Development and Application of Novel Analytical Methods to the Identification, Formation and Fate of Two Important Wine Aroma Compounds

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A thesis submitted for the degree of Doctor of Philosophy

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January 2012
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Review of the literature & summary of research aims.

Chapter 2
Evolution and occurrence of 1,8-cineole (eucalyptol) in Australian wine.

Chapter 3
Vineyard and fermentation studies to elucidate the origin of 1,8-cineole in Australian red wine.

Chapter 4
Application of a modified method for 3-mercaptohexan-1-ol determination to investigate the relationship between free thiol and related conjugates in grape juice and wine.

Chapter 5
Analysis of precursors to wine odorant 3-mercaptohexan-1-ol using HPLC-MS/MS – Resolution and quantitation of diastereomers of 3-S-cysteinylhexan-1-ol and 3-S-glutathionylhexan-1-ol.

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A compilation of additional co-authored publications generated throughout candidature.
Wine flavour is complex and encompasses a wide variety of compounds with very different sensorial properties. It is only through detailed investigations, however, that knowledge of aroma compounds is improved. One aroma compound of inconclusive origin and requiring further study was 1,8-cineole, which was especially relevant to the Australian viticultural landscape. Another group of important flavour compounds found in wine that required greater understanding are the sulfur compounds. Varietal thiols, in particular 3-mercaptohexan-1-ol (3-MH), have some of the lowest aroma thresholds of any food or beverage component. Knowledge of thiol precursors is also important for understanding the formation of the corresponding varietal aroma compounds during winemaking, since they are released from odourless precursors in grape juice through fermentation.

To enable a better understanding of the formation and fate of the two important aroma compounds chosen, novel analytical methods using stable isotope dilution analysis (SIDA) were developed and thoroughly validated for the quantification of 1,8-cineole and 3-MH in grapes and wine. In addition, a SIDA method was developed and validated for the analysis of the diastereoisomers of the precursors, 3-S-cysteinyhexan-1-ol (Cys-3-MH) and 3-S-glutathionylhexan-1-ol (Glut-3-MH) to complement the studies on 3-MH.

1,8-Cineole was found to be predominantly present in red wines, being extracted during fermentation rather than forming from terpene precursors as previously proposed. Extension of this research revealed that Eucalyptus trees in the vineyard had a strong influence on the concentration of 1,8-cineole in wine. The incorporation of grape leaves and stems, and in particular Eucalyptus leaves into red must fermentations significantly elevated the level of 1,8-cineole concentrations in the wine. Additionally, this study revealed a surprising increase in rotundone concentrations when grape leaves and stems were included during fermentation.

An analytical method was developed for 3-MH determination that used conventional electron ionisation GC-MS and eliminated the use of mercuric compounds for thiol isolation. A 3-MH precursor analytical method was also developed which provided the first method where both diastereoisomers of Cys- and Glut-3-MH could be
analysed individually in one run. This was improved further by the addition of Cysgly-3-MH into the method following the identification of this compound in grapes for the first time. These methods were applied to wines and grape juices to investigate factors which might affect their concentrations, such as freezing grapes and juice, grape processing and fruit transportation. These studies highlighted the dynamic nature of these precursor compounds. In addition a new conjugated aldehyde which was the obvious missing link between the reaction of (E)-2-hexenal and glutathione in the formation of Glut-3-MH was identified for the first time.

The development of the new analytical methods discussed, together with their applications has contributed considerably to our understanding of various aspects of wine flavour. This work has uncovered the origin of 1,8-cineole in red Australian wine. It has also greatly enhanced our knowledge of 3-MH and its precursors so that we can understand how these compounds are formed and what affects their concentrations in juice and wine. Ultimately, this knowledge will enable wine producers to have greater control over the aroma profile of their wines.
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Publications

This thesis is a collection of manuscripts that were published or were submitted for publication in various refereed Journals during candidature. Most of the manuscripts were published in The Journal of Agricultural Food Chemistry (JAFC) and in 2010 the impact factor of JAFC according to ISI statistics was 2.816, and the 5-year impact factor was 3.209. JAFC, a high impact ACS journal, is ranked number 2 for the Agriculture - Multidisciplinary, and is in the top 10 for Chemistry – Applied (number 2 if only considering wine science) and in the top 10 for Food Science & Technology, with the greatest number of published articles and total citations in these subject categories.

