Characterisation of lees and Novel uses for Yeast Lees to Create New Wine Styles.

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Doctor of Philosophy

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Abstract

When wine is aged on lees, the process of lees autolysis causes the release of a range of constituents including mannoproteins, polysaccharides, amino acids and fatty acids that interact with the fermented wine leading to changes in the final flavour and sensory aspects of the wine. There is no doubt that ageing wines on lees for extended periods of time, adds beneficial organoleptic qualities to a finished wine. Furthermore, whilst the interactions of wine lees on phenolics, lipids and mannoproteins released have been widely studied, exactly how all these factors and constituents influence final wine quality is not totally clear. It can be said that there is still a poor understanding of how lees impacts on wine aroma. Moreover, the addition of autolytic enzymes to speed up lees breakdown or the addition of inactivated dry yeast (IDY) preparations to enhance the release of mannoproteins and glucans so that the perceived benefits of lees exposure are achieved in a shorter timeframe is only a recent advance over the last decade or so. Consequently, more research is needed in this field in order to further clarify the mechanisms and factors that lead to these perceived changes in a wine upon ageing on lees.

To this end, the Introduction for this PhD thesis provides a complete summary of the current state of play in terms of knowledge as to the scientific and practical potential of ageing wines on lees. It begins with a detailed account into the history of winemaking in Australia and the current state of play in terms of how Australian winemakers perceive utilising lees exposure to prepare their wine styles. Autolysis is a slow process and often is conducted over periods of years. Coupling this with the fact that wines are produced from a range of grape varieties; employ a range of different yeasts and utilise different winemaking protocols, results in a complex field of research study in which it can often be difficult to draw clear conclusions between the research studies and into the mechanisms and factors that lead to these perceived changes of a wine upon ageing on lees. Consequently, the Introduction provides a detailed account of all major research studies completed over the last few decades. The Introduction then concludes with an introduction to the aims of the research to be carried out within this PhD thesis.

Chapter two describes an exploratory study into the effect of lees exposure on both a Pinot Noir wine and a Chardonnay wine over a two-year period. A recent survey into current Australian practices showed that the ageing of Pinot Noir on lees is highly employed in Australia thus leading us to include this variety in this study. The use of lees ageing for the preparation of Chardonnay wines is typical throughout the World. Furthermore, the project was enlarged to include a number of wine and lees treatments (including the addition of commercial enzyme preparations). A range of chemical parameters were measured over the two-year period in order to evaluate further the importance of lees exposure on these wines. Some of the parameters measured, such as levels of polysaccharides or changes in colour, were those that have been shown to alter over time upon lees ageing, thus comparisons were able to be drawn between the work conducted here and that already reported in the literature. Moreover, we also examined other parameters which are not normally associated with lees autolysis such as possible changes in overall viscosity and metal concentrations in the wines to see if further information on how lees ageing impacts on a wines final quality could be gleaned. Unfortunately, the autolytic enzyme utilised in this study was found to be not very active at enhancing the autolysis rate in our wine treatments, thus the results found were not entirely as we hoped, although some new preliminary findings were captured. Clearly further highly focussed research is needed to unravel this complicated field of wine research.

Based on the results of the above study we then found ourselves in a position to devise a new alternative strategy to avoid the uncertainties associated with traditional lengthy lees exposure times, the results of which are described within Chapter three of this thesis. Instead of simply leaving the wines on lees for extended periods of time, we explored the concept that exposure of lees to microwaves for a short period of time may accelerate autolysis and when the treated lees is added back to a base wine for a short time, could the same perceived benefits of lees ageing be observed. This new technological approach for the preparation of new wine styles, coupled with both chemical evaluations and formal sensory trials was evaluated and it was found from our initial studies that there appears to be clear evidence that such a process leads to wines in which there is a perceived difference in the organoleptic properties of the microwaved lees wines when compared to the control wines. Further studies are needed in order to clearly define the key constituents that are altered in terms of their concentrations when such an approach is employed, however, it can be concluded that microwave assisted lysis of yeast lees appears to be a

new method for the accelerating of yeast autolysis and thus allows for a shortening of wine/lees exposure time needed to achieve the perceived organoleptic benefits of lees exposed wines.

