A STUDY OF SHIRAZ GRAPE BERRY COMPOSITION IN RELATION TO THE QUALITY OF TABLE WINE

A Thesis
Submitted in Partial Fulfilment
of the Requirements for the
Degree of
Doctor of Philosophy

by
Nerida Anne Abbott
(BSc Melb.)
Department of Horticulture, Viticulture and Oenology
The University of Adelaide
1991

TABLE OF CONTENTS

ABSTRACT

Shiraz grapes of preassigned quality were sourced from three regions over three years. Quality assignments of the fruit were made on the basis of winemakers' evaluation of the fruit, commercial and show records of wines from the vineyards, and the viticultural characteristics of the vineyards. A second set of samples was sourced from a South Australian Department of Agriculture field trial.

A qualitative and quantitative study of the free and glycosidically bound fractions of juice and skin samples from high and low quality Shiraz grapes was carried out. The fractions were analysed by gas chromatography-mass spectrometry; 176 compounds were identified. Most of these compounds belong to three biogenic categories, derived from fatty acid, phenylpropanoid and terpene metabolism. A quantitative study of the free and glycosidically bound composition of samples sourced from vineyards known to produce grapes, and hence wine, of a consistently high or low quality, was conducted over three years. The results showed that there was a greater overall concentration of bound volatiles present in the high quality grape samples.

Sensory analysis, duo-trio difference tests and formal descriptive analyses, were performed on mild acid and also glycosidase enzyme hydrolysis of the bound fractions of juices from the 1988 and 1989 vintages. Similar studies on hydrolysates derived from wines of the 1989 vintage were also undertaken. The results showed that for one pair of wines, and all but one pair of hydrolysates, the quality differences could be distinguished and quantified. The glycosidic hydrolysates prepared by both enzyme and acid hydrolysis were found to contribute non-berry attributes to wine such as 'stalky', 'earthy' and 'cigar-tobacco'. Wines considered to be of high quality were rated higher in these non-berry attributes than their low quality counterparts. It may be deduced, therefore, that glycosidic hydrolysates contain aroma compounds that are important to high quality Shiraz wine.

A common feature of the glycosides present in Shiraz grapes, and fruit in general, is that they are glucosides, and the central glucose molecule may or may not be further substituted. A glucose molecule is released on hydrolysis of each glycoside. A three-part assay was developed to determine the concentration of released glucose involving: (a) isolation of juice glycosides; (b) release of glucose with a glycosidase enzyme; and (c) quantification of the released glucose with a hexokinase/glucose dehydrogenase system. The assay was applied to samples of known quality, and the data supported the hypothesis that high quality samples had a higher concentration of glycosidically-bound secondary metabolites.

The glucose assay developed in this thesis was applied to fruit sourced from a vine density trial, treated to produce fruit of varying quality. The measure of the concentration of fruit secondary metabolites as glycosyl-glucose was found to be proportional to the concentration of released volatiles across all treatments at both vine spacings. Such a relationship is encouraging for the use of the simple assay developed during this project to measure potential fruit flavour and therefore wine quality and also as a means of monitoring the changes in fruit flavour during viticultural trials without having to rely only on the inherently subjective assessment of wine quality by sensory analysis.

DECLARATION

I hereby declare that this thesis contains no material that has been accepted for the award of any other degree or diploma at any university. To the best of my knowledge and belief, no material described herein has been previously published or written by any other person except when due reference is made in the text.

If accepted for the award of Doctor of Philosophy, this thesis will be available for loan or photocopy.

Nerida Anne Abbott

ACKNOWLEDGMENTS

I wish to thank my two supervisors, Dr Bryan Coombe and Dr Patrick Williams for their advice throughout this thesis. The Director of the Australian Wine Research Institute and Professor of Oenology (Department of Horticulture, Viticulture and Oenology, The University of Adelaide), Professor Terry Lee has been most supportive during my project and is thanked for giving me the opportunity to complete this study at The Australian Wine Research Institute. Financial assistance from the Grape and Wine Research Council is gratefully acknowledged. The Australian Wine Research Institute is also thanked for its financial support while completing the written report.

Penfolds Wines, Mildara Wines and Wolf Blass Wines are acknowledged for their donation of grape and wine samples.

To Dr. Mark Sefton I extend my gratitude for his constant support and advice throughout this thesis research. I also wish to thank my colleagues at The Australian Wine Research Institute for their friendship, support and especially their help and patience during the sensory analysis.

I also wish to acknowledge the helpful discussion and enthusiasm of many wine industry members especially Prue and Stephen Henschke, Wendy Allen and Patrick Iland.

Thank you also to my family and friends without whose constant support, understanding and encouragement I would not have completed this work.

PUBLICATIONS

Part of the work described in this thesis has been reported in the following publications:

Abbott, N.A.; Coombe, B.G.; Sefton, M.A.; Williams, P.J. The composition of Shiraz grapes in relation to the quality of table wines. 4éme Symposium International d'Oenologie: Actualités oenologiques; 15-17 Juin, **1989**; Bordeaux, France, 94-99.

Abbott, N.A.; Coombe, B.G.; Williams, P.J. The flavour of Shiraz as characterised by chemical and sensory analysis. *Aust. N.Z. Wine Ind. Journal* **1990a**, *5*, 315-319.

Abbott, N.A.; Coombe, B.G.; Sefton, M.A.; Williams, P.J. The secondary metabolites of Shiraz grapes as an index of table wine quality. Williams, P.J.; Davidson, D.M.; Lee, T.H., eds. *Proceedings of the Seventh Australian Wine Industry Technical Conference*; 13-17 August, 1989; Adelaide, SA. Adelaide, SA: Australian Industrial Publishers; **1990b**, 117-120.

Abbott, N.A.; Coombe, B.G.; Williams, P.J. The contribution of hydrolysed flavour precursors to quality differences in Shiraz juice and wines: an investigation by sensory descriptive analysis. *Am. J. Enol. Vitic.* **1991**, *41*, 167-174.

Abbott, N.A.; Coombe, B.G.; Williams, P.J. Development of a rapid analytical technique for the estimation of the concentration of potential volatile flavour compounds and hence quality of *Vitis vinifera* Shiraz grapes. Provisional patent No. IRN 219000, 16th July 1991.

ABBREVIATIONS

%	percent
α	alpha
ΔA	change in absorbance
A	absorbance
AOV	analysis of variance
A_{max}	maximum absorbance
AF	adsorbed frees
AH	acid hydrolysis
β	beta
Bar	Barossa Valley
C18 RP	C18 reverse phase
^{o}C	degree Celsius
cm	centi (10^{-2}) metre
CV	coefficient of variation
CVA	canonical variate analysis
Cw	Coonawarra
et al.	and others
eg.	for example
EH	enzyme hydrolysis
eV	electron volts
F	free volatiles
γ	gamma
g	gram
g	acceleration due to gravity
GBG	glycosidically bound glucose
GC-MS	gas chromatography-mass spectrometry
HPLC	high performance liquid chromatography
HS	headspace
Hq	high quality
i.e.	that is
Lc	Langhorne Creek
Lq	low quality
L	litre

LSD	least significant difference
M	molar
Mq	medium quality
mg	milli (10^{-3}) gram
µg	micro (10^{-6}) gram
min	minute
mL	milli (10^{-3}) litre
µL	micro (10^{-6}) litre
mM	milli (10^{-3}) molar
µM	micro (10^{-6}) molar
mm	milli (10^{-3}) metre
µm	micro (10^{-6}) metre
MRA	multiple regression analysis
mV	milli (10^{-3}) volts
m/z	mass/charge
N_2	nitrogen gas
na	not available
nd	not determined
nm	nano (10^{-9}) metre
NS	not significant
RT	retention time
s	second
Sig	significance
Stdev	standard deviation
o	ortho
p	para
PCA	principal component analysis
ppb	parts per billion
PVPP	polyvinylpolypyrrolidone
V	volt
vol/vol	volume by volume
Wks	weeks
wt/vol	weight by volume

Chapter 1

INTRODUCTION AND LITERATURE REVIEW

Chapter 1

INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION AND AIMS

Traditional methods used in Europe for quality assessment of wine grapes, such as measures of acid and sugar concentration, have been found to be inadequate in Australia and provide only broad indices of fruit maturity (Coombe *et al.* 1980). Accordingly, viticulturists and winemakers have been forced to rely on sensory evaluation of juices before fermentation (Cootes *et al.* 1981), or more frequently of the finished wines, for quality evaluation of grapes. The sensory assessment of wines is an after-the-fact approach which must cope with the inherent variables of winemaking and the complications due to the introduction of fermentation products to the medium being evaluated.

The lack of objective measures of wine grape quality in Australia has led to a disparity in wine grape pricing. Traditional economic forces of supply and demand have been the major determinants of grape pricing, and in most cases independent of grape quality. To create an equitable system whereby grape-growers are rewarded proportionately to the value of wines made, it is necessary to develop objective measures of grape quality that correspond to the potential quality of wine which can be made from these grapes.

The general aim of this work, therefore, was to develop a rapid and reliable index of grape quality for determining the potential quality of wine that can be made from these grapes. However, as little is known about the composition of grapes of *Vitis vinifera cv* Shiraz, the variety under review, this project was divided

into five aims:

1. to develop methods for the qualitative and quantitative study of the volatile secondary metabolites in their conjugated and non-conjugated forms in Shiraz grape berries;

2. to identify the volatile flavour compounds present in Shiraz grapes and wines;

3. to determine if the volatile flavour compounds identified in Shiraz grapes had sensory properties relevant to the wines made from that variety;

4. to develop objective methods for measuring the quality of grapes delivered to the winery which would in turn indicate the potential quality of the wines made from these grapes; and

5. to apply the methods developed in 4) to a grape-vine planting density trial.

1.2 COMPOSITION OF THE GRAPE BERRY

The grape berry is a complex unit of which the flesh or pulp, skin and seeds provide the basic structure (Coombe and Hand 1987). The flavour compounds present within this structure can be divided into two broad categories: primary metabolites that have been defined as the essential elements required for the nutritional survival of a plant (Mann 1987) and secondary metabolites which are 'non-essential' for the plants' survival and which have no obvious metabolic function (Bu'Lock 1965, Williams, D.H. *et al.* 1989). These latter compounds have

a propensity to accumulate in particular organs or tissues of the host (Haslam 1975), yet the actual reasons for the production and accumulation of secondary metabolites in plants are not known. It has been suggested that secondary metabolites are formed as storage compounds for primary metabolites which have been produced in a disproportionate amount in the plant (Bu'Lock 1965). A more recent hypothesis, however, has suggested that 'secondary metabolites are a measure of the fitness of an organism to survive and have been synthesised through evolution to attract or repel other organisms' (Williams, D.H. *et al.* 1989).

Acids and sugars in grape berries are regarded as primary metabolites as these compounds are ubiquitous to all grape varieties, and although they are important for the production of a stable and balanced wine, they do not contribute to the varietal flavour characteristics of the ensuing wine. The secondary metabolites, in contrast, include those compounds that confer the specific varietal characteristics, such as colour, aroma and flavour to each individual grape variety.

1.2.1 Primary metabolites

1.2.1.1 Sugars

The principal sugars present in the grape berry are D(+)-glucose and D(-)-fructose. Glucose predominates at the beginning of ripening (Peynaud 1984), while at maturity the ratio of the two sugars is about unity (Kliewer 1966). In overripe berries the concentration of fructose may exceed that of glucose (Pongracz 1978). Other sugars are also present in the grape berry at low

concentrations, and do not contribute to the characteristics of the grape (Kliewer 1966).

Sugar concentration in the juice can be measured by either hydrometry or refractometry and expressed as °Baumé or °Brix (1°Baumé = 1.80°Brix). °Brix values are a direct measure of total soluble solids, while °Baumé gives an indication of the potential alcohol that will be formed from complete fermentation of the juice. The sugar concentration is therefore a basic index of grape maturity and potential wine style, as a minimum concentration of sugar is required to produce a biologically stable wine of a particular type.

1.2.1.2 Acids

The principal acids present in the grape berry are L(+)-tartaric and L(-)-malic acids which together account for 90% of the total acidity (Winkler *et al.* 1974). Minor amounts of citric, ascorbic and phosphoric acids make up the other 10%.

The acidity of grape juice is usually measured by either pH or titratable acidity (TA). Measures of both pH and TA are important during vinification and are used in conjunction with the sugar content as maturity indicators. The acidity of grape juice is important for the balance of flavour in a finished wine and provides a natural means by which the growth of moulds and bacteria and oxidation of phenolics during aging can be limited (Jakisch 1985, Peynaud 1987, Singleton 1987). The pH also influences the colour of red wines as the equilibrium of anthocyanins between their red/brown and blue/green forms is pH-dependent (Somers 1971).

1.2.1.3 Primary metabolites for the assessment of grape quality

Sugar concentration has been used as an index of quality in most parts of Europe, and for many years has been the sole criterion used for remunerating growers for their grapes (Champagnol 1977, Huglin 1977, Hoare 1988). In France, Champagne is one of the few areas that does not use sugar concentration to determine grape prices, since the quality of the style of wine made does not relate to percent sugar; growers in this area are paid according to weight of grapes harvested with bonuses awarded depending upon the grape variety and the position of the vineyard (Hoare 1988).

In Switzerland, tartaric acid concentration has been used for the evaluation of quality when calculated as a proportion of the total acid content (Eggenburger 1988). This quality parameter was suggested because of the ability of tartaric acid to complex with potassium and calcium ions in the juice (ions that are thought to be detrimental to the quality of wine), thus removing them as a precipitate (Ribéreau-Gayon, J. *et al.* 1975).

In Germany, the validity of sugar concentration as the only index of wine quality has been questioned by Pigott (1988). This author believes that both area and yield influence the potential quality rating of a wine and has proposed a vineyard classification system based upon continuous tastings of wines from each area, with a review of the quality assignment each year.

Some pricing systems in California are linked to a bonus system which takes into account the acid content of the fruit and the position of the vineyard. In many

cases a contractual arrangement exists between the grapegrowers and winemakers, which can be either in the form of a complex legal document, or simply 'word of mouth'. Both parties have the right to invite an inspector to the winery to arbitrate if any problems arise (Hoare 1988).

In Australia, high must pH is a factor detrimental to wine quality; pH has been suggested as a possible indicator of quality for red wines, as a small change in pH has a marked affect both on colour and taste (Somers and Evans 1974, Somers 1977, Glories 1987), and in warm climates wine quality has been demonstrated as being inversely proportional to pH (Somers 1975, Hepner and Bravdo 1985).

Several combinations of pH, °Brix and titratable acidity have been suggested as measures of grape maturity and grape quality (Ough and Singleton 1968, Ough and Alley 1970, Sinton *et al.* 1978, Coombe *et al.* 1980, Du Plessis 1982). A quality index suitable for Australian wine grapes that combines the European quality parameter of sugar concentration with pH, has also been explored by Coombe *et al.* (1980). Some of these indices have been useful as a measure of grape maturity, however, the industry consensus appears to be that they do not adequately delineate either grape or wine quality.

According to Clingeleffer *et al.* (1987), wine quality is dependent upon a combination of acid, sugar and flavour in the grapes, highlighting the complexity of the search for a single index of quality. Another research group related the climate, sugar content, total acidity and tannin concentration to grape and wine quality (Seigel and Tatter 1961).

There is no set standard for grape quality assessment in Australia. Three large South Australian wineries, however, have their own grape quality assessment schemes set within their company infrastructure and tailored to their specific needs. The only scheme documented to any extent is the Grape Quality Assessment Scheme (GQA) developed by Yalumba Wines (S. Smith and Son Pty Ltd) and outlined by Cootes *et al.* (1981) and further explained by Wall (1986). The categories assessed within this scheme and the respective bonus percentage points for each category are given in Table 1.1. Sugar, pH and titratable acidity account for 15% of the total points awarded for grape quality. A major proportion of the bonus points is related to the sensory assessment of aroma and taste of juice based on harvested samples.

Table 1.1 Categories and the respective bonuses paid for grapes according to the Grape Quality Assessment Scheme used by a Barossa Valley winery (Cootes et al. 1981)

Category	Maximum percentage points
Aroma and taste of the grape juice	40
Altitude of the vineyard	20
Chemical analyses	
Baume	5
Titratable acidity	5
pH	5
Physical condition of grape sample (MOG)*	10
Sulfur dioxide content of grape sample	5

* MOG = matter other than grapes

A broad relationship exists between grape primary metabolites and quality of the ensuing wine, as a certain level of sugar (approximately 12 °Baumé) and acid (pH 3.1-3.6) is considered desirable to produce a stable and balanced table wine. However, two separate lots of grapes harvested at the same pH and °Baumé and treated in the same manner can produce two totally different wines. Furthermore, measures of sugar and acid concentration account for neither the special flavour characteristics of particular varieties, nor the variations among wines of the same variety but of a different quality. Therefore, the concentration of primary metabolites should only be considered as broad indices of fruit maturity rather than measures of grape, and potential wine, quality.

1.2.2 Secondary metabolites

The secondary metabolites in grapes are present in both a non-volatile and volatile form. The non-volatile secondary metabolites are mainly responsible for the colour, longevity and 'mouth-feel' of wine whereas the volatile secondary metabolites contribute to the flavour of wine grapes and wine. This latter group includes compounds such as esters, aldehydes, ketones, hydrocarbons, pyrazines, volatile phenols, alcohols and other nitrogenous compounds.

