Characterisation of the wine meta-metabolome:

linking aroma profiles to yeast genotype

A thesis presented in fulfilment of the

requirements for the degree of

Doctor of Philosophy

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Table of contents

Abstract

This thesis outlines the development of a high throughput headspace solid phase micro-extraction gas chromatography mass spectrometry (HS-SPME GC-MS) method to analyse aroma compounds in white wine which are considered important to the overall aroma of the 'fermentation bouquet'. Important aroma compounds were determined as those which have previously been included in 'fermentation bouquet' aroma studies in white wine and have odour activity values (OAV) greater than 1; compounds which are found naturally at concentrations higher than their odour perception thresholds. The designed method was then used to create aroma profiles of fermentations formed with a *Saccharomyces cerevisiae (S. cerevisiae)* overexpression library to explore the relationship between the overexpressed genes and the aroma profile of the wine. The overexpression library includes approximately 1500 plasmids contained in an *Escherichia coli (E. coli)* host which required extraction and purification prior to transformation into a wine strain of *S. cerevisiae*. Multivariate analysis of the resulting datasets narrowed down the search field from ca. 1500 clones to 87 clones. After further analyses, a relationship between the genes overexpressed in the yeast and the aroma profile displayed was discovered, leading to a hypothesis for future research.

Chapter 1 of this thesis contains an introduction to the current literature relating to the formation and importance of aroma compounds in white wine and previous research in the area of metabolomics in regards to aroma compounds in wine. Chapter 1 provides the background knowledge to the research and provides context for the findings.

Chapter 2 details the research into the development of the HS-SPME GC-MS method required for this thesis. This chapter describes the proposal of a novel scoring system for choosing the correct

SPME fibre for volatile studies in young white wines. This scoring system is based on the coefficients of determination of the linear curve associated with standard curves formed in 10% ethanol solutions. Using this new method, two out of the five fibres studied were determined as the best for use in the development of a high throughput method to be applied in larger wine studies. The best fibres were chosen utilising the proposed novel scoring system to rate their overall ability to extract the compounds of interest while taking into consideration peak symmetry and sensitivity. This chapter was published in the *Australian Journal of Grape and Wine Research* in 2014.

Chapter 3 continues on from Chapter 2 and describes the final selection of the best fibre for use in studies looking at the 'fermentation bouquet' aroma compounds found in white wines. The selection of the 65 µm PDMS/DVB fibre was made after optimising the analytical parameters used in typical HS-SPME GC-MS methods with regards to the compounds of interest. This fibre was then used in the development and validation of a semi-quantitative method to use in high throughput analyses of 'fermentation bouquet' aroma compounds in white wines. The method was validated through a thorough examination of standard curves formed in three different media; a bag-in-box white wine, CDGJM-Leu fermentation using the parental strain to be used in the final overexpression screen with a blank plasmid (isoC9d ΔLeu + pGP564), and in 10 % ethanol model wine. The results showed that only one internal standard was needed for consistent results and that there were limited differences in the line of best fit seen for each aroma compound in the different media analysed. This chapter was published in the *Australian Journal of Grape and Wine Research* in 2015.

Chapter 4 follows the formation of the important aroma compounds in the 'fermentation bouquet' in white wine throughout the entire fermentation timeline in a CDGJM-Leu medium using the isoC9d ΔLeu + pGP564 yeast. This chapter outlines the similarities and differences of the progression of

the aroma compounds within the CDGJM-Leu as compared to previous real wine and other model media (MS300) studies. The results indicate that fermentations using CDGJM-Leu media using the isoC9d ΔLeu + pGP564 yeast show similar trends in the formation of aroma compounds as a conventional ferment, or a ferment with MS300, with the exception of the compounds related to the biosynthesis of leucine, which fluctuated in concentration until the end of fermentation. This chapter was accepted for publication in the *American Journal of Enology and Viticulture* in late 2015.

Chapter 5 describes the major research undertaking of this project. Specifically, the preparation and testing of the overexpression library of ca. 1500 clones, which was screened along with 20 commercially available yeast (Laffort) for their aroma profile. The library and commercial strains were set up as five replicates. The fermentations were followed according to fermentation progress of the parental strain and a set of replicates sacrificially sampled at the designated time-point, for analysis of total sugars (glucose and fructose) via enzymatic methods and then frozen until aroma analysis. The final sampling was performed at four days after the parental strain had finished fermentation. Only fermentations which were dry at each specific time-point were analysed for their aroma profiles. The results of the screen showed 51% of the library finished fermentation within the allocated time period and that of these 737 clones and 19 Laffort yeasts, we were able to show that 92 clones differed to the rest of the library with respect to their aroma profile. Of these interesting clones, 87 were overexpression clones and 5 were commercial yeasts. It was also hypothesised that for a yeast to retain its plasmid throughout the experimental fermentations, the LEU2 marker is not sufficient and a faster growth rate would increase the rate of plasmid rejection, hence more cells will die due to a lack of nutrients. For the plasmid to be retained, either a beneficial gene, or a gene which when overexpressed decreased vegetative growth also needed to be present. These findings will be beneficial for future studies using or creating overexpression libraries for fermentation studies.

