Contribution of Grape Berry Lipids to Wine Aroma



FINAL REPORT to

GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: **GWT 1218**

Principal Investigator: **Christopher Blackford**

Research Organisation: **CSIRO Plant Industry/Murdoch University**

Date: **24/10/2013**

Abstract

This report outlines the objectives and outcomes of attending the 64th American Society for Enology and Viticulture National Conference held in Monterey, California. The project was jointly funded by the GWRDC and CSIRO Plant Industry. The goals of attending this conference were to present aspects of my PhD research, become familiar with up-and-coming technical and research areas to inform my ongoing research. Information from a selection of conference presentations, as well as my poster abstract and presentation "Contributions of Grape Berry Compounds to Wine Aroma" are reported.

Executive Summary

The GWRDC travel grant provided $1,600 (GST exclusive) which was used to part-fund the flights to and from the USA for attendance at the 64th American Society for Enology and Viticulture National Conference in Monterey. The balance of the funding for the flights was provided by CSIRO Plant Industry for as was the funding for the conference fees and accommodation.

The American Society for Enology and Viticulture National Conference is an annual conference at which researchers and industry representative meet to exchange and discuss the most recent developments in oenology and viticulture. The conference in 2013 represented the 64th time this meeting had been held. If attracts the best North American scientists working in the research fields of oenology and viticulture and often has a number of keynote speakers from around the world presenting the latest research from their laboratories.

Many presentations had impact on my ongoing research, including those that had developed methodologies for analysing membrane lipid composition. Attending the conference also gave me the opportunity to present the findings of my research to date in poster sessions and informally during other conference events.

Attending the ASEV National Conference was a great advantage to me because it improved my knowledge of the state of research in the field in which I work, it provided me with feedback on my research, and lead to connections being made with leading scientists in the field.

Travel Report

The GWRDC travel grant of $1,600 provided partial funding for me to attend the 64th American Society for Enology and Viticulture National Conference, held in Monterey, California in June, 2013. The objective of attending the conference was to present the current results of my PhD project and to increase my knowledge of the latest research developments in grape and wine research, associated technologies and the important industrial themes of today.

The ASEV National Conference was attended by about 600 delegates from 12 different countries, and featured more than 200 presentations. There were 13 session streams:

1. Flavor/Analysis
2. Micro/Molecular Biology
3. Sensory/Sensory Impacts
4. Impact of Yeast & Bacteria
5. Wine Stability & Oxidation
6. Tannins
7. Pests & Diseases
8. Rootstocks
9. Environmental Impacts
10. General Viticulture
11. Cultural Practices
12. Water Relations
13. Red Blotch

Additionally, there were seminars primarily pitched for industry dealing with topics such as sustainable water management, preparation of labs for harvest and bottling line sanitation. The key note address was given by Dr Jim Wolpert and addressed the challenges and opportunities that face the research and extension communities, particularly in California.

The two presentations I found most useful and interesting were surprisingly not part of the flavour and aroma areas that I anticipated. Firstly, there was the very interesting presentation by Dr David Block, of UC Davis, on how temperature changes lead to alteration of yeast lipid composition and the links to fermentation outcomes. Yeasts that are able to alter the lipid composition of their membranes are able to better stay activated in, and tolerate, stressful temperature and ethanol conditions. Interestingly, in the strains investigated, they found that the increase in ergosterol was only slightly important in finishing fermentation. Other lipids seemed to be more significantly involved temperature survival. Increases in phosphotidylinositides were correlated with fermentations at the end of exponential growth and transitions into stuck fermentations, and high levels of this type of lipid in early fermentation could be used to indicate possible problem ferments. Lipid species containing very long fatty acid chains 34:1 and 36:1 were strongly correlated with 35 °C ferments. In15 °C ferments, the yeast had higher levels of tighter curving phosphatidylethanolamines, but it was too difficult to say why this would be an advantage at low temperatures. On the other hand, other lipid groups that favour tight acyl packing were more concentrated at the low temperatures, perhaps because the chains allow the yeast to maintain fluidity of the membrane in the cooler temperatures. From this presentation I was lead to the idea of including lipid composition sampling of yeast in fermentation to the planned pre-fermentation analysis of lipid composition of grapes in my PhD research. The group had developed an extraction modification for taking yeast from ferments. I was

also exposed to the technique of fluorescence anisotrophy, which could be used to evaluate the changes in lipid membrane fluidity.