1.2.2.1 Non-volatile secondary metabolites

The non-volatile secondary metabolites comprise mainly phenolic constituents, i.e. anthocyanins, flavonoids and tannins that are responsible for the colour, astringency and maturation potential of wine (Somers 1975, Singleton 1982, Ribéreau-Gayon and Glories 1987). These compounds are present in seeds (64%), skins (33%) and juice (3%) (Meyer and Hernandez 1970). The stalks are also an

important source of the more astringent phenols (tannins) and, depending on the technique of vinification, can affect the tannin level in a finished wine (Singleton and Noble 1976). During ripening the level of phenols increases in the skins, decreases in the seeds and stays constant in the stems (Ribéreau-Gayon and Glories 1982). Due to the high concentration of phenolics in the skins and seeds, the phenolic concentration in wine can be manipulated by the amount of skin contact and the extent of pressing during vinification.

The phenols that contribute to the colour and astringency of wine are: flavonols that are responsible for the yellow pigment in the skin of black berries with only trace amounts being found in white berries (Ribéreau-Gayon, P. *et al.* 1975); anthocyanins that are present mainly in the skin (Somers 1967) (as monoglucosides in *Vitis vinifera* grapes) and contribute to the colour of the berry; and the procyanidins or tannins in the skin, seeds and stalks that contribute to the astringency of wine.

Although the structure of the tannins during the aging of wine is well understood (Somers 1967, Ribéreau-Gayon and Glories 1987), little is known about the reasons for the variation in 'mouth-feel' of young wines. Recently, however, it has been demonstrated that these compounds do contribute to the bitterness and astringency of wines (Lea 1990, Robichaud and Noble 1990). Astringency generally decreases during aging as a result of increasing condensation of tannins present in the wine (Somers 1977). Both polymerisation of the tannins to form larger tannin polymers and condensation reactions between tannins and anthocyanins have been shown to lead to a greater colour stability during aging

and a decrease in astringency (Ribéreau-Gayon and Glories 1987).

1.2.2.2 Non-volatile secondary metabolites for the assessment of grape and wine quality

The anthocyanin composition of the berry has been suggested as a possible index of black grape quality (Somers 1975, Kliewer 1977, Jackson *et al.* 1978, Timberlake *et al.* 1978, Ribéreau-Gayon and Glories 1987). Somers and Evans (1974, 1977) found no relationship between wine quality and either colour density or anthocyanin content. They did find, however, that there was a correlation between the degree of ionisation of anthocyanins (α) and colour and the overall quality of red wines made from Cabernet Sauvignon and Shiraz. A relationship between grape colour and wine score has recently been established for Pinot Noir grapes and wine (Iland and Marquis 1990); the colour of Pinot Noir grapes was positively correlated to the wine score for a particular site.

1.2.2.3 Volatile secondary metabolites of fruit

The low molecular weight secondary metabolites of fruits occur not only as free, volatile compounds but are also bound as non-volatile flavourless glycoconjugates.

The volatile aroma component of many fruits and spices has been studied, and a synopsis of these data has been given in Table 1.2. In the past, such studies have concentrated on the identification of the free aroma volatiles in these foodstuffs in an effort to find key aroma compounds for use as flavourants. These key free volatile compounds have been useful in monitoring changes in the volatile

Table 1.2. Some examples of foods and beverages whose free and/or bound volatiles have been analysed.

Food or beverage		Free volatiles	Bound volatiles	Ref
Wine	Chardonnay	*		r
	Riesling	*		h, s, dd
	Shiraz	*		b, u, x
	Terret, Ugni Blanc, Clairette, Sauvignon blanc, Picpoul, Cinsaut, Grenache, Cabernet Sauvignon, Shiraz	*		b
	Gamay, Shiraz, Malbec, Carignan, Pinot	*		u
Grapes	Riesling		*	o,t,y
	Chardonnay	*	*	q, t, z
	Muscat	*	*	n, t, aa
	Sauvignon Blanc		*	z
	Semillon		*	z
	Traminer, Rulander, Muller Thurgau, Scheurebe, Optima, Rieslander	*	*	o
Other fruits and spices	Apple		*	ff
	Apricot, yellow plum, peach		*	i
	Blackcurrant	*		j
	Celery	*	*	w
	Elderberry	*		m
	Feijoa	*		p
	Ginger	*	*	dd
	Honey	*		v
	Lemon peel	*		d
	Marjoram		*	k
	Passionfruit	*	*	f, cc
	Pineapple	*	*	ee
	Quince	*	*	bb
	Raspberry		*	l
	Strawberry	*		e
	Strawberry jam	*		a
	Black and green tea	*	*	g
	Tomato		*	c

[a]Barron and Etiévant 1990, [b]Baumes et al. 1986, [c]Buttery et al. 1990, [d]Chamblee et al. 1991, [e]Douillard and Guichard 1990, [f]Engel and Tressl 1983, [g]Fischer *et al.* 1987, [h]Güntert et al. 1986, [i]Krammer et al. 1991, [j]Nijssen and Maarse 1986, [k]Nitz et al. 1985, [l]Pabst et al. 1991, [m]Poll and Lewis 1986, [n]Ribéreau-Gayon, P. et al. 1975, [o]Schreier et al. 1976a, [p]Shaw et al. 1990, [q]Sefton et al. 1991, [r]Simpson and Miller 1984, [s]Simpson and Miller 1983, [t]Strauss et al. 1987b, [u]Symonds and Cantagrel 1982, [v]Tan et al. 1989, [w]Tang et al. 1990, [x]Vernin *et al.* 1988, [y]Williams, P.J. et al. 1982a, [z]Williams, P.J. et al. 1989, [aa]Wilson et al. 1984, [bb]Winterhalter and Schreier 1988a, [cc]Winterhalter 1990, [dd]Wu et al. 1990, [ee]Wu et al. 1991, [ff] Schwab and Schreier 1990a.

composition of fruits after processing, e.g. freezing (Douillard and Guichard 1990). Wine made from different grape varieties has not been able to be differentiated in terms of the qualitative composition of their free volatiles (Baumes *et al.* 1986). However, with the application of discriminant analysis to a small number of components and a large number of samples, quantitative differences in composition amongst wines made from different grape varieties have been demonstrated (Symonds and Cantagrel 1982, Rapp 1988a). Etiévant (1991) has recently published a summary of the compounds that are considered to be characteristic of either particular varieties or certain technological processes. Where possible, sensory threshold data for these compounds have also been provided.

1.2.2.4 Classes of volatile secondary metabolites present in grapes and grape products

The volatile secondary metabolites so far identified in either free or bound forms in grape juice and wine can be divided into three broad biogenic categories: terpenoids, substituted benzene derivatives and aliphatic compounds.

1.2.2.4.1 Terpenoids

The terpenoids are a large and structurally diverse group of secondary metabolites derived from mevalonic acid (Mann 1987) with structures made up of C_5 isoprenoid units. In *Vitis vinifera* grapes, only the monoterpenes (C_{10}), norisoprenoids (C_7 C_8 C_{11} and C_{13}), sesquiterpenes (C_{15}) and carotenoids (C_{40}) have been identified as natural products.

- *Monoterpenes*

Monoterpenes are the most extensively studied group of secondary metabolites in *Vitis vinifera* grapes and wines. These compounds have been identified as important contributors to the aroma of the essential oils of many plant species, where they are often present at high concentration and have characteristic odours.

The monoterpene composition of grapes and wines has been thoroughly surveyed in several reviews:
Marais 1983, Rapp *et al.* 1984, Strauss *et al.* 1986b, Rapp 1988b, most of which provide a comprehensive list of all terpenes and terpene derivatives. Marais (1983) and Rapp *et al.* (1984) described the changes in the concentration of terpenes in grape berries during ripening and the effect of grape maturity, fermentation, aging and technological treatments on the terpenes in grapes and wine. Furthermore, Rapp *et al.*(1984) illustrated the use of terpene profiles to distinguish Riesling from other white wine grape varieties, and in addition Strauss *et al.* (1986b) reviewed the effects of fermentation, aging and winemaking techniques on monoterpene concentration as well as methods for the analysis of free and bound monoterpenes and their significance in winemaking. As part of a review on wine aroma, Rapp (1988b) listed 50 known monoterpene oxides and alcohols identified up to 1988 as well as the changes in monoterpene composition brought about through infection of grapes by the fungus *Botrytis cinerea*.

Three new monoterpene alcohols have been recently identified as grape conjugates in Riesling wine, the uroterpenol ***(1)*** and 3,7-dimethyloctane-1,7-diol ***(2)*** and

(Z)-3,7-dimethyloct-2-ene-1,7-diol ***(3)*** (Winterhalter *et al.* 1990). These latter two compounds, ***(2)*** and ***(3)***, had previously only been observed as free volatiles in Muscat (Rapp *et al.* 1983, Strauss *et al.* 1986a). (*For structures of compounds see chapter 5)*

- *Norisoprenoids*

The second group of terpenoids, the C_{13} norisoprenoids, is one of the most important classes of flavour compounds identified in nature. Many such compounds have attractive aromas and low odour threshold values. These compounds are used extensively in the perfume industry and as food flavourants (Weeks 1986). Although not proven, carotenoids are regarded as the precursors of norisoprenoids in tobacco (Enzell 1981, Wahlberg and Enzell 1987), grapes (Sefton *et al.* 1989, Williams, P.J. *et al.* 1991), tomatoes (Cabibel and Nicolas 1991) and fruits (Winterhalter and Schreier 1988b). This hypothesis is based on structural relationships amongst the compounds as well as the identification of both carotenoids and norisoprenoids in these plant products. However, Eugster and Märki-Fischer (1991) have recently demonstrated that carotenoid degradation in roses occurs according to the following scheme:

$$C_{40} \longrightarrow C_{13} + C_{27} \longrightarrow C_{13} + C_{14}$$

Eugsters' group has identified many C_{27} and C_{13} compounds in roses as well as one C_{14} compound, rosaflurene to support this hypothesis.

In grape extracts, most of the major C_{13} norisoprenoids identified to date have

been postulated as arising from the enzymatically catalyzed oxidative cleavage of the 9,10 and 9',10' double bonds of the six carotenoids identified in *Vitis vinifera* grapes (Gross 1987, Razungles *et al.* 1987, 1988). The C_{13} norisoprenoids formed from the oxidative cleavage of these double bonds can be classified firstly in terms of their likely biogenesis from carotenoid precursors, and secondly in terms of their carbon skeleton; i.e. compounds with an intact megastigmane skeleton or non-megastigmanes formed from megastigmane precursors by acid-catalysed cyclization and rearrangement reactions (Sefton *et al.* 1989).

-Sesquiterpenes

C_{15} terpenoids have been identified as important components of the aroma of many essential oils (Ramaswami *et al.* 1986). Although more than 200 sesquiterpenes have been found in nature (Ramaswami *et al.* 1986) few have been identified in grapes or wine. Schreier *et al.* (1976a) tentatively identified several sesquiterpenes in grape juice, of which fourteen were later confirmed (Schreier *et al.* 1976b). Several other sesquiterpenes have been cited in a review on terpenes in grapes and wine, but with no comment as to their importance or origin (Marais 1983).

1.2.2.4.2 Substituted benzene derivatives

The majority of the benzene derivatives or their glycoconjugates identified in grapes are presumed to be derived, at least in part, from the shikimic acid pathway. This pathway produces an extremely diverse range of compounds, including the aromatic amino acids, cinnamic acids and polyphenols (Mann 1987), with a variety of hydroxy and methoxy substitution patterns on the aromatic

ring, and one- to four- carbon side chains. Of these volatile phenols, 4-ethylguaiacol and 4-ethylphenol are thought to be amongst the most important flavour components of red wine (Etiévant 1981) and are also thought to be shikimate derived.

The vinyl phenols may be formed by the enzymatic decarboxylation of the parent cinnamic acid derivatives by yeasts or bacteria (Schreier 1979) or by simple heating. Care must be taken, therefore, in assigning importance to the concentration of the vinyl phenols found in gas chromatograms of extracts of grapes or wine, as they may be present due to thermal decarboxylation in the GC injector block. Ethylphenols, in contrast, are thought to be formed by bacteria during malolactic fermentation (Dubois 1976) and therefore would not be expected as grape components.

The role of benzene derivatives in wine has been mentioned in several general and specific reviews (Maga 1978, Schreier 1979). The thresholds of simple phenols in food, their origins, mechanisms of formation and occurrence in foods and beverages have been presented (Maga 1978). Schreier (1979) indicated that most of the known volatile phenols were present in wines rather than grapes; this observation was consistent with the view that the volatile phenol composition of wine originated from use of oak during fermentation and maturation (Dubois and Dekempe 1982). However, most of the volatile phenols identified as wood components have also been isolated from wine made with no wood contact (Etiévant 1981) proving that the volatile phenols can also be of grape origin.

Most phenols are present in grapes and wines at a concentration below their individual sensory thresholds. The aroma of these phenols is considered to be additive and, as a consequence, they may make a significant contribution to the aroma of some wines (Singleton and Noble 1976).

1.2.2.4.3 Aliphatic compounds

Most of the straight chain, aliphatic, acidic compounds that have been identified as wine constituents have been postulated as yeast fermentation products (Schreier 1979), however, a minor concentration of several of these compounds has been observed in a study of Chardonnay juices and hydrolysates (Sefton *et al.* 1991a).

Maga (1976) outlined the importance of lactones as aroma compounds for different foodstuffs. The C_4-C_{10} γ-lactones have also been found in grapes and wines (Schreier 1979, Sefton *et al.* 1991a) and, as they are potent odour compounds (Stahl 1973), they may make an important contribution to wine aroma either singularly or as a group. The lactones in wine have been considered to originate either from the acid catalyzed cyclization of unsaturated fatty acids or during fermentation via reactions involving 4-oxobutyric acid and 2-oxoglutaric acid (Muller *et al.* 1973). Neither of these sources accounts for the presence of 4-alkyl γ-lactones in grapes. In milk, the formation of lactones is considered to arise from the hydrolysis of triglycerides containing a hydroxyacid residue (Kinsella *et al.* 1967), a process which may also occur in wines.

1.2.2.5 Volatile secondary metabolites for the assessment of grape and wine quality

Few data are available on the potential of volatile secondary metabolites as an index of grape and wine quality except for the quantification of monoterpenes for flavour evaluation of floral varieties which has been suggested for the objective assessment of grape quality (Dimitriadis and Williams 1984). Quantification of monoterpenes as an indicator of quality is restricted, however, to floral varieties, which contain a relatively high concentration of monoterpenes (Williams, P.J. *et al.* 1987).

It is generally considered that the key to an objective measure of grape quality that correlates with wine quality lies with the measurement of flavour compounds. At this stage, however, no such correlations have been demonstrated.

Chapter 2

PRELIMINARY WORK: FRUIT AND WINE SAMPLES AND DEVELOPMENT OF ANALYTICAL TECHNIQUES

Chapter 2

PRELIMINARY WORK: FRUIT AND WINE SAMPLES AND DEVELOPMENT OF ANALYTICAL TECHNIQUES

2.1 ABSTRACT

Isolation of the glycosidically conjugated volatile components of Shiraz juice via adsorption onto C_{18} reversed phase (C_{18} RP) adsorbent cased in a *Sep-pak* cartridge was more efficient than adsorption of these compounds onto a large column of C_{18} RP adsorbent. The reproducibility of the total concentration of enzyme- and acid-catalysed hydrolysates was judged to be acceptable with a coefficient of variation of less than 13%. Quantification of the individual acid-released volatile compounds was more reproducible for a greater number of compounds than quantification of the individual enzyme-released compounds.

2.2 INTRODUCTION

The analysis of flavour compounds in any fruit involves a series of steps: extraction (removal of volatile and conjugated aroma compounds from the fruit or fruit product), concentration, separation and finally identification of the volatile aroma compound (Williams, A.A. 1982).

2.2.1 Extraction of free volatile compounds

Procedures for the extraction of free volatile compounds include steam distillation, liquid/liquid extraction and headspace techniques using either solvents (Rapp and Knipser 1980) or polymeric adsorbents (Jennings *et al.* 1972, Bertuccioli and Montedoro 1974, Williams and Strauss 1977, Noble and Murakami 1979) to trap the compounds. The type of solvent or adsorbent

chosen is dependent upon both the type of compounds being extracted and the matrix in which they are present. For the extraction of free volatiles from fermentation products, nitrogen entrainment of the volatiles onto polymeric adsorbents is advantageous in that the adsorbents generally have a minimum retention for ethanol and water. Most adsorbents, however, have a limited capacity to adsorb volatiles over a wide range of boiling points and are therefore of little value for complete analysis of the volatile composition of fruit products (Williams, P.J. *et al.* 1982b).

The use of dynamic headspace extraction with nitrogen, coupled to continuous liquid/liquid solvent extraction, as described by Rapp and Knipser (1980), has enabled the identification of a range of free volatile compounds with a single method of analysis. Etiévant *et al.* (1986) used a standard solution of twelve compounds to compare the Rapp and Knipser method of headspace analysis with other headspace techniques, such as purge and cold trap injection, static headspace (with and without preconcentration) and also with direct liquid injection. The dynamic headspace technique combined with liquid/liquid extraction was the most sensitive. The reproducibility of this headspace technique was further examined by quantification of a model system of sixteen compounds (Guichard and Ducruet 1984); the Rapp and Knipser method was effective for apolar compounds but not for polar compounds, which were not recovered quantitatively. This limitation applies not only to the Rapp and Knipser headspace technique but to all headspace analyses, as the coefficient of activity of polar substances is low and their recovery takes much longer than that for apolar substances.

The solvent chosen for any method of extraction must have a high degree of purity and a low boiling point to reduce the possibility of artefacts in the aroma extract (Rapp *et al.* 1982). The free volatile compounds present in grape juice are most commonly extracted by continuous liquid-liquid extraction using either Freon (Rapp *et al.* 1980, Williams, P.J. *et al.* 1980) or dichloromethane (Ribéreau-Gayon, P. *et al.* 1975).

