King Valley Vignerons

GROWING GREAT GRAPES

SEMINAR

Featuring

Dr Mike Trought

Marlborough Wine Research Centre
New Zealand



Shed Wine Show:
Sunday 2nd October 2005
Gracebrook Stables
King Valley. 11 am - 4.30 pm

Dinner:
Sunday 2nd October 2005
Swinburne Pavilion, Whitfield
King Valley. 7.00 pm

Seminar:
Monday 3rd October 2005
Swinburne Pavilion, Whitfield
8.45 am - 4.45 pm

King Valley Vignerons
Growing Great Grapes Seminar

Ripe for the picking

Bitterness and phenolics in grapes and wine

Nick Bulleid

Introduction

Grape phenolics are some of the most important components of wines. Although usually present in red wines in concentrations of less than six grams per litre, they have a role in the overall balance and wine style which rivals that of acidity and alcohol. They also play a role in the quality and style of some whites.
This paper will examine

- the sources of phenolics and how desirable phenolics may be achieved at harvest time,
- how the desired extraction levels may be achieved, as appropriate for the style of wine desired,
- how undesirable phenolics may be avoided or ameliorated, and
- the consumer's perception of phenolics.

It will examine these from the point of view of the winemaker/blender/taster.

Phenolics

Grape phenolics include the anthocyanins (coloured or colourless pigments), the tannins and a group of other related compounds such as catechins and flavonols. The desirable sources of phenolics are the skins and seeds and, to a limited extent, the stems (although other materials in the grape bin may also contribute unwanted phenolics). The fermenting of red wines on skins seeks to extract these into the finished wine. Whites are fermented without skins present, but must or juice handling before fermentation may be managed to increase phenolic levels when these suit a particular wine style.

Skin and seed phenolics

These are part of an extremely complex group of compounds, most of which are highly reactive. So complex are they that it is no exaggeration to say that most of what we know about their behaviour in wine comes anecdotally and from the experience of winemakers, rather than from scientific research. While research has demonstrated the behaviour of phenolic compounds in simple solutions, the changes in them during the ripening process and the reactions between hundreds of them in wine are difficult to characterise. Even a reproducible analytical technique for tannins is proving elusive (Smith et al, 2005).

Tannins are formed during berry growth and maximum concentration is reached by veraison and before colour development. The concentration of anthocyanins in skins has been found to increase with ripening after veraison to a maximum at about 22°

Brix, while overall polymeric material continued to increase two-fold to 26° Brix (Iland et al, 1998). Seeds were found to contain catechin, epicatechin and proanthocyanidin polymers. While catechin remained constant during ripening, epicatechin decreased by half from 19 to 26° Brix.
Seeds in fermenting wine have been found to increase the quantity of total tannins, catechins and total phenolics, and to increase stability of colour to SO_2, (Peng et al, 1998).
Winemakers relate green, bitter tannins to unripe grapes and particularly seeds, but it is not known whether this effect is caused by higher concentrations of bitter compounds in unripe grapes, the presence of specific bitter compounds or the increased masking effect of bitterness by other compounds as grapes ripen further (Herderich et al, 2004). Nevertheless, winemakers recognise that seeds with pale colour – lime green or blonde – and with grape pulp strongly attached produce wines with more bitterness than seeds with a dark tan colour, with pulp which separates well.
Winter et al (2005) surveyed 25 Shiraz vineyards in northeast Victoria and found relationships between size of canopy, vine balance, cane lignification, fruit exposure to morning sun and bunch-zone temperature with degree of flavour ripeness, bitterness and overall wine quality.
White wines present a different case entirely. With most varieties, it is important to avoid phenolic characters, as these can interfere with the balance and clean finish of the wine. Sunburnt grapes, as a result of exposure to strong sunlight, and long periods of skin contact for mechanically harvested grapes should be avoided, particularly with Riesling and Semillon. Some varieties, such as Chardonnay, Pinot Gris and Gewürztraminer can benefit from skin contact for increased flavour and texture, provided there is enough fruit intensity and body to maintain balance or when the phenolics can be ameliorated by fining or oxidative handling of the juice. Whole bunch pressing is effective when low phenolic extraction is required, as in sparkling bases and some styles of Chardonnay.
Whites which carry high levels of phenolics into bottle usually deepen in colour and develop quickly, becoming "blown out" in flavour and showing bitterness.

Ferment and phenolic extraction
During ferment skins and seeds are initially brought to the surface by bubbles of carbon dioxide gas but, as the pulp breaks down the seeds sink to the bottom of the ferment. Colour extraction from the skins is often complete when the ferment is about half-way through, but tannin extraction continues until close to dryness. Wines that are made for early drinking should therefore be pressed off skins earlier than those which are destined for bottle-age. It has been recognised (Singleton and Draper, 1964) that, when seeds are ripe, they may make a useful contribution to the desirable phenolics in the wine. However, unripe seeds can lead to high levels of bitterness, so early pressing is again recommended. Alternatively, their effect can be reduced by removing them from the fermenter. Flushing green seeds out from the sloping bottom of a fermenter as part of a rack-and-return process works well, but seeds should not be pumped out, as this will abrade the seed's wax layer increasing bitter tannins in the wine that is returned to the fermenter.
Don't allow seeds to be crushed, as this can lead to increased bitterness in wines.

If maximum extraction of colour and tannins is important, the winemaker should maximise the length of time on skins. This could include a cold soak before

fermentation, as is common with Pinot Noir and some Shiraz ferments. The benefit of extended maceration on skins after ferment cannot be predicted, except through the winemaker's experience with particular grape varieties and sources. While extended maceration may not necessarily increase total extraction, with some varieties, notably Cabernet sauvignon, it may reduce bitterness and improve phenolic texture and colour stability.

Stems, petioles and leaves

Stems are often included in red ferments in Europe to increase phenolics, notably with Pinot Noir in Burgundy, and Shiraz and Grenache in the Rhône Valley. However, these stems typically show some lignification and do not contribute as much bitterness as they may in Australia, where it is rare to find lignified stems. Inefficient destemmers can allow fragments of stem and pedicel to enter the ferment, leading to increased bitterness, particularly in Cabernet Sauvignon (which usually destems well, however) and Merlot.

Petioles in mechanically harvested grapes can lead to high levels of bitterness in wine. Winter et al, (2005) relate bitterness in several wines surveyed to petioles in mechanical harvester skips. In a recent classification tasting of 35 Pinot Noir ferments in Tasmania, the author found that several wines which showed higher levels of bitterness were made from mechanically harvested blocks which had begun to drop leaves in autumn, leading to increased petioles in the skips and the ferments. (Overall, phenolic ripeness was the single most important factor in determining the quality of these wines.)

Leaves in the ferment may also lead to increased bitterness.

Removal of bitterness

Bitterness and green tannins are best avoided in the first place, through appropriate vineyard management, fruit exposure and timing of picking. However, they can be reduced in wine through a combination of barrel ageing, splash-racking and micro-oxygenation.

Fining agents can also be successful.

The consumer's view

The author is not aware of any consumer research specifically designed to evaluate the consumer's preference in tannins. However, anyone who has poured wine at cellar-door or at an exhibition, such as *Wine Australia*, will know that one of the greatest attributes the uninvolved drinker can give a red wine is that it is "smooth". We can take that to mean that the tannins are even in the mouth, not aggressive and are in balance with the other components in the wine.

Good quality red wines at any price-point and from most varieties have tannins which coat the mouth and tongue evenly and continuously from start to finish, with no abrupt transitions. Green, unripe tannins often give a strong front-of-mouth harshness around the lips and a bitter sensation on the finish. (Some varieties, such as Cabernet sauvignon, Nebbiolo and Sangiovese obviously produce stronger astringency than others, but their wines should not be bitter.)

Red wines that age well in bottle, with tannins that are in balance with the other components of the wine, also start their lives as balanced wines. Young, harsh, bitter and unbalanced wines, develop into old that are harsh, bitter and unbalanced wines. Indeed, green flavours and bitterness remain after sweet, primary fruit flavours have diminished with age.

References

Herderich, M.J., Bell, S.J., Holt, H., Ristic, R., Birchmore, W., Thompson, K. and Iland, P.J. (2004) Grape maturity and tannins: the impact of viticultural treatments on grape and wine tannins. Proc. Twelfth Aust. Wine Ind. Tech. Conf. Melbourne, pp 79 – 84.

Iland, P.G., Peng, Z.K., Pfenning, G., Ford, C.M., and Høj. P.B. (1998) Changes in the phenolic composition of Shiraz grapes during ripening. Poster, Proc. Tenth Aust. Wine Ind. Tech. Conf.

Peng, Z.K., Waters, E.J., Sefton, M., and Iland, P.G. (1998) Effect of grape seeds on the phenolic profile of Shiraz wine: a preliminary investigation. Poster, Proc. Tenth Aust Wine Ind. Tech. Conf.

Singleton, V.L., and Draper, D.E. (1964) The transfer of phenolic compounds from grape seeds into wines. Am. J. Enol. Viticult. 12:152-158.

Smith, P.A., Herderich, M.J., and Pretorius, I.S. (2005) Unravelling the mysteries of red wine tannins. Aust. NZ Wine Industry J. 20(1): 18-24.

Winter, E., Lowe, S., Bulleid, N.C and Harvey, J. (2005) Sitting in the Morning Sun - Shiraz Benchmarking in northeast Victoria, 2005. Proc. *Growing Great Grapes* Seminar, Whitfield, King Valley, Vic.

The Australian Wine Research Institute

A Mouthful
Measuring Tannins in grapes and Red Wine

Paul A. Smith, Caroline J. Sarneckis, Patrik Jones, Bruce Kambouris*, Robert G. Dambergs and Markus J Herderich

Australian Wine Research Institute
Cooperative Research Centre for Viticulture
*McGuigan Simeon Wines

3 Streams of AWRI Tannin Research

- Broad knowledge of the principles associated with tannin and phenolics in red wine and grapes.
- Structure-function relationships between phenolics
  - Colour and sensory properties
- Analysis of phenolic compounds
  - Reference and 'field' methods
  - Targeted identification of wine pigments and tannins
- Validation of research concepts and methods
  - Viticultural and winemaking studies
  - Linking grape properties to wine composition



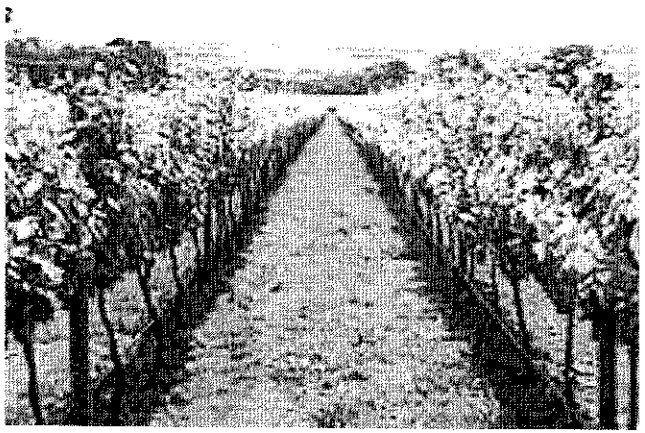
Introduction

- **Analytical method which is robust, simple and inexpensive**

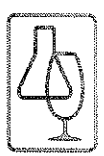
- **Allow wineries to have a better control of wine style**

You can't control

what you can't measure



Analytical measurements of grape and red wine phenolics



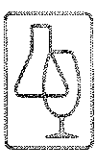
Previous Tannin Analysis methods

- Precipitation of tannins by proteins
- Precipitation of tannins by material other than protein
  - metals, polymers
- Colorimetric assays
  - Indirect and non-specific

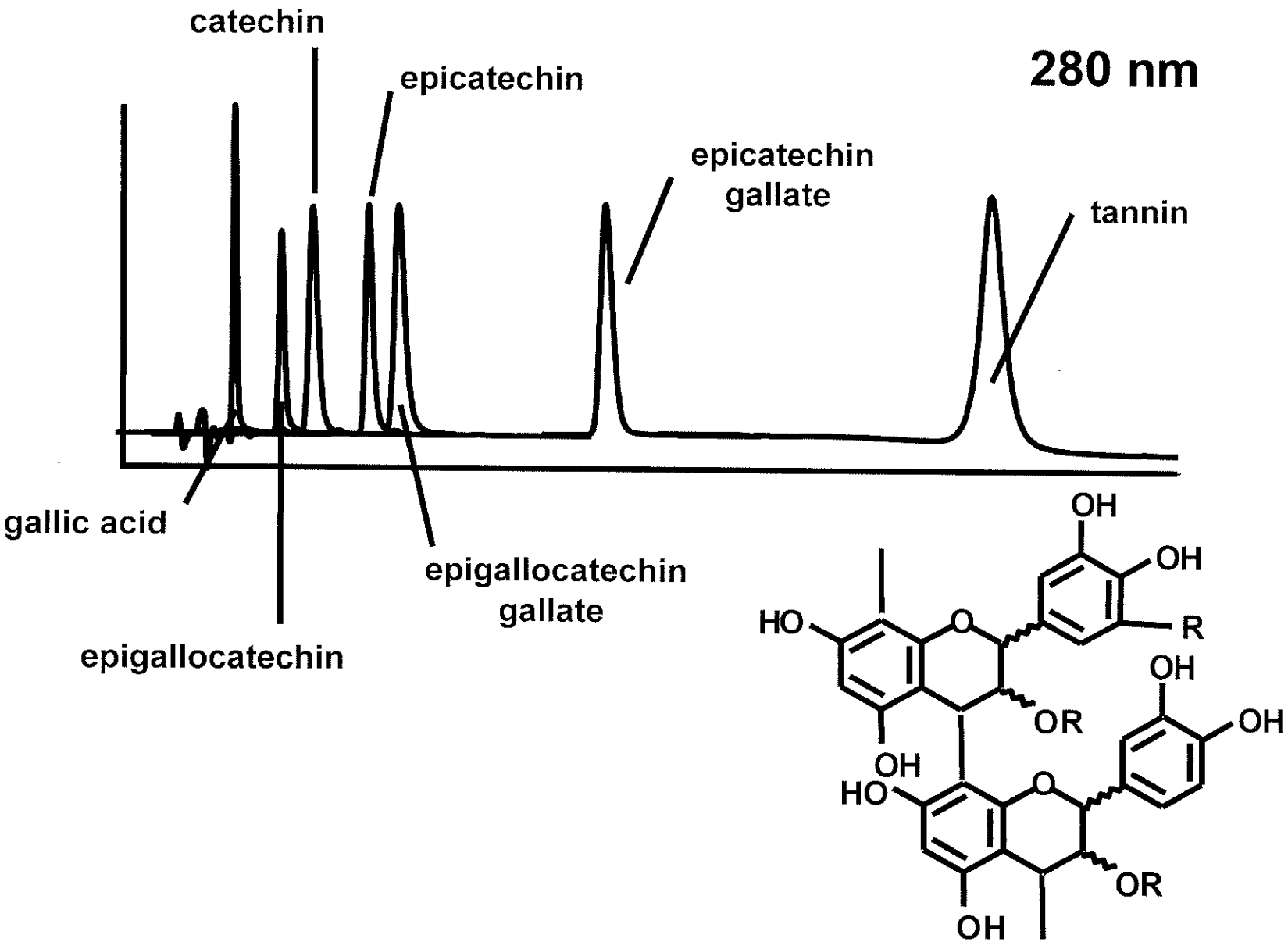
Challenges

- Can be complicated and laborious
  - Good research tools
  - Winery application mid-vintage?

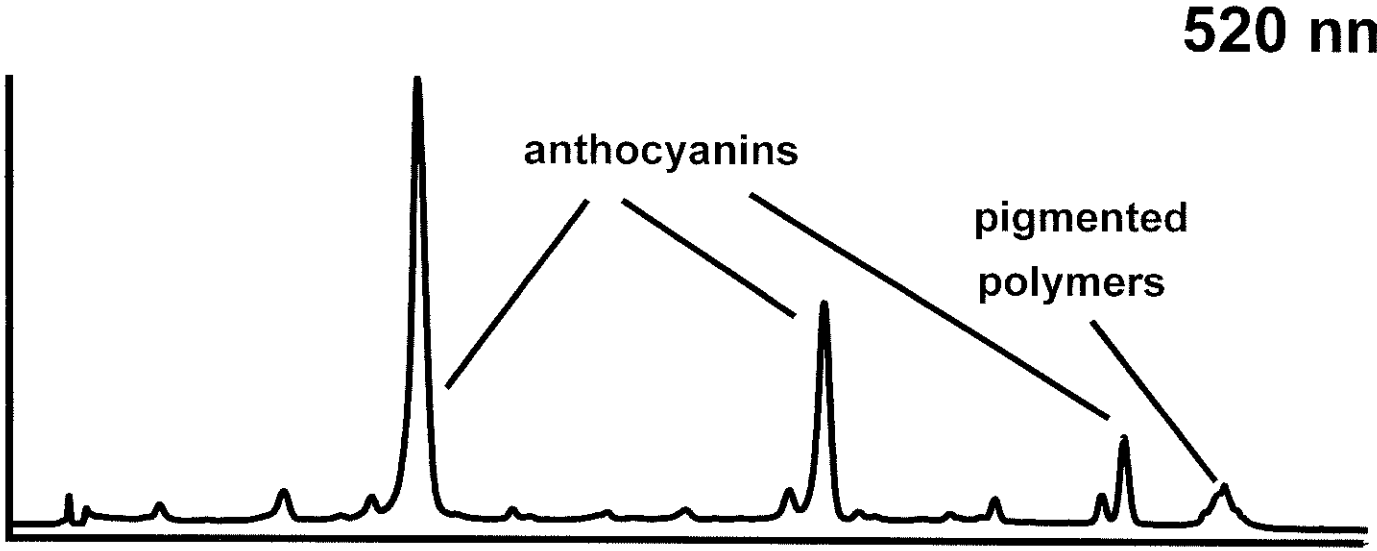
- Can be affected by pH, ionic strength and presence of organic solvents etc

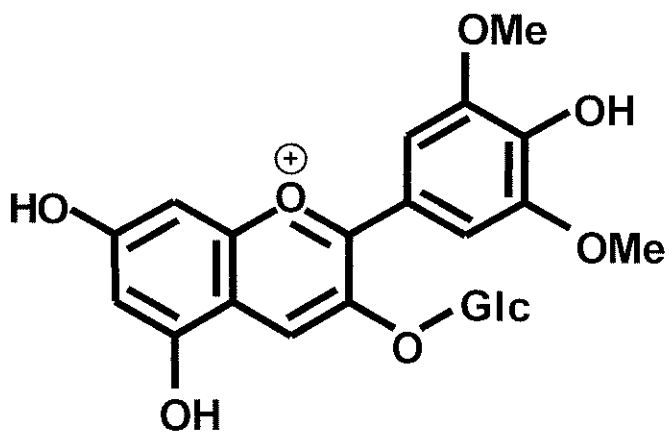


HPLC of phenolics



HPLC of phenolics

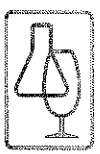




A quotation .....

"We can routinely measure sugar, acid and anthocyanins in grapes – we need a simplified, reasonably specific tannin assay to at least differentiate tannins from non-phenolic UV absorbing material and low MW phenolics, but ideally to differentiate skin and seed tannin"

Bob Dambergs, Riverlink Cabernet quality workshop 29 July 2003



Project aims

- This project aimed to further develop and validate an analytical assay to quantify the amount of tannin in wine and grapes
  - Red wine
  - 50% ethanol grape homogenate extract

- Allow for better understanding and control of tannins

- Aid decision making during winemaking including;
  - tannin additions
  - potential wine style
  - processing options

UV-Vis Tannin assay

- Measure A_{280} (i.e. total phenolics) of homogenate extract or wine
  - anthocyanins, flavanols and tannin

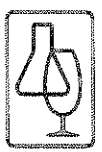
- Add polymer precipitant

- Measure A_{280} (post-precipitation and centrifugation)

A_{280} (total phenolics) - A_{280} (supernatant after precipitation of tannin) = A_{280} (tannin)

General protocol

- Prepare control and treatment sample in 10 mL centrifuge tube
  - tannin solution
  - polymer solution
  - saturated ammonium sulphate solution
  - Milli-Q water
  - Centrifuge for 5 minutes at 4000 rpm
  - Measure 280 nm absorbance of supernatant (A_{280} supernatant) and control (A_{280} control)

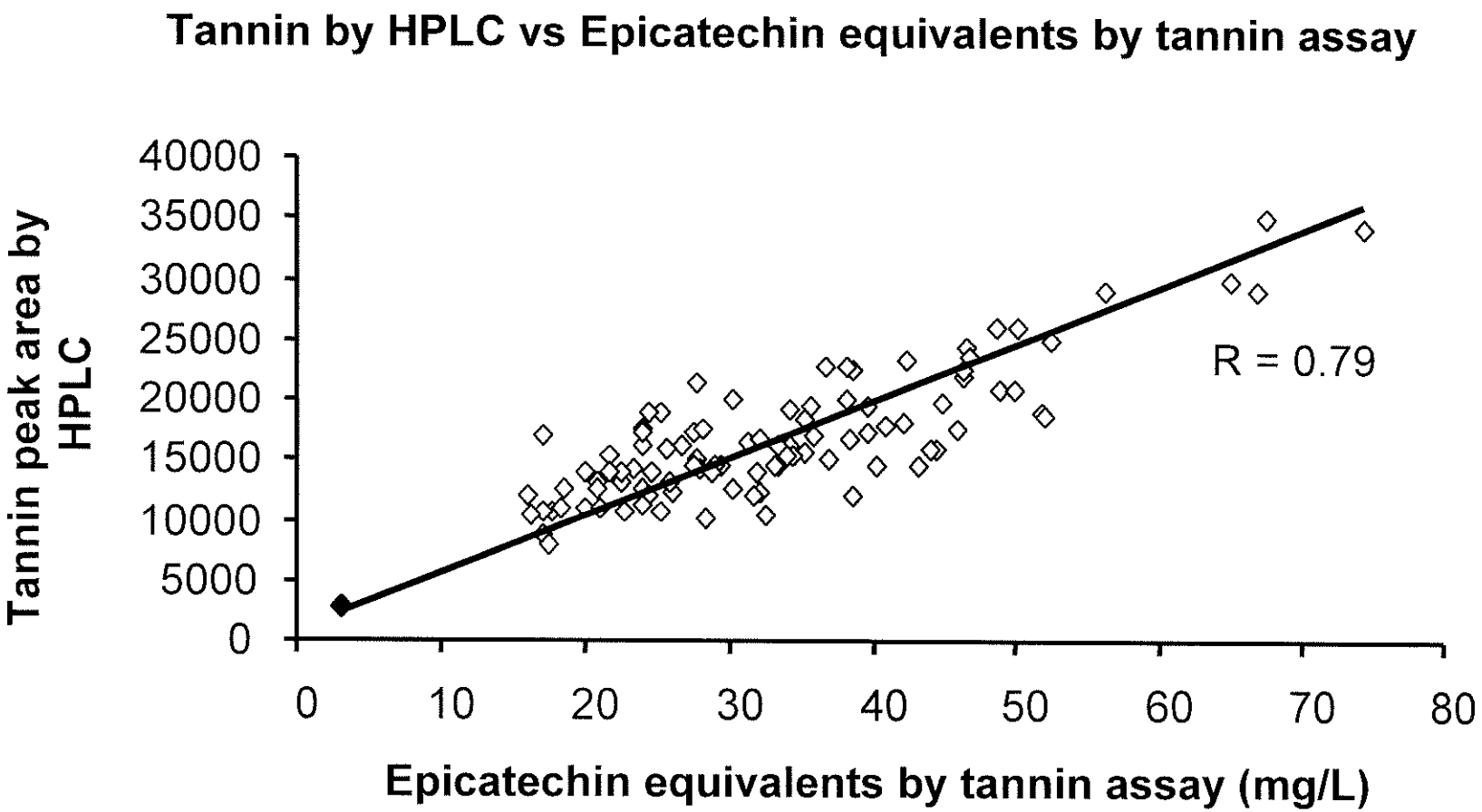


Red wine and Grape Homogenate Extract

- Complicated matrix
  - Anthocyanins
  - Sugar
  - Ethanol
  - pH

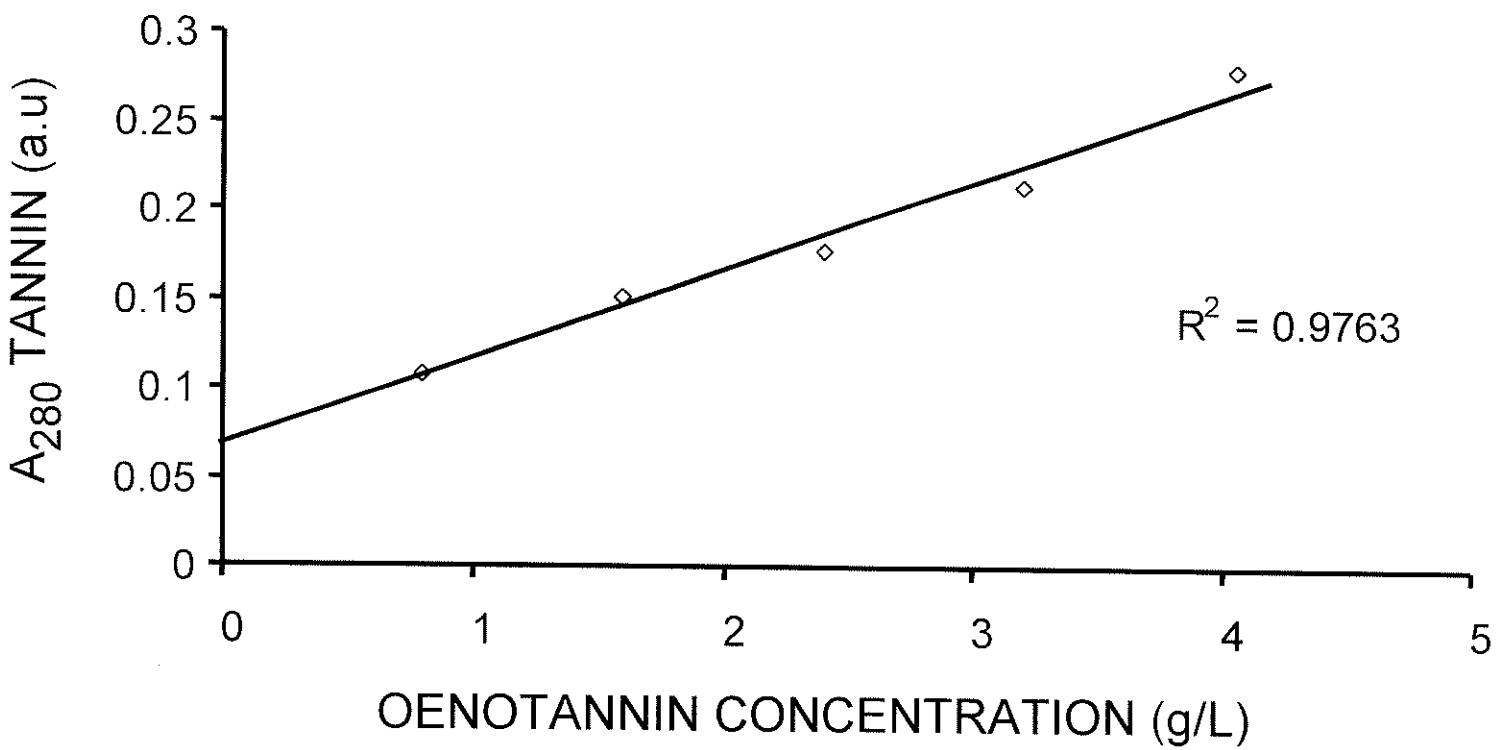
- Would the polymer:
  - Precipitate tannins in a complicated matrix;
  - Be selective and only precipitate tannins and not other A_{280} absorbing phenolics?