Another presentation I found interesting was a poster from the Bisson group at UC Davis discussing the changes in *Saccharomyces cerevisae* when a [GAR+] prion is active. Prions are proteins that have at least one conformational state that is self-templating. The yeast [GAR+] prion behaves like a switch that turns off the acute preference that yeast have for consuming fructose and glucose over other carbon sources. Activation of the stable, dominant and heritable [GAR+] prions leads to yeast utilizing alternative carbon sources such as glycerol and maltose, and taking on morphology resembling stationary growth phases and production of pseudohyphae. I was particularly interested in the change of the yeast lipid membrane with phosphoinositides increasing in the prion affected yeast. The [GAR+] prion is thought to be a possible cause of some sluggish and arrested fermentations, often ceasing at 3 Brix. However, it is thought that the prion provides yeast with improved viability by allowing it to 'have eggs in many baskets'. I was involved in a discussion that approached the possibilty that the prions were a way by which the metabolism of yeast could be altered by other microbes (such as lactic acid bacteria) in order to free up more glucose and fructose for consumption by those microbes. Like the previously mentioned presentation, this poster has lead to me thinking about producing hypotheses for lipid changes that I should expect to see in grapes and yeast in my own research.

There were many other presentations that had smaller implications on my research or were outside my research area altogether. Here is a selection of some of those that I found interesting:

The presentation by Dr Cheynier from the INRA in France was an enlightening window into the colour and phenolic composition of Rose wines. Using a large chemometric data set generated largely by high through put UPLC-MS/MS and colour characterisation, they found that oxidation and extraction were the major impacts on colour and composition of the Rose wines. There was an in depth discussion of the various pathways leading to colour formation through hydroxycinnamic acids, ethyl anthocyanins and pyranoanthocyanins.

Dr Tomasino from Oregan State University presented interesting research into a problem that particularly affects growers in Oregon. Brown marmorated stink bugs not only damage grape berries, but presence alongside grape processing can lead to significant negative sensory impacts that can reduce the quality of wine.

Another interesting talk was from Frida Gustafsson from University of British Columbia, who has been using quantum dots conjugated to glutathione to track different strains of *Saccharomyces cerevisae* throughout fermentation. The quantum dots have their colours altered by the different environments in the yeast strains and this can be quantified with confocal microscopy. As a result the entire yeast population can be quantified. The cost was less than half of that of similar results done by microsatellite analysis, despite quantifying 50 times more yeast cells.

During the conference I met many new researcher contacts, including Mark Eltom of Lincoln University, Hildegarde Heymann of UC Davis, and Mark Krasnow of the Eastern Institute of Technology. I was able to network with industry and other researchers at the poster session and received valuable comments and feedback on the PhD research in my poster in the discussions that I had.

Addenda

1. Conference Poster Abstract

Contribution of Grape Berry Compounds to Wine Aroma

Christopher L Blackford, Eric G Dennis, Robert D Trengove, Paul K Boss

Aroma compounds have been shown to have major influence over consumer preferences for wine. This has led to interest in understanding development and final composition of wine aroma compounds. Understanding the origin of important wine aroma compounds could allow interventions in grape growing and wine production that improve characteristics that are desired by wine consumers.

Grapes contribute to the composition of final wine, not only through varietal "impact" compounds, such as terpenes and methoxypyrazines, but also through non-varietal aroma compounds, such as C_6-alcohols and some esters of higher alcohols. A recent study in our group showed that several additional aroma compounds increased in model ferments as the proportion of Riesling or Cabernet Sauvignon grape juices increased.