A recent comparative study of extraction methods and solvents for the extraction of volatile components of grape juice demonstrated that dichloromethane was the most suitable solvent for isolating volatiles from grape juice and that steam distillation-extraction was the most accurate and precise method for their quantitative analysis (Blanche *et al.* 1991).

2.2.2 Isolation and analysis of glycosidically conjugated volatiles

The above procedures have focused on the identification of the free volatile compounds present in extracts of fruit and fruit products. However, the paucity of the free compounds in juices of some grape varieties, added to the fact that these compounds have not been useful to discriminate amongst wines made from different varieties with obviously different aromas, raised the question about the nature of compounds responsible for these differences and the origins of the perceived aroma of the juice and wine (Etiévant *et al.* 1983).

The presence of glycosidically conjugated volatile compounds in grapes was first suggested by Cordonnier and Bayonove (1974) and later confirmed by Williams, P.J. *et al.* (1982a). This discovery has since led to the development

of techniques to isolate glycoconjugates from grape juice (Williams, P.J. *et al.* 1982b, Günata *et al.* 1985b) and to release the conjugated volatiles by either acid or enzyme hydrolysis (Strauss *et al.* 1986b).

Intact glycosides, isolated from grape juice on C_{18} RP adsorbent (Williams P.J. *et al.* 1982b), have been further separated using droplet counter-current chromatography (DCCC), a technique that has enabled the flavour chemist to identify both the volatile and non-volatile moieties of grape glycosides (Strauss *et al.* 1987b, Winterhalter *et al.* 1990). Günata *et al.* (1985b) used solvents of increasing polarity to separate the glycosides, isolated from grape juice on XAD adsorbent, into broad categories prior to analysis. HPLC (Bitteur *et al.* 1989) and HPLC in conjunction with soft-ionisation mass spectrometry (Salles *et al.* 1988) have also been used for the elucidation of glycosides.

2.2.3 Hydrolytic liberation of flavour compounds from glycoconjugates

The volatile moiety of the conjugate can be characterised only after its free form is liberated; this can be achieved by either enzyme- or acid-catalysed hydrolysis of the glycosidic fraction.

2.2.3.1 Acid hydrolysis

Acid hydrolysis of glycosides occurs naturally in grapes and wines due to their low natural pH; the rate of hydrolysis is dependent upon processing or storage temperatures (Günata *et al.* 1986, Voirin 1990). Deconjugation of grape or wine glycosides by thermal treatment with acid at grape and wine pH,

therefore, gives the same array of compounds that one would expect after several years of maturation of a wine.

Some grape glycosides are reactive under acidic conditions (Strauss *et al.* 1984) and can be deconjugated by heating either whole juice or glycosidic extracts at pH 1 (Williams, P.J. *et al.* 1982b). In one such experiment, similar patterns of monoterpenes were released by both enzyme- and acid-catalysed hydrolysis of the glycosidic fraction (Williams, P.J. *et al.* 1982b), with additional compounds identified amongst the acid hydrolysis products. These additional products were formed by chemical rearrangements. A further experiment demonstrated that heating a juice of low pH can lead to extensive changes in the volatiles creating difficulties in the identification of the precursor aglycones (Williams P.J. *et al.* 1985a).

Therefore, acid hydrolysis provides a means of understanding the flavour development that may be expected on cellaring a wine, it does not, however, provide a means of positively identifying the precursor aglycone glycoconjugated in the fruit.

2.2.3.2 Enzyme hydrolysis

Enzyme hydrolysis of fruit glycosides, in contrast to acid hydrolysis liberates the intact aglycone without rearrangement, thereby providing a means of direct identification of the volatile compound that was previously glycosidically bound in the fruit.

Enzyme hydrolysis of the glycosides to their respective volatile aglycones involves a two step process: initial cleavage of the secondary inter-sugar link followed by hydrolysis of the aglycone-glycosidic linkage by enzymes with a ß-glucosidase function. The enzyme system employed for hydrolysis of these disaccharide glycosides must therefore be multifunctional (Günata *et al.* 1988).

2.2.3.2.1 Natural grape glycosidases

Natural grape glycosidases have been isolated and characterised and shown to have ß-glucosidase, ß-galactosidase and α-arabinosidase activities (Aryan *et al.* 1987). These glycosidases, however, have limited hydrolase activity *in situ* and hydrolyse only a small proportion of the total monoterpene glycosides in juice (Aryan *et al.* 1987, Günata *et al.* 1990). Grape-derived glycosidases are also constrained by their aglycone specificities, and have limited capacity to hydrolyse conjugates of tertiary alcohols (Aryan *et al.* 1987).

2.2.3.2.2 Release of flavour by glycosidases during vinification

The extent to which the natural glycosidases present in grapes hydrolyse glycosides during vinification is unknown. Most of the glycosidase activity of these enzymes would be expected to be inhibited by the high sugar concentration and the low pH (3-4) of grape juice. The optimum conditions for the glycosidase activity of these enzymes is at pH 5. It is possible that these enzymes regain some of their activity as the sugar concentration decreases during fermentation, but again it is not known to what extent or even if this occurs in the latter stages of fermentation and during maturation. If, however, an enzyme could be found that is active under these conditions, the

oenologist would have a means of modifying and 'enhancing' flavour. A fungal enzyme preparation, *Novoferm 12*, has been developed for the release of flavour in commercial winemaking and has known ß-glucosidase, α-arabinosidase and α-rhamnosidase activities (Canal Llaubères 1990). The preparation is inhibited by high glucose concentration but regains full activity towards the end of fermentation at 10 g/L glucose (<1 Baumé). The glycosidase activity of *Novoferm 12* is not inhibited by the alcohol concentration in wine and will continue to liberate flavour compounds in the finished wines (unless the enzyme is removed by fining). For sweeter style table wines with retained 18-25 g/L sugar the utility of *Novoferm 12* is questionable.

2.2.3.2.3 Glycosidases in flavour research

Flavour researchers have overcome the difficulties caused by the inhibition of glycosidase activity by sugar in grapes and wines through the development of methods for extracting the intact glycosides from juice and wine (Section 2.2.2 above). Enzyme preparations derived from both plant and fungal sources have been used for research into the identification of both the aglycone and sugar moieties of glycosides in grapes.

A plant-derived glycosidase, almond emulsin, has been used extensively in the study of grape glycosides (Williams, P.J. *et al.* 1983, Wilson *et al.* 1984, Günata *et al.* 1985b, Aryan *et al.* 1987). This enzyme has specific ß-glucosidase (Günata *et al.* 1990) with some ß-galactosidase (Aryan *et al.* 1987) and ß-xylosidase activities (Schwab and Schreier 1990b). Almond emulsin, however, has a limited capacity to hydrolyse conjugates of tertiary alcohols

(Aryan *et al.* 1987).

Fungal-derived enzymes provide a further source of natural glycosidase enzyme preparations, most of which exhibit a high level of ß-D-glucosidase, α-L-arabinosidase and α-L-rhamnosidase activities (Williams, P.J. *et al.* 1991). Cordonnier *et al.* (1989) compared the hydrolase activities of 34 enzyme preparations tested in juice or marc extracts; the array of aglycones released was variable, highlighting the variation in glycosidase activities present in the enzyme preparations. Two fungal-derived enzyme preparations *Pectinol VR* and *Rohapect C,* which have been used extensively in the study of fruit glycosides, have been shown to be non-specific with regard to aglycone structure (Günata *et al.* 1985a, Aryan *et al.* 1987). Furthermore, *Rohapect C* completely hydrolysed the glycosidic fraction of a Riesling wine (Winterhalter *et al.* 1990). The completeness of the hydrolysis was demonstrated in a control experiment where acid hydrolysis of the aqueous residue remaining after removal of the aglycones given by enzymic hydrolysis released no further volatiles (Winterhalter *et al.* 1990).

Variables to be considered in the hydrolysis of a glycosidic fraction of any fruit include not only the choice of enzyme but also the concentration of enzyme. Sefton and Williams (1991) have found that a high concentration of three fungal enzyme preparations can produce artefacts due to oxidation of some aglycones.

2.2.4 Analysis and identification of volatile compounds

Gas chromatography is the method most often employed for the separation of volatile aroma compounds present in an aroma extract. The development of high-efficiency glass capillary columns and the optimization of flow rate of carrier gas, the split ratio in split/splitless injectors and temperature programs have enabled the resolution of hundreds of compounds present in the one sample without pre-separation of any volatile compounds (Rapp *et al.* 1982). Mass spectrometry coupled with gas chromatography is the most common method used for the identification of volatile aroma compounds present in a crude aroma extract.

The odour qualities and relative intensities of odour components in complex mixtures can be described by a method known as gas-chromatographic sniffing (GC-sniff) (Christophe and Drawert 1985). The separated volatile components eluting in the effluent of a gas-chromatographic column are identified simultaneously by a flame ionization detector (FID) and a human nose located at a sniffing port. GC-sniff has been used for the detection of undesirable or off-flavour components in aroma extracts, such as the strawberry-like flavour found in wines made from *Vitis labrusca* grape varieties (Rapp 1988a) and the catty odour found in blackcurrant products (Nijssen and Maarse 1986). GC-sniff has also been used to identify the aroma impact compounds of foods and beverages; Poll and Lewis (1986) identified, by GC-sniff, five distinct regions in a gas chromatogram of an elderberry extract that corresponded with an elderberry-like aroma. They subsequently identified the compounds responsible for these aromas. Furthermore, compounds present at a

concentration below the detection limits of a FID detector have also been characterised by GC-sniff techniques (Christophe and Drawert 1985).

2.2.5 Choice of Shiraz grapes as the subject of this study

The flavour composition of many floral and non-floral white grape varieties is reasonably well understood; in contrast the flavour composition of the black *Vitis vinifera* varieties has received little attention. This has possibly been due to the distinctive and simple aroma of the white floral varieties first studied, e.g. cv. Muscat of Alexandria, as well as the complicating factor of skin contact during red wine vinification. The free and bound volatile composition of the non-floral white grape varieties Chardonnay, Semillon and Sauvignon Blanc (Sefton *et al.* 1989, Williams, P.J. *et al.* 1989) and the floral but non-muscat variety Riesling (Winterhalter *et al.* 1990) has recently been investigated. Development of methods for analysing the glycosidically bound volatiles in grape juice has led to the elucidation of many different classes of compounds and has demonstrated that the aroma of these varieties is dependent not upon monoterpenes but on other classes of compounds such as norisoprenoids and substituted benzene derivatives (Williams, P.J. *et al.* 1989).

The *Vitis vinifera* variety Shiraz was chosen for this study as it is a significant red wine grape in Australia and one from which a range of wine styles, from bulk to premium, can be produced. Furthermore, several viticultural trials have demonstrated that the yield and quality of Shiraz grapes can be manipulated by differing crop levels, canopy management, rootstock, water

status and other viticultural practices (Freeman *et al.* 1979, 1980, Hedberg and Raison 1982, McCarthy 1983, McCarthy and Staniford 1983, Cirami *et al.* 1984, Smart *et al.* 1985a, 1985b, Cirami *et al.* 1987, McCarthy 1988).

The table wines produced from this variety are generally intensely coloured, with a full-bodied palate structure. Shiraz wines have a characteristic aroma often described as violet, raspberry and iris root (Lichine 1979), blackberry, cherry, plum, spice, pepper, earthy and tobacco (Henschke *et al.* 1990).

The volatile composition of Shiraz wine has been investigated in two general studies of the volatile constituents of wines from different cultivars (Symonds and Cantagrel 1982, Baumes *et al.* 1986). Both groups found that the qualitative composition of Shiraz wine did not differ from that of other red wines. Application of discriminant analysis to six compositional variables, however, demonstrated that Shiraz wine could be quantitatively separated from wines made from other black grape varieties (Symonds and Cantagrel 1982).

Approximately 200 compounds have been identified in a volatile extract of Shiraz wines including alcohols, esters, acids, ethers and ketals, carbonyl compounds, amides and phenols, hydrocarbons and heterocyclic compounds (Vernin *et al.* 1988). Stepwise discriminant analysis was used in this study to reduce the number of variables (compounds) needed to classify Shiraz wines according to their origin. The evolution of the volatile compounds in Shiraz berries during maturation was also studied by these authors. The main

components identified were C_6 aldehydes and their corresponding alcohols, of which *n*-hexanol was found to increase and both *trans*- and *cis*-3-hexen-1-ol were found to decrease during ripening (Vernin *et al.* 1988). These authors also studied the composition of the skins of Shiraz berries and found that several sesquiterpene hydrocarbons and geraniol were the major constituents of the skin extract, with the latter compound decreasing during ripening.

The evolution of geraniol and ß-carophyllene in the skins and *n*-hexanol and *trans*-hex-2-enal in the juice were found to be useful discriminants in distinguishing the composition of one clone (clone 73) from the other two studied (clones 99 and 100) (Boniface *et al.* 1987).

Five carotenoids, ß-carotene, lutein, neoxanthin, 5,6-epoxylutein and violaxanthin have been identified as components of Shiraz skin and pulp samples but not of the juice (Razungles *et al.* 1988). In another study these authors found that Shiraz grapes had a concentration of the major carotenoids, ß-carotene and lutein, higher than that of the other black *Vitis vinifera* varieties (Razungles *et al.* 1987). During maturation the carotenoid concentration in both the skin and pulp of Shiraz berries was found to decrease (Razungles *et al.* 1988).

Shiraz grape juice, which is relatively deficient in monoterpenes, was used for the study of volatile C_{13} compounds released by acid hydrolysis of the C_{18} RP isolate (Strauss *et al.* 1984). Vitispirane was found to be the major volatile norisoprenoid in the Shiraz grape juice analysed, with minor amounts of

hydroxydihydroedulan and 8-hydroxytheaspirane identified after heating the juice.

2.3 MATERIALS

2.3.1 General

pH 3.2 tartrate buffer: an aqueous solution of saturated potassium hydrogen tartrate, at room temperature, was adjusted to pH 3.2 with saturated aqueous tartaric acid.

pH 5.0 citrate phosphate buffer: prepared from a mixture of 0.1M aqueous citric acid solution (48 mL) and 0.2M aqueous sodium hydrogen orthophosphate (52 mL).

D0, D3 and D7 *Ekwip* filter pads were purchased from Industrial Equipment (Asia) Pty Ltd, Australia, and the 0.45 µm membrane filters were purchased from Millipore, Australia.

The C_{18} silica gel reversed phase adsorbent, (C_{18} RP adsorbent) pore size 100/200A°, particle size 120-160 µm was purchased from Alltech Australia Pty, Ltd. *Sep-pak* cartridges containing C_{18} RP adsorbent (360 mg), poresize 125A°, particle size 55-105µm, were purchased from Waters® Millipore, Australia.

The commercial pectinase enzyme preparation, *Rohapect C* (Röhm, Darmstadt, Germany), was kindly donated by Enzymes Australia.

All water used in these experiments was pre-treated by a *Milli-Q* (Millipore Corporation, Sydney, Australia) system.

All solvents were of high purity at purchase and were redistilled before use. Freon refers to Freon F11, b.p. 24°C.

2.3.2 Fruit and wine samples

The samples of Shiraz fruit were selected from three viticultural regions and from vineyard blocks of three qualities, 'high', 'medium' and 'low'. The assignment of quality was obtained from:

(a) the winemakers' evaluation of the fruit;

(b) the commercial and, where appropriate, show records of wines made from the vineyards; and

(c) the viticultural characteristics of the vineyards - the yield from the vineyards assigned as being of low quality was two to three times greater than that from the high quality vineyards for both regions and years.

The samples of high, medium and low quality fruit from each of the three regions were coded as outlined in Table 2.1. Throughout this thesis the juice, skin and wine derived from these samples will be referred to by using the codes.

Wine was prepared commercially in 1989 from fruit from the high and low quality Coonawarra and Barossa vineyards described above. The wines were sampled directly after fermentation and had received no wood treatment.

Table 2.1 Identification codes for the samples of high, medium and low quality Shiraz grape berries

Code	Sample identification
HB	High quality Barossa
MB	Medium quality Barossa
LB	Low quality Barossa
HC	High quality Coonawarra
MC	Medium quality Coonawarra
LC	Low quality Coonawarra
HL	High quality Langhorne Creek
ML	Medium quality Langhorne Creek
LL	Low quality Langhorne Creek

2.4 EXPERIMENTAL

2.4.1 Fruit processing

Approximately 15 kg of grapes as whole bunches, were randomly picked from each vineyard on the day prior to commercial harvest. Samples of 400 berries each were taken at random from each vineyard sample, weighed immediately and frozen. The remaining fruit was crushed and pressed in a water-inflated bag press, the press cake broken and pressed again. Sodium metabisulfite was added to give a total sulfur dioxide concentration of 50 mg/L.

The juice was pressed directly into glass jars (5 L) and settled overnight in a cool room set to -7°C; the clear juice was decanted and frozen under nitrogen before storage at -7°C. For all preliminary work juices from the 1988 vintage were thawed at room temperature and sequentially filtered through D0, D3 and

D7 *Ekwip* filter pads and then through a 0.45 µm membrane before analysis. Whole bunch samples (5 kg) and residual marc from the pressings (500 g) were also kept frozen at -7°C.

The medium quality fruit sourced from the Barossa Valley in 1988 (MB 1988) was used for all preliminary work except where comparative analyses were made.

The codes referring to the different volatile fractions extracted from the juice, skin and wine samples are given in Table 2.2. Results of investigation into the most suitable extraction techniques for each of these fractions shall be given in detail in the following sections.