Tannin by HPLC vs Tannin by precipitation assay

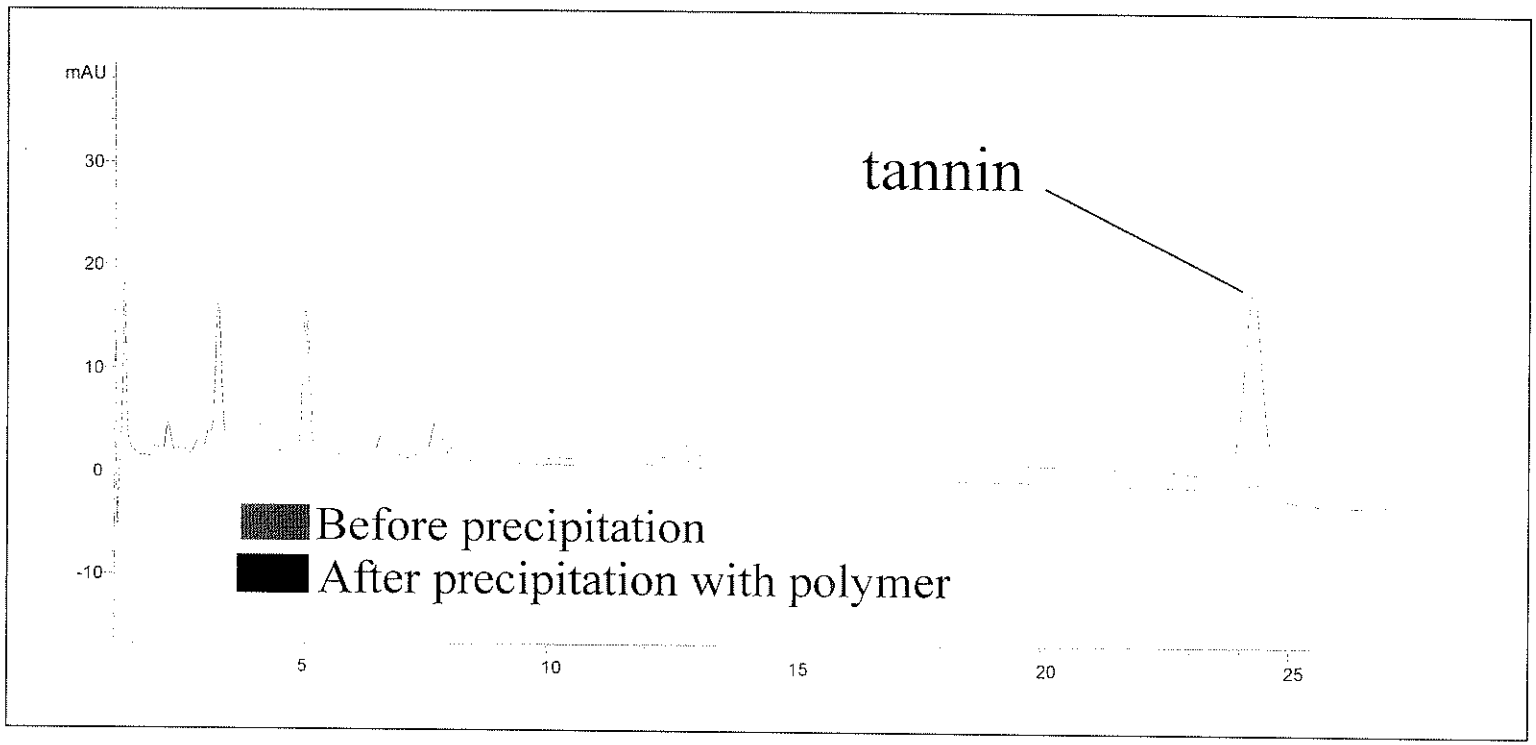


Recovery

50% ETHANOL GRAPE HOMOGENATE EXTRACT

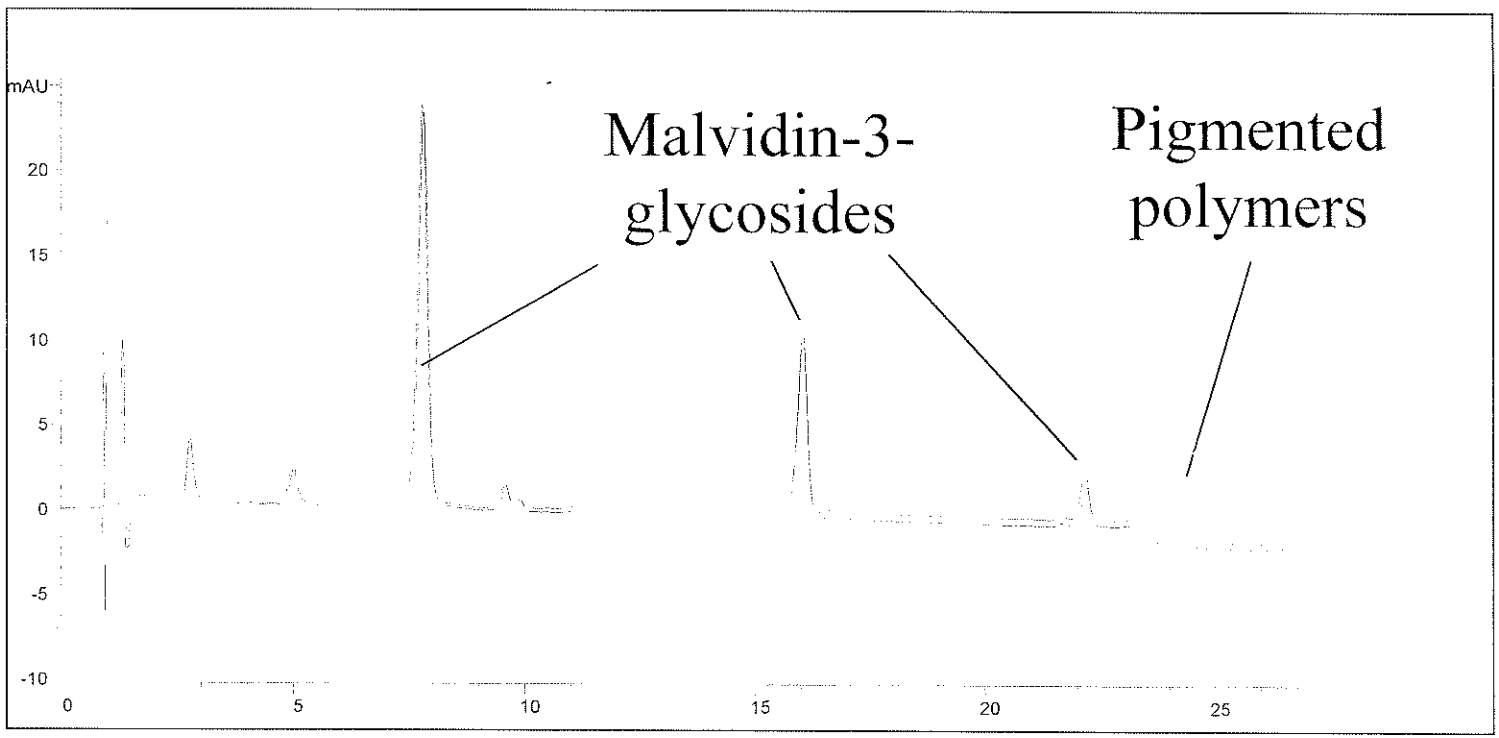


Model tannin



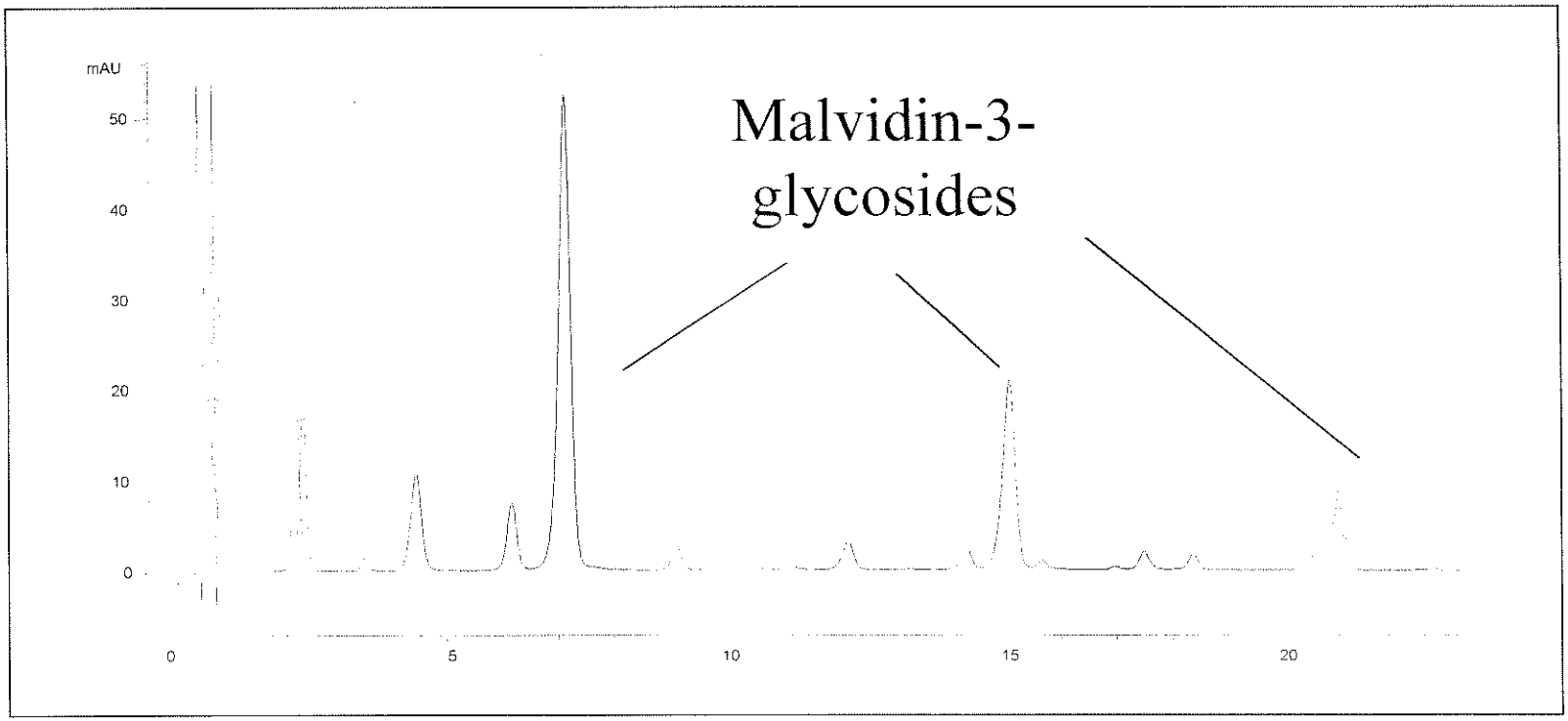
- **Assay performance was monitored using HPLC**
  - C18 column
  - Reverse phase

Red wine



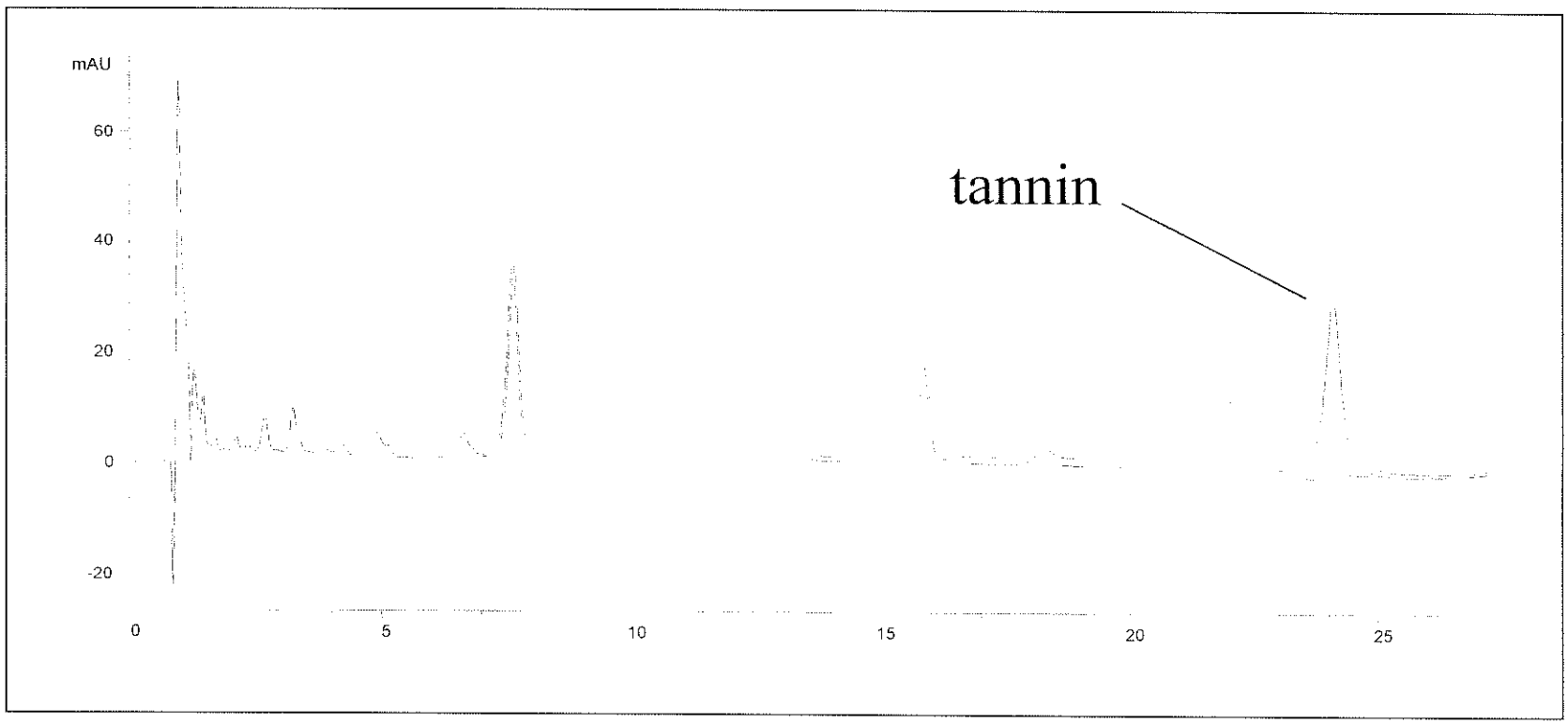
- **520 nm HPLC chromatogram**

Homogenate extract



- **520 nm HPLC chromatogram overlay**

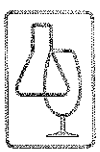
Homogenate extract



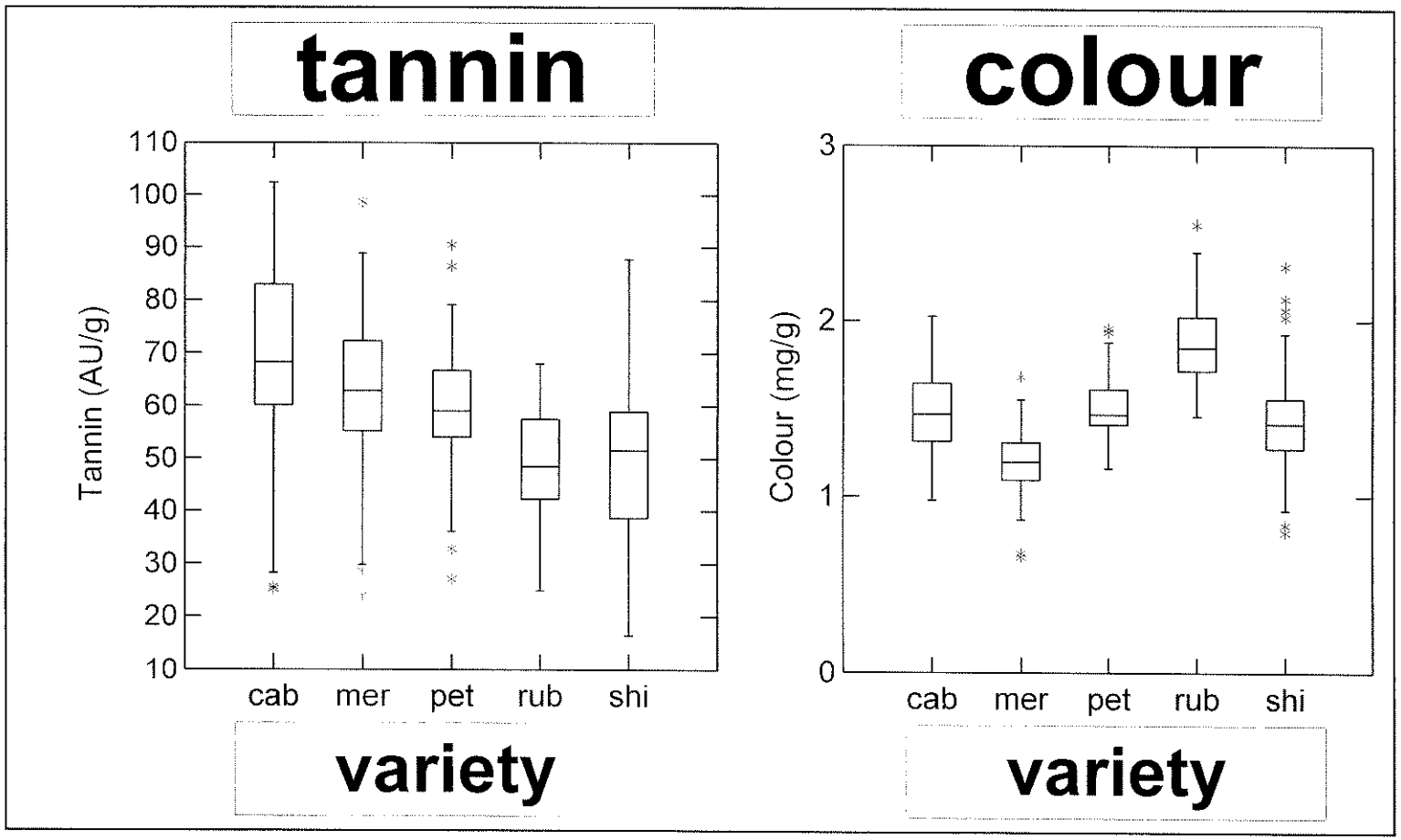
- **280 nm HPLC chromatogram**

We have a lot of colour data on grapes ;

We now have the ability to also accumulate a lot of tannin data but why do we want tannin as well as colour?

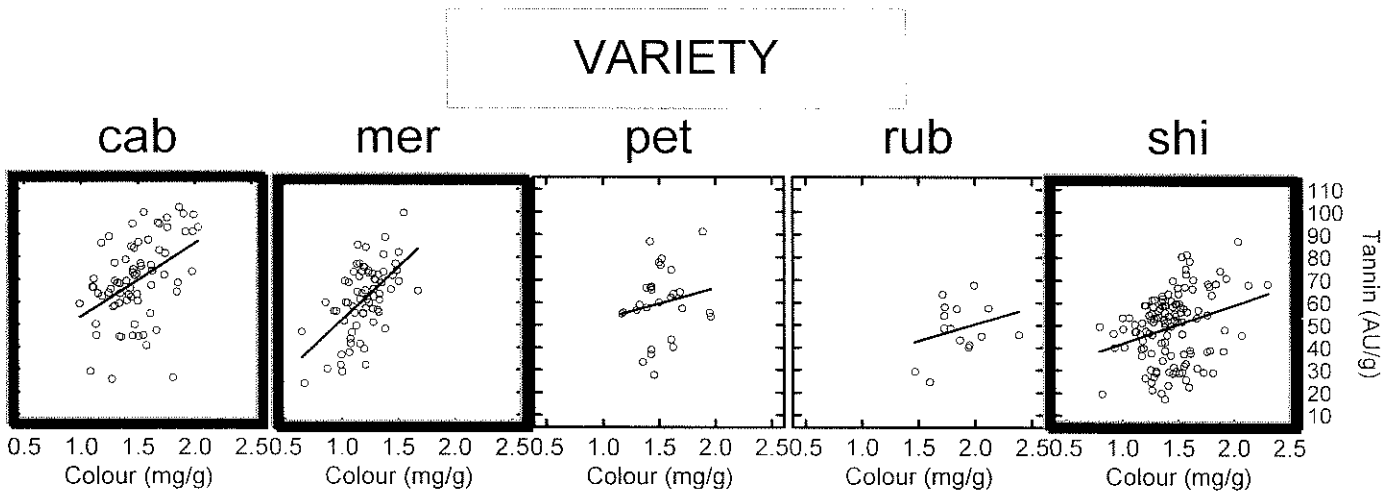


Tannin and Colour Vary With Variety



Bob Dambergs & Bruce Kambouris, Christine Casey, Carla Temby

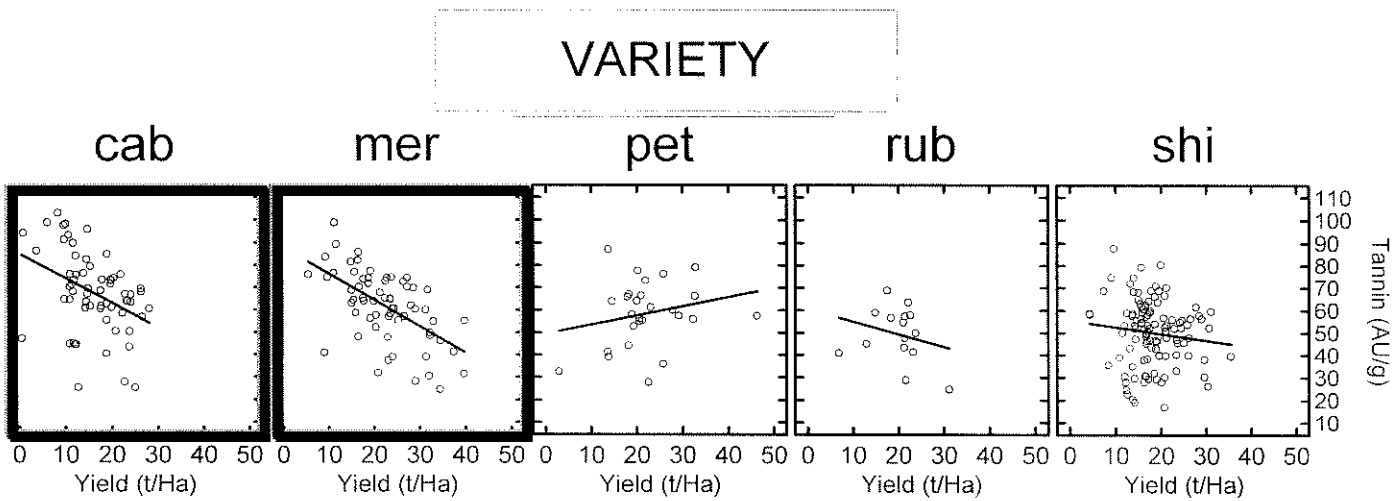
Grape Tannin x Colour



	cab	mer	pet	rub	shi
r	0.48	0.57	0.05	0.45	0.27
p	<0.001	<0.001	ns	ns	0.001

Bob Dambergs & Bruce Kambouris, Christine Casey, Carla Temby

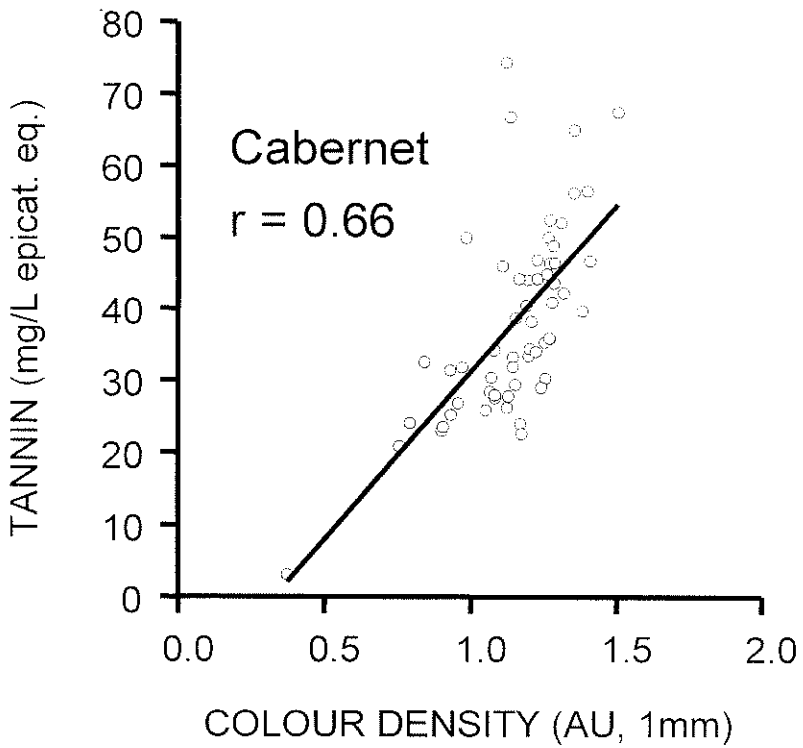
Grape Tannin x Yield

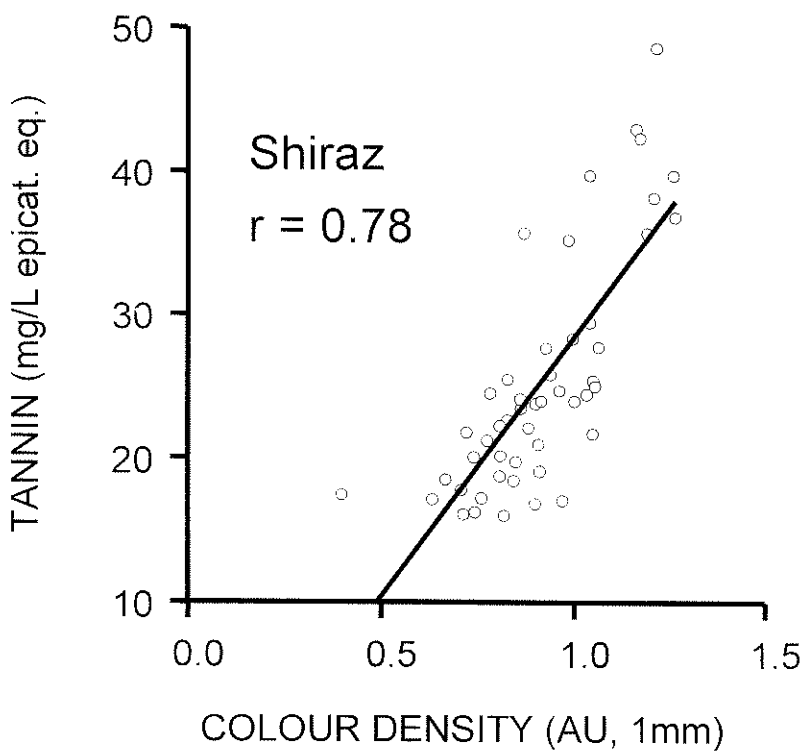


	cab	mer	pet	rub	shi
r	-0.44	- 0.56	0.22	-0.25	-0.13
p	0.001	<0.001	ns	ns	ns

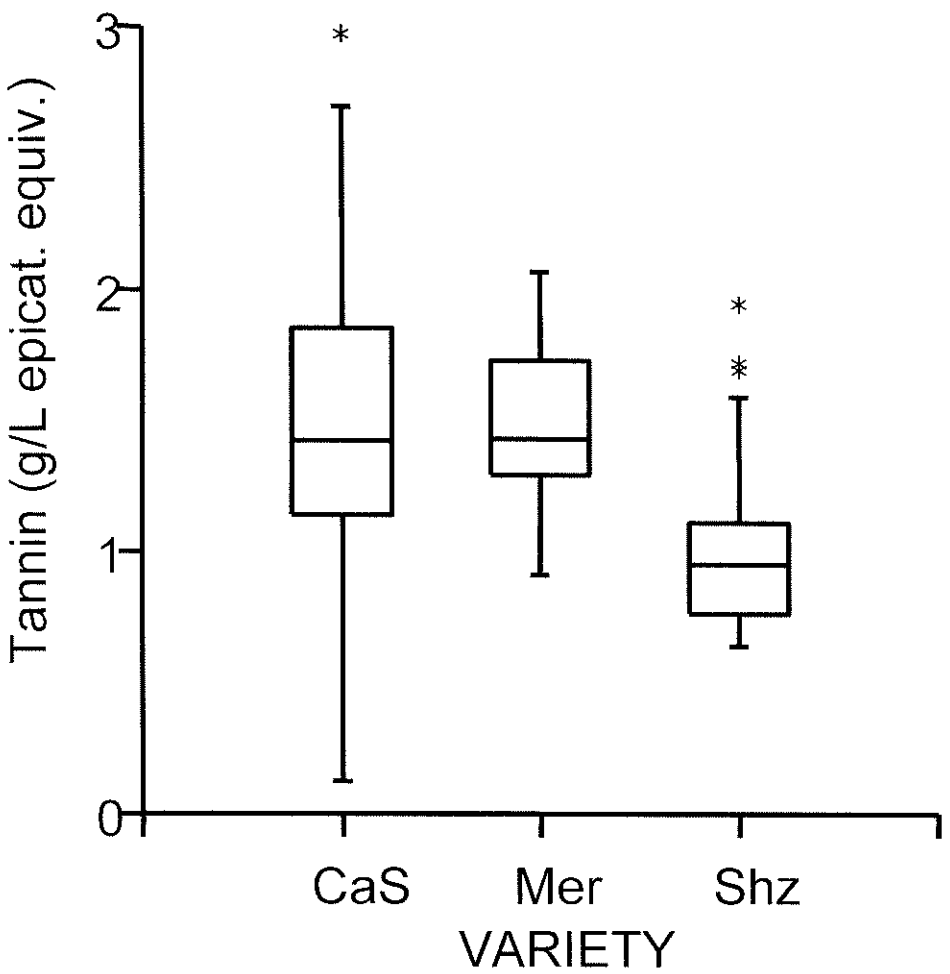
Bob Dambergs & Bruce Kambouris, Christine Casey, Carla Temby

