{2},k+\frac{1}{2}} \end{array} \right]$$

The above set of linear equations can be solved in exactly identical fashion as the SIMPLE method yielding pressure correction which can then be used to update pressure and velocity until a converged solution is reached.
Appendix VIII  Detailed Drawing of Oxygenation Cell
M42 x 1.5

To be mached from PVC, Delrin or nylon and attached to the glass cell with epoxy type adhesive or similar.

---

**OXYGENATION CELL**

**THREADED COLLAR DETAIL**

**DRAWN BY:**
S. Dykes

**DESIGNED BY:**
S. Dykes

**SCALE:** 1:20

**DMC NO.:** DCELL-CELL TC-004
Conclusion
REFERENCES


Appendices


Appendices


Appendices


Appendices


Vidal, S., Francis, L. and Cheynier, V. (2002). The effect of tannins, anthocyanins, ethanol and polysaccharides on red wine mouth-feel. Use of Gases in Winemaking, South Australia, ASVO.


Appendices