Table 2.2 Codes referring to the volatile extracts isolated from samples of Shiraz juice, skin and wine

Codes	Volatile extract
AF	Adsorbed Frees, i.e. free volatiles adsorbed onto C18 RP adsorbent
FV	Free volatiles
EH	Enzyme hydrolysis products
AH	Acid hydrolysis products
HS	Head space

2.4.2 Extraction of free volatiles from Shiraz juice

The free volatiles in the Shiraz juice (200 mL) were extracted by continuous liquid/liquid extraction with dichloromethane (CH_2Cl_2) (150 mL) for 16 h. The CH_2Cl_2 extract was concentrated by distillation through a column of Fenske's helices to approximately 100 µL and stored at -20°C prior to analysis by GC-MS.

2.4.3 Optimisation of extraction of glycosidically conjugated volatile compounds from Shiraz juice by C_{18} RP adsorbent

2.4.3.1 Column procedure

2.4.3.1.1 Preparation of the C_{18} RP adsorbent

A pyrex column (250 x 15 mm) was packed with C_{18} RP adsorbent (28.6 g) as a dry powder. The adsorbent was washed with methanol (100 mL) followed by water (200 mL). After elution of the C_{18} RP isolate in each experiment as outlined below, the C_{18} RP adsorbent was washed with further aliquots of methanol (200-500 mL) and water (500 mL).

2.4.3.1.2 Efficiency of the elution of glycosidically bound material with methanol

Shiraz juice (500 mL), under a headspace of nitrogen, was peristaltically pumped at a rate of 2 mL/min through a column of C_{18} RP adsorbent. The juice eluate was retained and frozen until required. The column was washed with water (500 mL) and flushed with nitrogen to remove residual water. The bound volatiles were progressively eluted from the column with methanol (11 x 25 mL aliquots). Four further aliquots of methanol (75 mL, 200 mL, 2 x 50 mL) were passed through the column until a negligible weight of eluted material was obtained. Each aliquot was reduced to dryness under vacuum and then flushed with nitrogen until a constant weight was recorded.

This experiment was repeated as described above except that the bound volatiles were progressively eluted from the column with methanol (3 x 100 mL

aliquots followed by 1 x 300 mL aliquot). Each aliquot was evaporated to dryness under vacuum and then flushed with nitrogen until a constant weight was recorded. The C_{18} RP isolates prepared in these experiments were resuspended in buffer (pH 5, 10 mL) and frozen until required for further analysis.

2.4.3.1.3 Efficiency of extraction of glycosidically bound material by C_{18} RP adsorbent

The juice eluate (500 mL) was peristaltically pumped through the washed column of C_{18} RP adsorbent as described above, except that the column was washed with water (500 mL) and the bound material was eluted with methanol (500 mL) in one aliquot. The methanol eluate was evaporated under vacuum and flushed with nitrogen until a constant weight was recorded. The juice eluate was then passed back through a clean column of C_{18} RP adsorbent, the column washed and the bound material eluted as described above.

2.4.3.1.4 Selectivity of the C_{18} RP adsorbent for glycosidically conjugated material

Shiraz juice (500 mL) was pumped through the main column as described above, and followed by a water wash (500 mL); the bound material was eluted with methanol (500 mL). The methanol eluate was evaporated under vacuum and the C_{18} RP isolate was resuspended in water (10 mL). The free volatiles were removed from the resuspended C_{18} RP isolate by washing the solution with Freon (5 x 10 mL) in a separating funnel. Each Freon washing was collected and pooled. The aqueous phase was then washed with

dichloromethane (5 x 10 mL) in a separating funnel and the aliquots pooled. Both the Freon and the dichloromethane extracts were concentrated by distillation of the solvent through a column of Fenske's helices to approximately 100 µL. Each concentrate was further reduced to approximately 10 µL prior to analysis by GC-MS.

2.4.3.2 Sep-pak *procedure for the extraction of glycosidically conjugated volatiles from Shiraz juice*

The C_{18} RP adsorbent in the *Sep-pak* cartridge was prepared by washing with methanol (50 mL) followed by water (50 mL). Shiraz juice (50 mL) was passed through a prepared cartridge by means of a 50 mL glass syringe. Unadsorbed material was removed by washing the cartridge with water (50 mL); the glycosidically bound material was eluted with methanol (50 mL). The methanol eluate was evaporated to dryness under vacuum and the C_{18} RP isolate redissolved in either pH 3.2 or pH 5 buffer (40 mL) prior to enzyme- or acid-catalysed hydrolysis of the glycosides as described in sections 2.4.4.1 and 2.4.4.2 respectively.

The efficiency of the retention of intact glycosides onto the C_{18} RP adsorbent in a *Sep-pak* cartridge was determined by extracting one juice aliquot (50 mL) through a series of five prepared cartridges. The methanol eluate from each cartridge was evaporated to dryness under vacuum and the C_{18} RP isolate was subjected to enzyme hydrolysis as described in section 2.4.4.1.

2.4.4 Optimisation of hydrolytic techniques for the release of glycosidically bound compounds from the C_{18} RP isolates

Aglycones were released from their conjugates by either enzyme- or acid-catalysed hydrolysis as described in the following sections.

2.4.4.1 Enzyme hydrolysis

A commercially available pectinase preparation *Rohapect C* (200 mg) was dissolved in citrate/phosphate buffer (pH 5, 100 mL).

The *Rohapect C* solution (10 mL of a 2 mg/mL stock solution) and citrate/phosphate buffer (pH 5, 30 mL) were added to the resuspended C_{18} RP isolate to give a final volume of 50 mL. The solution was transferred to a long-necked round-bottom flask (50 mL) and flushed with nitrogen. The flask was sealed and incubated at 37°C for 16 h.

2.4.4.1.1 Enzyme activity

The glucosidase activity of three samples of *Rohapect C* was verified by the method of Leung and Bewley (1981) using *p*-nitrophenyl-ß-D-glucopyranoside as substrate. *Rohapect C* (200 mg) was dissolved in buffer (100 mL) pH 5.5, prepared from 3 g/L glacial acetic acid, adjusted to pH 5.5 with 2M sodium hydroxide solution. *p*-Nitrophenyl-ß-D-glucopyranoside (1 mL of a 0.3 mg/mL solution of substrate in buffer pH 5.5), buffer (1 mL) and *Rohapect C* (1 mL) were incubated at 37°C for 35 min. The reaction was stopped after this time by the addition of 0.1M Na_2CO_3 (1 mL). Enzyme activity was measured by the absorbance at 410 nm of the *p*-nitrophenolate anion from the aglycone released

by enzyme hydrolysis. Dilutions of the *Rohapect C* stock solution (2 mg/mL) were prepared to give 1, 0.5, 0.25, 0.05 and 0.025 mg/mL, and these were analysed as outlined in section 2.4.4.1.1.

2.4.4.2 Acid hydrolysis

The glycoconjugates from Shiraz juice (500 mL) were isolated as described in section 2.4.3.1.3 and the C_{18} RP isolate resuspended in tartrate buffer pH 3.2 (80 mL). Eight aliquots (10 mL) of the resuspended C_{18} RP isolate were placed in glass ampoules (50 mL). Nitrogen was bubbled through the solutions for 1 min, and the ampoules were sealed and placed in a preheated oven at 50 °C. Two ampoules were removed after heating for 1, 2, 4 or 8 weeks, respectively.

In a separate experiment the hydrolysis was carried out as described above except that one ampoule was not flushed with nitrogen before being sealed and the ampoules were heated at 50°C for 28 days.

2.4.4.3 Extraction of released volatiles

The volatiles released by enzyme or acid hydrolysis of the C_{18} RP isolate were extracted from the aqueous solution by continuous liquid/liquid extraction with Freon (150 mL) or dichloromethane (150 mL) for 16 h. The solvent was concentrated by distillation through a column of Fenske's helices to approximately 50 µL. This concentrate was further reduced to about 10 µL prior to analysis by GC-MS.

2.4.5 Gas chromatography-mass spectrometric analysis (GC-MS)

The volatiles isolated from the juice extracts by GC-MS were separated and identified with a Finnigan 4021 mass spectrometer coupled to a Varian 3300 gas chromatograph. The gas chromatograph was equipped with a 30 m J&W DB 1701 fused-silica column, 0.25 mm i.d. and 0.25 µm film thickness. Helium was used as the carrier gas at a linear velocity of 40 cm/s. Split-splitless injections were made with a split ratio of 1:10. The column was held at 60°C for 1 min, programmed at 4°C/min to 250°C and held at this temperature for 20 min. Electron impact mass spectra were taken at 70 eV.

GC-MS analysis of the extracts of grape samples from the 1990 vintage was undertaken with a Finnigan TSQ 70 instrument coupled to a Varian 3400 gas chromatograph under the same conditions as described above.

2.4.6 Internal standards

The compounds in Table 2.3 were screened as internal standards appropriate for the quantification of the free volatiles and the volatile compounds released by either enzyme- or acid-catalysed hydrolysis of the C_{18} RP isolate.

A glycoside was required as an internal standard to add to the juice before the isolation of the juice glycosides on the C_{18} RP adsorbent, and a second volatile internal standard was required to monitor the continuous liquid/liquid extraction of the released volatiles. The ten compounds listed in Table 2.3 were dissolved in dichloromethane (1 mg/mL) and their retention times and chromatographic properties determined by GC-MS.

Table 2.3 Compounds screened for use as internal standards

Retention time (sec)	Compound	Corresponding glycoside
488	Salicylic acid	Salicin
534	n-Octanol	n-Octyl-β-D-glucopyranoside
539	Hydroquinol	Arbutin
1958	p-Nitrophenol	p-Nitrophenyl-β-D-glucopyranoside
211	Cyclohexanol	
351	Octan-2-ol	
364	Butylbenzene	
928	2-Phenoxyethanol	
1258	n-Dodecanol	
1634	α - Napthol	

A standard solution of the four glycosides (0.5 mL of a 1 mg/mL solution of each glycoside) was added to Shiraz juice (500 mL) to give a final concentration of 100 µg/L before extracting the glycosides from this solution by passage through the large column of C_{18} RP adsorbent.

Alternatively, an aliquot of the standard solution was added after the glycosides were extracted from an aliquot of Shiraz juice (500 mL) and before the C_{18} RP isolate was hydrolysed by *Rohapect C* as described above.

2.4.7 Analysis of phenolics in Shiraz juice and C_{18} RP isolates

The concentration of total phenolics in the juice, C_{18} RP isolate and skins was determined according to the method of Somers and Verette (1988). Aliquots of the juice (1 mL) or C_{18} RP isolates (*section 2.4.1 and 2.4.3.2)* (1 mL) were

diluted with concentrated hydrochloric acid (10 mL) and their absorbance spectrum read from 250 to 720 nm.

Skins from 100 Shiraz berries were steeped in methanol/concentrated hydrochloric acid solution (200 mL of a solution prepared by adding 3 mL hydrochloric acid to 1 L methanol) and macerated in a *Waring* blender for 1 min. The liquor was decanted, MeOH/HCl solution (200 mL) added and the skins macerated again for 1 min. This step was repeated once more and the three MeOH/HCl aliquots pooled and made up to 1 L with MeOH/HCl solution. The solution was further diluted (1 mL/100 mL) and its absorbance spectrum read from 250 to 720 nm.

2.5. RESULTS AND DISCUSSION

2.5.1 Quality assignment of grape samples

The quality of any food product is difficult to rigidly define because of the subjectivity inherent in any such rating. The word 'quality' as used in this thesis relates to expected market value as wine. This avoids the problems inherent in allocating quality ratings based on measurements made on the grapes, even though it is accepted that the grapes required to produce a superior quality wine must be of a significantly different composition than that of the grapes used to produce wine sold in bulk.

As the main aim of this study was to determine if there were analytical differences between Shiraz grapes from which wines of different market values were produced, it was important to choose grape samples from vineyards that

produced grapes of a known and consistent quality. Grapes were sourced, therefore, from vineyards owned or managed by three large Australian wine companies which had been producing both bulk and premium Shiraz wines for many years. The three areas chosen for this study, the Barossa Valley, Coonawarra and Langhorne Creek, are all established grape growing regions in South Australia.

Viticultural and oenological data over the three years for the nine chosen vineyards are presented in Table 2.4. There was a large yield difference between the three quality ratings of the Barossa and Coonawarra vineyards. In the former area the yield difference was associated with different water regimes, whereas in the latter area the yield difference was due to canopy management.

The grapes sourced from the Barossa region were from established vineyards with the vines grown on a traditional T-trellis (Figure 2.1a). The 'high' quality vines were low yielding and grown on sandy soil without supplementary irrigation. The 'medium' and 'low' quality vines were medium to high yielding, irrigated and grown on red loam/clay. The vines producing the grapes assigned as high quality were hand pruned compared with the other vines which were machine pruned (Figure 2.1b). All three vineyards formed part of a project undertaken by the winery to link the yield per hectare from each vineyard and the production costs of winemaking to the market value of the resultant wine (*Chapter 6*).

Table 2.4. Viticultural details of Barossa (B), Coonawarra (C) and Langhorne Creek (L) vineyards from which grape samples were obtained in 1988, 1989 and 1990.

Sample	Baume			pH			TA (g/L)		
	1988	1989	1990	1988	1989	1990	1988	1989	1990
HB	14.2	14.0	13.3	3.7	4.0	3.8	5.0	5.2	5.2
MB	14.6	14.3	12.4	3.6	3.6	3.5	7.5	8.3	6.5
LB	15.1	14.8	12.6	3.9	3.6	3.6	5.5	7.0	5.5
HC	13.3	12.2	13.9	3.5	3.5	3.4	8.7	6.5	9.8
MC	12.3	9.2	12.5	3.4	3.3	3.5	7.8	7.9	7.6
LC	12.8	11.1	11.8	3.6	3.1	3.5	7.2	9.3	8.1
HL				3.3			4.9		
ML				3.4			3.9		
LL				3.3			4.4		

Sample	Yield (t/ha)			Berry weight (g/berry)			Water regime
	1988	1989	1990	1988	1989	1990	
HB	2.6	3.5	3.5	0.8	0.8	1.1	No irrigation
MB	6.7	8.0	6.7	0.9	0.6	1.0	Winter top up
LB	10.0	10.8	6.3	1.0	0.6	1.0	Double irrigation
HC	5.7	9.4	6.4	1.2	1.5	1.5	Supplementary water from overhead sprinklers in January and early March if required
MC	9.1	20.0	14.8	1.3	0.8	1.1	
LC	12.5	14.0	20.0	1.2	0.8	1.0	
HL	12.5			1.5			Drip irrigation 8 L/week
ML	12.8			1.3			Ground water/ supplementary drip irrigation
LL	14.1			1.2			

2.1a

2.1b

Figure 2.1a and b *An example of a) the hand pruning technique applied to the vineyard assigned as producing high quality grapes in the Barossa Valley; b) Machined pruned vines in the vineyard assigned as producing low quality grapes in the Barossa Valley.*

The grapes sourced from the Coonawarra region were from a viticultural trial implemented by the winery to produce grapes for different Shiraz wine qualities from premium to bulk. The three vineyards from which grapes were sourced in this area were pruned in a cyclic fashion over a three-year period as follows:

Year one: vines were minimally pruned = medium yield/hectare and assigned as producing medium quality fruit.

Year two: the vines that had been minimally pruned in year one were minimally pruned for a second year = high yield/hectare and assigned as producing low quality fruit.

Year three: the vines that had been minimally pruned for two years were machined pruned in the third year = low yield/hectare and assigned as producing high quality fruit.

The production staff from this winery reasoned that the minimally pruned vines had a large leaf surface area which resulted in greater root and canopy capacity and probably greater carbohydrate reserves as a consequence. When the vines were mechanically pruned in the third year it was postulated that this should lead to improved colour, flavour and a more synchronous ripening pattern (G. Hogg pers. comm).

Shiraz grapes were sourced in the Langhorne Creek area from three vineyards. The grapes were used in blends of premium to low quality wines of a large wine producer. None of the vineyards was owned by the company, however, the owners of the vineyards had supplied fruit to the winery for many years for various blends. There was no significant difference in the yields from the three vineyards, and preliminary work confirmed that in 1988 the fruit was not significantly different in terms of either the chemical or the physical characters measured (Table 2.4, Figure 2.2).

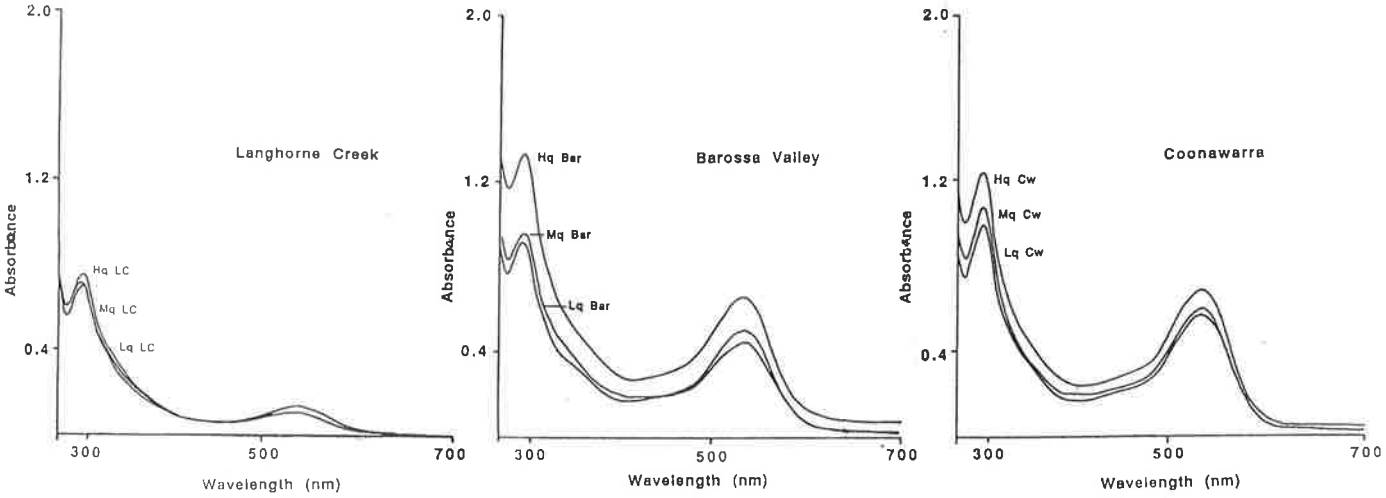


Figure 2.2 *Total phenolics present in Shiraz juice assigned as high medium and low quality from the Barossa Valley, Coonawarra and Langhorne Creek.*

These measures applied to grapes from the other two regions in this year were significantly different. Therefore, the fruit from Langhorne Creek was not sampled after 1988, and the results from Langhorne Creek grapes are not discussed further in this thesis.