Chapter four describes a study on the use of commercial inactivated dry yeast (IDY) preparations and their ability to alter the properties of a fermented Chardonnay wine and a browning model wine system containing (+)-catechin, iron(II), copper(II), and acetaldehyde aover a short period of time. Such yeast derived preparations are now being used as an alternative technique to ageing wines on lees, because they permit a quicker release into the wine of yeast compounds such as mannoproteins and glucans and as such the perceived benefits of lees exposure are achieved in a shorter timeframe. We analysed 10 commercial IDY preparations and evaluated their effectiveness at altering the base wines composition and the browning model wine system by a range of analytical measurements. Importantly, it was found that a range of the IDY preparations were able to substantially inhibit oxidative browning with the amount of soluble proteins being released correlating well with their preventative browning abilities. Such a finding is yet to be reported in the literature. Undoubtedly further scientific research will aid in unlocking the mysteries of IDY preparations and the host of positive effects that they can have on a finished wine.

Finally, Chapter five contains a detailed description of all the experimental methods and analyses utilised throughout these studies.

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, contains no material previously published or written by another person, except where due reference has been made in the text.

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Yuanyuan Wang

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Abbreviations

μM	Micromolar
11D	Lees racked off on the 11th day after primary fermentation
3D	Lees racked off on the 3rd day after primary fermentation
4-EP	4-Ethylphenol
4-EG	4-ethylguaiacol
5'-GMP:	5'-Guanosine monophosphate
5'-IMP	5'-Inosine monophosphate
AA	Amino acids
Alc	Ethanol concentration
ANOVA	Analysis of variation
AOC	Appellation d'origine contrôlée
AWRI 1503	Yeast strain *Saccharomyces cerevisiae* x *Saccharomyces kudriavzevii*
AWRI Fusion	AWRI 1502: yeast strain *Saccharomyces cerevisiae* x *Saccharomyces cariocanus*
BA	Biogenic amine
Be	Baume
BSO_2	Bound sulphur dioxide
C	Control
CCBG250	Commasie brilliant blue 250
CHAR	Wine made from Chardonnay grape variety
CIE*lab*	Commission Internationale de l'Éclairage
DAD	Diode array detector
DPPH	Diphenylpicrylhydrazyl
E	Enzyme
SEM	Scanning electron microscope
EU	European Union
FLD	Fluorescence detector
FRAP	Ferric reducing ability of plasma
FSO_2	Free sulphur dioxide
g	Gram(s)
GC	Gas chromatography
GRAS	Generally regarded as safe

GSH	Glutathione
h	Hour(s)
hL	Hectolitre(s)
HPLC	High pressure liquid chromatography
IDY	Inactivated dry yeast preparations
kg	Killogram(s)
KHT	Potassium hydrogen tartrate
L	Litre(s)
LAB	Lactic acid bacteria
M	Molar
min	Minutes
mg	Milligram(s)
mL	Millilitre
mmol	Millimoles
MLF	Malolactic fermentation
mM	Millimolar
MS	Mass spectrometer
Mw	Molecular weight
NMR	Nuclear magnetic resonance
non-GMO	Non-genomic modification organisms
OIV	International organisation of wine and vine
OTC	Ochratoxin
PDM	Yeast strain *Saccharomyces cerevisiae* (variety: *bayanus*)
PMS	Potassium metabisulphite
PN	Wine made from Pinot Noir grape variety
ppm	Parts per million(s), mg/L
PPO	Polyphenol oxidase
SD	Standard deviation
SEM	Scanning electron microscopy
SO_2	Sulphur dioxide
TA	Titratable acidity
TEM	Transmission electron microscope
KHT	Potassium hydrogen tartrate
Trolox	6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid

TSO_2	Total sulphur dioxide
VA	Volatile acidity
Vc	L-Ascorbic acid or vitamin C
v/v	Volume/volume

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In Adelaide, I had the darkest experience ever in my life. I wish I could live a healthier and happier life afterwards.