# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>i</td>
<td>TABLE OF CONTENTS</td>
<td>i</td>
</tr>
<tr>
<td>vii</td>
<td>ABSTRACT</td>
<td>v</td>
</tr>
<tr>
<td>ix</td>
<td>DECLARATION</td>
<td>x</td>
</tr>
<tr>
<td>x</td>
<td>ACKNOWLEDGMENTS</td>
<td></td>
</tr>
<tr>
<td>xi</td>
<td>PUBLICATIONS</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>LITERATURE REVIEW</td>
<td>1</td>
</tr>
<tr>
<td>1.1</td>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>1.2</td>
<td>GRAPE PHENOLICS</td>
<td>1</td>
</tr>
<tr>
<td>1.2.1</td>
<td>Anthocyanins</td>
<td>3</td>
</tr>
<tr>
<td>1.2.2</td>
<td>Flavan-3-ol monomers and oligomers</td>
<td>5</td>
</tr>
<tr>
<td>1.2.3</td>
<td>Polymerisation reactions during wine making, maturation and aging</td>
<td>6</td>
</tr>
<tr>
<td>1.2.3.1</td>
<td>Formation of pyranoanthocyanins</td>
<td>6</td>
</tr>
<tr>
<td>1.2.3.2</td>
<td>Formation of wine polymeric phenols</td>
<td>7</td>
</tr>
<tr>
<td>1.2.3.2.1</td>
<td>Model wine studies of polymeric pigment formation</td>
<td>12</td>
</tr>
<tr>
<td>1.2.3.3</td>
<td>Polymerisation due to polyphenol oxidase (PPO)</td>
<td>13</td>
</tr>
<tr>
<td>1.3</td>
<td>ISOLATION OF POLYMERIC PHENOLS</td>
<td>13</td>
</tr>
<tr>
<td>1.4</td>
<td>CHARACTERISATION AND IDENTIFICATION OF POLYMERIC PHENOLS</td>
<td>15</td>
</tr>
<tr>
<td>1.4.1</td>
<td>Spectrophotometric analysis</td>
<td>15</td>
</tr>
<tr>
<td>1.4.2</td>
<td>Chromatographic and acid hydrolysis analyses</td>
<td>17</td>
</tr>
<tr>
<td>1.4.2.1</td>
<td>Mass spectrometry analyses</td>
<td>20</td>
</tr>
<tr>
<td>1.5</td>
<td>SENSORY PROPERTIES OF GRAPE AND WINE PHENOLICS</td>
<td>22</td>
</tr>
<tr>
<td>1.5.1</td>
<td>Contribution of flavonoids to wine bitterness and astringency</td>
<td>22</td>
</tr>
<tr>
<td>1.5.2</td>
<td>Factors affecting perceived astringency and bitterness</td>
<td>23</td>
</tr>
<tr>
<td>1.5.3</td>
<td>Sensory analysis of astringency attributes</td>
<td>25</td>
</tr>
<tr>
<td>1.6</td>
<td>CONCLUSION</td>
<td>26</td>
</tr>
<tr>
<td>2</td>
<td>PIGMENT TRANSFORMATIONS IN MODEL SYSTEMS</td>
<td>27</td>
</tr>
<tr>
<td>2.1</td>
<td>INTRODUCTION</td>
<td>27</td>
</tr>
<tr>
<td>2.2</td>
<td>MATERIALS AND METHODS</td>
<td>29</td>
</tr>
<tr>
<td>2.2.1</td>
<td>Preparation of grape seed extract</td>
<td>29</td>
</tr>
<tr>
<td>2.2.2</td>
<td>Determination of the phenolic content of the extract by Folin-Ciocalteu</td>
<td>29</td>
</tr>
</tbody>
</table>
2.2.3 Isolation of anthocyanin extracts 30
2.2.3.1 Methanol extraction 30
2.2.3.2 Iso-amyl alcohol extraction 30
2.2.4 HPLC analysis 31
2.2.5 Ultrafiltration 31
2.2.6 Experiments 31
2.2.6.1 Experiment A 32
2.2.6.2 Experiment B 32
2.2.6.3 Experiment C 32
2.3 RESULTS 33
2.3.1 Experiment A 33
2.3.2 Experiment B 36
2.3.3 Experiment C 37
2.3 DISCUSSION 39
2.4 CONCLUSION 41