2.5.2 Extraction of free volatile compounds from Shiraz juice

The lack of free volatiles in Shiraz juice relative to the abundance of the conjugated flavour precursors has been demonstrated (Abbott *et al.* 1989). There was no significant difference amongst the free volatile composition of different quality grapes either from the same region or between different regions. The free volatile composition of Shiraz grapes, although analyzed in subsequent years, was not used for the comparative studies.

2.5.3 Optimization of the extraction of glycosidically conjugated aroma compounds from Shiraz juice with C_{18} RP adsorbent

2.5.3.1 Elution of bound material from the C_{18} RP adsorbent with methanol

The glycosidically conjugated material in a sample of Shiraz juice (500 mL) adsorbed onto a column of C_{18} RP adsorbent was eluted sequentially with aliquots of methanol to determine the volume of methanol that was required to elute all bound material from the column. The majority (83% by weight) of the material bound to the C_{18} RP adsorbent was eluted in the first two methanol eluates (2 x 25 mL) (Table 2.5a). The remaining 17% of the weight of bound material was eluted from the column with a further 13 aliquots of methanol. Analysis of the enzyme-released volatiles from the first two eluates by GC-MS demonstrated that the first extract had a greater concentration and the same number of volatiles as the second eluate (data not shown). There were no volatiles identifiable by GC-MS in the last fraction (F15), and eluate 14 (F14), contained two unknown compounds with mass spectra *m/z* 147, 133 and *m/z* 75. The mass of bound material and the chromatograms of the enzyme-released products of these first two eluates suggested that the majority (>95%) of the

glycosidically bound volatiles were eluted from the column with 50 mL methanol.

In a second experiment, Shiraz juice (500 mL) was passed through the column and the bound material eluted with methanol (3 x 100 mL + 1 x 300 mL) (Table 2.5b). The first two aliquots accounted for 92% by weight of the total bound material eluted from the column. However, to ensure that all the bound volatiles were released from the column in all subsequent experiments, the same volume of methanol as juice which had been passed through the column was used to elute the bound material. The column was washed with a further aliquot of methanol (500 mL) and water (500 mL) before reuse.

Table 2.5 Mass of C18 RP isolates progressively eluted from the column with an increasing volume of methanol

2.5a Experiment 1			2.5b Experiment 2		
No.	Volume methanol (mL)	Weight C18 RP isolate (mg)	No.	Volume methanol (mL)	Weight C18 RP isolate (mg)
F1	25	238	F1	100	288
F2	25	97	F2	100	17
F3	25	9	F3	100	7
F4	25	6	F4	300	18
F5	25	3			
F6	25	6	Total	600	330
F7	25	5			
F8	25	6			
F9	25	5			
F10	25	5			
F11	25	4			
F12	75	10			
F13	200	5			
F14	50	3			
F15	50	1			
Total	650	403			

* The same juice was used for both experiments. The discrepancy observed in the weights of glycosides eluted from the column is possibly due to traces of solvent residue remaining in the fractions in experiment 1.

2.5.3.2 Selectivity of the C_{18} RP adsorbent for glycosidically bound material

The free volatiles adsorbed onto the C_{18} RP column (*adsorbed frees*) were investigated by extracting these volatiles from the first C_{18} RP fraction (F1, Table 2.5a) with Freon (5 x 10 mL) in a separating funnel. Five major volatile components were present in the first fraction eluted from the column. The main ion fragments from the mass spectrum of each peak are given in Table 2.6. The second C_{18} RP isolate (F2) was treated in the same way as the first fraction (F1) and had a composition identical to that of F1. Fifteen further fractions were eluted from the column in 25-200 mL methanol aliquots; the adsorbed frees from the 14th and 15th fractions were analysed. Only the last two compounds with RT 1778 and 1880 seconds (Table 2.6) were present in the 14th fraction. No volatile compounds were identified by GC-MS in the last fraction.

Table 2.6 Mass spectra of the major ions of uncharacterised compounds observed in the chromatogram of the free volatiles adsorbed onto the C18 RP adsorbent

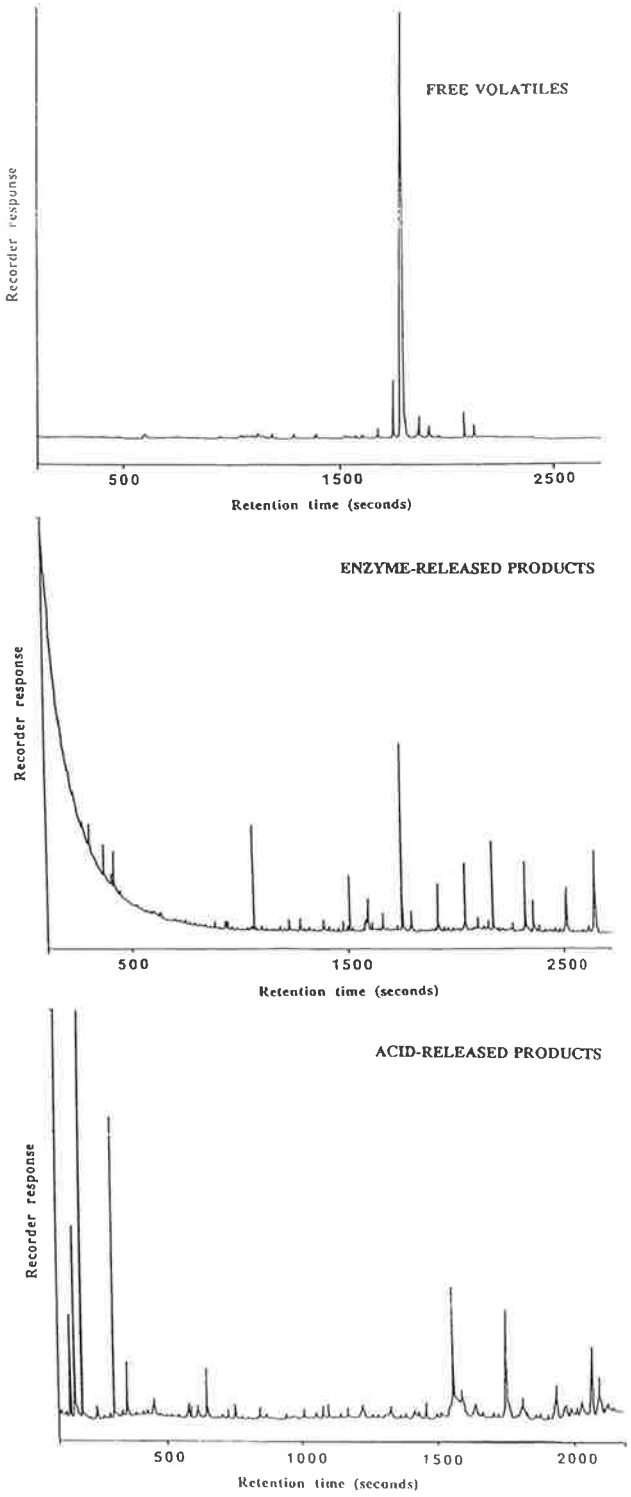
Retention time (seconds)	Mass spectra m/z
890	59(100), 95(67), 110(31), 150(12)
976	43(100), 121(51), 163(23)
1744	89(100), 327(6)
1778	147(100), 133(35)
1880	75(100), 313(5)

Extraction of the free volatiles present in C_{18} RP isolates, F1, F2 and F14, with dichloromethane (5 x 10 mL) yielded the same group of compounds as were previously extracted with Freon but present in a different ratio.

From this experiment it was concluded that it was not necessary to remove the free volatiles adsorbed onto the C_{18} RP adsorbent and subsequently present in the C_{18} RP isolate as the overall concentration of these compounds was minor compared to that of the volatile components present in the enzyme- or acid-liberated products. The concentration of these compounds was further minimised by isolating the glycosides through a *Sep-pak* cartridge (section 4.3.2).

2.5.3.3 Efficiency of the C_{18} RP adsorbent to adsorb glycosidically bound volatiles from Shiraz juice

The eluate of a juice that had been passed once through a column of C_{18} RP adsorbent was passed through a second column to determine if glycoconjugates were still present in the eluate. The methanol washing from this second column gave, after drying, a waxy red substance (263 mg) which had no aroma. The adsorbed volatiles were extracted from this C_{18} RP isolate by continuous liquid/liquid extraction with Freon and analysed by GC-MS (Figure 2.3a). The compounds identified in this extract were mainly phthalate esters and an unknown compound with *m/z* 147, 133. The solvent-stripped C_{18} RP isolate was split into two aliquots (10 mL). One aliquot, which was hydrolysed by a non-specific glycosidase enzyme (*Rohapect C*) gave a sweet/fruity smelling extract. Analysis by GC-MS identified a series of phthalates, hydrocarbons



Figures 2.3 *Gas chromatograms of (a) the adsorbed frees, (b) enzyme- and (c) acid- released products from a C_{18}RP isolate obtained from a juice already prestripped by an earlier passage through a C_{18} RP column.*

and an unknown compound with *m/z* 147, 133 (Figure 2.3b). No volatile aroma compounds identified as enzyme released products were identified in this extract. The second aliquot was hydrolysed under acidic conditions and analysed by GC-MS (Figure 2.3c). 5-Hydroxymethyl-furfural was identified in this extract along with several unknown compounds, one of which may be a monoterpene (RT =269 seconds). Most of these compounds, however, were present at a concentration too low to be positively identified.

One pass of an aliquot of juice (500 mL) through a column of C_{18} RP adsorbent at approximately 2 mL/min was therefore found to be sufficient to isolate most of the glycosidically bound secondary metabolites of interest to this study. These compounds are discussed further in Chapter 5.

2.5.3.4 Enzyme hydrolysis

The glycosidically bound volatiles were released by enzyme-catalysed hydrolysis at 37°C over a period of 16 h. These conditions were chosen as work previously conducted in the laboratory had demonstrated that they were optimum for the liberation of these glycosidically bound volatiles.

Investigation of the compounds generated by enzyme hydrolysis of the glycosidically bound volatiles demonstrated that Shiraz juice contains an abundance of bound volatiles in comparison to the free volatiles described above (Abbott *et al.* 1989). The enzyme added, *Rohapect C*, was a commercial preparation with a range of non-selective glycosidase activities (Aryan *et al.* 1987).

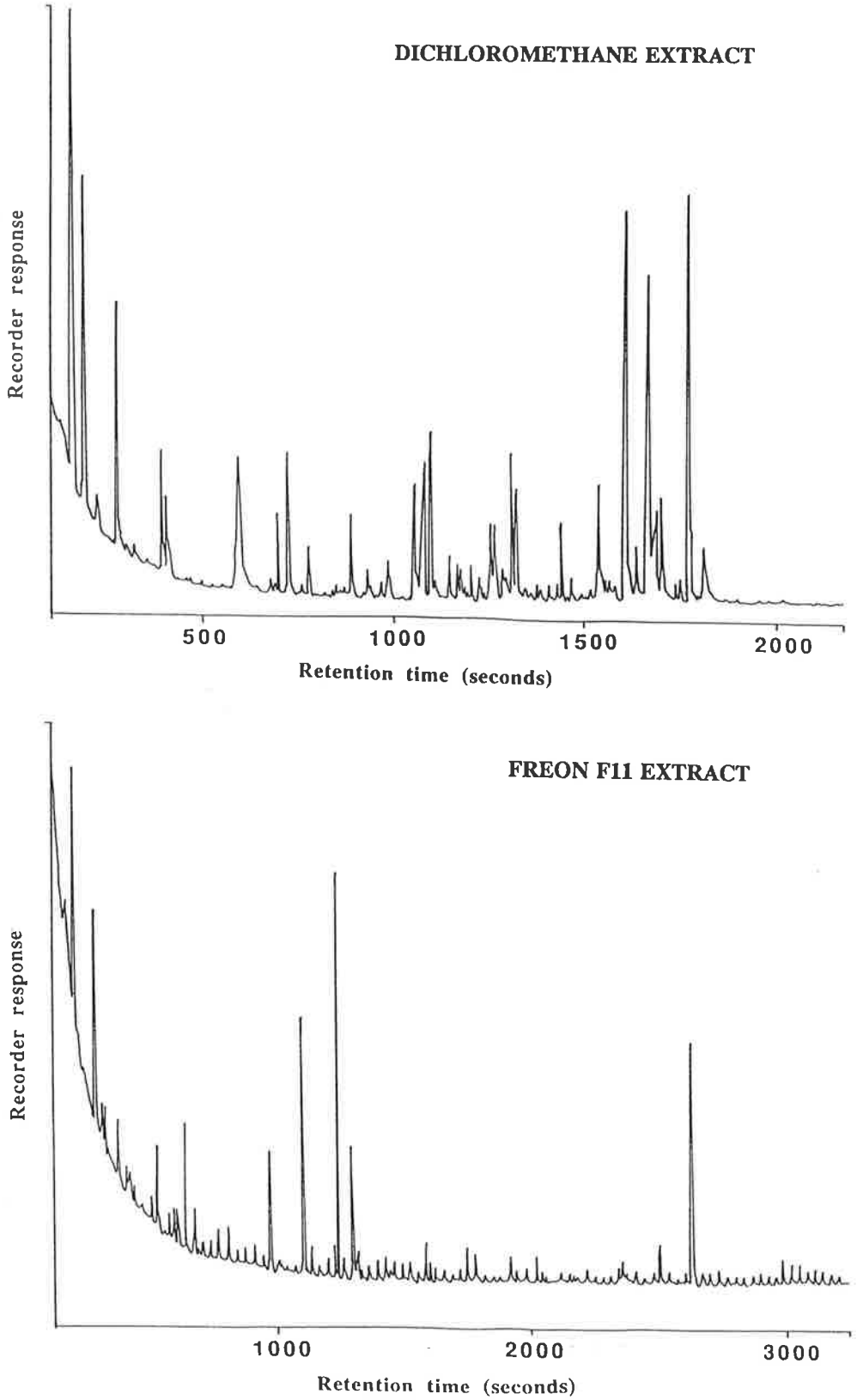


Figure 2.4 *Gas chromatograms of the enzyme-released volatiles extracted by continuous liquid/liquid extraction with (a) dichloromethane and (b) Freon.*

Continuous liquid/liquid extraction of the released volatiles with dichloromethane gave both a greater number and a greater concentration of volatiles than when the volatiles were extracted with Freon (Figure 2.4a and b). This result supports the findings of Blanche *et al.* (1991) that dichloromethane was the most suitable solvent for isolating volatile compounds from grape juice. Dichloromethane is a more polar solvent than Freon and would therefore be expected to extract a greater array of compounds than a less polar solvent.

The glycosidase activity of three different batches of *Rohapect C* was measured. The results (Figure 2.5) illustrate the importance of checking the activity of each new batch of enzyme preparation before use. Batch number 1 was used for all subsequent experiments as this was the most active at all concentrations.

2.5.3.5 Acid hydrolysis

Investigation of the compounds generated by acid hydrolysis of the glycosidically bound volatiles showed that, in comparison to the free volatiles described above, Shiraz juice contained an abundance of bound volatiles that were acid labile and that could be released by heating the C_{18} RP isolate from Shiraz juice at pH 3.2.