Wine Colour Density and Total Wine Tannin

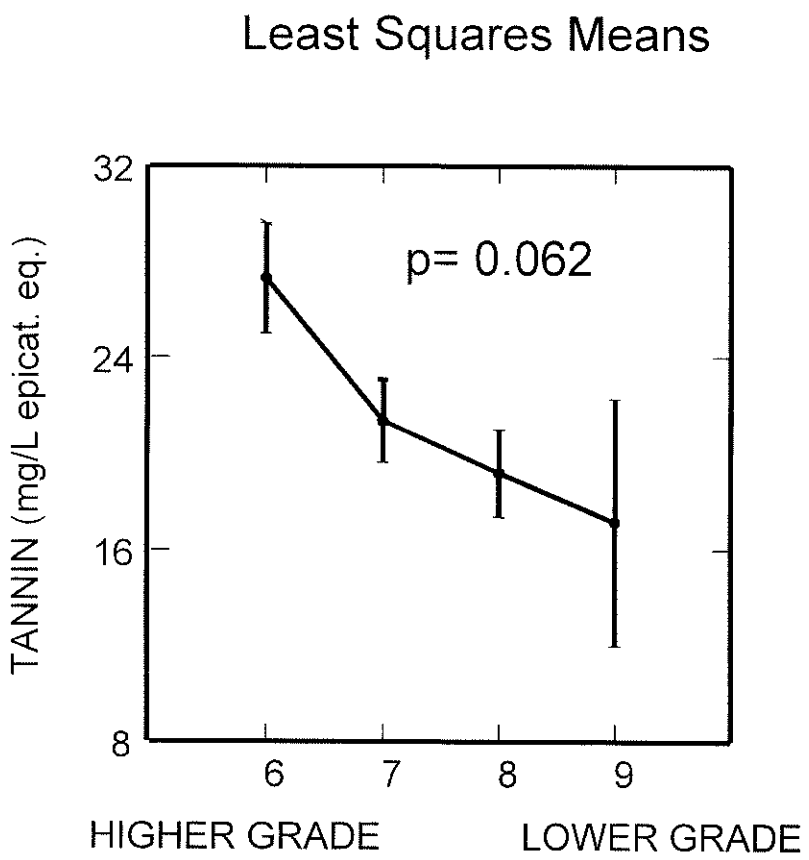


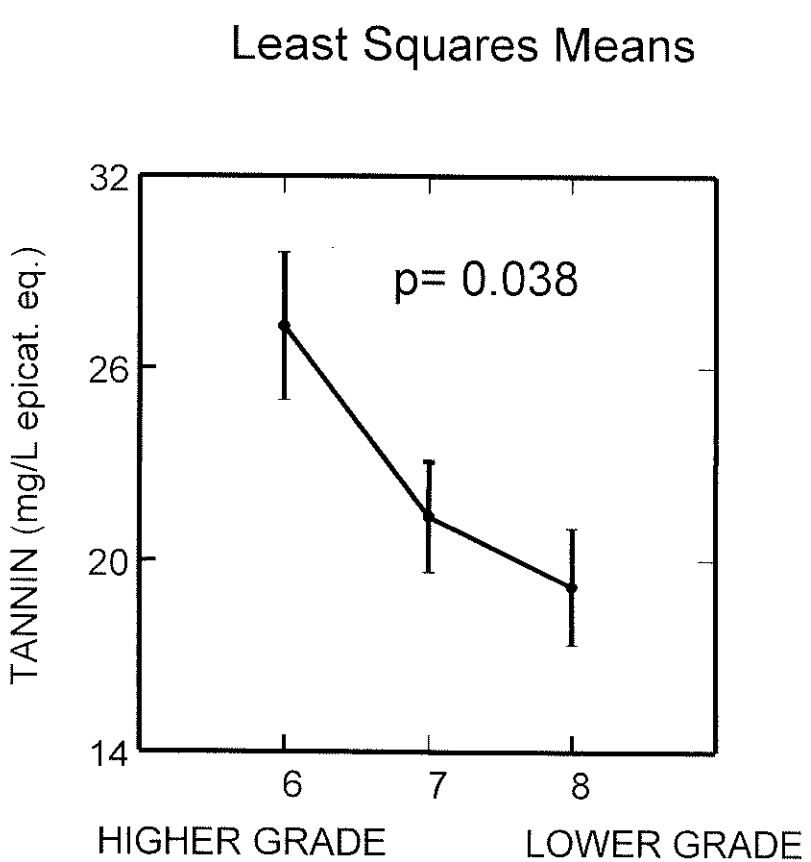


Wine Tannin and Variety



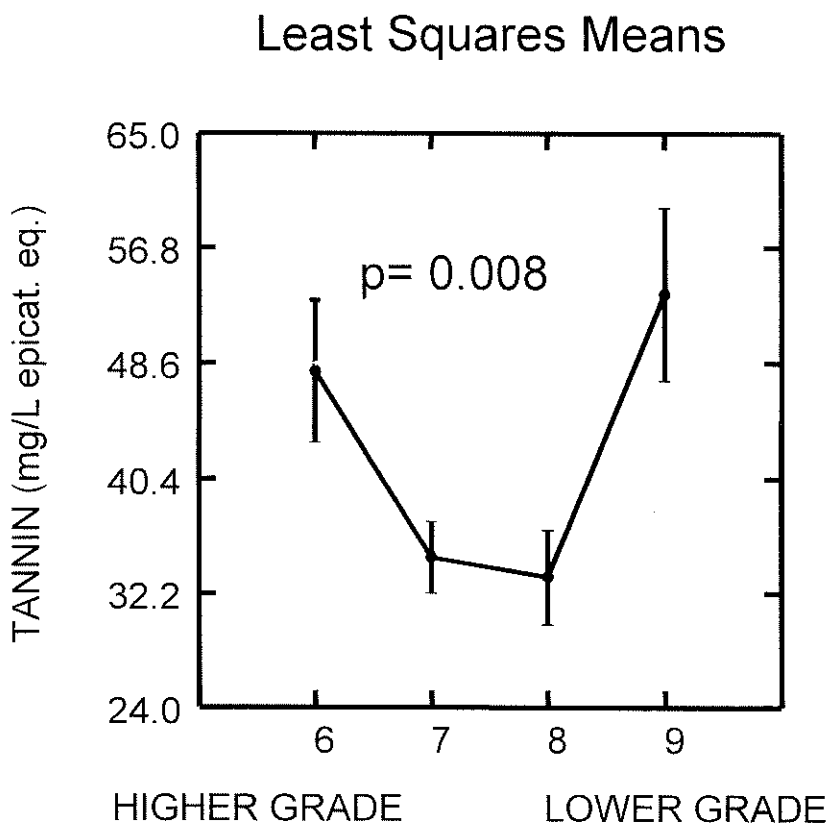
Shiraz:
Wine Tannin and Wine 'Grade'





'GRADE' = POST-VINTAGE WINE ALLOCATION

Cabernet:
Wine Tannin and Wine 'Grade'

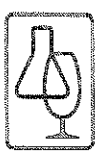


'GRADE' = POST-VINTAGE WINE ALLOCATION

Summary

- The challenge with tannin analysis stems from structural complexity and their similarity to other phenolics
- Presented a polymeric precipitation assay format that is
  - simple and robust
  - red wine, 50% ethanol grape homogenate extract
- Allows objective data for use in a winery setting for decision making and management
- Preliminary Australian data on grapes and red wine
- Tannin concentration, composition and extractability – grape & wine quality?

…the world of tannin awaits



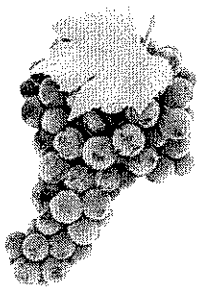
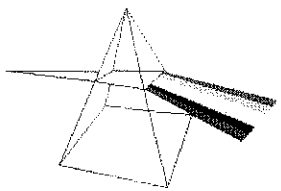
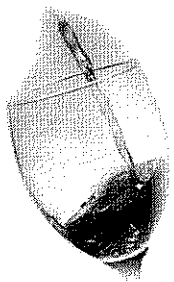
Acknowledgements

- KVV organising committee
- This work was financially supported by Australia's grapegrowers and winemakers through their investment body the Grape and Wine Research and Development Corporation, with matching funds from the Australian Government, and by the Commonwealth Cooperative Research Centres Program.
- McGuigan-Simeon Wines (Buronga Hill Winery)
- Hardy Wine Company
- AWRI Rapid Analytical Methods team
- AWRI Tannin team



The Australian Wine Research Institute

The relationship of grape and wine colour with quality

Bob Dambergs

Growing Great Grapes
King Valley
3 October 2005

Acknowledgments

Many wineries who have participated with our projects but in particular:

Hardy Wines: Chris Bevin, Kylie Goldsack, Audrey Lim

McGuigin-Simeon: Bruce Kambouris, Brenton Porter

HARDY
WINE COMPANY

The AWRI RAMS

"Rapid Analytical Methods" team



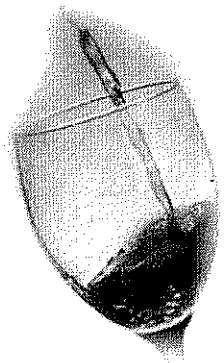
Wies Cynkar Mark Gishen Daniel Cozzolino
Bob Dambergs Les Janik

This work was financially supported by Australia's grape growers and winemakers through their investment body the Grape and Wine Research and Development Cooperation, with matching funds from the Federal Government, and by the Commonwealth Cooperative Research Centres Program.

GWRDC

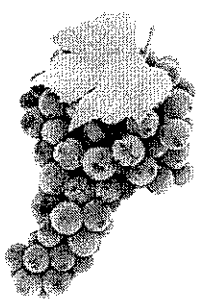
CRCV

Grape and wine quality

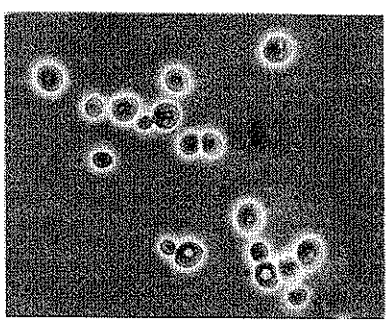


There are many factors that will determine and describe the final wine quality involving complex interactions between:

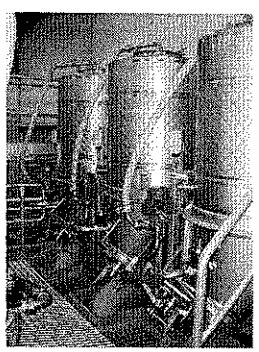
grapes



yeast

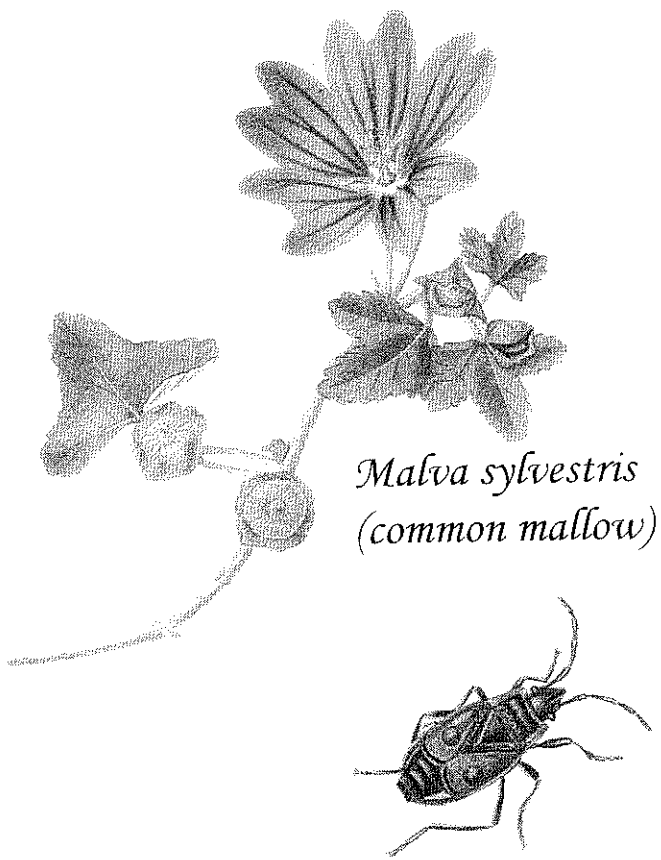


the winemaking process



But I will concentrate on grape and wine colour

- What is grape colour?
- Why is colour important?
- An overview of colour analysis methods by NIRS

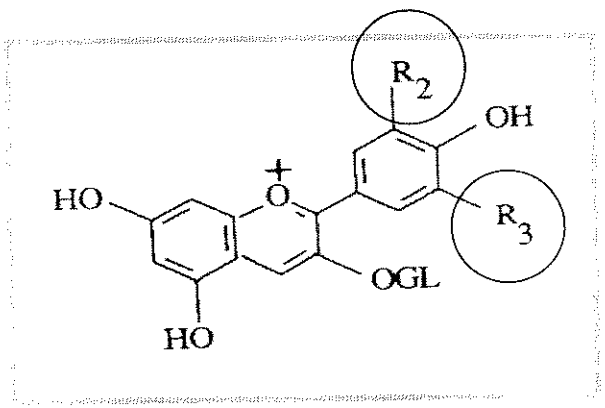


What is grape colour?

Colour is due to anthocyanins which occur in many plants and are a major source of natural colour

Malvidin – the major anthocyanin in grapes derives its name from the common mallow from which it was first identified

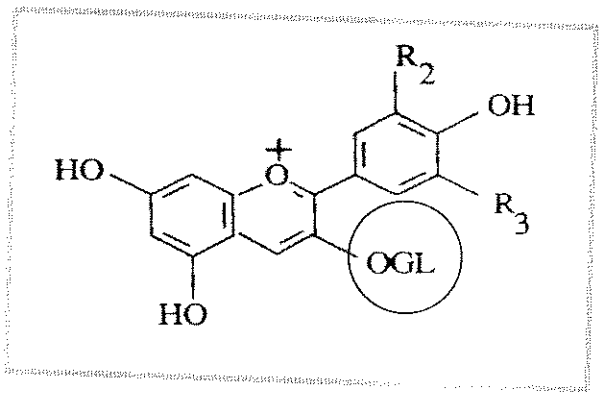
The structure of anthocyanins



Different types depending on the number of hydroxyl groups and the degree of methylation

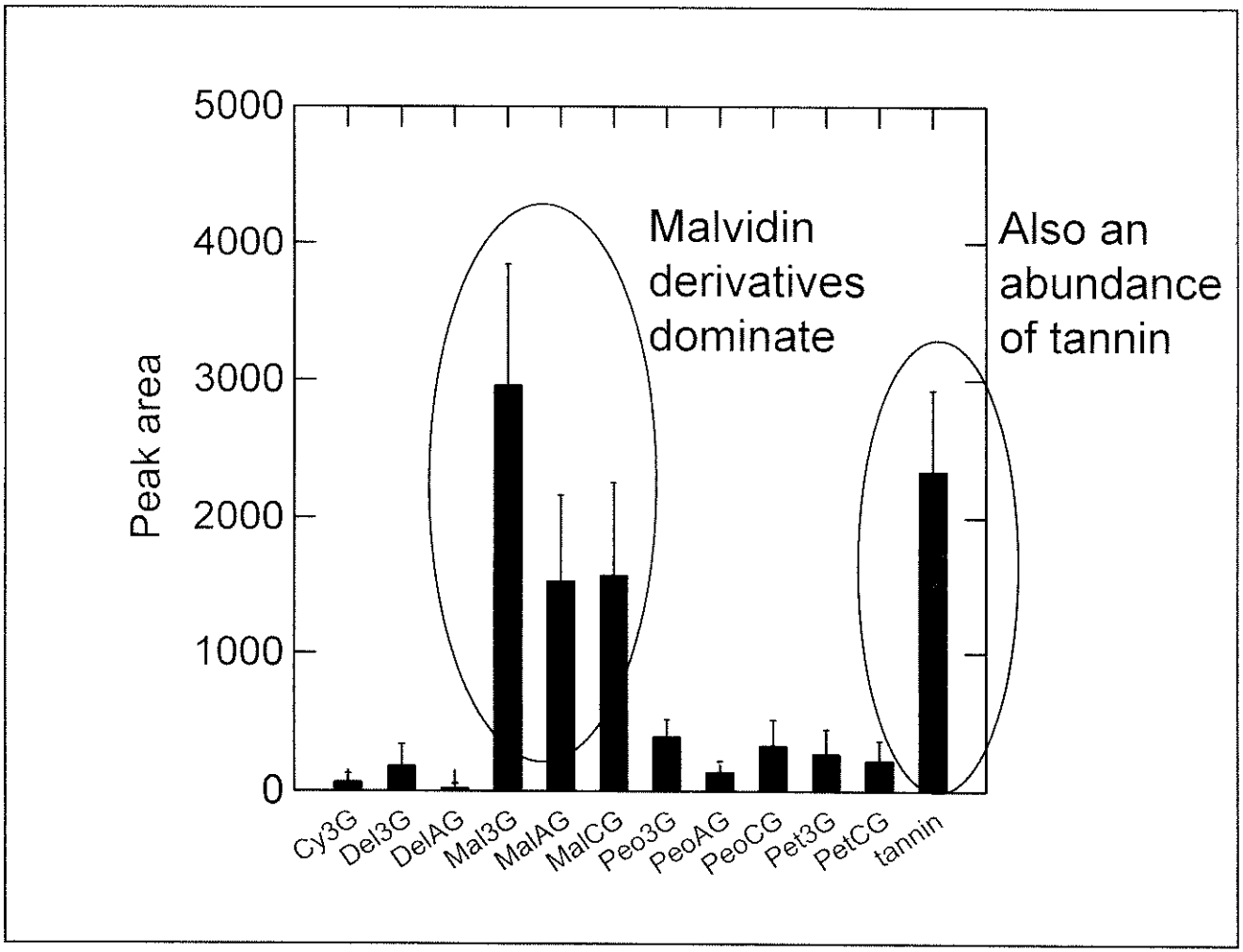
	R_2	R_3
Cyanidin	OH	H
Peonidin	OMe	H
Petunidin	OMe	OH
Malvidin	OMe	OMe

Anthocyanins normally occur as glucosides

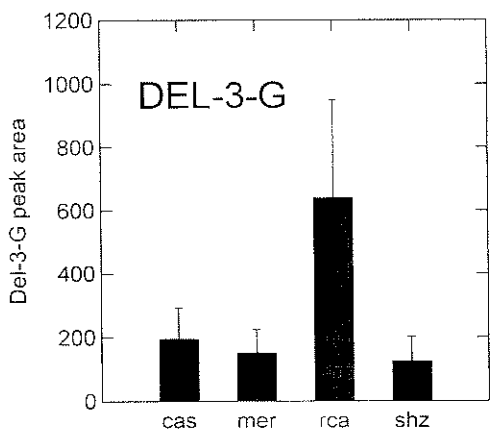


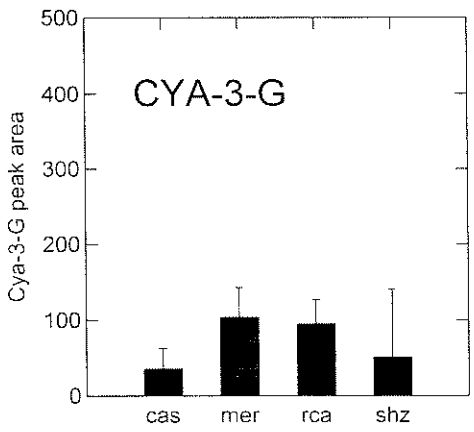
- Anthocyanins normally have a glucose molecule attached via a glycosidic bond
- Glucosylated anthocyanins are more stable
- Anthocyanins contribute to the GG content of red grapes
- Anthocyanins also exist as acetyl and courmaryl derivatives

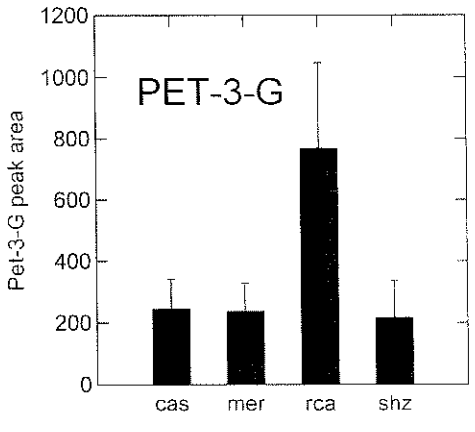
Types of anthocyanins in grapes?

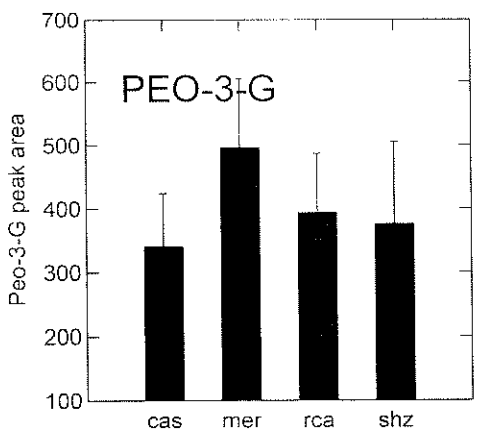


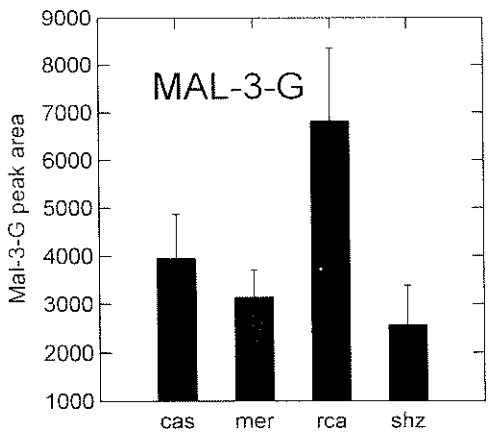
Anthocyanin glycosides by variety











Anthocyanin profiles vary with variety

Where is colour in the grape berry?

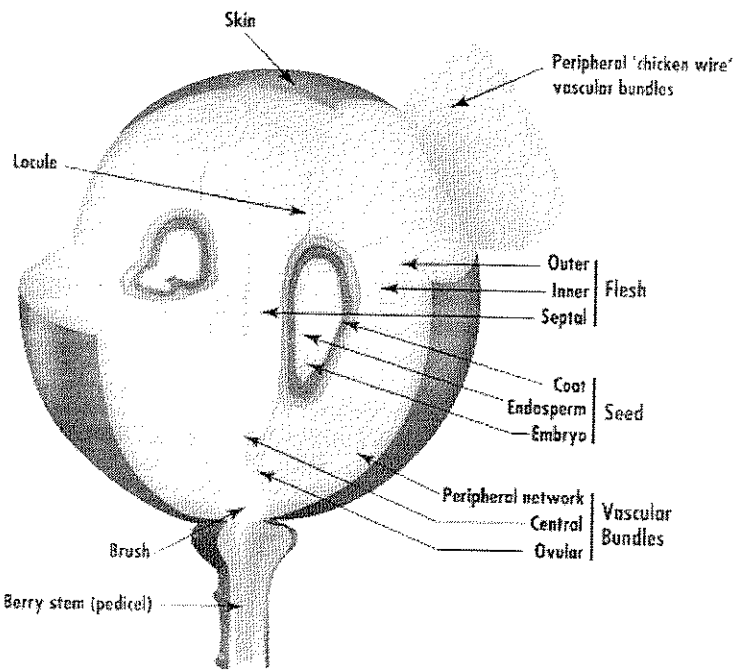


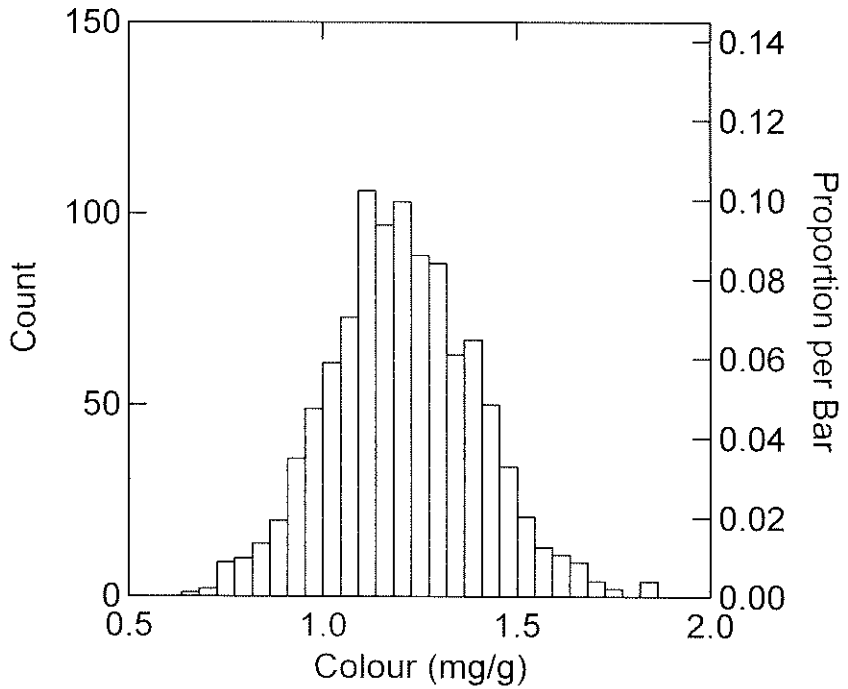
Figure 1: Structure of a ripe grape berry partially sectioned on the long and central axis to show internal parts. Illustration by Jordan Koutroumanidis, Winetitles.

