

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

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Thesis Summary

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-cysteinylhexan-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.

Declaration

I declare that this thesis is a record of original work and contains no material which has been accepted for the award of any other degree or diploma in any University or other tertiary institution. To the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference has been made in the text. The publications included in this thesis have not been previously submitted for the award of any degree at the University of Adelaide or other University.

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Dimitra Capone

Date

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 2. Dimitra L. Capone, Katryna Van Leeuwen, Dennis K. Taylor, David W. Jeffery, Kevin H. Pardon, Gordon M. Elsey, and Mark A. Sefton. Evolution and occurrence of 1,8-cineole (eucalyptol) in Australian wine. *J. Agric. Food Chem.* **2011**, 59: 953-959.
- Chapter 3. Dimitra L. Capone, David W. Jeffery and Mark A. Sefton. Vineyard and fermentation studies to elucidate the origin of 1,8-cineole in Australian red wine. *J. Agric. Food Chem.* **2012**, 60: 2281-2287.
- Chapter 4. Dimitra L. Capone, Mark A. Sefton and David W. Jeffery. 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. *J. Agric. Food Chem.* **2011**, 59: 4649-4658.
- 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

3-S-cysteinylhexan-1-ol and 3-S-glutathionylhexan-1-ol. *J. Agric. Food Chem.* **2011**, *58*: 1390-1395.

Chapter 6. Dimitra L. Capone and David W. Jeffery. Effects of transporting and processing Sauvignon blanc grapes on 3-mercaptohexan-1-ol precursor concentrations. *J. Agric. Food Chem.* **2011**, *59*: 4659-4667.

Chapter 7. Dimitra L. Capone, Kevin H. Pardon, Antonio G. Cordente and David W. Jeffery. Identification and quantitation of 3-S-cysteinylglycinehexan-1-ol (cysgly-3-MH) in Sauvignon blanc grape juice by HPLC-MS/MS. *J. Agric. Food Chem.* **2011**, *59*: 11204-11210.

Chapter 8. Dimitra L. Capone, Mark A. Sefton and David W. Jeffery. Analytical investigations of wine odorant 3-mercaptohexan-1-ol and its precursors. In "Flavor Chemistry of Wine and Other Alcoholic Beverages". 2011, American Chemical Society: Washington, DC; (Accepted).

Chapter 9. Dimitra L. Capone, Cory A. Black and David W. Jeffery. Effects of 3-mercaptohexan-1-ol precursor concentrations from prolonged storage of Sauvignon blanc grapes prior to crushing and pressing. *J. Agric. Food Chem.* **2012**, *60*: 3515-3523.

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-mercaptohexan-1-ol 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-mercaptohexan-1-ol 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-mercaptohexan-1-ol' and 'The origin of eucalyptol in Australian wine'.

Panel of supervisors

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