2.5.3.5.1 Rate of formation of acid hydrolysis products

Thirty compounds were quantified after 1, 2 and 4 weeks of heating a resuspended C_{18} RP isolate in pH 3.2 buffer at 50 °C. The concentration for most compounds except that of vanillin, vanillic acid and vitispirane (Table

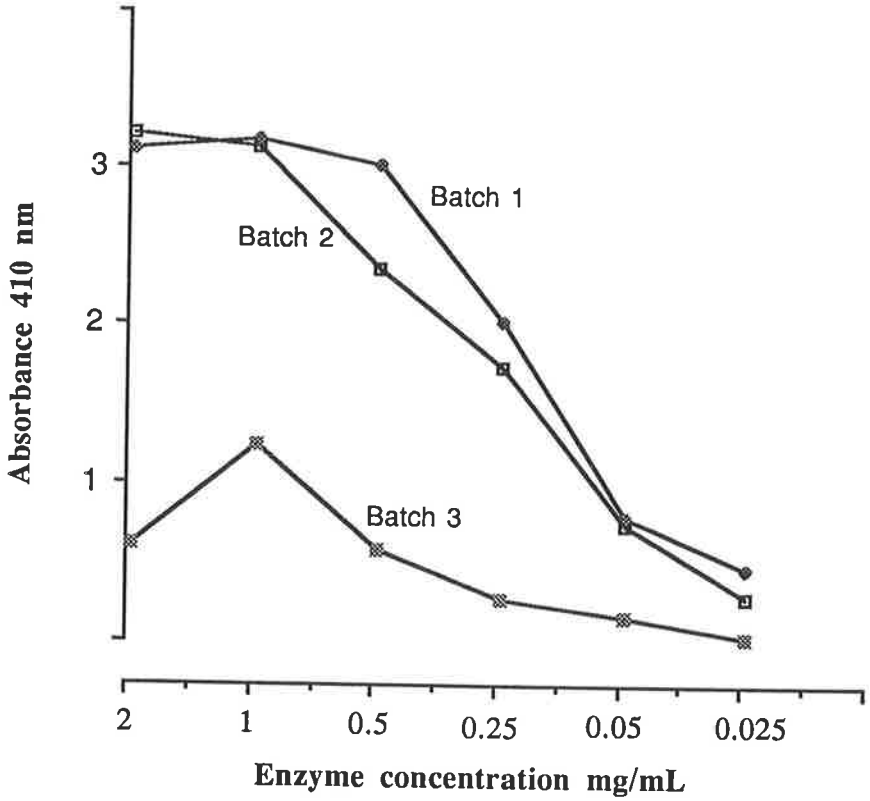


Figure 2.5 *Activity of two batches of* Rohapect C, *measured by* A_{410} *according to the method of Leung and Bewley (1981), batch 1 had been kept in two different containers; batch 1a was used continuously in the laboratory and batch 1b was kept sealed in the chemical store. Batch 2 was purchased approximately one year after batch 1.*

2.7) which appeared to decrease in week 2 before reaching a peak in week 4, increased with time of heating. Analysis of a further sample after 8 weeks of heating at 50°C indicated that upon prolonged heating the concentration of most compounds detected appeared to significantly decrease. Only one replicate of this sample, however, was analysed and therefore the results have not been presented here. Acid hydrolysis of the glycoconjugates were conducted in buffer (pH 3.2) at 50°C for 28 days for all subsequent comparative work.

Table 2.7 Concentration of volatile compounds identified as acid hydrolysis products at 50 degrees celsius for one, two and four weeks, respectively.

Compound	Concentration (ug/L)		
	Week 1	Week 2	Week 4
Furan linalool oxide: isomer 1	4	10	23
Furan linalool oxide: isomer 2	2	6	14
Benzyl alcohol	2	2	25
2-Phenylethanol	6	8	23
Vitispirane	3	1	14
2,6-Dimethyloct-7-ene-2,6-diol	6	26	44
2-Ethyl-3-methylmaleimide	3	4	8
4-Vinyl guaiacol		<1	12
4-Vinyl phenol			25
1- Terpineol		1	7
Actinidol: isomer 1	2	8	11
Actinidol: isomer 2	4	14	20
Vanillin	2	1	3
2-(3-Hydroxybut-1-enyl)-2,6,6-trimethylcyclohex-3-en-1-one	2	8	19
Methylvanillate		1	5
3,4-Dihydro-3-oxoactinidol		1	14
Methoxytyrosol			14
Methyl-4-hydroxybenzoate			2
3-Hydroxy-β-damascone			5
Vanillic acid	4	2	17
Tyrosol propionate		<1	15
α-Ionol			8
4-Hydroxybenzoic acid			8
Acetosyringone			13
Propiosyringone			6
2-Oxo-2-(4-hydroxy-3-methoxyphenyl)-acetic acid			8
Vomifoliol		2	25
Syringic acid			17
Dehydrovomifoliol			15
trans-Hydroxycinnamic acid		6	17

[1]Compounds were assigned mass spectrometrically (Chapter 5); [2] Concentrations were determined from peak area in relation to an internal standard; [3] Data are the result of a single analysis.

2.5.4 Internal standards

Once it had been determined that volatile flavour compounds, both free and glycosidically bound, could be extracted from Shiraz juice and identified by GC-MS, it was necessary to find a suitable internal standard to enable quantification of the data. A series of experiments was conducted to determine the suitability of the compounds listed in Table 2.3 for use as internal standards.

The response of seven volatile standards, cyclohexanol, octan-2-ol, *n*-butyl benzene, *n*-octanol, 2-phenoxyethanol, *n*-dodecanol and α-naphthol, was determined in relation to each other by analysing several dilutions of the stock solution by GC-MS. Butylbenzene and *n*-octanol were the most reliable of the seven volatile standards tested. The ratio of butylbenzene and *n*-octanol did not vary with varying concentration, and these compounds did not interfere with other compounds in the chromatogram of the volatile extracts from Shiraz juice. Butylbenzene was therefore used to check the efficiency of the retention and hydrolysis of *n*-octyl-ß-D-glucopyranoside. *n*-Octanol was used as a standard to check the efficiency of retention and hydrolysis of the other glycosides listed in Table 2.3.

Two glycosides, arbutin and *p*-nitrophenyl-ß-D-glucopyranoside, were poorly recovered by the protocol. These results suggested that the glycosides of hydroquinol and *p*-nitrophenol are either not retained or are poorly retained by the C_{18} RP adsorbent, are water soluble and eluted during washing of the column with water, are not hydrolysed by *Rohapect C* or have poor

chromatographic properties. In contrast *n*-octyl-ß-D-glucopyranoside was completely retained by the C_{18} RP adsorbent and completely hydrolysed by *Rohapect C* as the peak size was the same as that expected from the equivalent concentration of *n*-octanol.

n-Octanol and its glycoside, *n*-octyl-ß-D-glucopyranoside, were therefore chosen for use as internal standards for the quantification of volatile aroma compounds from the enzyme- and acid-catalysed hydrolysis of the juice extracts. *n*-Octyl-ß-D-glucopyranoside (100 µg/L) was added to the juice prior to its passage through the C_{18} RP isolate and followed by enzyme hydrolysis. *n*-Octanol was added to the solution containing the acid hydrolysis products before extraction of the released volatiles by continuous liquid/liquid extraction with dichloromethane. Butylbenzene was chosen to monitor the performance of the *n*-octanol and *n*-octyl-ß-D-glucopyranoside and was added prior to extraction of the volatile compounds released by either enzyme- or acid-catalysed hydrolysis of the C_{18} RP isolate.

2.5.5 Alternative methods for the extraction of glycosidically bound material from grape juice

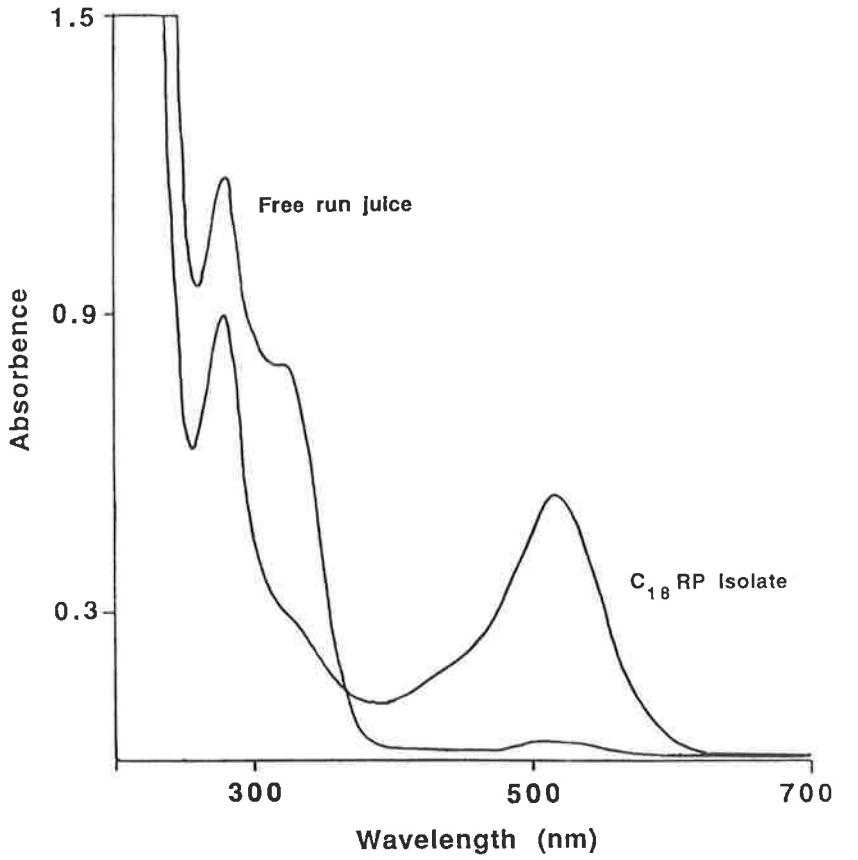
The column of C_{18} RP adsorbent started to block after approximately eight months use, irrespective of either how often the column was repacked or whether the C_{18} RP adsorbent was washed thoroughly with methanol, acetonitrile and water and dried before being repacked into the column.

Initially it was thought that the high phenolic content of Shiraz juice was

causing the column to block by adsorbing onto the C_{18} RP adsorbent. The total phenolic concentration of the juice, the C_{18} RP isolate and the juice eluate was therefore measured to determine if phenolic material was accumulating on the C_{18} RP adsorbent and creating an impermeable barrier through which the juice could not pass. The results indicated that the phenolic material present in Shiraz juice was not being retained on the C_{18} RP adsorbent (Figure 2.6a). Allowing for losses of hydroxycinnamates in the juice eluate (Figure 2.6b) the recovery of phenolic material in the methanol eluate was high (~95%) and therefore suggests that some other particulate matter, or reaction occurring on the column, was causing the blockage.

A disposable guard column (25 x 15 mm) containing C_{18} RP adsorbent was introduced to protect the main column from blocking. However, even when the C_{18} RP adsorbent in the guard column was replaced before each extraction, blockage of both columns still occurred. The adsorbent in the guard column was replaced with Amberlite XAD but this led to even greater problems, the pressure causing the ends to be forced off the columns after only 50 mL of juice had been passed through the column.

The juice was subsequently passed through the guard column and main column, both of which contained C_{18} RP adsorbent, under a stream of nitrogen which appeared to alleviate many of the problems previously faced. It was found that by extracting only 50-100 mL of juice at a time under nitrogen, with new C_{18} RP adsorbent in the guard column, the column did not block.



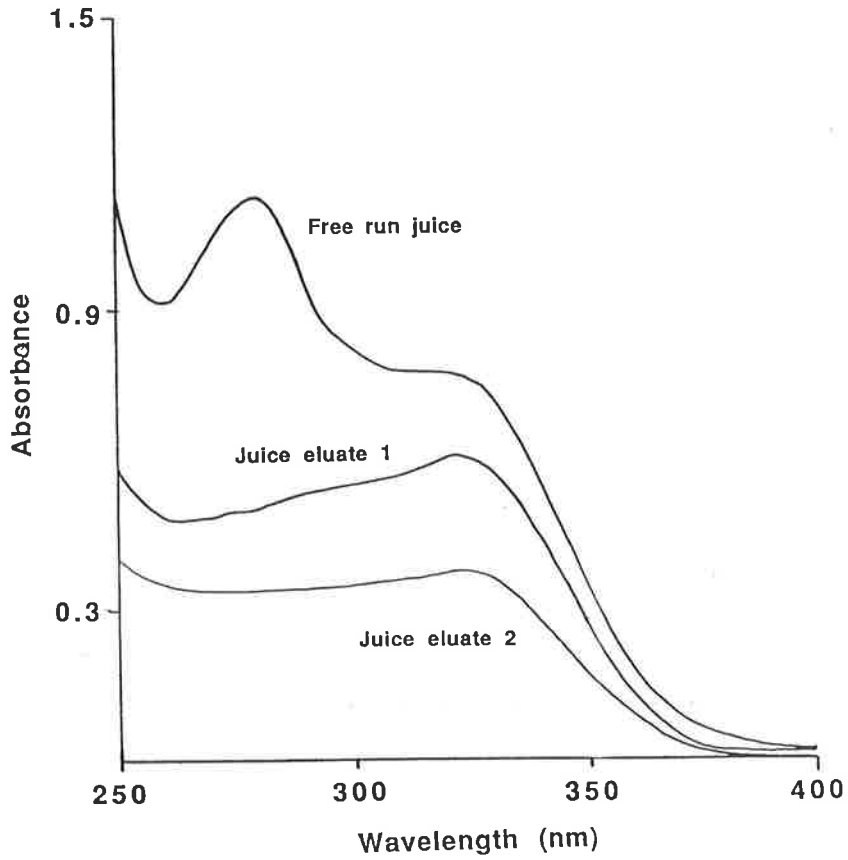


Figure 2.6 *Total phenolic concentration of (a) the free run juice and C_{18} RP isolate and (b) free run juice, juice eluate 1 and juice eluate 2 (eluate of 1) measured by $A_{250-700}$, to determine if phenolic material was being left on the column.*

2.5.5.1 Use of Sep-pak *cartridges*

An alternative method for the extraction of glycosides from juice samples was sought due to the cost of renewing the C_{18} RP adsorbent in the guard column before each extraction and the frequent blockages of the column that were still occurring.

An alternative source of C_{18} RP adsorbent used for the extraction of glycosides was found in prepacked, *Sep-pak* cartridges. Preliminary experiments demonstrated that the same glycosides from Shiraz juice could be adsorbed onto the C_{18} RP adsorbent present in the cartridge as were isolated by passing the juice through C_{18} RP adsorbent present in a glass column. It appeared that the cartridges were more efficient in retaining glycosidically bound material from the Shiraz juice for most of the compounds quantified (Table 2.8). This increase in efficiency may have been due to the more uniform size of the C_{18} RP adsorbent contained in the cartridge compared to the bulk C_{18} RP adsorbent that was packed into the columns. Furthermore, an aliquot of Shiraz juice (50 mL) could be extracted efficiently, with up to twenty juice samples being processed in approximately three hours, and with no wastage of juice due to blockage of the C_{18} RP adsorbent. The *Sep-pak* cartridges used for the extraction of glycosides were recycled once without any apparent loss of effectiveness.

Table 2.8 Concentration of enzyme-released volatile compounds isolated from Shiraz juice by passage through either a Sep-pak cartridge or a column containing C18 RP adsorbent

Compound	Sep-pak extraction (ug/L)	Column extraction (ug/L)
Benzyl alcohol	15	<1
2-Phenethanol	8	31
4-Vinylphenol	131	29
E-2,6-Dimethylocta-3,7-diene-2,6-diol	3	4
Z-2,6-Dimethylocta-3,7-diene-2,6-diol	8	13
Vanillin	<1	-
Methyl vanillate	4	-
Tyrosol	1	10
Butyrovanillone	1	3
3-Hydroxy-β-damascone	3	4
2,5-Dihydroxybenzoic acid methyl ester	<1	1
3-Oxo-α-damascone	6	-
cis-Coniferyl alcohol	<1	-
Dihydroconiferyl alcohol	3	3
9-Hydroxymegastigma-4,6-dien-3-one	14	6
9-Hydroxymegastigma-4,6,7-trien-3-one	<1	
3-Oxo-α-ionone	6	-
2-(4-Hydroxy-3-methoxyphenyl)-2-oxoethanol	<1	1
Methyl syringate	3	2
trans-Coniferyl alcohol	<1	2

[1]Compounds were assigned mass spectrometrically (Chapter 5); [2] Concentrations were determined from peak area in relation to an internal standard; [3] Data are the result of a single analysis.

2.5.5.2 Efficiency of retention of intact glycosides

The efficiency of the retention of the glycosides onto a *Sep-pak* cartridge containing the C_{18} RP adsorbent was determined by passing one juice sample through a series of five cartridges and quantifying the concentration of glycosides retained by each cartridge. The concentration of volatiles extracted through each cartridge was compared with a sample of the same juice that had been passed twice through the same cartridge. This latter procedure was undertaken to ensure that there was no error introduced due to the rate of

passage of the juice through the column.

Table 2.9 contains the list of compounds released by enzyme hydrolysis of each methanol eluate and indicates that the glycoconjugates that were not totally adsorbed by the C_{18} RP adsorbent during the previous run. The concentration

Table 2.9 Concentration of enzyme-released volatile compounds isolated from Shiraz juice (50mL) by passage through a series of four Sep-pak cartridges

Compound	Retention time (sec)	Concentration (ug/L) Cartridge number 1	2	3	4
A fatty acid	637	316	28	tr	
Benzyl alcohol	692	387	194	tr	6.6
Phenol	721	17	8		
2-Phenethanol	821	181	56	10	tr
Methyl salicylate	868	62	21		
Vitispirane	913	40	tr		
4-Vinylguaiacol	1201	157	66	6	tr
4-Vinylphenol	1230	59	14	3	tr
Methyl vanillate	1615	52	15		
Acetovanillone	1621	23	11		
A phthalate	1705	266	129		
Methoxytyrosol	1732	99	174	31	
2,5-Dihydroxybenzoic acid methyl ester	1823	55	40		
Megastim-4-en-3,9-dione	1866	55	24		
3-Oxo-α-damascone	1881	51	31		
Vanillic acid	1895	60	7		
Methyl syringate	2054	56	43		
Total volatile concentration		1936	858	49	6.6

[1]Compounds were assigned mass spectrometrically (Chapter 5); [2] Concentrations were determined from peak area in relation to an internal standard; [3] Data are the result of a single analysis.

of compounds adsorbed by passing the juice through a cartridge only once accounted for 85% of the volatiles observed when the juice was extracted twice through the same cartridge. Extraction of the eluate through a second clean cartridge isolated a further 10% of volatiles, therefore at least 95% of the

volatiles could be accounted for by extraction of the juice and juice eluate through the first two cartridges. A further 1.5% was extracted by passing the juice eluate through the third cartridge and no known volatiles were isolated by the fourth cartridge except for the compounds listed in Table 2.9 which were identified in trace amounts. No known volatiles were identified from the fifth extraction of the juice eluate.