In this presentation are outlined the further investigation that has been undertaken in our group on the origin of aroma compounds that are dependent on grape composition. Particular focus is given to fatty acid ethyl esters and lactones. Spike model grape juice ferments and isolation of products of grape berries that influence wine aroma production have led us to hypothesise that grape berry lipids play a role in the production of some wine aroma compounds. Labelled substrates spiked into model grape juice ferments give further understanding about the mechanism by which this occurs.

2. Conference poster

Contributions of Grape Berry Compounds to Wine Aroma

Christopher L Blackford,[1,2] Eric G Dennis,[3] Robert D Trengove,[4] Paul K Boss[1]

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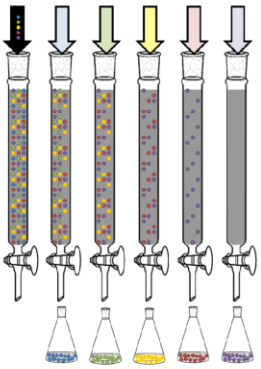
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Aroma compounds have major influences on consumer preferences for wine. This has led to interest in understanding the development of wine aroma compounds and variables affecting the final volatile composition of wines. Understanding the origin of important wine aroma compounds could allow interventions in grape growing and wine production that improve characteristics that are desired by wine consumers. This work has taken a natural products separation approach and aims to purify compounds in grapes that influence the production of volatile compounds in wines.

Fractionation and fermentation

Whole frozen grapes were extracted with acetone and extract loaded onto a reversed-phase open column of polystyrene-divinylbenzene (HP20) by iterative steps of passing through the column and dilution with water.[1,2] Five fractions were produced by eluting with increasing proportions of acetone in water (20, 40, 60, 80 and 100% acetone). Fractions were dried by evaporation under low pressure and freeze drying.

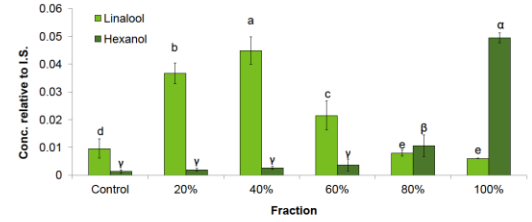
Model grape juice must (MGJM) was prepared to simulate the primary metabolites necessary for EC118 yeast fermentation. 50 mL MGJM was spiked with fraction aliquot equivalent to 50 g of fresh weight grape berries. Fermentation was carried out to dryness and the wines analysed by HS-SPME GCMS.



The model wines differed in volatile profile.

The fractionation separated known components of grapes that influence wine aroma. Terpenoids (e.g. linalool) tended to accumulate in wines spiked with polar fractions. C6 compounds (e.g. hexanol) tended to accumulate in wines spiked with non-polar fractions.

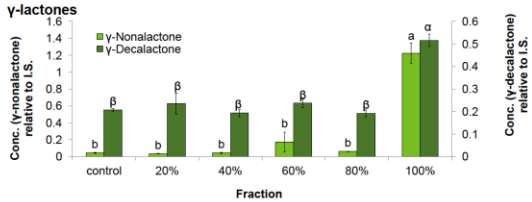
Linalool and hexanol



Non-polar fractions increase gamma-lactone levels.

Gamma-lactones have aromas with odour descriptions ranging from stone-fruits like apricot through to coconut and fatty. They can be synthesised by yeast de novo. However the compounds that eluted in the 100% acetone fraction greatly increased their abundance in the model wine.