3 METHOD DEVELOPMENT FOR THE SEPARATION OF PIGMENTED POLYMERS IN WINE 42
3.1 INTRODUCTION 42
3.1.1 Spectroscopic methods 42
3.1.2 Chromatographic methods 43
3.1.3 Fractionation methods 44
3.2 MATERIALS AND METHODS 47
3.2.1 General material and methods 47
3.2.1.1 Experimental wines 47
3.2.1.2 Somers’ measurements 47
3.2.1.3 Ultracentrifugation 48
3.2.2.1 RP-HPLC analysis 48
3.2.2.2 Calibration 49
3.2.2.1 Preparation of polymer pigmented standard 49
3.2.2.2 Preparation of polymer seed standard 50
3.2.2.3 Comparison with C_{18} column 51
3.2.2.4 Semi-preparative chromatography 51
3.2.3 Preparation of grape seed extract 51
3.2.4 Ultrafiltration

3.2.5 Fractionation with Sephadex LH-20

3.2.6 Investigation of sulphoxy ethyl cellulose (cation-exchange) column

3.2.7 Investigation of Licrosorb Si-100 NP-HPLC column:

3.2.8 Investigation of combination of fractionation methods

3.2.9 Investigation of CCC solvent systems for normal-phase separation

3.2.10 Preparative normal-phase medium pressure liquid chromatography (NP-MPLC)

3.2.11 Combination of NP chromatography methods for the isolation of polymeric pigment from wine

3.2.12 Investigation by electrospray ionisation mass spectrometry (ESI-MS) analysis

3.2.12.1 Direct injection ESI-MS analysis

3.2.12.2 Liquid chromatography (LC)/ESI-MS analysis

3.3 RESULTS

3.3.1 Quantitative separation of pigmented polymers

3.3.2 Investigation of the isolation and purification of pigmented polymers in wine

3.3.2.1 Ultrafiltration

3.3.2.2 Sephadex LH-20

3.3.2.3 Sulphoxy ethyl cellulose fractionation

3.3.2.4 Investigation of Licrosorb Si-100 NP-HPLC column

3.3.2.5 Identification of fractions obtained by multiple fractionation techniques

3.3.2.5.1 ESI-MS analysis

3.3.2.6 Investigation of fractionation with preparative normal-phase chromatography

3.3.2.6.1 Investigation of Thin-Layer Chromatography (TLC) solvent systems

3.3.2.6.2 Fractionation on normal-phase medium pressure liquid chromatography (NP-MPLC)

3.3.2.6.3 Isolation of pigmented polymers by thin-layer chromatography (TLC)

3.3.2.6.4 Mass spectrometry analysis of isolated TLC fractions

3.4 DISCUSSION

3.5 CONCLUSION

4. CHARACTERISATION AND IDENTIFICATION OF POLYMERIC PIGMENTS

4.1 INTRODUCTION

4.2 MATERIALS AND METHODS

4.2.1 Red wine fractionation
4.2.1.1 NP-MPLC of six month and five year old Shiraz wine 84
4.2.1.2 Separation and extraction on TLC plates 85
4.2.2 RP-HPLC 85
4.2.3 Paper-electrophoresis 85
4.2.4 UV-vis/CieLab analysis 86
4.2.5 Infra-red analysis 86
4.2.6 Acid hydrolysis 86
4.2.7 Liquid chromatography electrospray ionisation mass spectrometry (LC/ESI-MS) analysis 87
4.2.8 Sample clean-up 87
4.2.9 MALDI-TOF analysis 87
4.2.10 Separation of sugars and phenolics 88
4.2.11 Carbohydrate analysis 88
4.2.11.1 Carbohydrate hydrolysis 89
4.2.11.2 Reduction of monosaccharides 89
4.2.11.3 Acetylation 89
4.2.11.4 GC-MS of alditol acetates 90
4.2.11.5 Glycosyl-residue composition (Methanolysis) 90
4.2.11.6 Carboxyl reduction 92
4.2.11.7 Preparation of the NaOH slurry in dimethoxysulfoxide (DMSO) 92
4.2.11.8 Methylation of polysaccharides 92
4.2.11.9 Hydrolysis, reduction and acetylation of the methylated polysaccharides 93