The series of unknown compounds with *m/z* 147, 133 were identified only in the C_{18} RP isolates of the last two extracts. This compound has been observed in most C_{18} RP isolates so far studied. It is thought to be formed from compounds present in the juice once they are in contact with the C_{18} RP adsorbent *(Chapter 3)*.

2.5.5.3 Reproducibility of extraction of glycosides through a Sep-pak *cartridge*

2.5.5.3.1 Enzyme hydrolysis products

The reproducibility of the extraction and quantification methods employed in this study was determined by GC-MS. Quantification of the total concentration of volatile compounds released by enzyme hydrolysis of the C_{18} RP isolate was found to be reproducible with a coefficient of variation (CV) of 12% for the three replicates analysed. The within-sample variation, i.e. when the same sample was analysed in duplicate, was also low with a coefficient of variation of 8%. However, the coefficients of variation for the quantification of the individual compounds identified in the volatile aroma extracts was <20% for only 46% of the identified compounds. Of the identified volatiles 65% had a CV < 30%

and 74% a CV < 35% (*Appendix A.1*).

2.5.5.3.2 Acid hydrolysis products

Quantification of the total concentration of volatile compounds that were released by acid hydrolysis of the C_{18} RP isolate was also found to be reproducible with a coefficient of variation of 8% for the three replicates analysed. As for the enzyme-released products, the within-sample variation was also low (CV = 5%). The error associated with the quantification of the individual acid-released compounds was less than 11% for 26% of the compounds identified as acid-released products and a CV less than 20% for 59% of these identified compounds (*Appendix A.2*).

2.6 CONCLUSION

The use of *Sep-pak* cartridges for the isolation of Shiraz glycoconjugates alleviated many of the problems associated with the large column of C_{18} RP adsorbent. The cartridges did not block, the glycosides were more efficiently retained by the C_{18} RP adsorbent in the cartridge, and the time required for the isolation of the glycosides from each juice sample was reduced even though each sample was passed twice through the same cartridge to ensure complete extraction of the glycosides.

Quantification of the total concentration of volatile compounds that were released by both enzyme- or acid-catalysed hydrolysis of the C_{18} RP isolate eluted from a *Sep-pak* cartridge was found to be reproducible with a coefficient of variation of less than 13%. Quantification of the individual acid-

released volatile compounds was more reliable for a greater number of compounds than quantification of the individual enzyme-released compounds.

Chapter 3

ARTEFACT ANALYSIS

Chapter 3

ARTEFACT ANALYSIS

3.1 ABSTRACT

The extraction, isolation and concentration procedures by which the volatile extracts are prepared prior to analysis by GC-MS generated relatively few artefacts. The compounds that were observed as artefacts were mainly phthalate esters, silyloxy compounds and straight chain hydrocarbons. A series of unknown compounds with major ions at *m/z* 147, 133 were also observed.

3.2 INTRODUCTION

Gas chromatography- mass spectrometry is a sensitive analytical technique with the ability to detect compounds present in grape and wine extracts at a concentration of 10^{-4} to 10^{-9} g/L (Rapp 1988a). Accordingly, the isolation and concentration of aroma volatiles requires extreme care to ensure that results are free from artefact interference. Each analytical technique used for the analysis of free and bound aroma compounds of grapes and wine should therefore be carefully monitored to ensure that the compounds identified by GC-MS originate from the samples under investigation and are not introduced from an extraneous source.

Every aspect of the sample processing used in this research was investigated for possible generation of artefacts that may interfere in the volatile GC profile of Shiraz. The aim of the artefact analysis was to develop a library of all possible artefacts that may be introduced into the system from an extraneous source. Many of the extraction procedures utilised to extract potential artefacts were more

rigorous than those that normally take place during the isolation and analysis of Shiraz volatiles and in many cases present the 'worst case'.

3.3 MATERIALS

All materials used for the artefact analysis were the same as those described in Chapter 2.

3.4 EXPERIMENTAL

3.4.1 General procedures

All glassware was soaked overnight in *Pyroneg*®, washed in hot tap water then rinsed twice with purified water. Before use, all glassware was rinsed with redistilled ethanol and then redistilled methanol or dichloromethane, depending on the use of the glassware. Plastic containers were avoided for all aspects of the work.

3.4.2 Artefact analysis

3.4.2.1 Filter pads used to filter juice prior to extraction of free and bound volatiles

A filter pad (D0 *Ekwip*) was soaked in water (100 mL) at room temperature (4 h). The filter pad was removed and the aqueous extract centrifuged at 16 000 *g* for 20 min. The volatile compounds were isolated from the aqueous extract by liquid/ liquid extraction with dichloromethane (150 mL) for 16 h. The solvent was concentrated by distillation through a column of Fenske's helices to a volume of approximately 100 µL and stored at -20°C prior to analysis by GC-MS.

3.4.2.2 Plastic tubing

The plastic tubing from:

a) the juice to the peristaltic pump, and

b) the soft plastic tubing inside the body of the pump

were analysed as a possible source of artefacts. Tubing that had not been previously used was soaked in methanol (50 mL) at room temperature (4 h). The methanol extract was then concentrated under vacuum, the concentrate resuspended in dichloromethane (1 mL) and stored at -20°C prior to analysis by GC-MS.

3.4.2.3 Parafilm covering

Dichloromethane (1 mL) was placed in two pear-shaped flasks (5 mL) which were used for the storage of extracts. One flask was sealed with a ground glass stopper and *Parafilm* plastic and the other flask closed with a ground glass stopper and no *Parafilm*. The flasks were left for one month at -20°C. The solvent was concentrated by distillation through a column of Fenske's helices to a volume of approximately 100 µL and stored at -20°C prior to analysis by GC-MS.

3.4.2.4 Buffers pH 3.2 and pH 5

Buffer, pH 3.2, (30 mL) was placed in a glass ampoule, flushed with nitrogen and sealed. The glass ampoule was left in a preheated oven (50°C) for 28 days. Buffer pH 5 (50 mL) was sealed under nitrogen in a long-necked round-bottom flask and incubated at 37°C for 16 h. The volatile compounds were extracted by continuous liquid/liquid extraction from both buffered solutions with dichloromethane for 16 h and the solvent concentrated as outlined above (*Section 3.4.2.3*).

3.4.2.5 Rohapect C

Rohapect C (100 g) was suspended in buffer (pH 5, 50 mL) and sealed under nitrogen in a long-necked round-bottom flask. The solution was incubated at 37°C for 16 h and the volatile compounds were extracted from the buffered solution, concentrated and analysed as outlined above (*Section 3.4.2.3*).

In another experiment *Rohapect C* solution (200 mg in 100 mL pH 5 buffer) was thermally deactivated by heating the solution at 90°C for 15 min. A C_{18} RP isolate from Shiraz juice (50 mL) was resuspended in water (10 mL) and deactivated enzyme solution (10 mL) added. Incubation and extraction of the buffered solution was carried out as described in section 3.4.2.5.

3.4.2.6 Sep-pak cartridges

A *Sep-pak* cartridge was soaked in methanol (25 mL) for 16 h. The methanol eluate was evaporated to dryness under vacuum then resuspended in dichloromethane (1 mL). The solvent volume was reduced and stored at -20°C prior to analysis by GC-MS.

3.4.2.7 Dichloromethane

Dichloromethane (150 mL) was refluxed overnight in the apparatus used for the liquid/liquid extraction of volatile compounds. The solvent was then concentrated to approximately 100 µL by distillation through Fenske's helices and stored at -20°C until analysis. A further aliquot of dichloromethane (150 mL) was concentrated to approximately 100 µL by distillation through Fenske's helices and stored at -20°C prior to analysis by GC-MS.

3.4.3 Gas chromatography-mass spectrometric analysis (GC-MS)

The stored concentrates were further concentrated to approximately 10-20 µL prior to analysis by gas chromatography-mass spectrometry (GC-MS). Gas chromatograms were obtained using 2 µL injections of the concentrated samples. All GC-MS analyses were carried out under the conditions outlined in Section 2.4.5.

3.5 RESULTS AND DISCUSSION

The mass spectral data for the compounds observed in Figures 3.1 to 3.5 are listed in Table 3.1.

3.5.1 Filter pads

The filter pads used to filter the pressed juice before extraction of either the free- or glycosidically bound-volatiles were considered a possible source of contamination because of their long exposure to the juice. Water extracts of the pads contained the volatiles shown in Figure 3.1. The mass spectra of seventeen of these compounds (Table 3.1) demonstrated that several hydrocarbons and phthalates could be extracted from the filter paper. The hydrocarbon **(1)** was not subsequently identified in the C_{18} RP isolates, hence is possibly extracted from the filter paper only after prolonged soaking.

Vanillin **(12)** was also identified as an artefact arising from the filter pads. This compound has been identified in Shiraz juice as a free volatile and as a glycoconjugate, released by both enzyme- and acid-catalysed hydrolysis of a C_{18} RP isolate (*Chapter 5, Table 5.2*). As a consequence, juice solids were

Table 3.1 Mass spectral data of the compounds observed in the chromatograms presented in Figures 3.1-3.5

Peak No.	Retention time (secs)	m/z (%of base peak)
Filter pad (Figure 3.1)		
1	295	43(100), 57(71), 71(29), 85(21), 99(3), 142(3)
2	345	109(100), 111(51), 83(26), 85(16), 73(2)
3	479	43(100), 57(67), 71(23), 85(14)
4	550	45(100), 59(14), 72(7), 84(7)
5	666	43(100), 44(94), 61(54), 79(41), 49(10), 81(7)
6	726	94(100), 66(52), 65(26), 39(43), 40(22), 38(8), 50(5), 55(5), 37(4), 51(4), 63(4), 49(2), 95(1)
7	839	57(100), 85(33), 43(17), 84(10), 116(8), 55(2), 71(1)
8	1043	No spectrum
9	1224	60(100), 41(90), 43(70), 73(64), 55(44), 57(42)
10	1262	43(100), 71(75), 41(35), 56(21), 83(19), 55(17), 98(10), 57(8), 89(8), 73(8)
11	1304	43(100), 71(78), 56(70), 89(41), 41(43), 55(13), 57(10), 73(10), 39(5)
12	1531	151(100), 152(98), 81(60), 109(27), 123(10) (Vanillin)
13	1769	149(100), 177(11)
14	1830	99(100, 55(12), 211(4)
15	2141	87(100), 45(49), 72(27), 41(29), 44(22)
16	2325	149(100), 41(13), 150(5), 223 (<1)
17	2553	60(100), 81(51), 41(44), 95(10), 136(5), 55(5), 122(4), 121(4), 135(3), 107(3), 108(3), 53(2)
Plastic tubing number 1 (Figure 3.2a)		
18	1454	43(100), 57(65), 41(38), 71(19), 85(15),
19	1628	43(100), 57(74), 41(51), 71(43), 85(20)
20	2256	No spectrum
21	2321	No spectrum
22	2393	73(100), 147(44), 221(28), 281(13), 207(7), 295(7), 267(2), 131(2), 132(1), 341(1)
23	2482	147(100), 133(21), 43(9), 148(3)
24	2618	No spectrum
25	2662	149(100), 57(35), 41(32), 43(28), 167(23), 71(17), 70(15), 55(13), 104(5), 113(4), 112(2)
Plastic tubing number 2 (Figure 3.2b)		
26	1082	43(100), 57(69), 41(57), 71(34), 85(14), 55(11)
27	1118	161(100), 133(39), 175(37), 91(58), 41(55), 105(34), 119(27), 148(27), 77(14), 189(13), 204(13), 55(12)
28	1157	41(100), 95(69), 91(58), 93(50), 161(43), 79(41), 105(40), 107(35), 55(31), 119(25), 81(21), 67(17), 133(15), 147(8)
29	1274	43(100), 57(74), 41(63), 71(41), 85(23), 99(1)
30	1388	43(100, 41(48), 69(21), 55(8), 57(4), 67(4), 71(4), 83(3)
31	1455	43(100), 57(84), 41(61), 71(55), 85(36), 99(5)
32	1543	73(100), 147(13), 281(8), 221(6)
33	1608	43(100), 59(23), 179(20), 91(14), 119(7), 161(7)

Table 3.1 Mass spectral data of the compounds observed in the chromatograms presented in Figures 3.1-3.5

Peak No.	Retention time (secs)	m/z (%of base peak)
Plastic tubing number 2 (Figure 3.2b) cont.		
34	1629	43(100), 57(97), 71(40), 85(38), 99(5)
35	1770	149(100), 177(18), 65(14), 76(13), 105(10), 104(9), 121(5),
36	1803	73(100), 147(20), 221(8), 207(2), 281(2)
37	2031	73(100), 147(12), 221(4), 207(1), 281(4), 131(<1)
38	2241	73(100), 147(30), 221(7), 207(2), 281(4), 131(<1), 133(<1)
39	2327	149(100), 41(17), 104(7), 105(2), 205(2), 223(2), 121(2), 122(1), 123(<1), 160(<1),
40	2434	73(100), 147(15), 221(8), 281(3), 207(2), 131(<1), 133(<1)
41	2497	73(100), 147(43), 221(23), 281(7), 207(6), 295(1)
42	2613	73(100), 147(17), 221(10), 281(5), 207(3), 131(<1), 133(<1)
Parafilm (Figure 3.3)		
43	2270	281(100), 135(71), 147(60), 148(49), 73(46), 282(41), 327(41)
44	2325	120(100), 119(30), 91(23)
45	2874	73(100), 281(97), 147(94), 221(81)
46	3073	43(100), 71(59), 49(41), 159(39), 55(30)
47	3362	No spectrum
48	3399	147(100), 43(95), 73(84), 57(81), 221(53), 85(51)
49	3820	137(100), 138(40), 182(40), 122(19), 164(10), 43(10), 41(11)
50	4106	43(100), 95(50), 109(49), 138(48), 135(36), 41(5)
51	4491	149(100), 205(6), 150(5), 223(3), 104(2)
Deactivated enzyme (Figure 3.4)		
52	150	41(100), 45(73), 55(63), 69(17), 83(43), 39(60), 57(35), 43(27), 56(10), 70(10), 97(6)
53	501	56(100), 41(96), 55(84), 70(64), 43(63), 69(51), 84(48), 83(40), 42(43), 57(28), 39(18)
54	1026	60(100), 73(59), 41(44), 43(31), 55(23), 115(14), 129(14), 98(7), 69(7)
55	1085	43(100), 87(96), 57(92), 41(29), 56(21), 45(17)
56	1463	71(100), 43(45), 111(4), 159(4), 83(4), 69(3)
57	2114	147(100), 178(59), 119(22), 91(18), 65(10), 118(5), 120(4), 148(5)
58	2308	147(100), 133(32), 148(14), 149(7), 43(6), 119(4), 73(3), 173(<1), 159(<1)
59	2394	75(100), 313(9), 4397, 61(7), 101(1), 85(1), 113(1), 129(<1)
Methanol extract of Sep-pak cartridges (Figure 3.5)		
60	2448	149(100), 205(6), 223(4), 56(3)
61	2978	149(100), 77(22), 133(20), 163(18), 41(15), 57(14), 207(10), 104(9), 160(3)

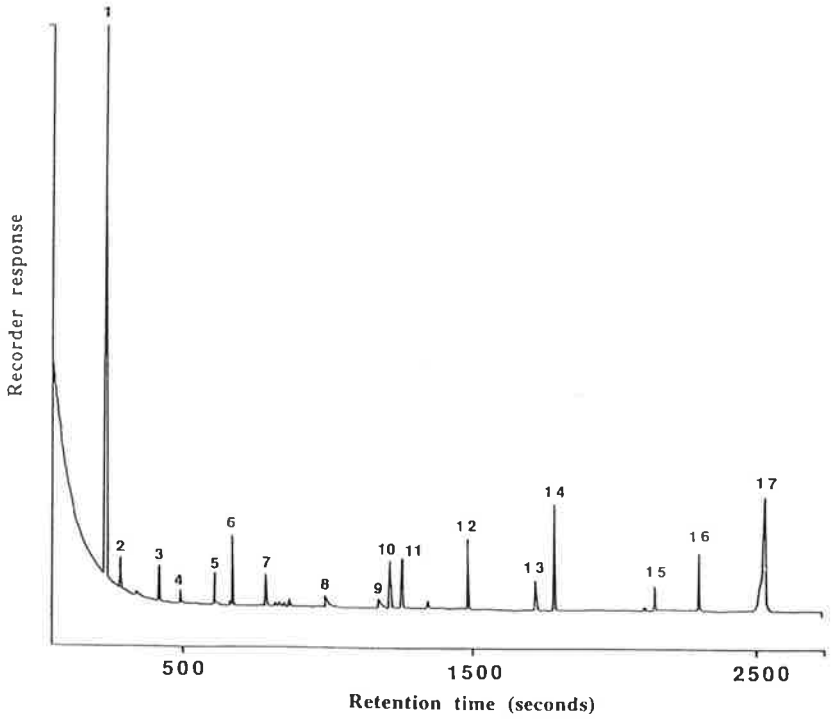


Figure 3.1 *Gas chromatogram of the aqueous filter pad extract*

subsequently removed by centrifuge rather than filtration before isolation of the volatile and glycosidically bound compounds from the supernatant.

3.5.2 Plastic tubing

There was a large number and high concentration of volatiles observed in the GC trace of the methanol extract of the plastic tubing associated with the peristaltic pump (Figures 3.2a and b). The volatile compounds present in these samples were mainly phthalate esters, straight chain hydrocarbons, silyloxy compounds and an unknown compound with major ions in the mass spectrum at *m/z* 147, 133 which was present in one of the plastic extracts (Figure 3.2a peak 23) in trace amounts. The mass spectra for the compounds observed in both methanol extracts are listed in Table 3.1.