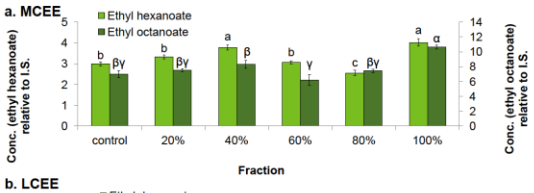
γ-lactones



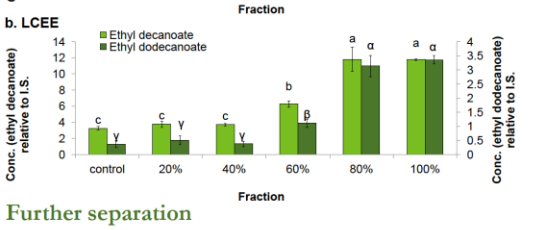
Ethyl esters influenced by grape components

Fatty acid ethyl esters (FAEE) are often considered to be "fermentation derived" esters; however, significant differences were observed in wines made from different grape fractions. Each FAEE was elevated in wine made from juice spiked with the 100% acetone fraction. The variation in each FAEE for the other fractions is different.

a. MCEE

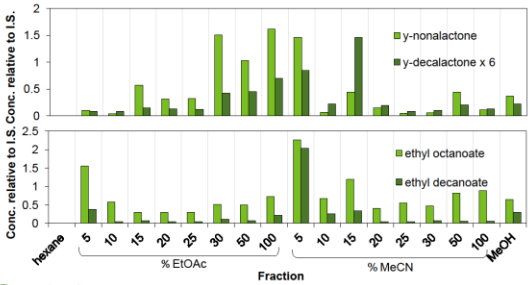


b. LCEE



Further separation

Once the sub-samples of fractions are assessed by model wine making, the fractions can be further separated. The 100% fraction was loaded on to a normal-phase DIOL column with hexane. The eluting solvent was increased in polarity from hexane up to ethyl acetate then to acetonitrile, followed by a methanol wash. Model wines were again made and volatiles analysed.



Conclusions

HP20 open column fractionation simplifies the grape matrix and the fractions have different potentials for volatile production when fermented. Further separation and spectroscopic identification of the components of these fractions is on going. Understanding the mechanisms by which these grape components influence wine aroma will inform strategies for grape-growers and winemakers to manage production to meet consumer preferences.

FOR FURTHER INFORMATION
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REFERENCES
1. West, L.M.; Northcote, P.T.; Battershill, C.N. *J. Org. Chem.* **2000**, *65*, 445-449.
2. West, L.M.; Northcote, P.T.; Hood, K.A.; Miller, J.H.; Page, M.J. *J. Nat. Prod.* **2000**, *63*, 707-709.

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3. Financial Report

END OF PROJECT FINANCIAL STATEMENT

Statement of Receipts and Expenditure

At the conclusion of each Project, the GWRDC requires a Statement of Receipts and Expenditure of GWRDC funds received in relation to the Project.

Project Title:	Contribution of Grape Berry Lipids to Wine Aroma

GWRDC Project Number	Project Start Date	Project End Date
GWT 1218	24/06/2013	28/06/2013

Financial Year (e.g. 2010-11)	(a) *Funds brought forward from previous year*	(b) #Approved GWRDC Budget (full year)	(c) Total budget available (a)+(b)	(d) Actual expenditure ($)	(e) Difference ($) (c)-(d)
Year 1: 2012/13	n/a	1,600.00	1,600.00	1,600.00	0.00
Year 2: 20__/__	0.00	0.00	0.00	0.00	0.00
TOTAL		1,600.00		1,600.00	0.00

* "Funds brought forward" refers to any unspent GWRDC funds from the previous year and approved by the GWRDC to be spent the following year (i.e. column (e) from previous year).
# "Approved GWRDC Budget" is the funding amount specified in the Project Agreement, including any approved Variations after Project commencement.

Was funding expended as approved in the Project Agreement? Yes ☑ No ☐

*If **NO**, please provide reasons:*

Has the cash and/or in-kind funding from Contributing Agencies been received and applied to the Project as approved in the Project Agreement? Yes ☑ No ☐

*If **NO**, please provide reasons:*

I hereby certify that this statement is true and accurate.

Signature of duly authorised representative) ..... Paul

PARUL RATHI — *Name:*
ACTING FINANCE MANAGER — *Title:*
18/10/13 — *Date:*