- Anthocyanins are located In the skin of Vinifera grapes

The implications of this are:

- increasing the skin to flesh ratio will concentrate colour and flavour
- the original skin to flesh ratio must be retained for accurate measurement of anthocyanin concentration in whole grapes

Colour increases with ripening but……



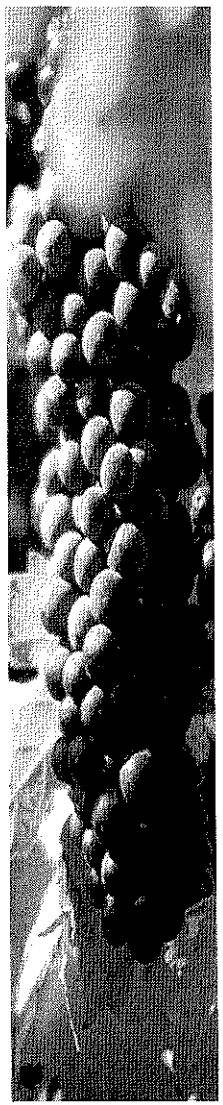
Grapes at the same Brix (24 – 24.5°) from different vineyards do not necessarily have the same colour

A very good reason why we should measure colour as well as Brix


Botryt
(also
Sh
h
incubated in
(Eile
TSS
(°Brix
Control
Inoculated
Uninoculated
(A520)
olics (AU)

Some ‘take home’ messages about colour development in grapes

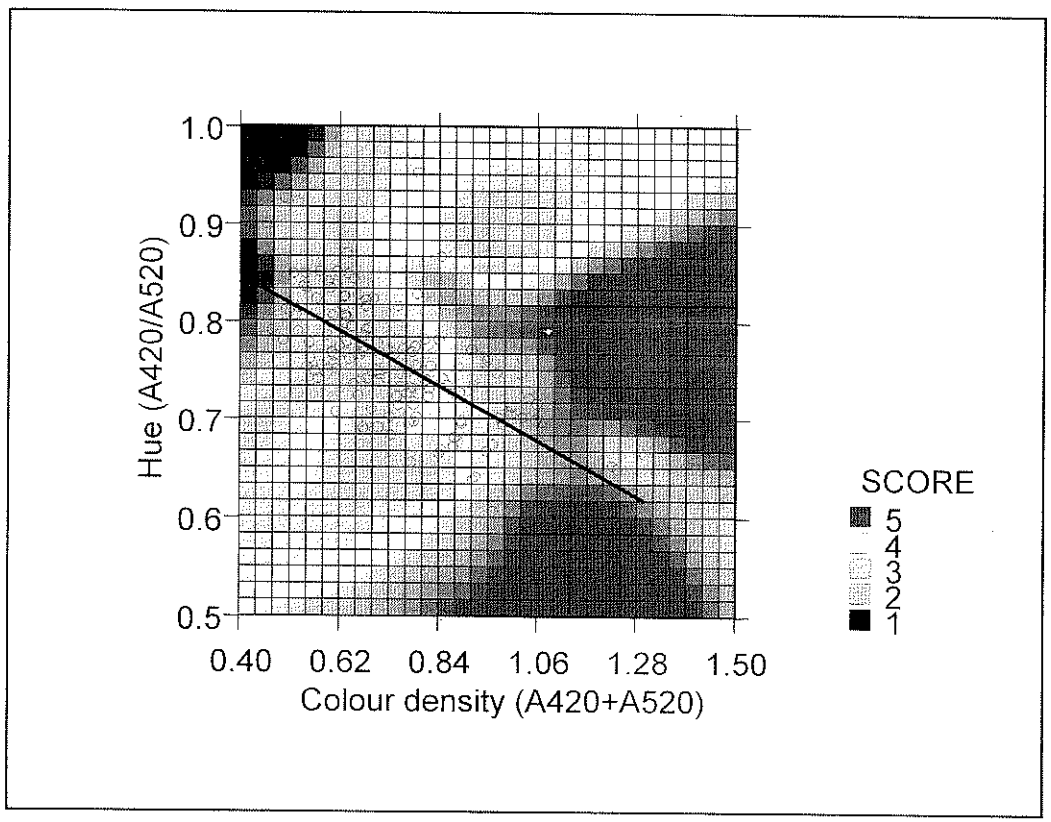
- For a given patch of grapes, colour correlates strongly with Brix, but this does not necessarily apply when comparing different patches
- Colour is in the skin, so for berries with the same amount of skin colour, overall colour concentration (colour per gram fruit weight) increases with smaller berries
- After berry ‘abscission’ colour can increase in concentration due to dehydration – the actual amount of colour per berry can decrease during this phase
- The best quality fruit is most likely to be when sugar, acid, colour and tannin reach optimal levels at the same time and before berry abscission (i.e. early ripening)



Part 2

- What is grape colour?
- Why is colour important?
- An overview of colour analysis methods by NIRS

There is a lot of data showing that wine colour correlates with wine quality e.g.

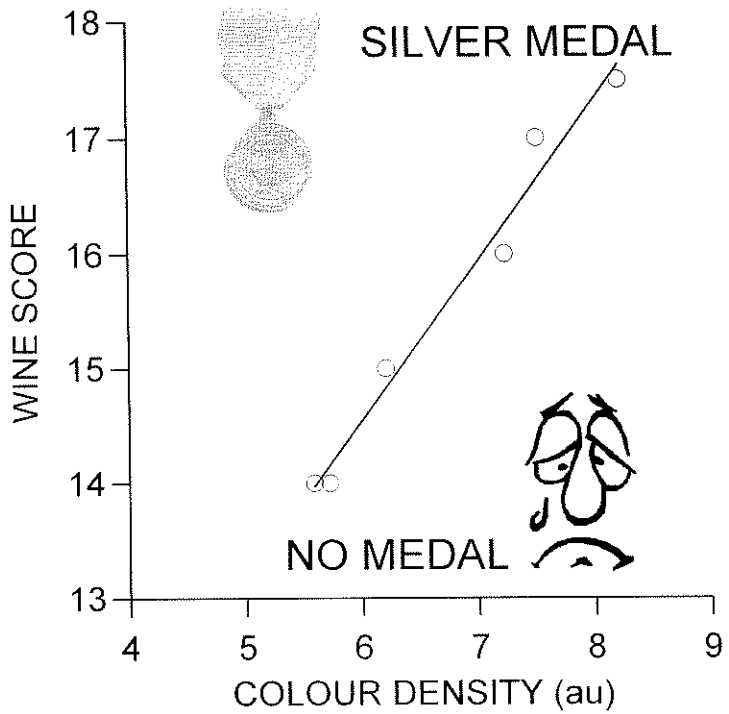


1999 Riverland Shiraz: 3D mosaic plot relating colour density, hue and commercial wine grading

1 is lowest quality

5 is highest quality

Wine score and colour density



Winemaker's scores
-averages of duplicate wines, tasted blind

(Garth Swinburne, Sunraysia Shiraz)

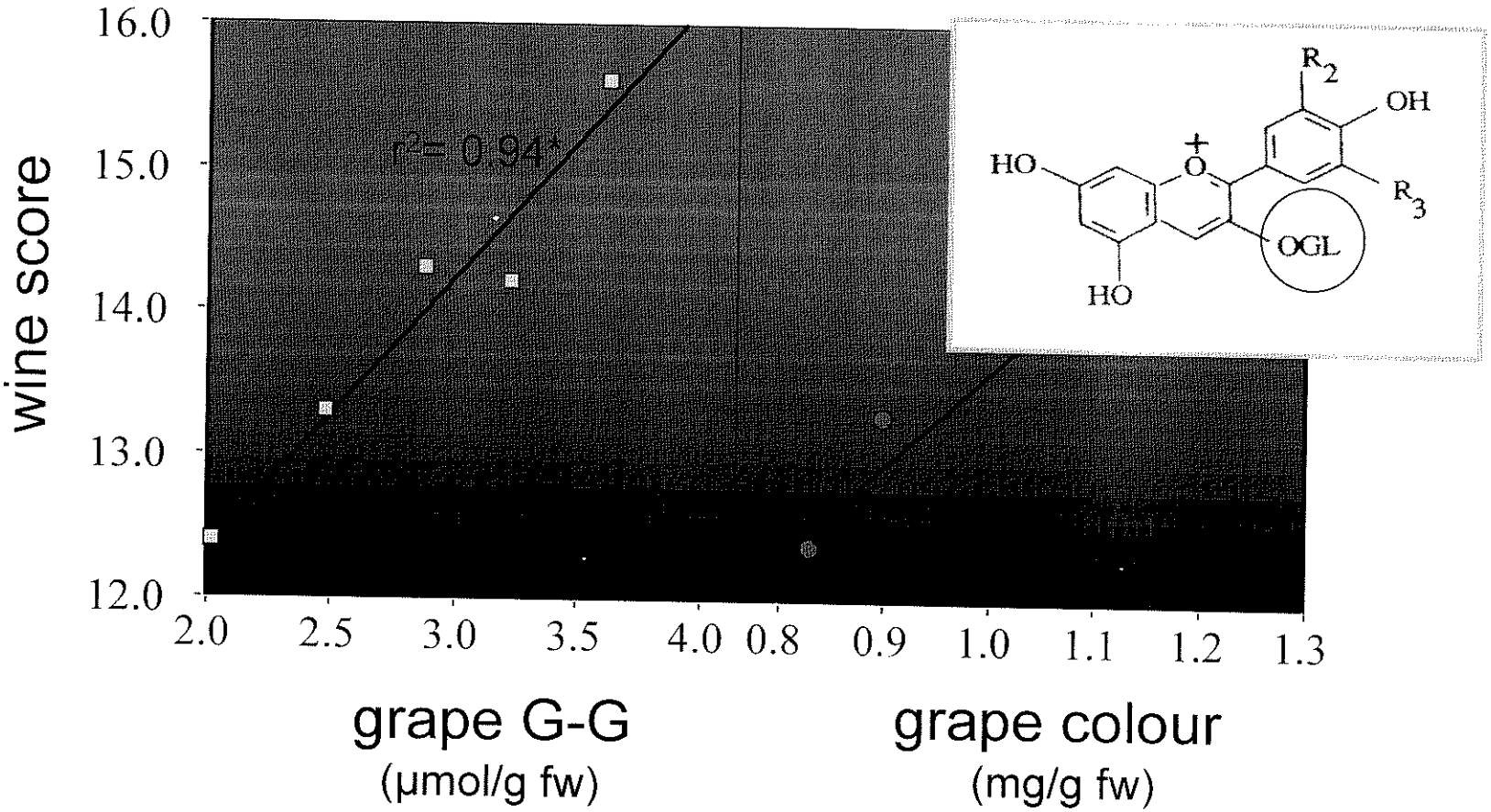
Scholefield Robinson
HORTICULTURAL SERVICES

Grape colour and glycosyl-glucose correlate with wine quality rating

Francis et al, AWITC 10

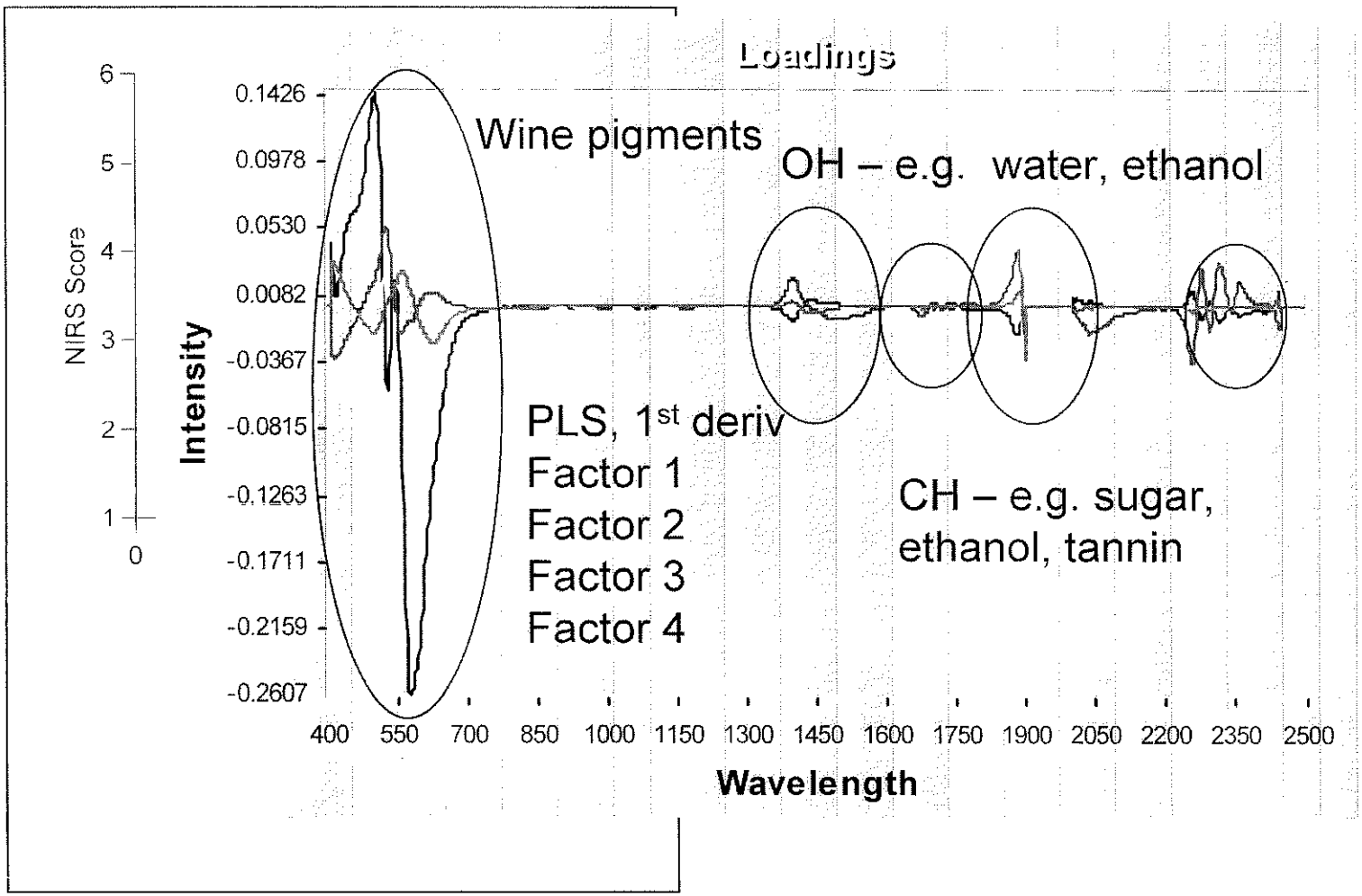
1995 Riverland Shiraz Trial
22.6 – 23.3 °Brix

but anthocyanins contain GG so just measure colour?

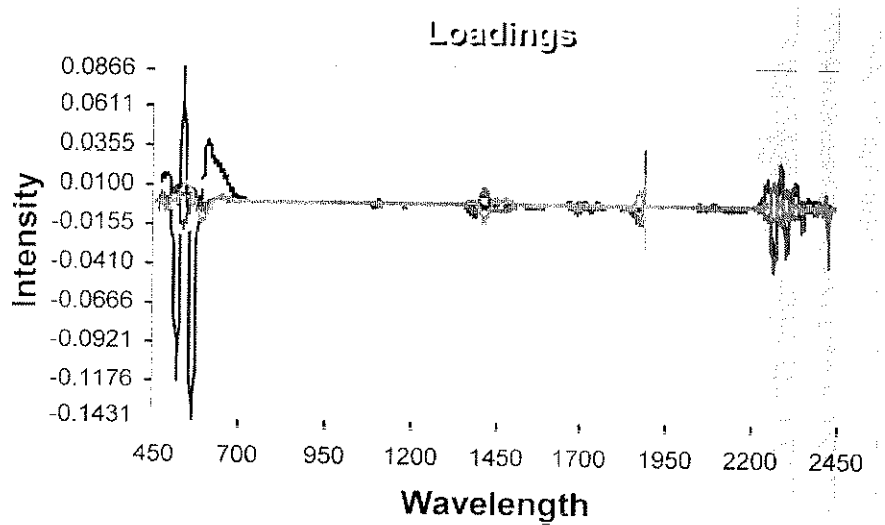


Spectral loadings for Vis-NIR quality calibration

Red wine commercial quality grading: Riverland Shiraz



2000 Cabernet and Merlot: predicting quality with Vis-NIR spectra

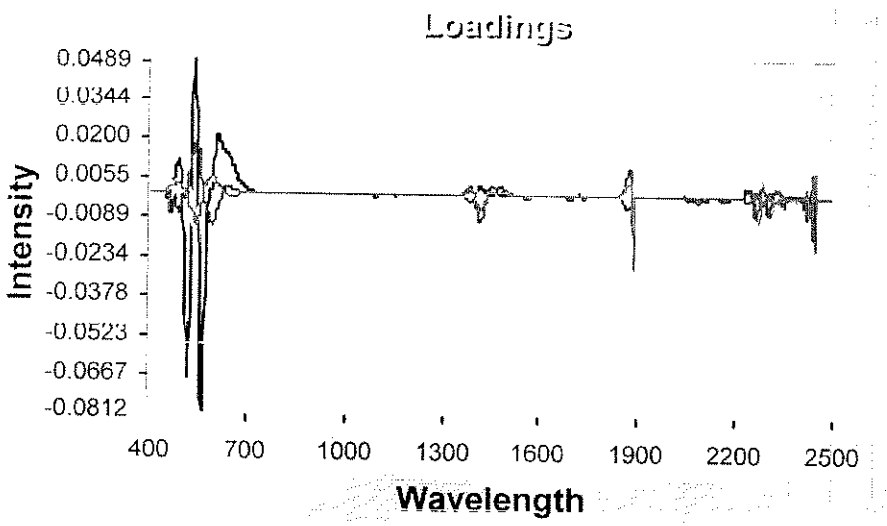


Cabernet Sauvignon

2nd derivative 450-1890, 2040-2450 nm

R^2= 0.79

SECV= 0.54



Merlot

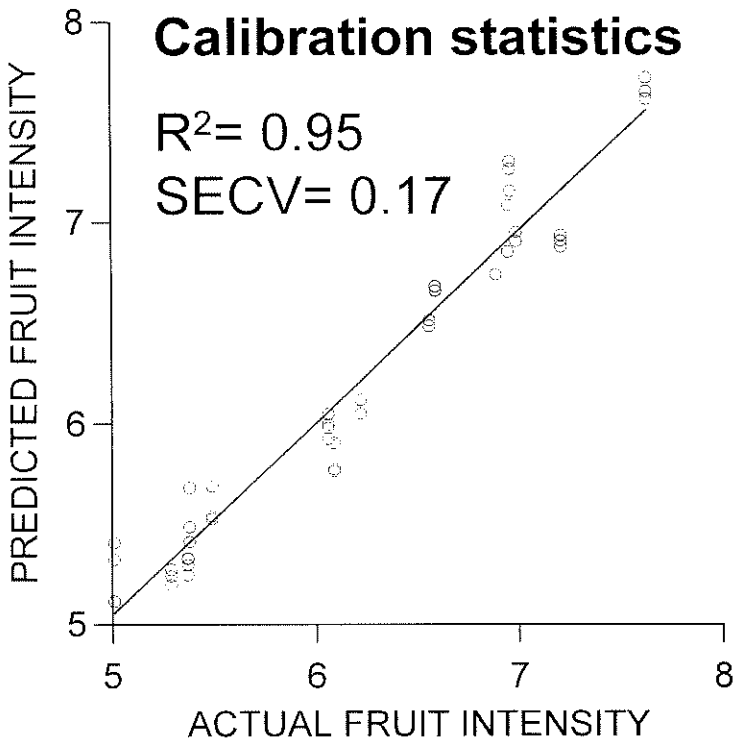
2nd derivative 450-1890, 2040-2450 nm

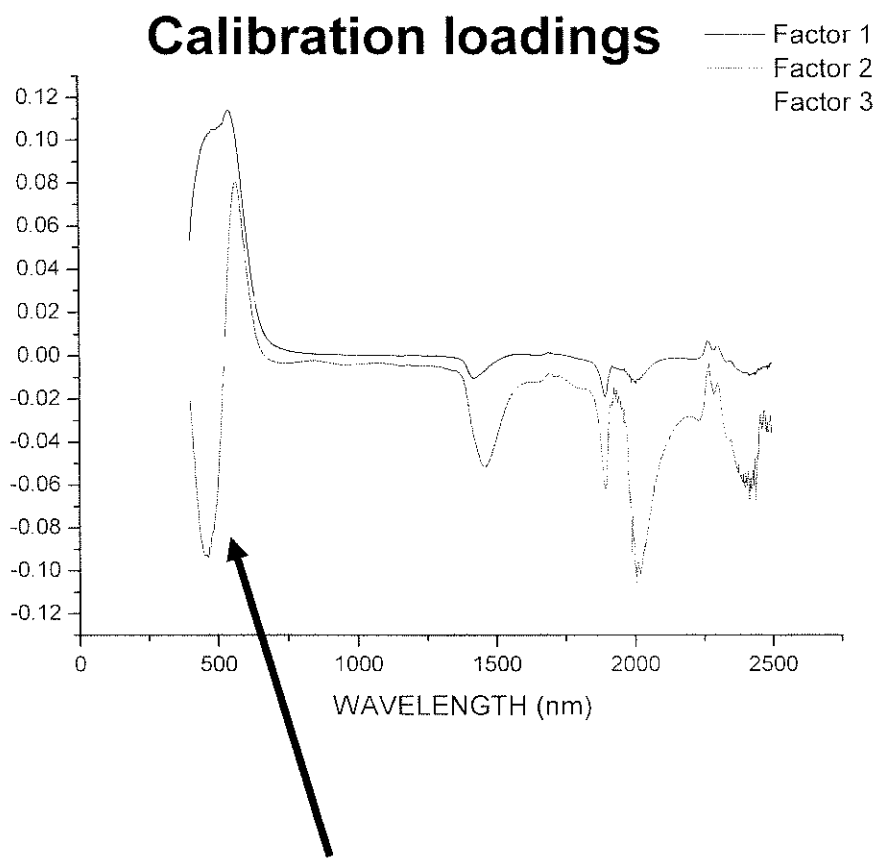
R^2= 0.82

SECV= 0.59


NEWS FLASH!