Chapter 6 details the synthesis of deuterated analogues of the aroma compounds studied which could be used as internal standards in future quantitative experiments. This synthesis chapter also details a new green method for the synthesis of ethyl esters and acetates. This method is particularly useful as it describes the purification of each compound to the point where they are pure enough for use as internal standards, which is difficult to achieve due to the low boiling point and high volatility of the compounds within this study.

Chapter 7 completes the thesis by giving an overall summary of the important aspects of this study and its potential impacts on the wine industry. This chapter also proposes future directions and research studies to follow on from this comprehensive work.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Publications

Haggerty, J., P. K. Bowyer, V. Jiranek and D. K. Taylor (2014). "Comparative study on the sensitivity of solid-phase microextraction fibre coatings for the analysis of fermentation bouquet compounds." Australian Journal of Grape and Wine Research **20**(3): 378-385.

Haggerty, J., P. K. Bowyer, V. Jiranek and D. K. Taylor (2016). "Optimisation and validation of a high-throughput semi-quantitative solid-phase microextraction method for analysis of fermentation aroma compounds in metabolomic screening studies of wines." Australian Journal of Grape and Wine Research: **22**(1), 3-10.

Haggerty, J., V. Jiranek and D. K. Taylor (2016). “Monitoring volatile aroma compounds during fermentation in a Chemically Defined Grape Juice Medium deficient in leucine”. American Journal of Enology and Viticulture: in press.

Haggerty, J., V. Jiranek and D. K. Taylor (2016). Characterisation of the wine metabolome: linking sensory attributes to genotype *Journal of Applied Microbiology and Biotechnology,* To be Submitted.

Conferences/ Presentations

Crush Conference, 28th-30th September 2011, Adelaide

Presented a talk entitled: “The biggest fermentation exo-metabolomic study to date”

University of Adelaide School of Agriculture, Food and Wine Postgraduate Symposium, 5th-6th October 2011, Adelaide

Presented a talk entitled: “Developement of a new HS-SPME GC-MS method to be used in quantification of the most important 38 aroma compounds”

3-Minute Thesis Competition, University of Adelaide, School of Agriculture, Food and Wine, Department of Wine Science and Business, Department Round, 2010, Adelaide

Presented a talk entitled: “Characterisation of the wine meta-metabolome: linking sensory attributes to yeast genotype”

3-Minute Thesis Competition, University of Adelaide, School of Agriculture, Food and Wine, Department of Wine Science and Business, Department and School Round, 2011, Adelaide

Presented a talk entitled: “Journey to the Holy Grail of Wine Aroma”

9th Annual Conference of the Metabolomics Society, 1st-4th July 2013, Glasgow

Presented a poster entitled: “Characterisation of the wine metabolome: linking sensory attributes to genotype”

Presentation to Funding Bodies, 17th June 2013, Laffort Oenology, Bordeaux

Presented a talk entitled: “Characterisation of the wine metabolome: linking sensory attributes to genotype”

Supervisory Panel

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Abbreviations

2,3-butOH	2,3-butandiol
2,3-butone	2,3-butanedione
2MBacid	2-methylbutanoic acid
2MBOH	2-methylbutanol
2MBA	2-methylbutyl acetate
IBacid	2-methylpropanoic acid
IBOH	2-methylpropanol
IBA	2-methylpropyl acetate
2PE	2-phenylethanol
2PEA	2-phenylethyl acetate
3MBacid	3-methylbutanoic acid
3MBOH	3-methylbutanol
3MBA	3-methylbutyl acetate
Acetal	acetaldehyde
AA	acetic acid

Acetoin	acetoin
ca.	approximately
Benz	benzyl alcohol
Bacid	butanoic acid
BOH	butanol
CW	Carbowax©
CAR	carboxen
CDGJM	chemically defined grape juice medium
CDGJM-Leu	chemically defined grape juice medium without leucine
Decacid	decanoic acid
°C	degrees celsius
DVB	divinylbenzene
eV	electron volts
EIB	ethyl 2-methyl propanoate
E2MB	ethyl 2-methylbutanoate
E3MB	ethyl 3-methylbutanoate
EA	ethyl acetate

EB	ethyl butanoate
ED	ethyl decanoate
EDod	ethyl dodecanoate
EH	ethyl hexanoate
EL	ethyl lactate
EO	ethyl octanoate
EP	ethyl propanoate
GC-MS	gas chromatography mass spectrometry
g	grams
HS	head space
Hacid	hexanoic acid
HOH	hexanol
HA	hexyl acetate
hr	hours
L	litres
MetOH	Methionol (3-(methylthio)propanol)
µg	micrograms

mg	micrograms
µL	microlitres
mL	millilitres
mmol	millimole
Min-DO	minimal dropout media
min	minutes
M	molar
mol	mole
nm	nanometre
POH	n-propanol
Octacid	octanoic acid
OAV	odour activity value
OD_{600}	optical density at wavelength of 600nm
PDMS	polydimethylsiloxane
Pacid	propanoic acid
rpm	rotations per minute
SIM	selected ion monitoring

SPME	solid-phase microextraction
SIDA	stable isotope dilution assay