4.2.11.10 Gas chromatography-mass spectrometry of monosaccharide derivatives 93
4.2.12 Determination of tannin mDP by phloroglucinolysis 93
4.2.13 Gel permeation chromatography 95
4.3 RESULTS 96
4.3.1 Isolation and characterisation of the polymeric fraction of a 6 month old Shiraz wine 96
4.3.1.1 Isolation of different polymeric pigment fractions by preparative NP chromatography and TLC 96
4.3.1.2 UV-vis/CieLab analysis 100
4.3.1.3 Normal phase HPLC fractionation 103
4.3.1.4 Polymeric nature of the isolated TLC bands 103
4.3.1.4.1 Electrophoresis 103
4.3.1.4.2 Infra-red spectral data 104
4.3.1.5 Acid hydrolysis in butanol and hydrochloric acid 106
4.3.1.6 LC-ESI-MS analysis 106
4.3.1.7 MALDI-TOF analysis 107
4.3.1.8 Carbohydrate analysis 111
4.3.1.9 Determination of tannin mDP by phloroglucinol 114
4.3.1.10 Gel permeation chromatography 115
4.3.2 Isolation and characterisation of the polymeric fractions of a 5 year old wine 116
4.3.2.1 Isolation of different polymeric pigment fractions by preparative NP chromatography and TLC 116
4.3.2.2 Analytical NP-HPLC analysis 121
4.3.2.3 LC/ESI-MS analysis 122
4.3.2.4 Determination of tannin mDP by phloroglucinolysis 129
4.3.2.5 Gel permeation chromatography 132
4.4 DISCUSSION 134
4.4.1 Characterisation of the polymeric fraction of a 6 month old Shiraz wine 134
4.4.2 Characterisation of the polymeric fraction of a 5 year old Shiraz wine 138
4.5 CONCLUSION 139

5 DEVELOPMENT OF A VOCABULARY TO DESCRIBE THE MOUTH-FEEL PROPERTIES OF RED WINE 141
5.1 INTRODUCTION 141
5.2 MATERIALS AND METHODS 142
5.2.1 Development of the descriptive analysis of mouth-feel properties 142
5.2.1.1 Deriving an astringency vocabulary 142
5.2.1.2 Panellist consistency and reliability in identifying astringent sub-qualities 152
5.3 RESULTS 153
5.3.1 Development of mouth-feel terminology 153
5.3.2 Panel performance and consistency 156
5.4 DISCUSSION 157
5.5 CONCLUSION 158

6 MOUTH-FEEL OF WHITE WINES MADE WITH AND WITHOUT POMACE CONTACT AND ADDED ANTHOCYANINS 159
6.1 INTRODUCTION 159
6.2 MATERIALS AND METHODS 161
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.2.1</td>
<td>Fermentation of treated juices and processing of the resulting wines</td>
<td>161</td>
</tr>
<tr>
<td>6.2.1.1</td>
<td>Extraction and purification of anthocyanin extract</td>
<td>161</td>
</tr>
<tr>
<td>6.2.1.2</td>
<td>Winemaking experiments</td>
<td>162</td>
</tr>
<tr>
<td>6.2.2</td>
<td>Chemical analysis</td>
<td>163</td>
</tr>
<tr>
<td>6.2.3</td>
<td>Quantitative sensory descriptive analysis</td>
<td>164</td>
</tr>
<tr>
<td>6.2.4</td>
<td>Data analysis</td>
<td>166</td>
</tr>
<tr>
<td>6.3</td>
<td>RESULTS</td>
<td>167</td>
</tr>
<tr>
<td>6.3.1</td>
<td>Wine composition</td>
<td>167</td>
</tr>
<tr>
<td>6.3.2</td>
<td>Colour measurements</td>
<td>168</td>
</tr>
<tr>
<td>6.3.3</td>
<td>Phenolic composition</td>
<td>170</td>
</tr>
<tr>
<td>6.3.4</td>
<td>Sensory analysis</td>
<td>172</td>
</tr>
<tr>
<td>6.3.4.1</td>
<td>Correlation between sensory and chemical data</td>
<td>175</td>
</tr>
<tr>
<td>6.4</td>
<td>DISCUSSION</td>
<td>179</td>
</tr>
<tr>
<td>6.5</td>
<td>CONCLUSION</td>
<td>181</td>
</tr>
</tbody>
</table>