Vis-NIR prediction of "overall fruit intensity"

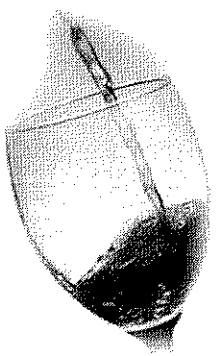


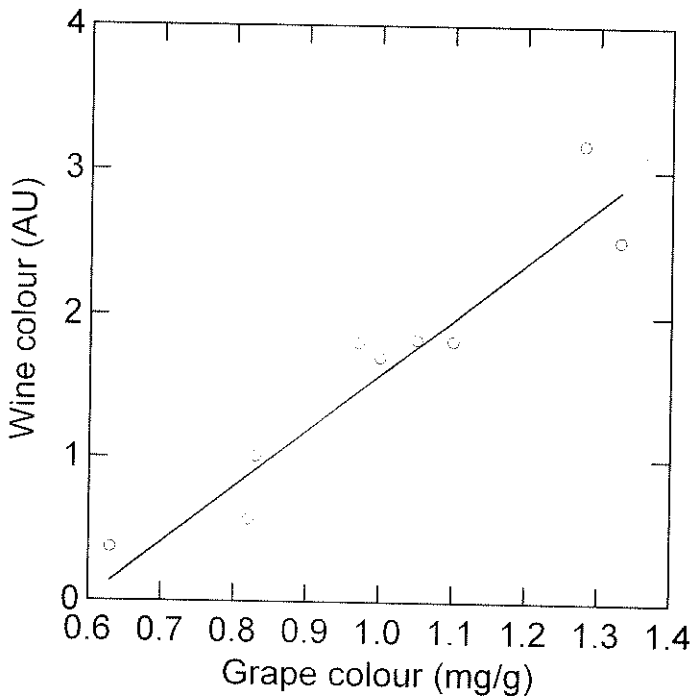


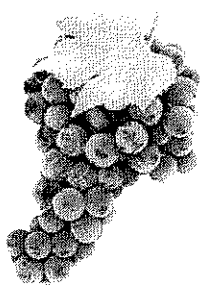
Commercial wines $9 - $35 rated (0-9) by an expert panel

Strongest loadings in the anthocyanin/pigmented polymer spectral region

Grape colour correlates with wine colour

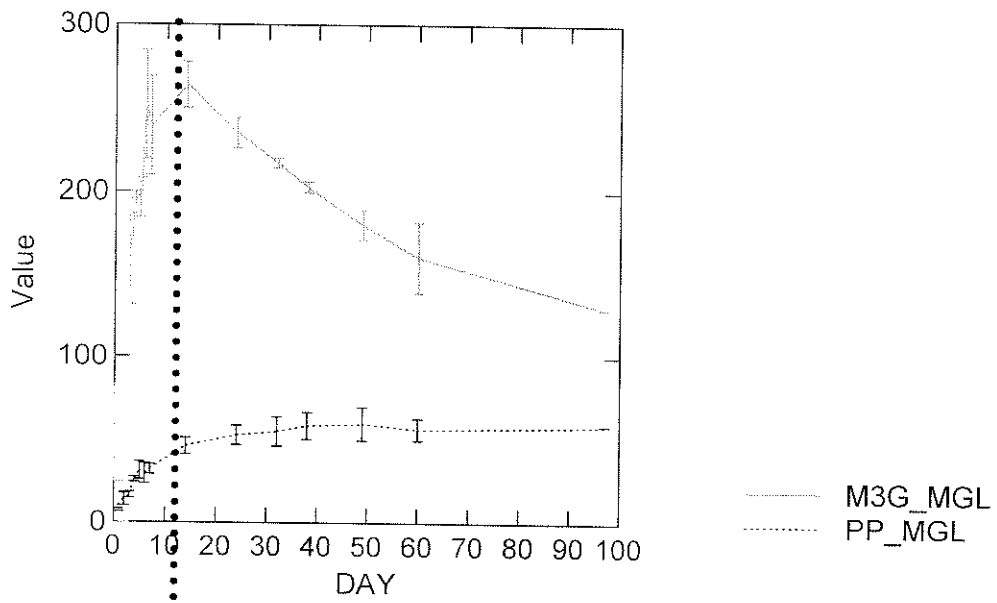






1995 Riverland Shiraz trial

But grape colour does not <u>equal</u> wine colour

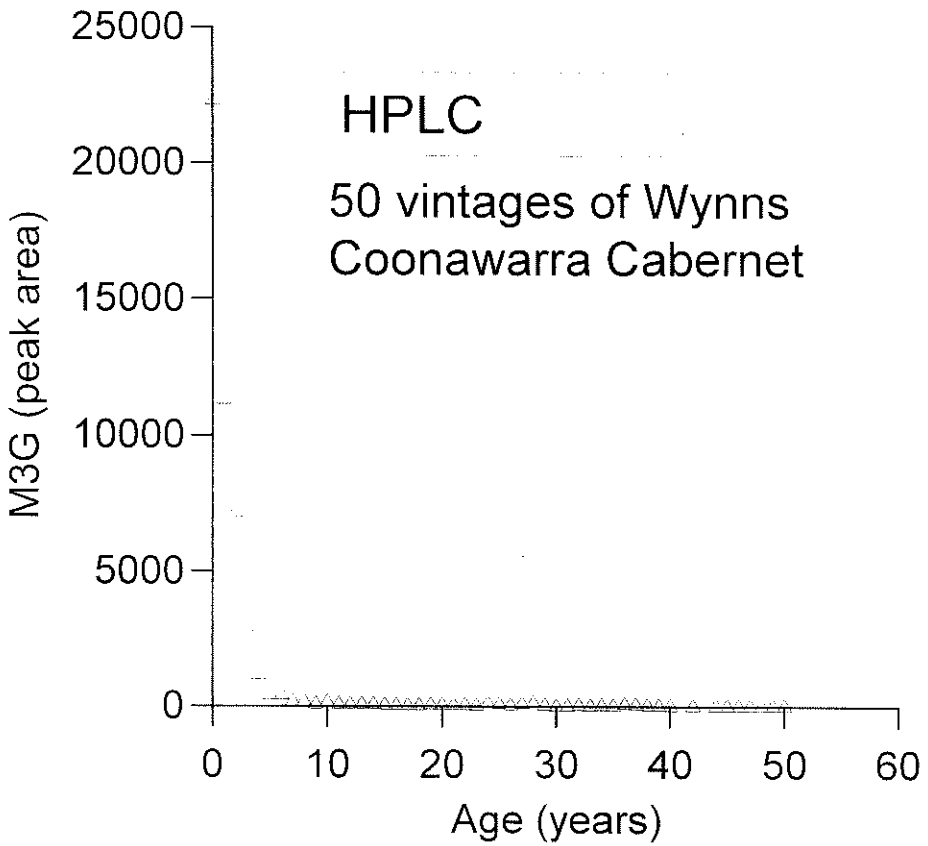


2001 AWRI fermentation trial

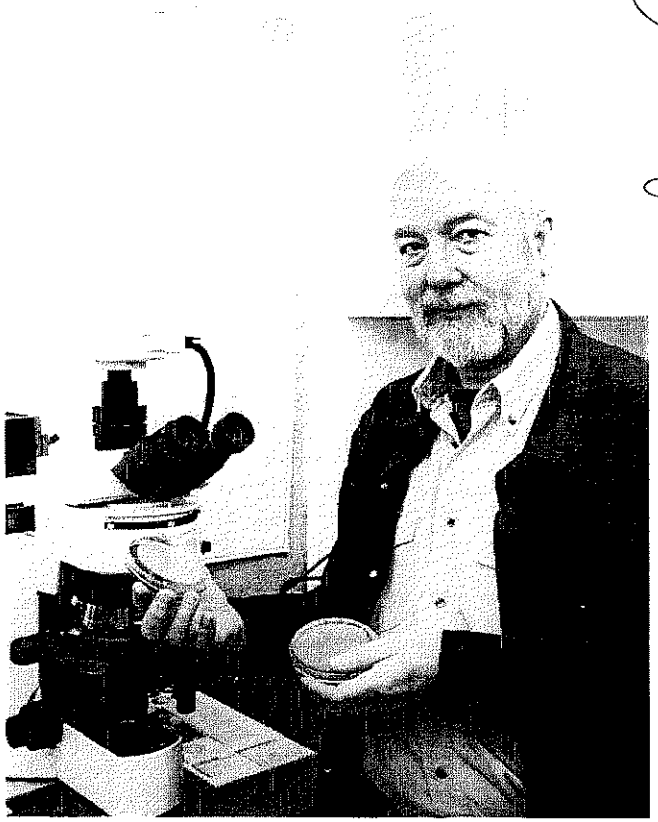
- The concentration of M3G increases during fermentation until taken off skins, then it rapidly declines, while pigmented polymer levels increase

- Anthocyanins are not the final stable colour in wine – they react with tannin to produce "pigmented polymers"

Free anthocyanin decreases with age



Some implications of pigmented polymer formation



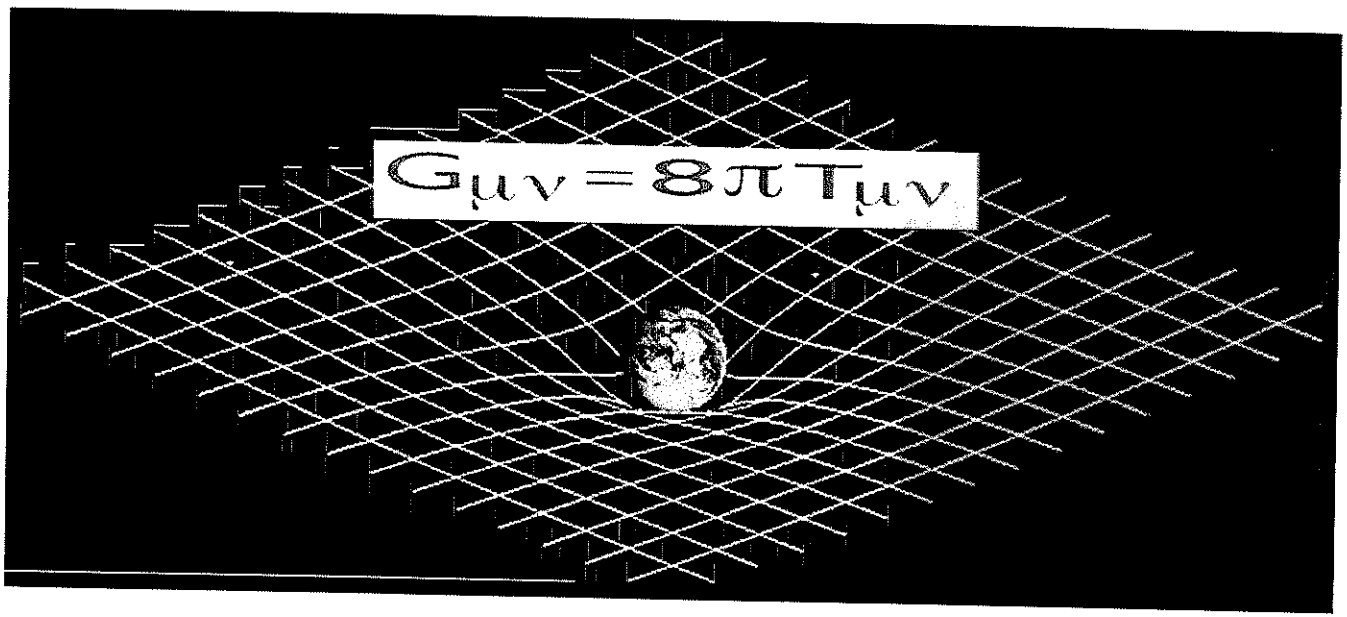
.....so, you can taste colour?

A tongue-in-cheek comment from a microbiologist
– Paul Henshke AWRI, 22 July 2003

YES, YOU CAN TASTE COLOUR
Anthocyanins in grapes combine with tannins to produce "pigmented polymers"

- this stabilises colour
- modifies mouthfeel of tannins?

Also, the physics of gravitation has "Einstein's equation for general relativity"



The chemistry of wine colour has the *"Dambergs-Herderich equation"*

[PP] = 0.06 [M3G] + 0.04 [T] - 2.88

R^2 = 0.92

PP: pigmented polymers

M3G: malvidin 3-glucoside

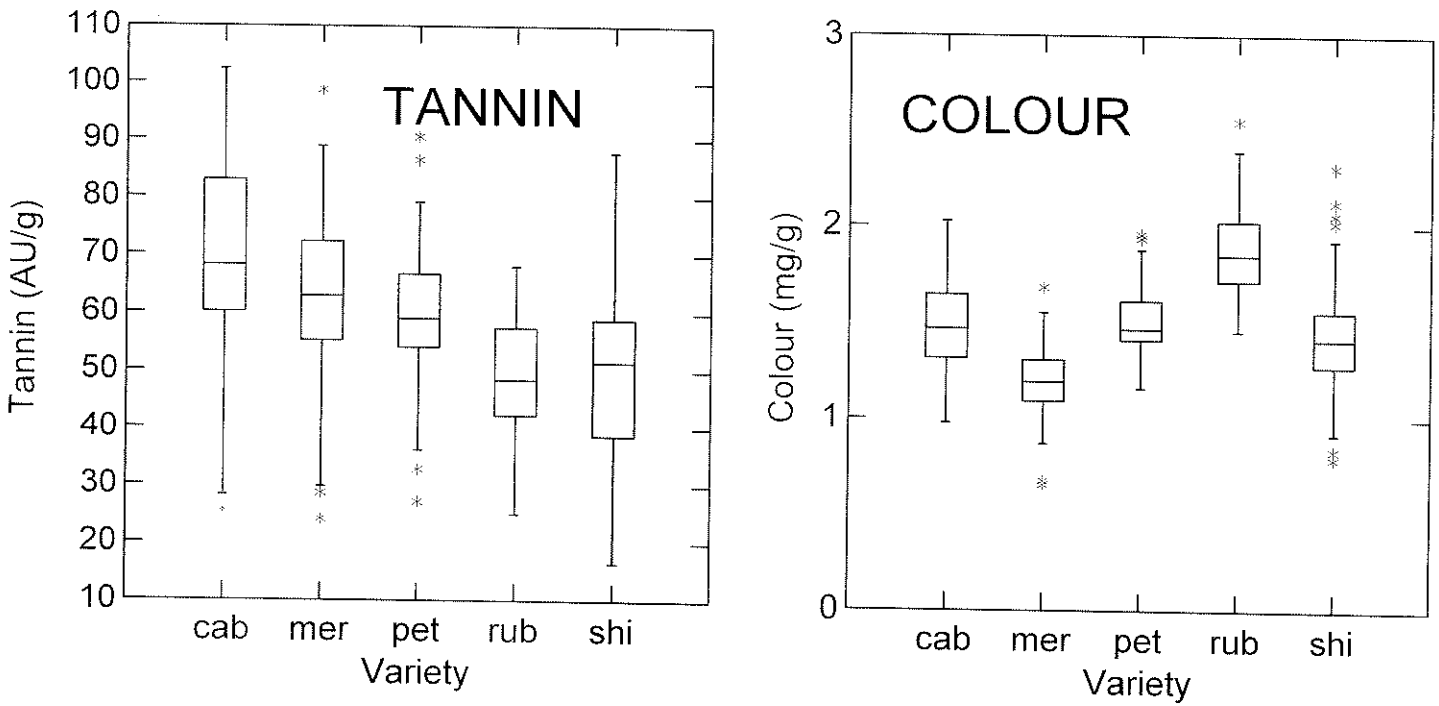
T: tannins

Extracted from the 2001 fermentation trial data

- ca. 250 samples analyzed
(1 yeast, 2 temperatures, 2 fermenter types, 10 ferments, n days)
- 22 variables from HPLC profiles

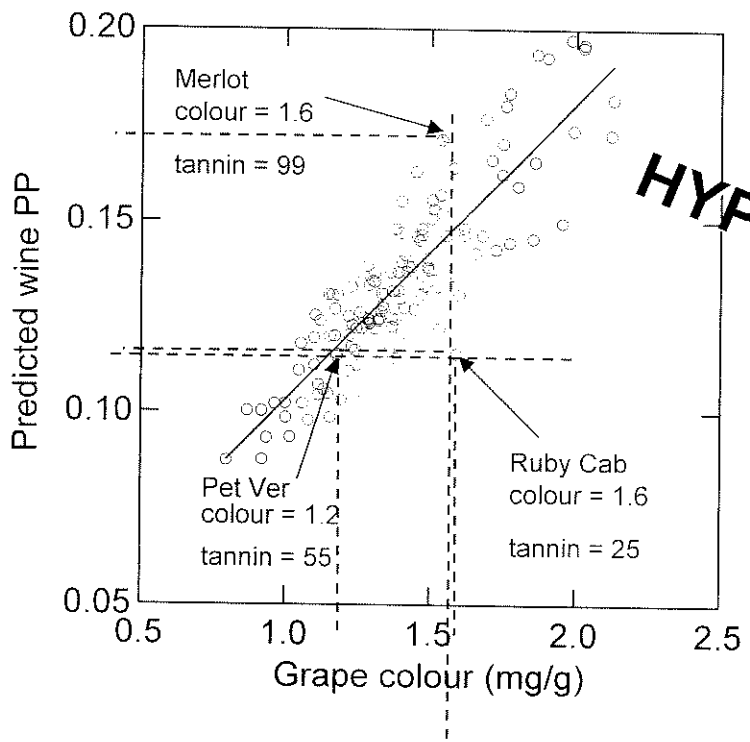
To predict pigmented polymers, the only significant variables are malvidin-3-glucoside and total tannin

Tannin and colour vary with grape variety



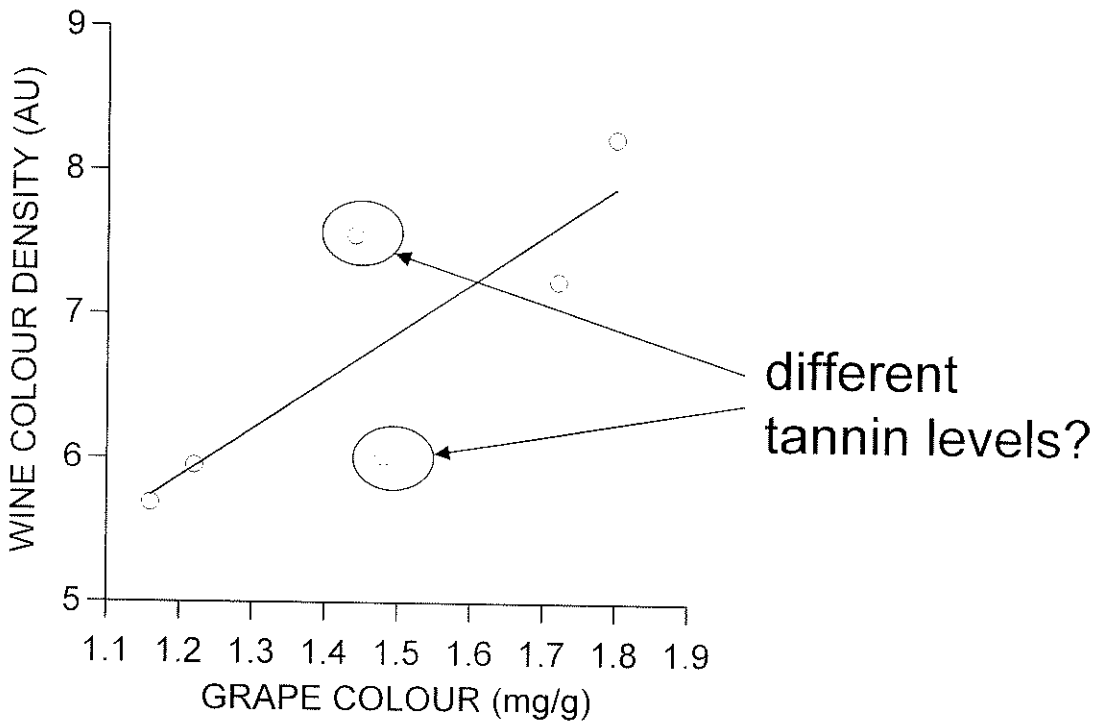
Sunraysia, 2004 vintage

Predicting wine pigmented polymers from grape anthocyanins and tannin



HYPOTHETICAL

Grape colour correlates with wine colour – tannin related outliers?



(Garth Swinburne, Sunraysia Shiraz)

Scholefield Robinson
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Colour expression in free anthocyanins is sensitive to pH and SO_2

Malvidin-3-glucoside

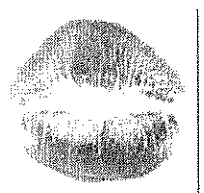


pH<1.5 **pH 3.7** **pH 3.7+SO_2**

Polymerised pigments are less affected by pH and SO_2 this differential sensitivity forms the basis of The "Somers measurements" which use a combination of absorbance at 420 and 520nm with and without acetaldehyde, SO_2 and low pH treatment

Correlation matrix – Somers & HPLC

	Col_Den	Anth_Som	A520_SO2	Phen	M3G	PP
Col. Density	1.00					
Antho_Somers	0.53	1.00				
A520 in SO2	0.82	0.03	1.00			
Phenolics	0.87	0.47	0.77	1.00		
M3G_HPLC	0.44	0.97	-0.04	0.34	1.00	
PP_HPLC	0.87	0.17	0.94	0.76	0.10	1.00
Tannin_HPLC	0.24	-0.39	0.58	0.32	-0.39	0.54



KISS

Somers total anthocyanins can replace HPLC anthocyanins
Somers colour density or A_{520} in high SO_2 can replace HPLC pigmented polymers

(and the new rapid tannin method can replace HPLC tannin?)

Somers' analyses and wine grade - multiple linear regression

Try to predict wine grade using the Somers' values, alcohol, S.G. pH and total acidity (a total of 11 parameters)

2001 Cabernet Sauvignon

Wine grade = 0.45(colour density*) –1.53

(*the only significant factor, p<0.05)

R^2= 0.54

SE= 0.57

So we have confirmed that…….

- **grape colour is correlated with wine colour**
- **wine colour is correlated with wine quality**

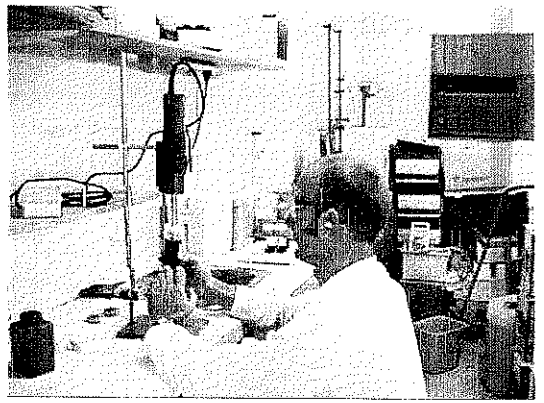
NOTE: this refers particulary to warm climates where temperature reduces the colour potential and therefore makes colour a limiting factor but low colour cool climtae fruit is also likely to be low quality

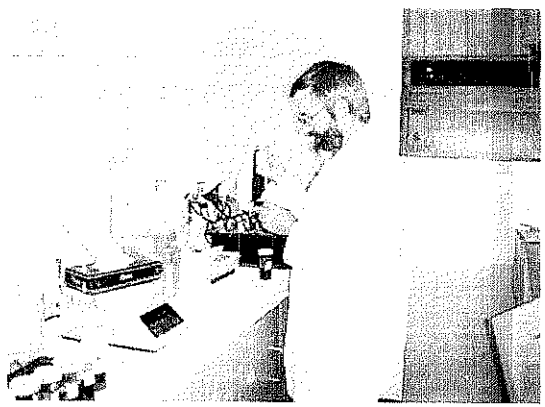
Part 3

- What is grape colour?
- Why is colour important?
- An overview of colour analysis methods including NIR specroscopy

Colour analysis- standard lab method

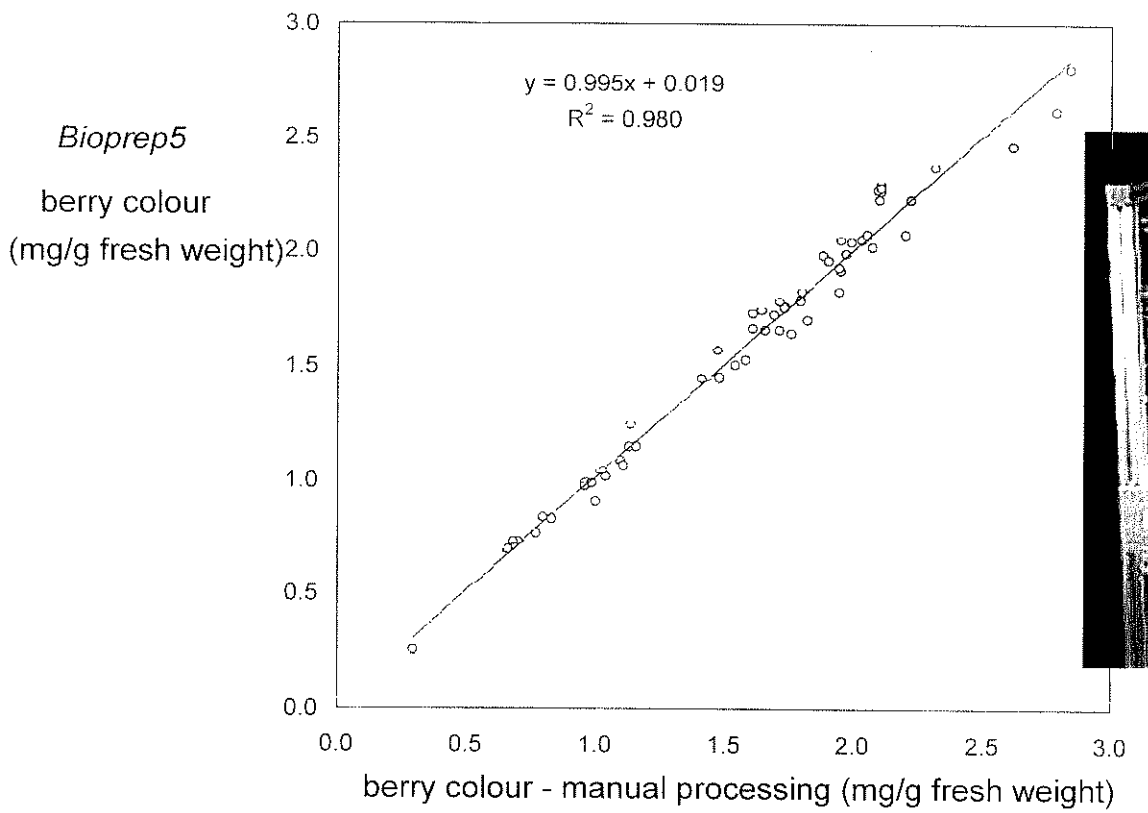
- High speed homogenise
- weigh out sub-sample
- extract in (acidified?) 50% ethanol
- centrifuge
- dilute supernatant in HCl
- read at 520 nm
- mg anthocyanin/g fruit =
  A_{520} x 20 x 1/1000 x 11 x extract vol x 1/fruit weight
- total time: usually 6-8 hours as a batch

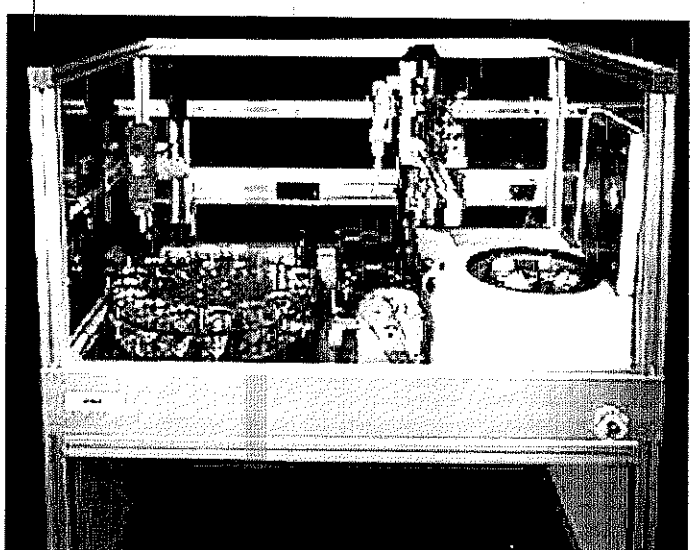




To analyse large sample numbers, we need a faster simpler method e.g. near infrared spectroscopy