The text and figures in these chapters contained different formatting, according to the various journal requirements. A Statement of Authorship, signed by all of the authors, listing individual contributions to the work is included at the beginning of each chapter.

The thesis is based on the following refereed publications.


Chapter 5. Dimitra L. Capone, Mark A. Sefton Capone, Yoji Hayasaka, and David W. Jeffery. Analysis of precursors to wine odorant 3-mercaptohexan-1-ol using HPLC-MS/MS – resolution and quantitation of diastereomers of


An additional 14 related publications co-authored by the candidate are given in the appendices.
Conferences

Crush, 28 to 30th September 2011, Adelaide.
Presented a talk titled ‘Studies on 3-mercaptopentanol and its conjugates in Sauvignon blanc juice and wine’.

The International Chemical Congress of Pacific Basin Societies (Pacifichem), 15 to 20th December 2010, Honolulu, Hawaii, USA.
Presented a talk in the session on: “Value-Added Food Products from Fruits and Vegetables” The talk was titled ‘Flavour precursors in Sauvignon blanc grape juice: the effect of fruit processing on thiol conjugate concentrations’.

University of Adelaide School of Agriculture, Food and Wine Postgraduate symposium, 21 to 22nd September 2010, Adelaide.
Presented a talk titled ‘The origin of eucalyptol in Australian wine’ and won the “Max Tate Award” for the best presentation at the symposium.

ACS Fall National Meeting, 22 to 26th August 2010, Boston, MA, USA.
Presented in the session of “The Chemistry of Alcoholic Beverages” This talk was titled ‘Analytical investigations to relate important wine odorant 3-mercaptopentanol to its precursors’.

Separation Science conference, 6 to 7th August 2010, Singapore.
Presented a poster titled 'Identification and analysis of new taint compounds using GC/MS/ODP and GC/MS/SIM'.

14th Australian Wine Industry Technical Conference, 3 to 8th July 2010, Adelaide.
Presented two talks at a flavour workshop. The presentations were titled ‘The origin of eucalyptol in Australian wine’ and ‘Monoterpenes in wine’. Also presented two posters titled ‘Quantification of the precursors to the wine odorant 3-mercaptopentanol-1-ol’ and ‘The origin of eucalyptol in Australian wine’.
Panel of supervisors

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Acknowledgements

I thank my supervisors Dr David Jeffery, Dr Mark Sefton and Professor Dennis Taylor for all of their support, advice and encouragement throughout my PhD. It was a pleasure working with each and every one of them. I would also like to thank my external advisor Professor James Kennedy for his valuable advice and encouragement.

I also thank the past and present staff at the AWRI for their on-going support and encouragement in particular Dr Leigh Francis, Natoiya Lloyd, Katryna van Leeuwen, Samantha Anderson and the various co-authors on the publications included in this thesis. I would also like to thank Dr Dan Johnson for his efforts in making this PhD possible.

I am extremely grateful to the many wine producers and wine makers across Australia who provided generous support of this research through the contribution of wine and grape samples and through openly providing detailed information about these included in this study. I would particularly like to thank Nick Bruer and Tim McCarthy of Orlando Wines and staff members from Casella Wines, in particular Steve Warne and Frank Mallamace.

On a personal note a special thank you goes to my husband Mauro Capone for his continued encouragement and support during this period. Also thank you to both sides of my family for their encouragement, interest and support. I would like to thank my niece Stefania Mercorella who assisted me with parts of the grape processing during vintage and my parents Niki and Theo Liacopoulos for their continued love and support.

This project was financially supported by:

The Australian Wine Research Institute, a member of the Wine Innovation Cluster in Adelaide, is supported by Australia’s grapegrowers and winemakers through their investment body, the Grape and Wine Research Development Corporation, with matching funds from the Australian government.