7 SUMMARY 183

BIBLIOGRAPHY 187

APPENDIX 207
ABSTRACT

Phenolic compounds play an important role in red wine colour, bitterness, astringency, as well as a range of other tactile or 'mouth-feel' characteristics. Progressive changes of phenolic compounds, initially extracted from grapes, occur during the storage and aging of red wines. The decrease of astringency occurring during wine aging has been considered as a result of mainly anthocyanin-flavanol condensation either directly or mediated by aldehydes. The contribution of these polymeric pigments formed during wine aging to the unique properties of red wine is an important question still unanswered.

Experiments were conducted to synthesise polymeric pigments in model wine solutions under different conditions in the absence of acetaldehyde to provide material for chemical and sensory studies. Only small amounts of polymeric pigments were formed in these experiments confirming that direct polymerisation is a slow process. The low yield of polymers made it necessary to investigate the isolation of polymeric pigments directly from wine. A preparative fractionation protocol was developed to obtain fractions enriched in different red wine pigment combinations for further investigation. A HPLC method was also developed that separated the pigmented and non-pigmented polymers, as well as the monomeric anthocyanins, flavanols (monomeric to trimeric), flavonols and hydroxycinnamic acids from each other. A 6 month old and a 5 year old Shiraz wine were fractionated and further analysed by acid hydrolysis in the presence of a nucleophile, gel permeation chromatography (GPC) and by different mass spectrometry techniques. The various fractions isolated from the 6 month old wine contained combinations of pentameric to dimeric pigments, while those from the 5 year old wine contained pigments with an average degree of polymerisation (DP) of at least 11 but possibly up to 32.

Experiments were conducted to determine the sensorial contribution of anthocyanins to wine as well as the effect of anthocyanin-flavanol polymerisation reactions taking place during maturation. It was shown that significant changes occur in the polymeric phenol composition and in the mean degree of polymerisation of Shiraz wine during aging. With aging more skin tannin were incorporated in the pigmented polymers and the percentage of galloylation in these polymers decreased.
In order to describe the sensory attributes of the polymeric pigments a refined vocabulary, describing the astringent and other mouth-feel sensations elicited by dry red table wines representing different styles was developed and called the mouth-feel wheel. The developed mouth-feel wheel was used in a study to investigate the contribution of anthocyanins and anthocyanin-proanthocyanidin reaction products to the mouth-feel properties of red wine. Wines were made from both red and white grapes with and without pomace contact, as well as with and without anthocyanin addition to the white grapes. The white wine made like a red wine did not exhibit the same mouth-feel sensory attributes of a red wine: it was lower in viscosity, less particulate in nature and lower in intensity for the astringency descriptors fine emery, dry and grippy. The presence of anthocyanins during fermentation appeared to increase the intensity of astringency related terms. Treatments with added anthocyanins increased the amount of polymeric phenols to twice that when compared to treatments without added anthocyanins.
DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying.

Anita Oberholster Date
I thank my supervisors, Dr Elizabeth Waters, Dr Leigh Frances, Dr Patrick Iland and Dr Graham Jones for their support. I am also indebted to Richard Gawel and Stella Kassara for their collaboration in respectively the development of the mouth-feel wheel and investigation of the model wine systems. I want to thank Dr Yogi Hayasaka for mass spectrometry analyses, as well as the mouth-feel sensory panel. Sincere thanks to Dr Robert Asenstorfer and Dimi Capone for their advice and support. I am grateful for the continual encouragement of many friends and family, especially Dimi Capone, Suné Beeselaar, Letitia Watson, Hanlie Swart and my brother, Michiel Smuts. I am eternally thankful to my husband Connie, for all his patience, support and love.

I acknowledge the Australian Wine Research Institute and the Grape and Wine Research and Development Corporation for funding this research, as well as KWV for making it all possible.
PUBLICATIONS