But first:
the ARRM Bioprep5 colour analysis robot

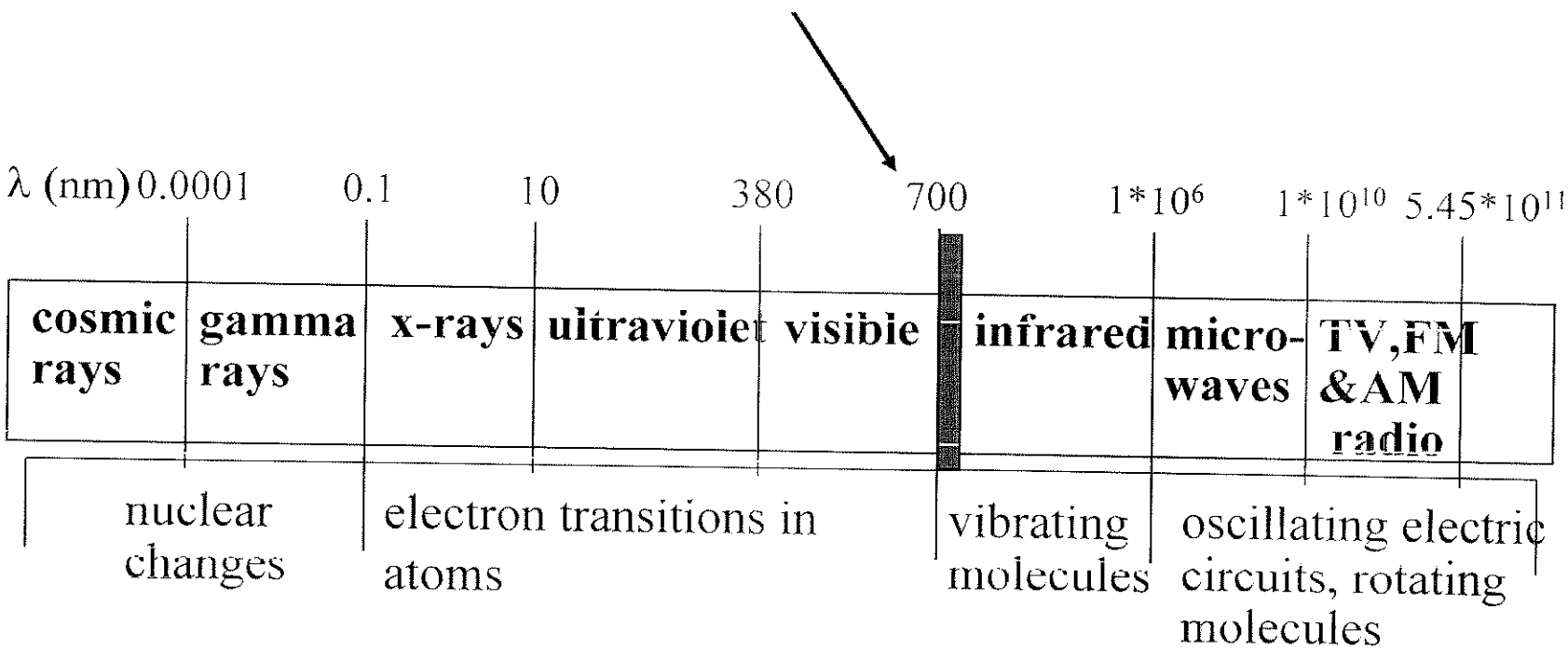




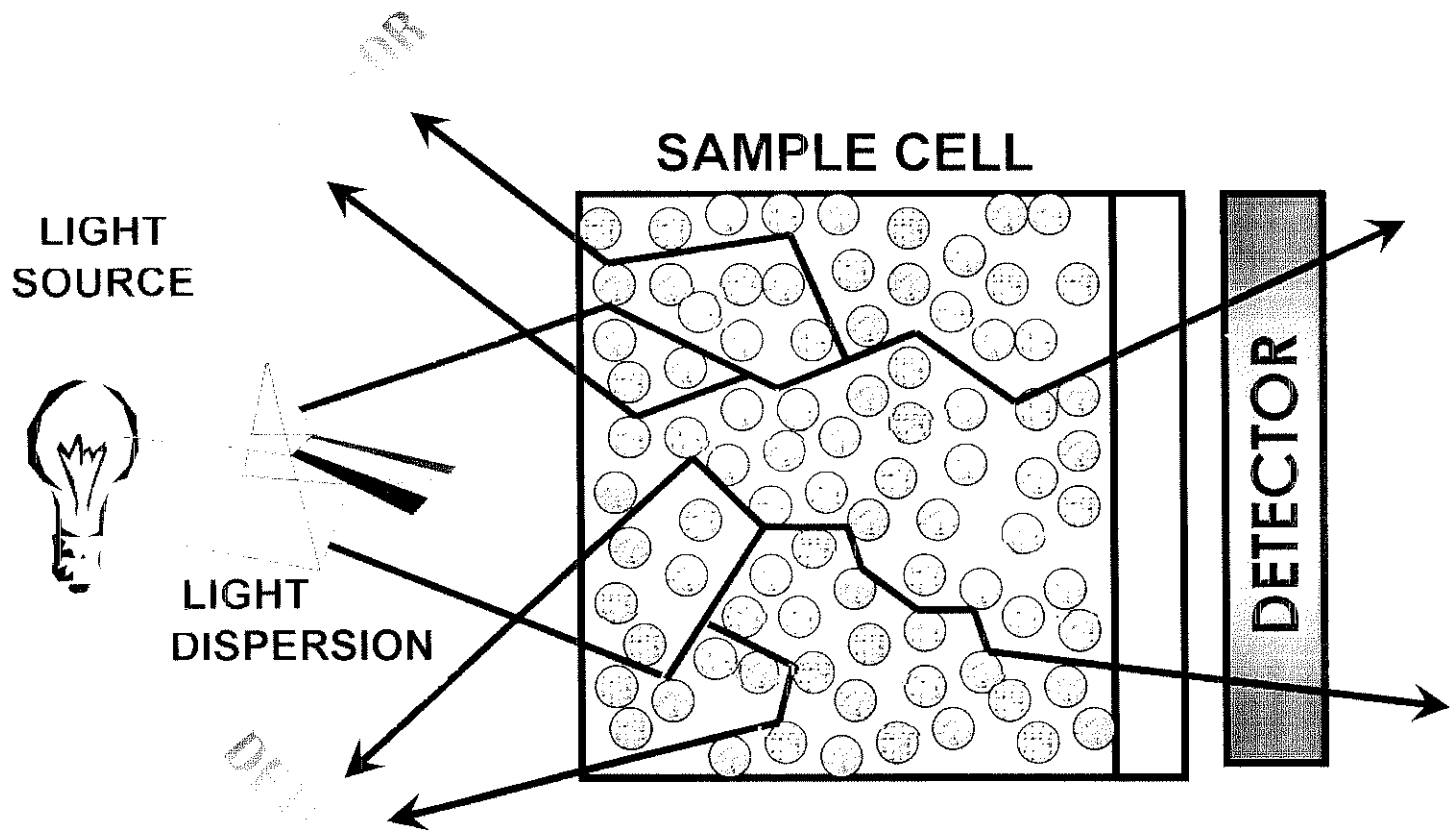
What is NIR specroscopy (NIRS) ?

NEAR INFRARED SPECTROSCOPY uses the ability of chemical bonds to absorb energy corresponding to the near infrared region of the spectrum

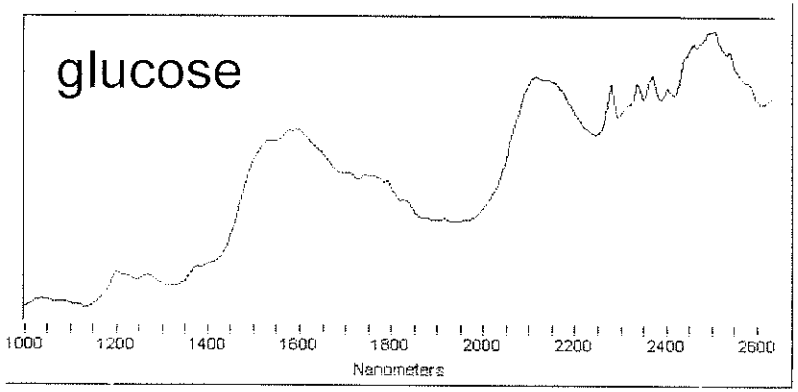
wavelength range: 700 – 2500 nm

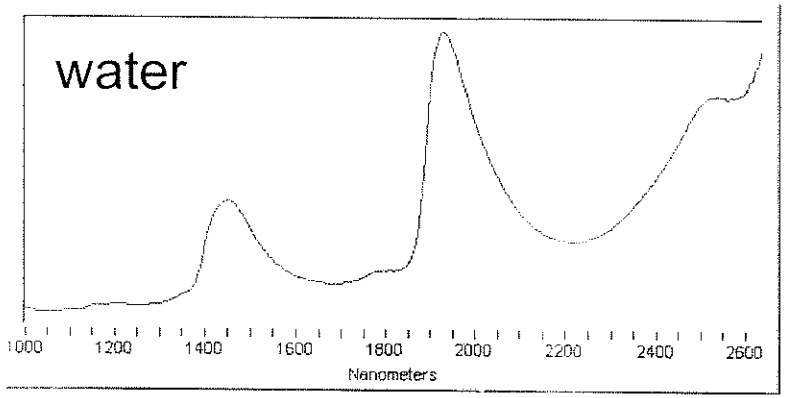


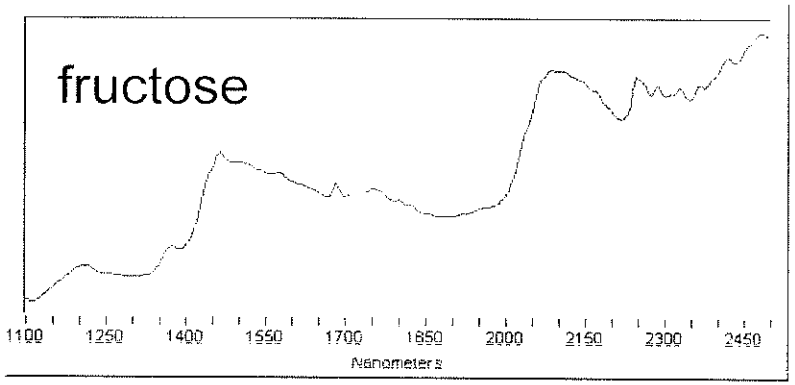
How do we measure NIR absorption?

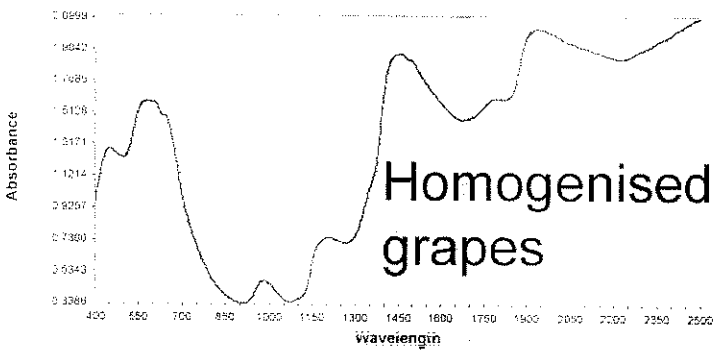


Examples of NIR spectra



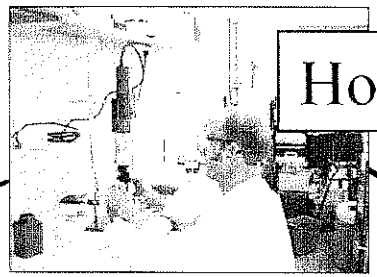




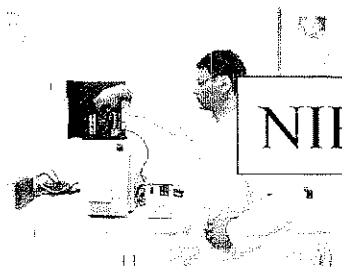


We use part of the visible and the NIR wavelength range (400 – 2500 nm)

NIR Calibration procedure



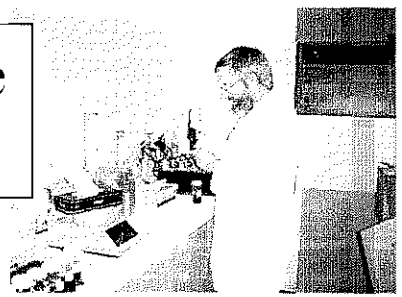
Homogenise



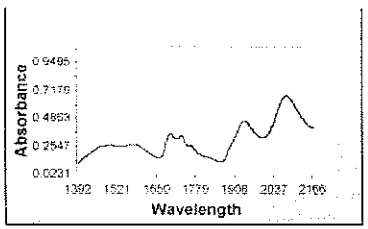
NIRS scan

1 minute

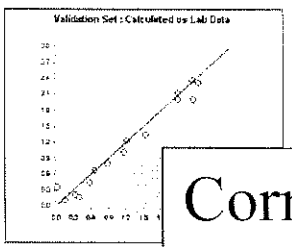
Reference method



1 day

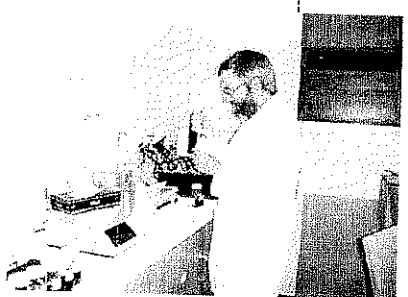


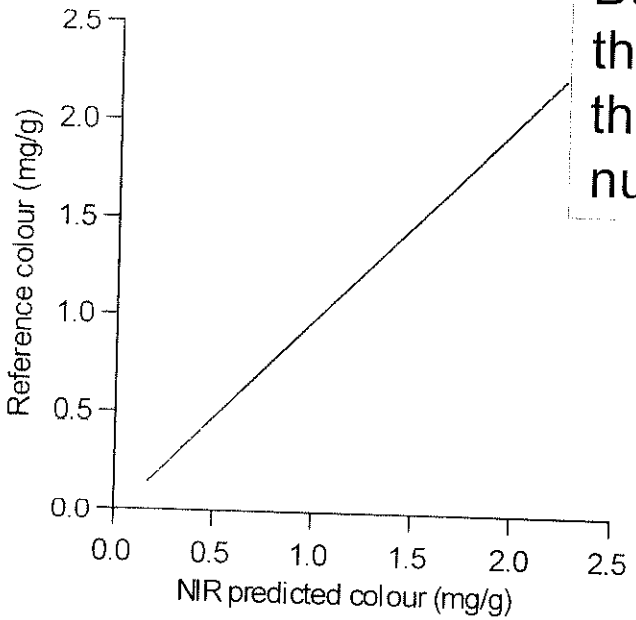
Chemometrics



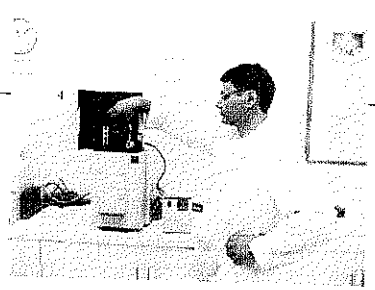
Correlative calibration

NIRS "predicts" the same colour value as the reference method

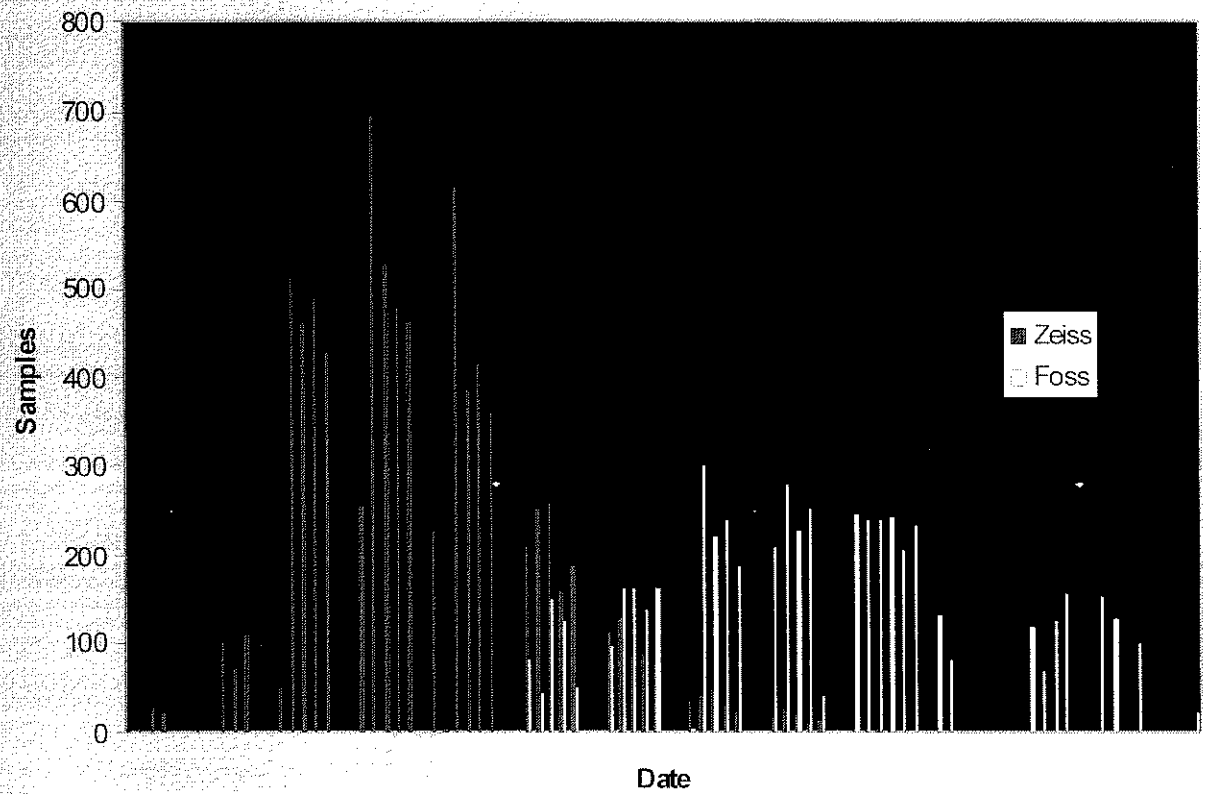




But with greater sample throughput so we now have the ability to analyse large numbers of samples



Samples Processed by NIR



$1 million dollars worth at commercial analysis rates

	Grower	Weighbridge	Total
2003 Vintage	11,304	5,808	**17,112**
2004 Vintage	9,859*	6,560	**16,419**

* Includes Petit Verdot

In summary, what simple colour measures can we perform on grapes and wine?

Grapes

- Total anthocyanins: *"wet chemistry" method or Vis-NIR*
- Total tannins: *methyl cellulose precipitation assay*

Wine

- Colour density (A_{520} + A_{420}) and /or A_{520} in high SO_2

Question time?



Size is not all – Skin to pulp ratios in ripe grapes

Dr M McCarthy
Senior Scientist Viticulture
SARDI

INTRODUCTION.

There is widespread belief in Australia that small berries will produce higher quality wine than large berries. This belief arose from research on irrigation management of Shiraz grapes in the irrigated inland regions in which it was demonstrated that water deficit after flowering could be used to reduce berry size, and in many cases, this resulted in better wine colour. This irrigation practice is commonly termed RDI. In the warm irrigated districts wine colour is often correlated with "quality", and there has been an improvement in red wine quality in the warm irrigated districts since the adoption of RDI (Figure 1).

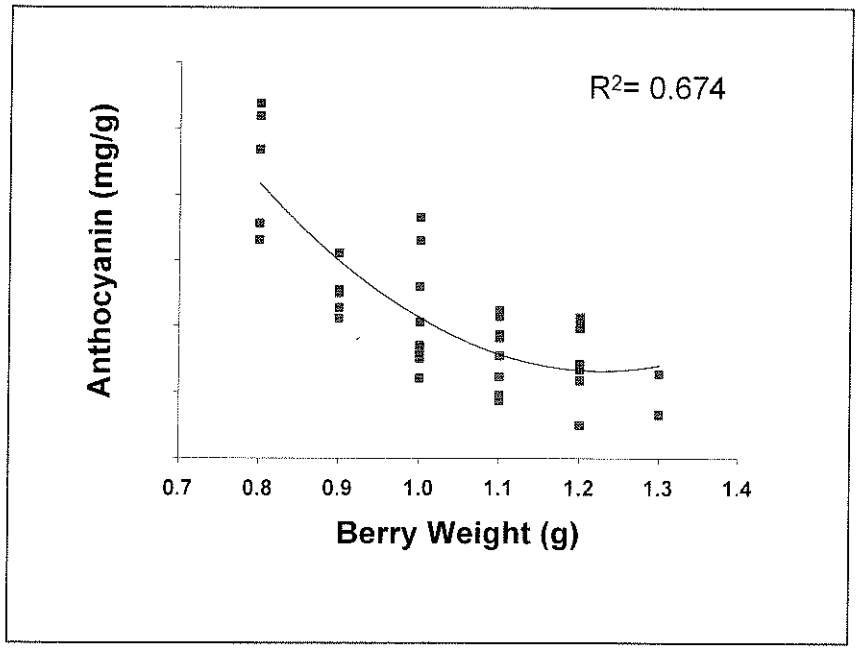


Figure 1 Relationship between berry weight and berry anthocyanin concentration (Data courtesy Dr Mark Krstic DPI Vic)

However, wine quality is not always correlated to berry size and there are examples of berries of different sizes resulting in wines of similar quality indices and wine score (Table 1). Although the berry weight of the heavier class was about 50% heavier the skin wt:fruit wt ratio and wine spectral measures of berries in these two classes were similar. Wines were equally scored, albeit only 13 on a 20 point scale.

Table 1 Quality indices for Shiraz wines made from berries from two weight range classes

Berry weight range (g)	0.8 - 0.9	1.2 - 1.3
Mean berry weight (g)	0.86	1.26
Skin wt : fruit wt ratio	0.14	0.14
Juice soluble solids (^{0}Brix)	24.8	24.9
Wine colour density (au)	2.99	3.11
Wine anthocyanins (mg/L)	343	352
Wine total phenolics (au)	29	29
Wine score	13	13

Shiraz berries - hand separated into two mass categories from minimally-pruned grapevines growing under full irrigation at Loxton SA 1989 vintage
Source:*Australian Journal of Grape and Wine Research* **11**, 2-8 (2005) Rob Walker et al. CSIRO Plant Industry Merbein Victoria

IS BERRY SIZE IMPORTANT IN NE VICTORIA?

Data in the recently reported RITA project on Benchmarking NE Victoria Shiraz indicates weak correlation between berry weight and berry phenolics per g fresh berry weight. Small berries tended to have higher phenolic content than larger berries, although not always, and there were many vineyards where berry weight did not appear to influence berry phenolics. Combining all the data collected in the benchmarking project into a single correlation matrix indicated there were few parameters strongly correlated with berry weight, either positive or negative. Berry weight was negatively correlated to berry flavanols, positively with E_{520} and $E_{520+420}$ and skin weight. There were no significant correlations between berry weight and many of the indices used to assess young wine quality or overall wine score. Wine "jaminess" was weakly correlated to berry weight. Overall wine score was most significantly correlated to "tannin softness", however there was no correlation between berry size and tannin softness. While there are some qualifications on the survey data collected in this benchmarking project it is apparent that for Shiraz grapes in NE Victoria small berries were not a pre-requisite for high wine quality. It should be noted that although the majority of the wines were below bronze medal score the highest rank wine was in the upper end of the berry weights recorded. The average berry in the benchmarking project was heavier than the upper weight class in Table 1 average wine scores were 2.2 higher (15.2 vs. 13) and is further evidence that in regions such as NE Victoria, within the range of wine scores reported, berry size was not the major determinant of young wine quality. Other speakers will allude to other possible drivers of wine quality in the region.

WHY MIGHT BERRY SIZE BE IMPORTANT?

Grape anthocyanins and other compounds accumulate in cells below the berry skin. Grape berry flesh primarily contains sugar(s) and water. The basis of the belief that berry size is important derives from the ratio of surface area of the skin (and its associated anthocyanins & other compounds) to the volume (the sugar and water). If a grape berry is assumed to be spherical, these parameters can be derived from the formulae below:

Berry volume $= {}^{4}/_{3}\pi r^{3}$

Berry surface area $= 4\pi r^{2}$

Where r is the radius of the berry

If the berry surface area is considered a surrogate measure of colour then colour will decrease at 3/r for every unit of increase in berry volume ie a small increase in berry volume (diameter) will result in a relatively larger reduction in the surface area/volume ratio.

Based on this, many believed that small berries were always better than large berries and, for example; some Chardonnay growers in warm irrigated districts implemented RDI in the belief it would improve "quality". Many were disappointed; apart from significant yield losses due to small berries, the wines were often of no better quality. In some instances, wines were of lower quality due to excessive sunburn and excessive phenolic character resulting from excessive exposure during ripening due smaller canopies.

The belief that smaller berries would result in higher quality wines was also widely accepted in many of the cooler/wetter grape growing regions. This assumption however ignored the fact that lack of colour is often not a major problem in many of the cooler grape growing regions. Many growers claimed to be using RDI in the belief that higher quality and hence greater financial returns would automatically result. However, once again, many have been disappointed and are now questioning the need to reduce berry size. The question arises whether the improvement in wine quality that may result from a reduction in berry size is in response to smaller berries *per se* or from a direct effect of water deficit/stress on berry structure &/or biochemical processes. The latter would suggest that any improvement in wine quality resulting from smaller berries would only be achieved if this reduction was through water stress, not from simply smaller berries.

Prof Mark Matthews and his students at the University of California Davis Campus (Roby et al 2004) evaluated the role of berry size and water deficit on aspects of fruit composition and reported that deficit produced an affect over & above what would be expected solely from a reduction in berry size. Field-grown Cabernet franc vines were subjected to three levels of water deficit and at harvest berries were separated into 6 weight classes. Berries from each weight class were measured for tannins and anthocyanins and skin and seed tannin was separately determined. Within each weight class the anthocyanin concentration of low irrigation vines was always higher than the other treatments (Figure 2) indicating that size alone was not the cause of the higher anthocyanin concentration.

Using this methodology, it was possible to separate the effect of berry size and water deficit on anthocyanins and tannins and it was concluded that the reported increase in these measures that accompanied water deficit appeared to result "more from differential growth sensitivity of inner mesocarp and exocarp than direct effects on phenolic biosynthesis". Roby et al (2004) reported that these effects were only associated with pre-veraison water deficit and post veraison deficit is likely to have little effect even though berry size might be reduced.

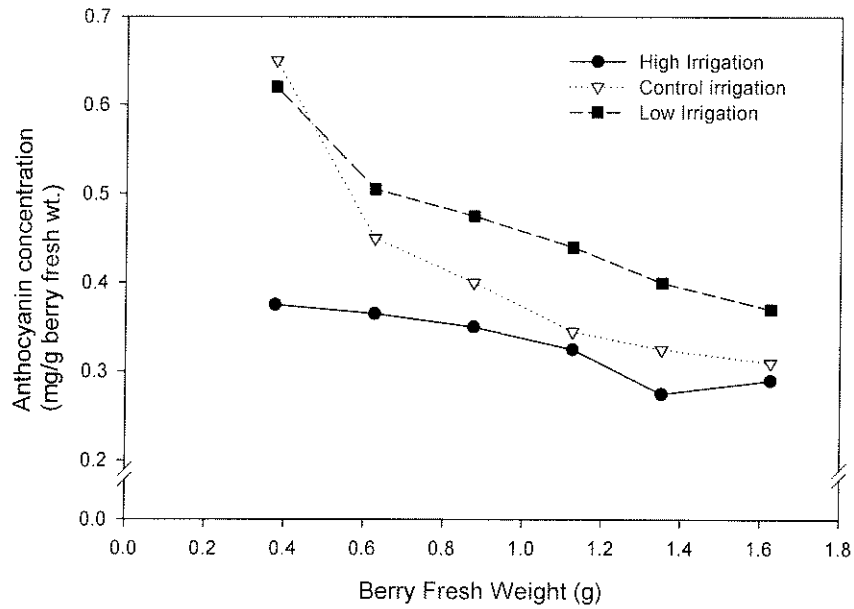


Figure 2 Anthocyanin concentration of Cabernet sauvignon berries from vines exposed to three levels of irrigation. (Redrawn from Roby et al. AJGWR (2004)

Similar data have been reported in irrigation experiments on Cabernet sauvignon grapes in Australia. Loveys & Bindon (pers comm.) reported an increase in anthocyanins and phenolics per g berry skin weight (Table 2) when berry size was reduced in response to water deficit.

Table 2 Effect of water stress on weight and composition of Cabernet sauvignon berries (Data reproduced with permission B Loveys & K.Bindon)

	Control	PRD
Berry fresh weight (g/berry)	0.82	0.71
Soluble solids (^{0}Brix)	26.7	26.1
Anthocyanins (mg/g skin wt)	11.31	14.36
Phenolics (A_{280} units/g skin wt)	7.34	9.16
PH	3.24	3.44

Summary

While the NE Victoria Benchmarking Project did not report significant effects of berry size on wine quality, there was some evidence of a relationship between berry size and phenolic composition. A wider range of berry size and wine quality scores may have produced stronger correlations. The work of Matthews and his students supports the hypothesis that berry size does play a role in wine quality, however, the mechanism by which berry size is reduced in critical. It is suggested that a reduction in berry size will only result in an improvement in wine quality if the reduction is brought about by water deficit before veraison. Other mechanisms by which berry size can be reduced (eg pruning, seasonal effects etc) may not result in higher wine quality.

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DOES SPEED MATTER?

- Are fast ripening grapes the best? -

Garth Swinburn
Senior Consultant
Scholefield Robinson Mildura

Scholefield Robinson
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Why are we interested in this question?



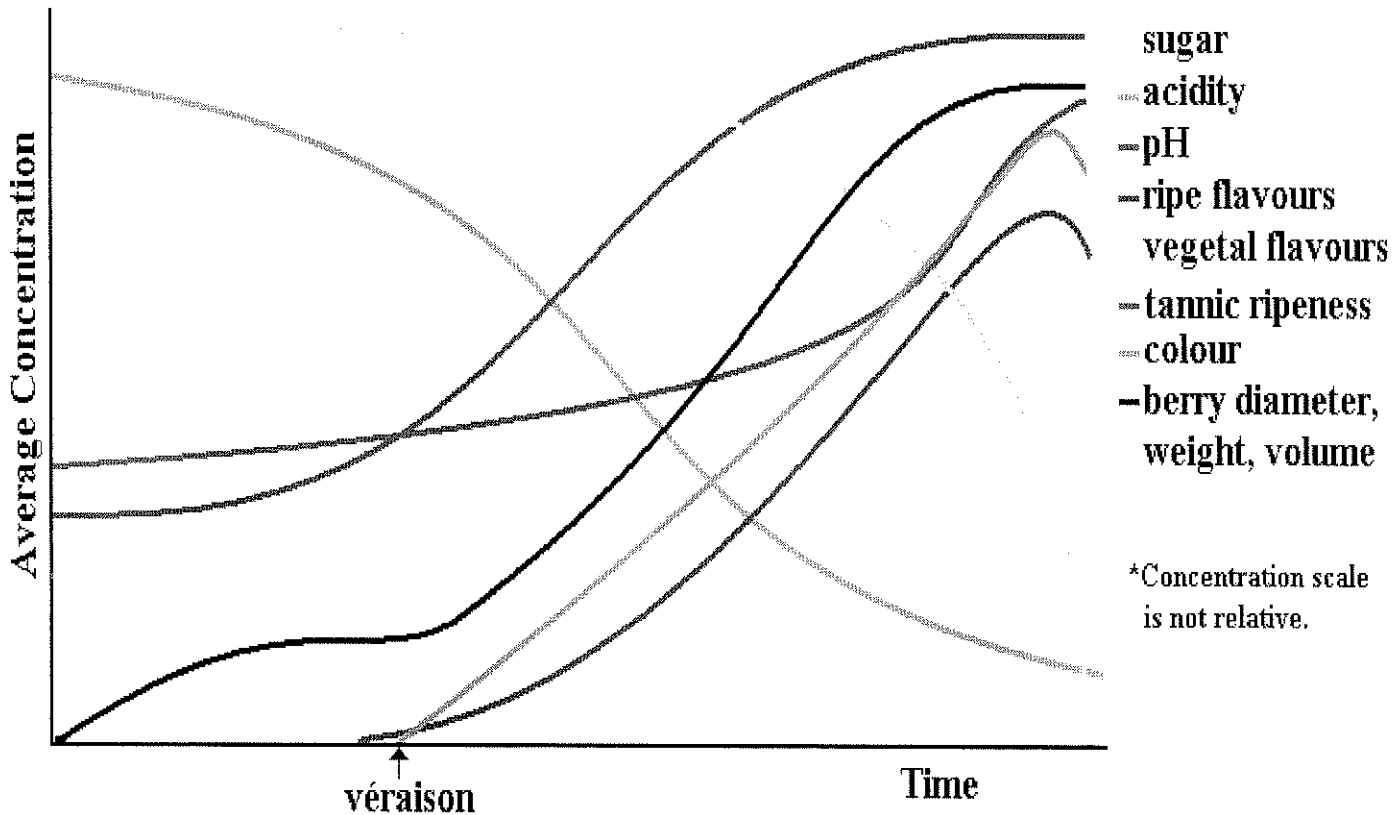
- To target harvest time to capture the best attributes of the grapes

Optimum Maturity

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Optimum Maturity





*Concentration scale is not relative.

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Optimum Maturity



Depends on

- Style of wine
- Working definition of quality
- Viticultural practices (esp. irrigation)
- Seasonal factors (heat)
- Variety (and rootstock)

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A Practical Perspective on Optimum Maturity



- Can you measure the quality or optimum maturity?
- How variable is it across the block?
- Can the harvester be booked at that time?
- Can the winery take the grapes at that time?

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Background to Early Ripening Project



- Many wineries were making the statement that the early fruit made better wine.
- Grape growers wanted to know whether this had any scientific basis.
- Grape growers wanted to know whether there were any benefits in trying to produce earlier ripened fruit.

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Project Methodology



- Literature review
  - What has already been researched, proven, trialed or documented in the literature.
  - What relevance does this information have to our region and grape varieties.
- Industry data
  - Field trials
- Interviews
  - Wine makers
  - Scientists

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Literature Reviews



Early Ripening = harvest date & days after 1 Jan.

Quality = baumé, colour, glycosyl-glucose (GG), acidity, phenolics

Most of the work in Australia has been done on Shiraz

50 references were reviewed, few addressed this specific topic

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Results from Literature

Holzapfel et al (1999)

- Early harvested Shiraz can be high quality but harvest date was not strongly correlated with berry colour

Gorman (2001)

- Early ripening Shiraz has a positive relationship with colour

Botting & Iland (1996)

- Early harvested Shiraz had better colour and GG. Late harvested Shiraz can achieve good colour and GG if the canopy is large but not dense.

Bravado et al (1985)

- Cabernet Sauvignon fruit maturation can be delayed by high crop loads or excessive irrigation but final wine quality is not affected by delayed harvest, unless it is caused by excessive irrigation.

Holzapfel & Rogiers (2001)

- Moderate yielding Shiraz (18-22t/ha) that are not over-vigorous can mature fruit without delay and with good colour. Harvest date is an unreliable measure of maturity.

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Industry data - field trials



- Scholefield Robinson Mildura (SRM) has been collecting data on berry composition and harvest dates for a number of seasons for Shiraz, Cabernet & Merlot
- Quality = baume + colour + TA + pH = **QIN**
- 2001 - no correlation
- 2002 - no correlation
- 2003 - QIN higher for early harvested fruit (SH & CS)
- 2004 - no correlation
- 2005 - QIN higher for early harvested fruit (ME)

Early harvested fruit in some years produces fruit (of some varieties) with higher colour, sugar and acid and lower pH (R= -0.40 to -0.53)

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Interviews



- Thirteen interviews conducted
  - Wine makers
  - Grower Liaison Officers
  - Consultants
  - Scientists (CSIRO, DPI)
- Half believed that early ripening resulted in better quality wine however there was little or no evidence provided to back up this opinion.
- The other half did not support the theory based on lack of evidence i.e objective data collected over a number of vintages.

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Believers



- What made the wine better?
  - Sugar/acid balance
  - Low pH
  - Better colour levels
  - Better flavour profile
  - Tannin structure
  - Possibly early fruit gets better treatment in the winery

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Summary



- Harvest time and fruit quality does not have a definable relationship.
- Delayed harvest often results in poorer quality fruit because of the management practices that cause the delay (is the converse true?)
- Wineries (& others) have the feeling that early fruit is best but have no evidence.
- The hectic pace of mid vintage can often adversely impact on the potential of the fruit (is the converse true?)

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Is Extended Maturation the Best?



'Hang Time' or Extended Maturation (EM) has been explored in California

- Proponents say
  - Higher baumé (15.5Be) for higher alcohol
  - Lower acid fruit
  - Lower vegetal flavours
  - Riper tannins
  - Soft fruity wines to drink earlier
- Critics say
  - Varietal boundaries are blurred
  - Wine unlikely to age
  - High alcohol overwhelms food
  - Not healthy or safe

Scent of heaven – why aroma must be grown in the vineyard

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http://www.wineresearch.org.nz/

When we unscrew the screw cap from a bottle of wine, pour the wine into a glass, swirl and evaluate the contents, we are assessing the culmination of considerable effort. The winemaker, viticulturist, vine and yeast all play their part in developing and releasing the flavour and aroma compounds that are released in the glass. It is the balance and intensity of these compounds that defines the character and style of the wine which when they meet or exceed the expectation of the consumer bring enjoyment and an appreciation of quality. When they do not meet the expectation, disappointment occurs.

Wine is a complex medium comprising of a non-volatile fraction (sugars, water, alcohol, phenolic compounds, etc.) and several hundreds of volatile compounds. These volatile compounds may range in concentrations from ng/L (that is parts per trillion) through to mg/L, and the olfactory impact they have on the wine will depend on the concentration, type and perception threshold of each compound. In addition, the presence of one compound may influence the sensory properties of others and as a result the absolute concentration of an aroma compound does not necessarily provide a good guide to the impact is has on the wine character. Add to this complexity, the varying ability of the consumer to smell, appreciate and recognize different compounds and one can start to appreciate the challenges of "getting it right".

The first step in successful winegrowing, is to manage the fruit in the vineyard to an appropriate composition. It is a wine industry adage that wines are made in the vineyard and then brought to fruition in the winery. The target fruit composition has many components which have to be managed by the viticulturist in a changeable environment. Short-term weather conditions, particularly at key phenological stages, can have a marked influence on fruit development, both the mean composition of the fruit and the variability around that mean. To achieve the target composition, viticulturists need to synthesize knowledge and predict the influence these changes have on subsequent fruit development and then adjust management accordingly.

Some important aromatic compounds in wine

Abbrev.	Perception threshold	Concentration in wine	Olfactory description
Thiols			
4MMP	0.8 ng/L	0-40	Broom, Box tree
3MH	60.0 ng/L	200-5000	Grapefruit, Passion fruit
3MHA	4.2 ng/L	0-500	Box tree, Passion fruit
4MMPOH	55 ng/L	0-150	Citrus zest
3MMB	1500 ng/L	30-150	Cooked Leeks
2FM	0.4 ng/L	0-20	Roasted coffee

Methoxypyrazine				
IBMP	2 ng/L	8-20		Green pepper
IPMP	2 ng/L			Green pepper, earthy
Monoterpenes		SBlanc	Riesl	
Linalool	50 µ/L	17	40	Rose
α-Terpineol	400 µ/L	9	25	Lily of the Valley
Citronellol	18 µ/L	2	4	Citronella
Nerol	400 µ/L	5	23	Rose
Geraniol	130 µ/L	5	35	Rose
Ho-trienol	110 µ/L	ND	25	Linden

Management factors influencing the concentration of some flavour and aroma compounds

In a short presentation, it is not possible to cover all factors determining the relative flavour and aroma concentrations, but a brief summary of some important characteristics are covered here:

Ripeness

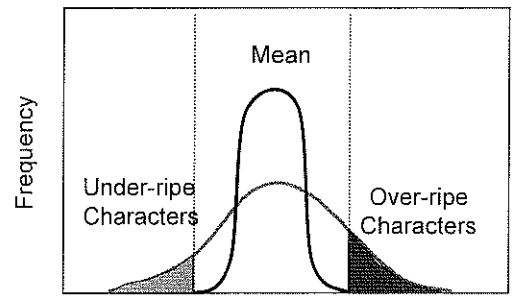
The main variable here is time. Changes in fruit composition as grapes ripen can be described in a general form (e.g. sugars, pH, monoterpenes, thiol precursors, anthocyanins increase, while acidity and methoxypyrazine fall); the relative concentrations vary, often reflecting changes in the fruit development during a particular season. However, a vineyard comprises of millions of berries (the average berry weight in a Marlborough Sauvignon Blanc vineyard is 1.93g, assuming 12T/ha, each hectare provides 6,217,617 berries/ha). Each contributes to the flavour and aroma profile in its unique way. Managing fruit composition in the vineyard involves managing the both the mean and variability around that mean.

The timing of the onset of ripening (at veraison) depends on the date of flowering, temperatures from flowering to veraison, crop load (higher crop loads delay the date of veraison on a particular site).

The variability in berry ripeness can be divided into three broad categories:

- Berry to berry differences
- Bunch to bunch differences
- Vine to vine differences

Conceptualization of impact of variability on
Flavour and aroma in wine



The uniformity of ripeness will depend on:

- The duration of flowering (where flowering occurs over a long time, we can anticipate greater variation in ripeness between individual berries),
- The number of bunches on individual shoots (apical bunches in general flower later and ripen later than basal bunches on the same shoot),
- On cane pruned vines bunches on shoots arising from terminal buds along the cane develop earlier than those on basal shoots close to the head.
- Between vine differences may be influenced by soil texture

Fruit shading

Sun exposed fruit will experience higher temperatures than shaded fruit. As temperature influences enzyme function we can anticipate the treatments that enhance fruit exposure (e.g. leaf removal in the fruit zone or training systems where the fruit is above the canopy layer) will influence the relative balance of flavour and aroma chemicals in the fruit. For example higher fruit temperatures from veraison to harvest generally increase the sugar, proline, quercetin and potassium concentrations in the fruit, while malic acid and methoxypyrazine concentrations are reduced. The anthocyanin concentration in Cabernet Sauvignon will increase in concentration up to about 25 °C, above this temperature it starts to decline again. Quercetin which acts as a natural "sun screen" in fruit co-bonds with anthocyanins in the wine making process, resulting in greater colour stability in the wine. It is the lack of adequate quercetin in shaded berries that may result in sunburn developing in fruit suddenly exposed to high light (for example after leaf removal) and the unpleasant phenolic concentrations in the skin.

Excessive canopy shading may result in premature leaf senescence, and higher potassium concentrations as inorganic nutrients are remobilized from the dying leaves to the developing fruit. Of greater importance, however, is the potential impact on disease levels, where for example Botrytis epidemics are generally worse on shaded fruit.

Grape nutrition

Soil nitrogen nutrition may have a direct or indirect influence on fruit composition. Many of the flavour compounds, in particular thiols, are derived from amino acids, which in turn appear to be influenced by the nitrogen status of the vine. From a winemaking point of view, low nitrogen can result in low yeast available nitrogen (YAN) in the juice and problem ferments. It is for this reason Di-ammonium phosphate is frequently added to juice during fermentation. Indirectly excessive nitrogen can increase canopy development and shading causing problems described above.

Potassium concentration in berries increases rapidly from veraison to harvest. Potassium deficient vines often show deficiency symptoms at this time, premature leaf senescence, which potentially reduces photosynthesis and stomata control. In contrast, excessive potassium results in high juice pH, thereby lowering fruit colour and colour stability in red wines. Magnesium and to a lesser extent Calcium are counter ions to potassium, and magnesium deficiency can be induced by high potassium.

In addition to soil fertility, rootstock selection can have an important role in determining the potassium nutrition of vines, for example vines growing on 420A rootstock are reported to have lower leaf and must potassium concentrations than those on SO4.

Berry size

Of the main yield components, (bunch number, berries per bunch and berry size), berry size appears to be the least elastic. However, berry size does vary in a vineyard, and potentially may have an important influence on the flavour and aroma concentrations in the fruit. For example, small berries will have a larger surface area to volume ratio, and as a consequence the concentrations of chemicals predominantly found in the skin will tend to be higher in juice, where skin contact is an important part of the wine making process. As a result, winemakers tend to

prefer small berries when making red wines. In contrast, however, iso-butyl methoxypyrazine (IBMP) concentrations also tend to be greater in the skin than in the flesh of the berry. It could be suggested that small berries may also have a greater herbaceous character.

Water stress, particularly early in the season, is reported to influence the potential size of berries and is potentially an important management tool in influencing fruit composition.

Summary

Before we can produce an ideal wine, we need ideal fruit. Knowing what makes up ideal fruit is the subject of much debate and research. The sensory properties of some important flavour and aroma chemicals in grapes and wine are intense and exceedingly low concentration, yet it is the role of the viticulturist to manage them to hit that ideal.

Crisp and Fresh - pH, Titratable acidity and Malic acid

Dr Patrick Iland
Patrick Iland Wine Promotions Pty Ltd

Introduction

The main acids of the grape are tartaric and malic acids. They are found in cells contained in the flesh and skin of the berry. When the berries are crushed (or pressed) these acids are released into the juice. Here they exist in a pH dependent equilibrium between free hydrogen ions and various forms of the acids. During berry ripening, the pH of the juice increases while the titratable acidity decreases. Three factors contribute to these changes:

1. dilution of the acids due to the uptake of water into the berry cells as a result of berry growth.
2. the respiration (breakdown/loss) of malic acid
3. changes in the forms of tartaric acid due to the uptake of potassium and sodium ions.

In this presentation I will focus on the respiration of malic acid and the effects of this on juice pH and titratable acidity. Although it must be appreciated that to fully understand the changes in pH and titratable acidity the other factors also need to be considered.

Assessment of berry flavour properties along with measures of juice pH and titratable acidity are used by winemakers to make decisions concerning the time of harvest. In some cases values of juice pH may be rising and the titratable acidity decreasing to levels which may be difficult to adjust with the addition of acid and a decision may be made to harvest the fruit earlier than if the timing were based mainly on flavour assessment. In other cases, low juice pH and high titratable acidity conditions may lead to wines having a tart or a sharp acid taste, being out of balance with other wine sensory characters. This latter situation is normally associated with cooler climates/seasons and is often a result of higher malic acid levels. Viticulturists and winemakers need to investigate means by which the malic acid and titratable acidity values can be lowered.

Modifying juice malic acid levels in the vineyard

Malic acid is accumulated in the berries pre-veraison. At veraison there is a dramatic change in metabolism and levels of malic acid decline due to respiration reactions. The rate of breakdown is temperature dependent, being greater at higher temperatures. Therefore, in cooler climates/seasons levels of malic acid can be higher than desirable for particular wine styles.

Case study 1: *Sauvignon Blanc, Marlborough, New Zealand*

Smith et al. (1988) conducted trials of different timings of leaf removal in the bunch zone of Sauvignon Blanc vines grown with vertical canopies in the Marlborough region of New Zealand. The data in Table 1 compares one of the leaf removal treatments (that conducted 2 weeks prior to veraison) with the control (no leaves removed). In this treatment the decrease in titratable acidity was the greatest, the titratable acidity at harvest being, 10.5 compared to 12.8 for the control (Table 1). There was no effect on juice pH values. The change in titratable acidity was related to both higher tartaric acid levels and lower malic acid levels. The leaf removal treatment resulted in a higher degree of bunch exposure and the decrease in malic acid was attributed to a temperature effect; the more exposed bunches being at a higher temperature and thus the rate of malic acid respiration was likely to be higher. There was a trend towards the levels of malic acid being lower in treatments where leaves were removed prior to veraison, rather than being removed after veraison. Comparing data from the 5 different treatments of leaf removal the reduction in titratable acidity was directly related to the extent of fruit exposure, assessed just prior to veraison.

Table 1. Effects of leaf removal on Sauvignon Blanc juice composition (adapted from Smith et al. 1988)

Treatment	°Brix	pH	TA (g/L) as tartaric acid	Tartaric acid (g/L)	Malic acid (g/L)	% Bunch exposure
Control	21.8	3.11	12.8	5.3	4.4	4
Leaf removed	21.8	3.12	10.5	6.2	3.7	52

The pH and titratable acidity values of the wines of both the control and the leaf-removed treatment were lower than those of the juice from which they were made (Table 2). This is likely to be due the effects of potassium hydrogen tartrate precipitation. This is an important aspect to consider when setting target juice pH and titratable acidity values; values should be set with knowledge of how they will change during the winemaking process.

Table 2. Effects of leaf removal on Sauvignon Blanc wine composition (adapted from Smith et al. 1988)

Treatment	pH	TA (g/L) as tartaric acid	Score
Control	3.00	10.4	14.1
Leaf removed	2.98	9.3	15.0

***Case study 2**: Sauvignon Blanc, Marlborough, New Zealand*

Naylor et al (2003) investigated the effects of row orientation and bunch exposure on light interception and fruit composition of Sauvignon Blanc vines in Marlborough New Zealand. Some of the data is summarized in Tables 3 and 4. Titratable acidity and malic acid levels were lower in the juice of berries from interior bunches of north-south rows compared to that of berries from interior bunches of east-west rows (Table 3). Titratable acidity and malic acid levels of juice of berries from exterior bunches on the north side of canopies of east-west rows was lower than that from the south side canopies of these rows (Table 4). The effects of bunch exposure were similar to those reported in the study by Smith et al. (1988), in that greater bunch exposure resulted in lower titratable acidity and malic acid levels (Tables 3 and 4). However, whereas the comparison in the data of Tables 1, 2 and 3 were made at a similar maturity level, as determined by juice °Brix, in the comparison of exterior bunches on north and south sides of east-west rows, fruit from the north side achieved a higher maturity, as determined by °Brix, indicating that achieving higher sugar maturity can also result in lower juice titratable acidity; and in this case the juice pH was higher.

Table 3. Effects of leaf removal on Sauvignon Blanc juice composition (adapted from Naylor et al. 2003). Comparison of east-west and north-south row orientation

Treatment	Berry weight (g)	°Brix	pH	TA (g/L) as tartaric acid	Malic acid (g/L)
East-west rows (interior)	1.89	20.6	3.23	9.2	4.9
North-south rows (interior)	2.03	20.6	3.27	8.6	4.6

Table 4. Effects of leaf removal on Sauvignon Blanc juice composition (adapted from Naylor et al. 2003). Comparison of north and south facing canopies of vines in east-west rows

Treatment	Berry weight (g)	°Brix	pH	TA (g/L) as tartaric acid	Malic acid (g/L)
Exterior north	1.73	22.7	3.38	6.8	2.6
Exterior south	1.91	19.9	3.08	8.9	4.4

Case study 3: — *Chardonnay Adelaide Hills*

A study conducted by Inca Lee and Andrea Hart, for their Masters in Viticulture and Bachelor of Agricultural Science-Viticulture, Honours, respectively, investigated the effects of leaf removal at set and veraison and bunch thinning at veraison on fruit composition of Chardonnay vines in the Adelaide Hills.

Although not statistically different, results showed a similar trend to those of the Sauvignon Blanc studies where leaf removal decreased juice titratable acidity levels. However, in this case levels were lower in the treatment where leaves were removed at veraison rather than at set. Bunch thinning also resulted in lower juice titratable acidity.

Table 5. Effects of leaf removal and bunch thinning on fruit composition of Chardonnay vines in the Adelaide Hills (adapted from Lee 1996)

Treatment	°Brix	pH	TA (g/L) as tartaric acid
Control	22.9	3.37	7.28
Leaf removed at set	22.5	3.34	6.78
Leaf removed at veraison	22.6	3.38	6.45
Bunch thinned at veraison	23.0	3.36	6.83

Table 6. Effects of leaf removal and bunch thinning on fruit composition of Chardonnay vines in the Adelaide Hills (adapted from Hart 1997)

Treatment	°Brix	pH	TA (g/L) as tartaric acid
Control	23.9	3.34	8.70
Leaf removed at set	23.8	3.30	8.82
Leaf removed at veraison	23.5	3.30	7.86
Bunch thinned at veraison	23.5	3.22	8.19

Understanding what is happening in the berry and in the juice

After veraison the concentration of malic acid in the juice declines. This is due to the combined effects of a loss in absolute amounts of malic acid and to dilution effects as the berry enlarges with the uptake of water. Even if no malic acid was respired in the berry cells, the concentration of malic acid in the berry cells (and thus in the juice expressed from these cells) would decrease due to the dilution effects. Hence to really understand what is happening you need to measure the amount of malic acid in the berry as well as the concentration in the juice. In some cases the decline in malic acid per berry does not occur until some time after veraison and when malic acid does start to decline, it appears that the rate of decline is not as fast as it would be if the decline had commenced at veraison or just after veraison. A delay in the timing when malic acid begins to be respired (ie a loss in absolute amounts) can lead to higher levels of malic acid in the berry and in the juice at harvest. Conditions associated with 'the phenomenon of delayed malic acid decline' appear to be more vigorous shoot growth leading to shaded canopy situations where both leaves and bunches are shaded.

Conclusions:

The Sauvignon Blanc study showed that

- the decrease in juice titratable acidity was due largely to decreases in malic acid
- the most effective treatments (lower juice titratable acidity and higher wine score) were those where leaves were removed prior to veraison.

The Chardonnay study showed that

- both leaf removal and bunch thinning treatments decreased titratable acidity
- there were no difference in the effects of removing leaves prior to or after veraison.

Summary:

Leaf removal around bunches can be a successful strategy for decreasing malic acid levels.

Trials need to be carried out in specific locations.

Avoiding shaded canopy situations can help to ensure that the respiration of malic acid occurs at or soon after veraison.

It is likely that the same principles would apply to black grape varieties as has been shown for the white varieties.

Monitoring juice malic acid levels to assess optimum harvest time

To answer this question the targeted style of wine needs to be identified and from this work backwards to the juice composition that gives this wine style. In the paper by Smith et al. (1988) the two leaf removal treatments resulted in lower juice titratable acidity, similar pH and lower malic acid levels. The wines from these treatments scored the highest in sensory evaluation and had descriptors such as red capsicum and tropical fruit characters compared to the control which was judged as being more grassy and herbaceous like. In this case lower malic acid levels tended to be associated with a greater intensity of red capsicum and tropical fruit characters.

In a study by Roujau de Boubee et al. (2000) a very good correlation was shown between the breakdown of malic acid and two-methoxy-3-isobutylpyrazine (IBMP) as the grapes ripened. (IBMP) is the compound identified as giving the green bell pepper aroma in Sauvignon Blanc and Cabernet Sauvignon wines. This character is often described as being 'green' or 'unripe'. Because of the close association of malic acid, IBPM and this unripe sensory character, the authors suggested that malic acid and IBMP may be used as markers for grape unripeness.

Conclusion:

Situations where juice malic acid levels are likely to provide a good indication of 'style specific characters' are —

cooler regions where malic acid levels are naturally high, and where microclimatic effects are driving differences in fruit composition. In this case, altering the microclimate in a particular vineyard site, by techniques such as leaf removal is more likely to lower the malic acid level and decrease the intensity of' 'unripe' fruit characters — hopefully leading to fruit and wines that are fresh and crisp but not too crisp.

References

De Boubée, D.R. Van Leeuwen, C. and Dubourdieu, D. (2000) Organoleptic impact of 2-methoxy-3-isobutylpyrazine on Red Bordeaux and Loire Wines, Effect of Environmental Conditions on Concentrations in Grapes during Ripening. J. Agric. Food Chem. 2000, 48, 4830-4834.

Hart, A. (1998) The effect of basal leaf removal and bunch thinning on berry and wine composition of *Vitis vinifera* L.cv. Chardonnay. Bachelor of Agricultural Science – Viticulture, Honours Thesis The University of Adelaide.

Lee, I.C. (1997) The effect of basal leaf removal and bunch thinning on the fruit composition of *Vitis vinifera* L.cv. Chardonnay. Master of Viticulture (Coursework) Thesis The University of Adelaide.

Naylor, A., Creasy, G.L. and Vanhanen, L. (2003) Effects of row orientation and cluster exposure on light interception and Sauvignon Blanc fruit composition. The Australian & New Zealand Grapegrower & Winemaker No. 474: 97-100.

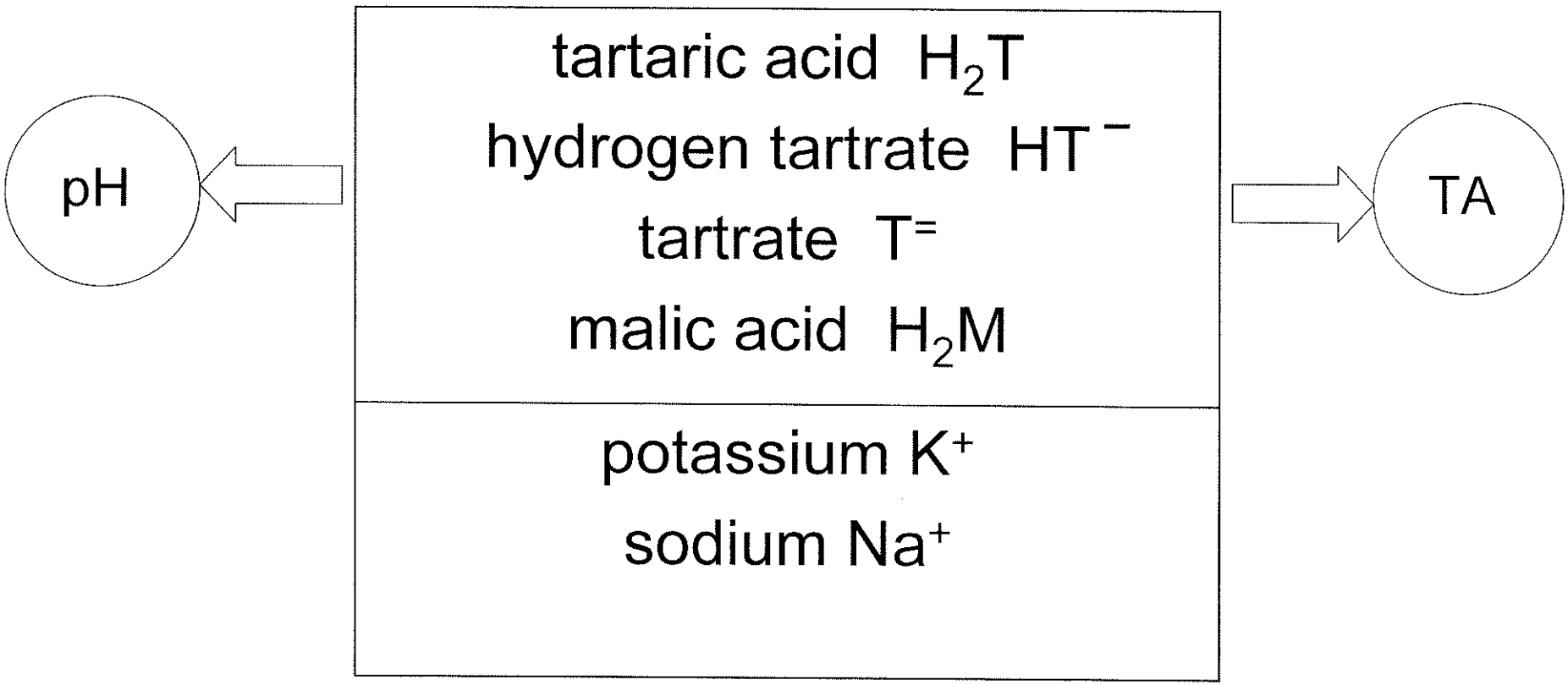
Smith, S., Codrington, I.C., Robertson, M. and Smart, R.E. (1998) Viticultural and eonological implications of leaf removal for New Zealand vineyards. Proceedings of the Second International Cool Climate Viticultural and Oenology Symposium, Auckland New Zealand: 127-133.

CRISP & FRESH

pH, TA
and
malic acid balance

pH ⇧ TA ⇩

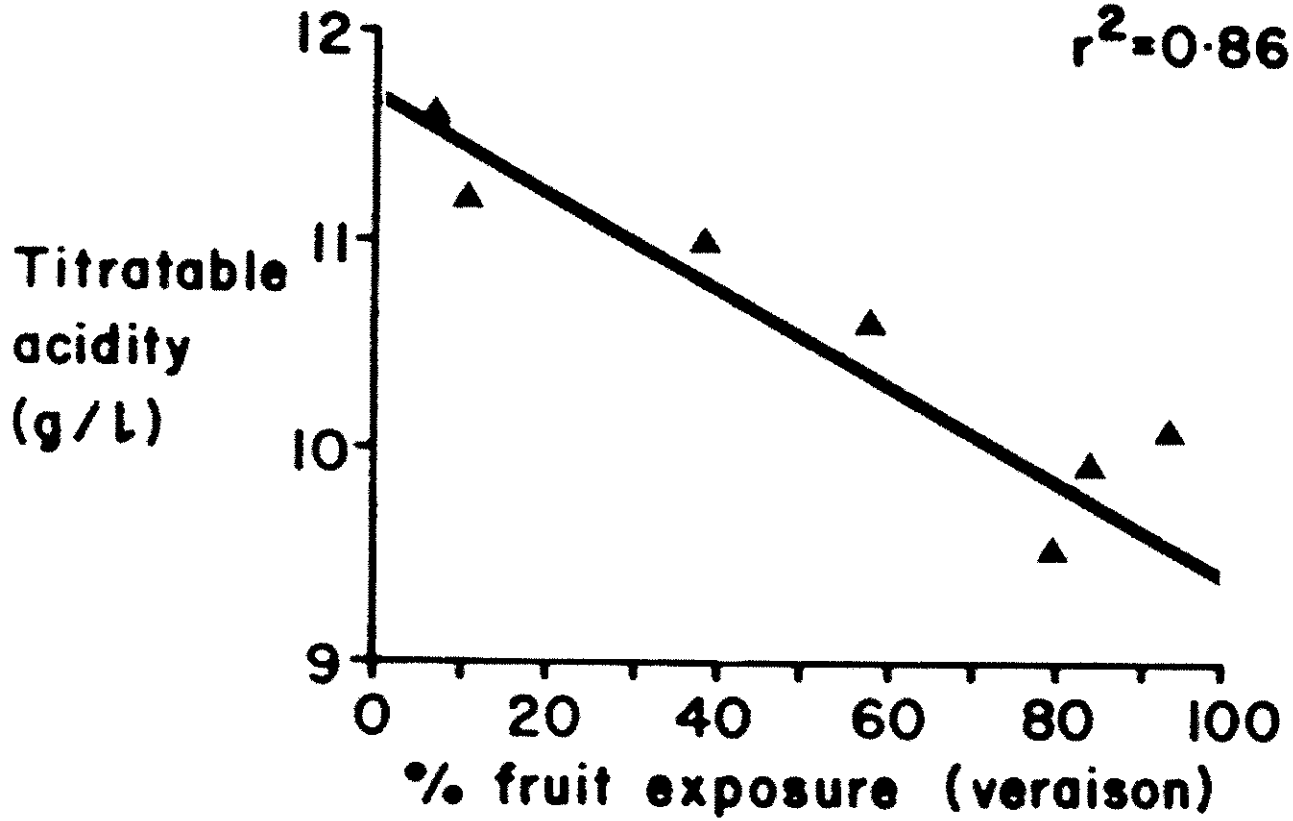
- Dilution of acids due to uptake of water
- Respiration of malic acid
- Changes in the forms of tartaric acid


pH
tartaric acid H_2T
hydrogen tartrate HT^-
tartrate $T^=$
malic acid H_2M
potassium K^+
sodium Na^+
TA

Effect of leaf removal in the bunch zone on Sauvignon Blanc juice composition

	JUICE COMPOSITION					
	°Brix	pH	TA (g/L)	Tartaric acid (g/L)	Malic acid (g/L)	% Fruit exposure
Control	**21.8**	**3.11**	**12.8**	**5.3**	**4.4**	**4**
Leaf plucked 19 Jan	**21.8**	**3.12**	**10.5**	**6.2**	**3.7**	**52**

Adapted from Smith et al. (1988)



Relationship between fruit exposure at veraison and titratable acidity at harvest, Sauvignon Blanc, Marlborough 1987

From Smith et al. (1988)

Effect of leaf removal in the bunch zone on Sauvignon Blanc juice and wine composition

	JUICE COMPOSITION					
	°Brix	pH	TA (g/L)	Tartaric acid (g/L)	Malic acid (g/L)	% Fruit exposure
Control	**21.8**	**3.11**	**12.8**	**5.3**	**4.4**	**4**
Leaf plucked 19 Jan	**21.8**	**3.12**	**10.5**	**6.2**	**3.7**	**52**

	WINE COMPOSITION		
	pH	TA	Score
Control	**3.00**	**10.4**	**14.1**
Leaf plucked 19 Jan	**2.98**	**9.3**	**15.0**

Adapted from Smith et al. (1988)

Table 2. Berry weight and compositional data for fruit from different locations in north-south and east-west rows.

	Berry wt (g)	°Brix	TA (g/l)	pH
E-W North	1.73	22.7	6.8	3.38
Interior	1.89	20.6	9.2	3.23
South	1.91	19.9	8.9	3.08
N-S East	1.86	21.4	7.3	3.27
Interior	2.03	20.6	8.6	3.27
West	1.94	21.2	7.2	3.28
Significance	ns	**	**	ns
LSD	0.13	0.80	0.7	0.14

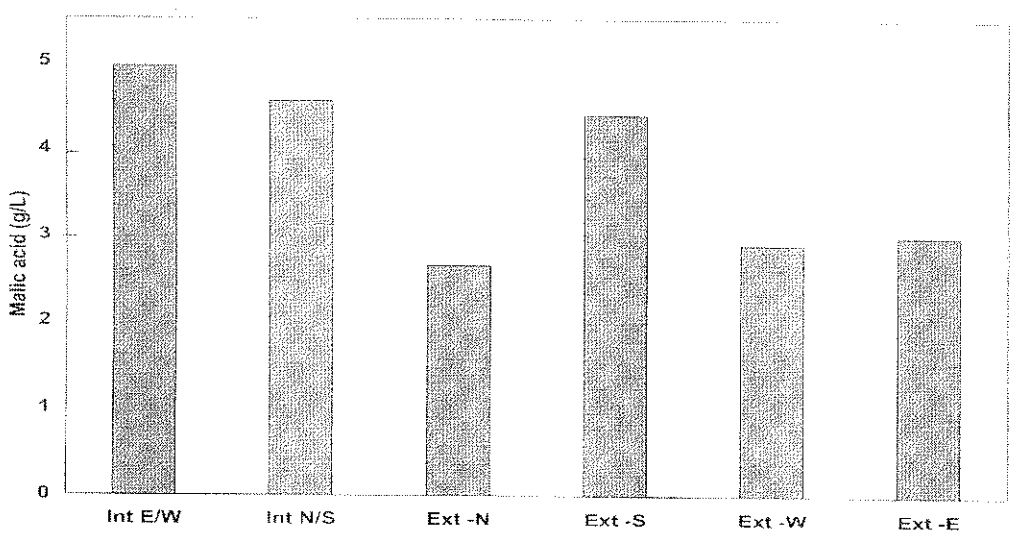


Fig. 3. Malic acid in fruit from different areas of north-south and east-west rows.

Effect of row orientation on Sauvignon Blanc juice composition

	Berry weight (g)	°Brix	pH	TA (g/L)	Malic acid (g/L)
E-W Rows Interior	**1.89**	**20.6**	**3.23**	**9.2**	**approx 4.9**
N-S Rows Interior	**2.03**	**20.6**	**3.27**	**8.6**	**approx 4.6**

Adapted from Naylor et al. (2003)

	Berry weight (g)	°Brix	pH	TA (g/L)	Malic acid (g/L)
Exterior North	**1.73**	**22.7**	**3.38**	**6.8**	**approx 2.6**
Exterior South	**1.91**	**19.9**	**3.08**	**8.9**	**approx 4.4**

Adapted from Naylor et al. (2003)

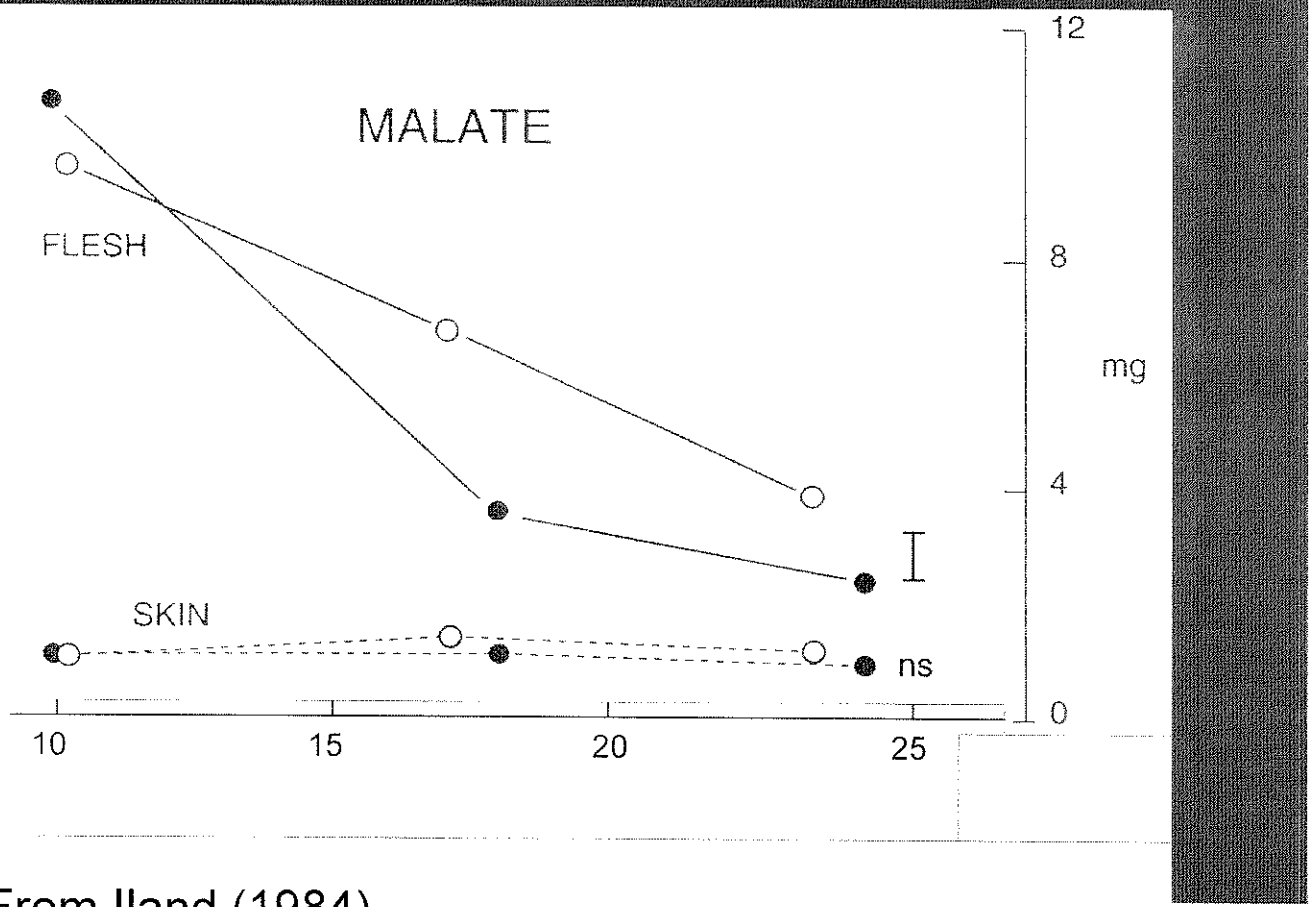
Other studies

Chardonnay Adelaide Hills 1996	°Brix	pH	TA
Control	**22.9**	**3.37**	**7.28**
Leaf plucked (Set)	**22.5**	**3.34**	**6.78**
Leaf plucked (veraison)	**22.6**	**3.38**	**6.45**
Bunch trimmed (veraison)	**23.0**	**3.36**	**6.83**

Chardonnay Adelaide Hills 1997	°Brix	pH	TA
Control	**23.9**	**3.34**	**8.70**
Leaf plucked (Set)	**23.8**	**3.3**	**8.82**
Leaf plucked (veraison)	**23.5**	**3.30**	**7.86**
Bunch trimmed (veraison)	**23.5**	**3.22**	**8.19**

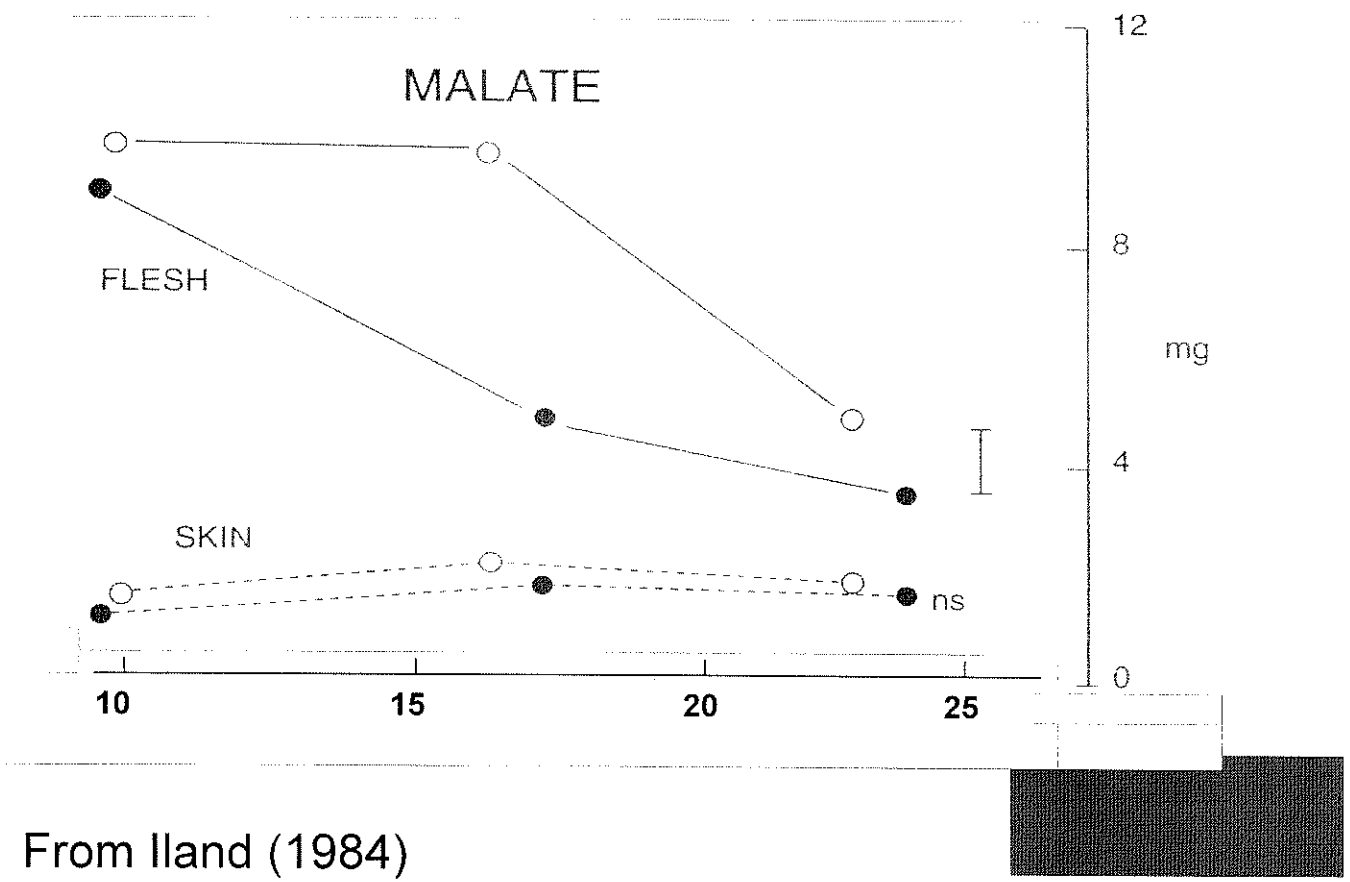
Adapted from Lee (1996) and Hart (1997)

Malic acid per gram berry weight



From Iland (1984)

Malic acid per berry



From Iland (1984)

Conclusions

Sauvignon Blanc studies showed that

- the decrease in TA was due to a decrease in malic acid
- the most effective treatment - leaf removal pre-veraison

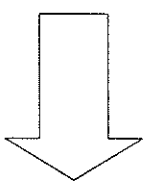
Conclusions

The Chardonnay studies show that

- leaf removal and bunch thinning decreased TA
- there was no difference between pre - and post - veraison leaf removal

Conclusions

Avoid situations which delay
the decline in malic acid



- Vigorous shoot growth
- Excessively shaded leaves and bunches
- Delayed ripening?

Question

Can monitoring juice malic acid levels help to assess optimum harvest time?

Sauvignon Blanc Marlborough New Zealand

Malic acid g/L	3.7	3.7	4.5	4.2	4.4
Aroma descriptor	Tropical/ red capsicum	Tropical/ red capsicum	Green/ red capsicum	Herbaceous asparagus	Herbaceous grassy

Adapted from Smith et al. (1988)

Question

Can monitoring juice malic acid levels help to assess optimum harvest time?

- Study of 2-methoxy-3-isobutylpyrazine (IBMP) in grapes during ripening
- Bordeaux and Loire

☆ Good correlation between

breakdown of malic acid	and	IBMP concentration

From De Boubee et al. (2003)

The answer

Can monitoring juice malic acid levels help to assess optimum harvest time?

- In some situations juice malic acid concentration can be used as an indicator of wine style

When?

- Within a vineyard site where microclimatic effects are driving changes in berry composition.

"Sitting in the Morning Sun"

Shiraz Benchmarking in NE-VIC 2005

Erika Winter, GrapeLinks, consultant; Stephen Lowe, Stony Creek Vineyard, field coordinator, Nick Bulleid, MW, Alan Buchanan, Myrrhee Laboratories and John Harvey, GWRDC, RITA project coordinator

Benchmarking vineyards before harvest according to detailed score systems was introduced in the late '90's and assisted grape quality judgement and crop streaming into quality brackets for different wine styles. The scoresheets were effective to detect major problems in vineyards and fruit. In a RITA initiative starting in 2001 these score systems were used by regional grower and winemaker groups to find cause and effect relationships between management practice, vineyard and fruit traits and wine quality ratings for Shiraz vineyards in Victoria. Results showed that there was a considerable seasonal variation in the correlation between the vineyard measurements and fruit quality parameters and that some traits of vineyards or fruit measured in the past lacked discriminating power due to the adoption of better management practice from good extension activities in the region. Furthermore, all regions were capable of producing medium and higher quality fruit or wine (*Rolley, 2002 and 2003, Lowe, 2004*). In a review of three years of Shiraz benchmarking in Victoria (*Winter 2005*) it became clear that a revised scoring system had to be elaborated. This was achieved by GrapeLinks with GWRDC Knowledge Management support and evaluated in NE-VIC in the 04/05 season.

It was important to recognise that, for full-bodied Shiraz, anthocyanins and total phenolics in fruit play an important role (*Somers, 1998*) as well as the fruit's mouthfeel and aroma characteristics and the standard fruit quality measurements of Bé TA and pH. The new scoring system also used fruit chemistry of grapes and young wines after ferment and Berry Sensory Assessment (BSA, *Winter et al. 2004*) as major benchmarking traits. For the vineyard assessments the new scoresheets contained the measure of vine balance (*Howell 2001*) at veraison (*Winter and Whiting 2004*) and cane lignification at that early stage (as in warm years this trait is not discriminatory before harvest). Furthermore, pre-harvest leaf condition and leaf cover over bunches was assessed on either side of the row. Most importantly, as it is known that colour and phenolics are heat sensitive (*Spayd et al, 2002*), bunch zone temperatures were monitored from veraison to harvest with data loggers attached to the posts at the fruit zone inside the canopies. Photographs of the vineyards were taken to support these observations. All vineyard assessments were done by the field coordinator so as to provide continuity over vineyards and seasons. In contrast to previous years, data were analysed for paired correlation coefficients by GrapeLinks and the data for each vineyard in the correlations was shown in the report to growers and winemakers to enable learning and individual adoption of targeted management.

Wine judgement showed that only the absence of bitterness, herbaceousness harsh tannins and overripe aromas led to good overall wine scores, which could be reached by 9 out of 19 vineyards having a large variation of vine balance, yields, Bé and TA, berry size as well as colour and phenolics in the fruit at harvest. A low degree of cane lignification at veraison was a pointer to unripe fruit and unbalanced wine.

Overall, good scores were observed in wines with grapes of moderately high temperature summation units from veraison to harvest provided there were no bitter phenolics through petioles in the bin or over extraction of unripe seeds.
Good wines were produced by site adapted canopy management which led to warm bunches in the morning but without afternoon heat loads. Cooler sites were able, additionally, to use hang-time to increase temperature summations. The optimum temperature summation window for fruity aromas is relatively small – below which there were herbaceous aromas in the wine and above which jammy aromas were noted. There was a high positive correlation between bunch zone temperature summations and tannin softness rating. The inexpensive temperature monitoring could be developed into a positive predictor system for grape quality. Research data are need to confirm the above findings and find the ripening warmth cut-off point above which the tannins in wines are harsher again as seen in the 3 wines from grapes grown in hot bunch zones.

Only negative quality pointers (lower scores when absent or low) could be drawn from correlations of young wine analysis and berry analysis to wine scores. However BSA ratings of ripe skins were a good positive predictor of tannin softness and BSA ratings for pulp aroma correlated with pleasant "red and black" fruit scores in the wines. Total phenolics or colour in grapes also were higher when bunch zone temperature summations were moderate but were less a predictor for wine scores than assumed in the literature. The relationship between colour and total phenolics in grapes was found over 2 years to be not linear and the ratio depended on the heat loads of the year and in the bunch zones with anthocyanins being the more heat-sensitive.

The very detailed survey showed that vineyards can reach higher phenolic, colour and aroma potential in grapes at all row orientations and in all regions by innovative vineyard scoring including BSA and inexpensive bunch temperature monitoring resulting in site adapted canopy management practices with an emphasis on letting the morning sun onto bunches and avoiding afternoon heat loads. If harvesting and winemaking processes are adapted to avoid MOG and unripe seeds, wines of all regions can at least win bronze medal status.

Howell, S. Sustainable grape productivity and the growth-yield relationship. Am. J. Enol. Vitic. 52(3) pp 165-174, 2001.

Lowe, S. North East Victoria Wine Grape Quality Measurement and Benchmarking. Vintage 2004 Report to GWRDC.

Rolley, L. Wine Grape Quality Measurement and Benchmarking. Central Victoria, Vintage 2002 and 2003 Reports to GWRDC.

Somers, C. The Wine Spectrum. Winetitles1998.

Spayd, S.E., Tarara, J.M., Mee, D.L. and Ferguson, J.C. Separation of sunlight and temperature effects on the composition of *Vitis vinifera* cv. Merlot berries. Am. J. Enol. Vitic. 53 (3), 2002.

Winter, E. Whiting, J. and Rousseau, J. Winegrape Berry Sensory Assessment in Australia. Winetitles, 2004.

Winter, E. and Whiting, J. Using leaf area to crop weight to determine vine balance. Australian Viticulture Jan/Feb pp 70-73, 2004.

Winter, E. Growing good Shiraz. Three years of Shiraz benchmarking studies in Victorian Vineyards. Australian Viticulture Jan/Feb. pp 61-64, 2005.