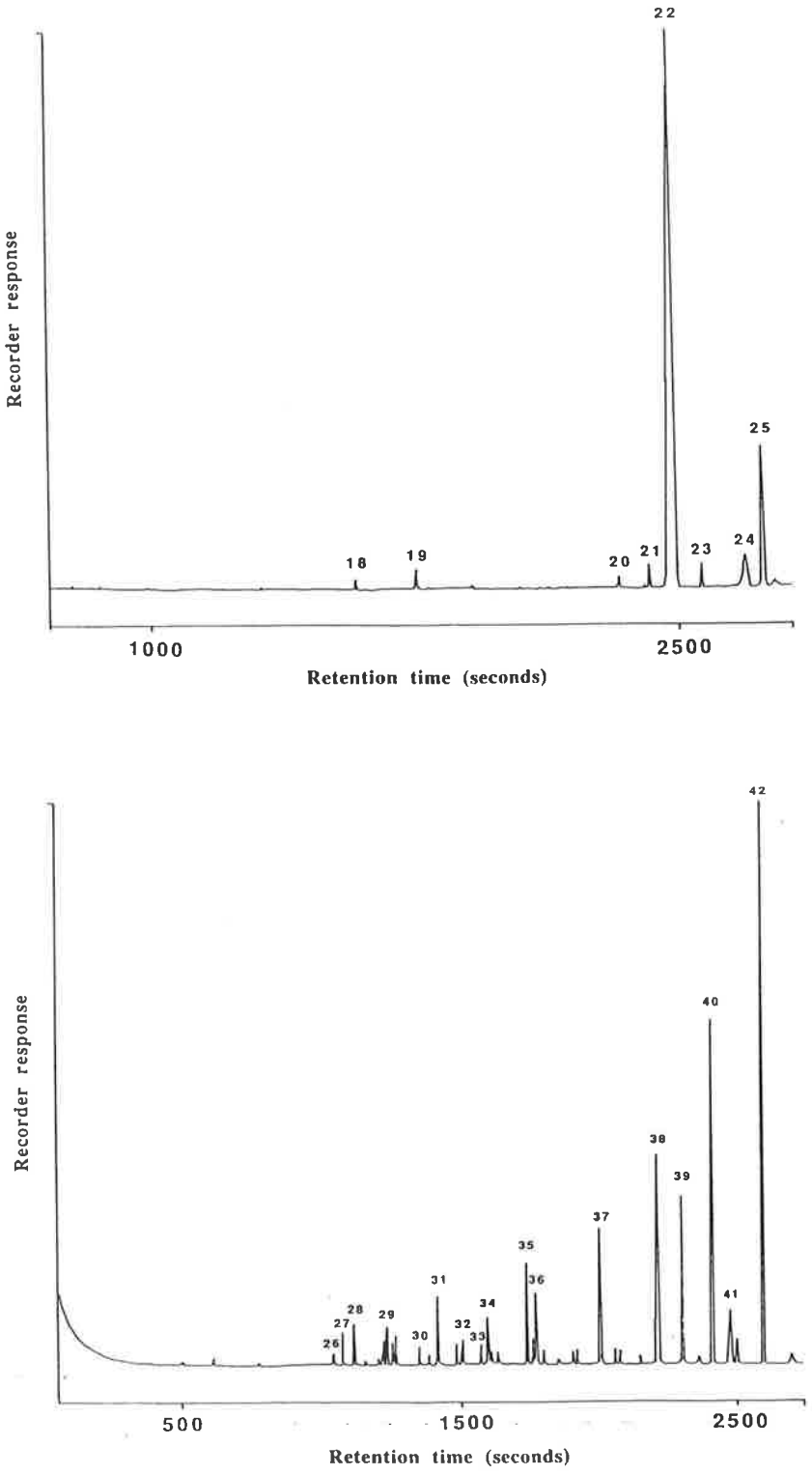


Figure 3.2a and b *Gas chromatogram of the methanol extract of plastic tubing (a)from the juice to the peristaltic pump and (b) inside the pump.*

3.5.3 Parafilm *covering*

The aroma concentrates from the juice samples were kept in pear-shaped flasks (5 mL) at -20°C for periods up to three months before analysis by GC-MS. These flasks were sealed with *Parafilm* to ensure that the solvent did not evaporate to dryness. Because of the volatility of dichloromethane and the solubility of *Parafilm* in this solvent it is possible that artefacts in the extracts may arise from the *Parafilm* during the opening and closing of the flasks or by migration of compounds from the *Parafilm* into the solvent. Although the evaporation rate of the solvent from the flask that had not been sealed with *Parafilm* was faster than from the flask that had been sealed with *Parafilm*, no volatile components were identified in the former solvent by GC-MS. On the other hand, the flask sealed with *Parafilm* was contaminated with several volatile compounds (Figure 3.3). The mass spectral data of these compounds is given in Table 3.1 .

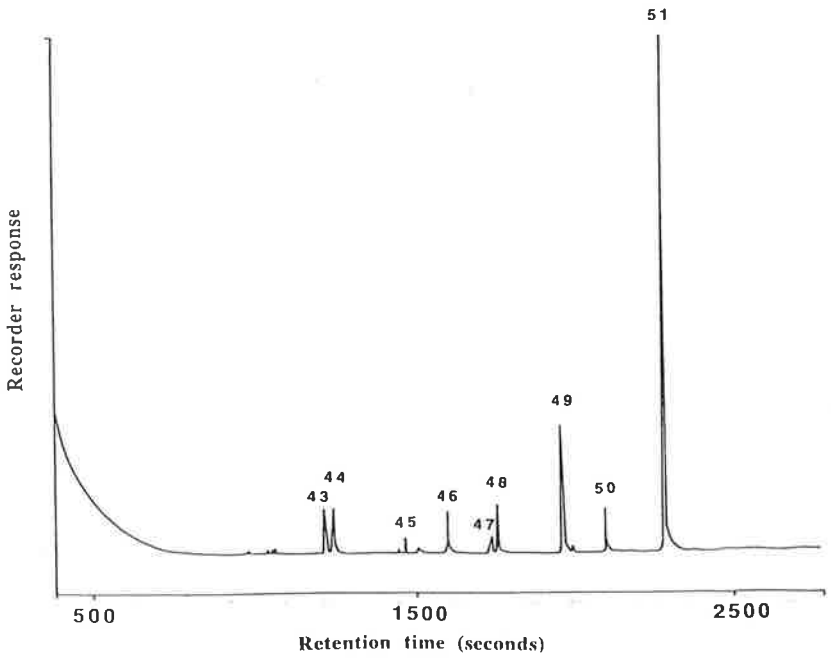


Figure 3.3 *Gas chromatogram of dichloromethane stored in a flask sealed with* Parafilm

3.5.4 Buffers pH 3.2 and pH 5

No detectable volatile impurities were identified in either of the two buffers used in this study (pH 3.2 and pH 5) after they had been heated at 50°C for 28 days and 37°C for 16 h, respectively. The compounds used to make up both buffered solutions, therefore, did not contain any impurities that gave rise to any volatiles, detectable by GC-MS, upon heating.

The lack of volatile products in these two buffers also suggests that the dichloromethane used for the extraction of the volatile compounds did not contribute any artefacts to the concentrated extract that were identifiable by GC-MS. Analysis of the two concentrated aliquots of dichloromethane described in section 3.4.2.7 by GC-MS confirmed the purity of this solvent as no volatiles were identified in the GC trace.

3.5.5 Rohapect C

Incubation of a solution of *Rohapect C* in buffer (pH 5) for 16 h did not lead to the formation of any volatile compounds detectable by GC-MS.

The C_{18} RP isolate incubated in the presence of thermally deactivated *Rohapect C* liberated several volatiles (Figure 3.4), the mass spectra of which are listed in Table 3.1. The major compound identified was an unknown volatile, *m/z* 147, 133 (compound 58), similar to compound 23 in the plastic tubing extract (Figure 3.2a).

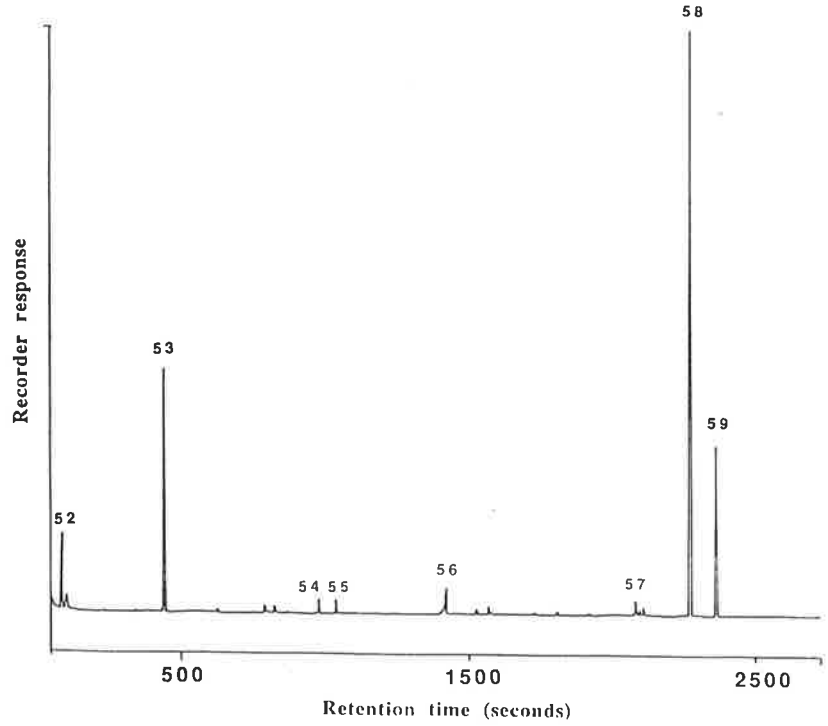


Figure 3.4 *Gas chromatogram of volatiles isolated from buffered solution after incubation of a Shiraz juice extract with deactivated enzyme*

3.5.6 Sep-pak *cartridge*

The gas chromatogram of the methanol extract of a *Sep-pak* cartridge is shown in Figure 3.5. Two phthalate esters (compounds **60** and **61**) were observed as possible artefacts that may arise by using *Sep-pak* cartridges for the isolation of glycoconjugates from Shiraz juice. These compounds, however, did not interfere in the chromatograms of the Shiraz juice, skin or wine extracts as their retention times are longer than those of the volatile compounds observed in the Shiraz extracts.

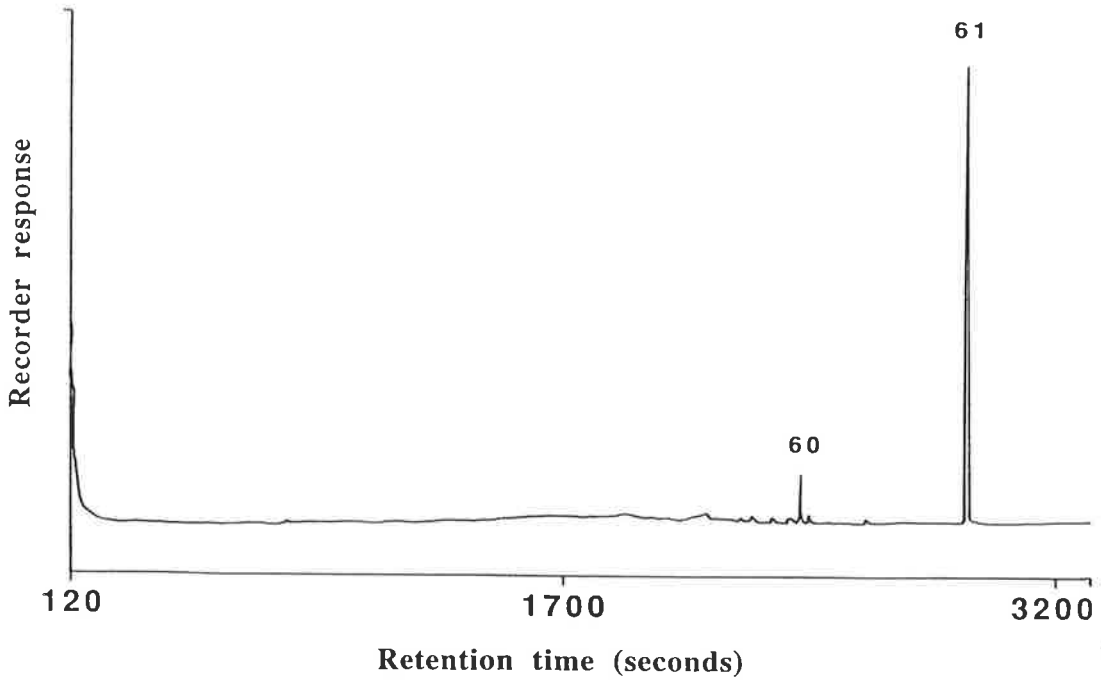


Figure 3.5 *Gas chromatogram of the methanol extract of a* Sep-pak *cartridge*

3.5.7 A series of unknown compounds with major ions at m/z 147, 133: artefacts or Shiraz volatiles?

A series of unknown compounds with a mass spectra of *m/z* 147, 133 was observed, one of which appeared as a major component in all the C_{18} RP isolates extracted through the large column of C_{18} RP adsorbent (Table 3.2) (*Section 2.4.3.1)*. This compound was also observed in trace amounts in the extract from the plastic tubing (Compound 22, Table 3.1).

The extracts in which these unknown *m/z* 147, 133 compounds have been observed are listed in Table 3.2. These compounds were seen in only some of the free volatile extracts of Shiraz juice in trace amounts and not in the wine headspace extract, suggesting that this compound is present in Shiraz juice predominantly

Table 3.2 Extracts in which an unknown *m/z* 147, 133 compound has been observed.

Origin	147, 133 compound present in GC-MS trace (✓) or not present (x)
[1]Free volatiles	tr
[2]Adsorbed frees	✓
Enzyme hydrolysates	✓
Acid hydrolysates	✓
Wine headspace	x
Wine enzyme hydrolysates	✓
Wine acid hydrolysates	✓
Plastic tubing	✓
Filter paper	x
Buffer pH 5, pH 3.2	x
Enzyme preparation	x
Deactivated enzyme + C_{18} RP isolate	✓
Methanol extract of *sep-pak* cartridges	x

[1] Free volatiles are those compounds isolated directly from the juice by continuous liquid/liquid extraction with Freon.
[2] Adsorbed frees are the free volatiles which are adsorbed onto the C_{18} RP adsorbent and are subsequently present in the C_{18} RP isolate. These compounds were extracted from the C_{18} RP isolate by liquid/liquid extraction in a separating funnel with both Freon and dichloromethane.

in a bound form or is an artefact from the plastic tubing or another unknown source.

The identification of these compounds as natural products is questionable as the concentration was found to fluctuate from zero to 300 µg/L between replicates, it was not formed through heating the juice at pH 3.2 and appeared to form only when the juice was in contact with the C_{18} RP adsorbent for prolonged periods. This compound appeared to build up on the column regardless of washing

procedures and was eluted with methanol only after a certain concentration of this unknown compound had been accumulated.

When the Shiraz glycosides were isolated by passing the juice through a *Sep-pak* cartridge, the unknown *m/z* 147, 133 compounds were not observed in the GC trace of the enzyme-released volatile compounds. However, when the same Shiraz juice was passed through a series of five prepared *Sep-pak* cartridges (*Section 2.4.3.2)* the concentration and number of these unknown *m/z* 147, 133 compounds was found to increase.

Although the unknown compounds were found in trace amounts in the free volatiles, the positive correlation between the time the juice was in contact with the C_{18} RP adsorbent and the concentration of these compounds suggests that they may be formed through condensation reactions of primary metabolites present in high concentration in grape juice or by degradation of the C_{18} RP adsorbant present in the column.

The *m/z* 147, 133 compounds are therefore regarded as an artefact and not a natural product of Shiraz grape berries or wine. The presence of this artefact was minimised with the use of *Sep-pak* cartridges for isolating the glycosidically bound material directly from Shiraz juice or from Shiraz skin and wine extracts.

3.6 CONCLUSION

Relatively few artefacts were observed as possible contaminants of the Shiraz volatile extracts as a result of the analytical procedure. Phthalate esters, silyloxy

compounds and straight chain hydrocarbons were identified as the major compounds present in the extracts studied as well as a series of unknown *m/z* 147, 133 compounds. These latter compounds are probably artefacts, but their nature and formation remains unknown. Use of *Sep-pak* cartridges for the isolation of Shiraz glycosides was found to minimise the concentration of this compound in the GC chromatogram. Filter pads, commonly used to filter juices in our laboratory were found to be a significant source of artefacts. Juices used in this study were therefore subsequently clarified by centrifugation, rather than filtration.

Chapter 4

ASSESSMENT OF QUALITY IN SHIRAZ JUICE AND WINE BY SENSORY DESCRIPTIVE ANALYSIS.

Chapter 4.

ASSESSMENT OF QUALITY IN SHIRAZ JUICE AND WINE BY SENSORY DESCRIPTIVE ANALYSIS

4.1 ABSTRACT

Volatile components liberated by hydrolysis of C_{18} RP isolates from *Vitis vinifera* cv. Shiraz juice were evaluated by sensory descriptive analysis. The isolates were subjected to hydrolytic treatment at pH 3.2 or to treatment with a non-selective glycosidase enzyme. Shiraz juices assigned as high and low 'quality', each sourced from two geographic regions and sampled in 1988 and 1989, were studied. Wines made from the 1989 juices were also subjected to descriptive analysis.

The quality difference for one pair of wines and for all but one pair of hydrolysates could be distinguished and quantified. The glycosidic hydrolysates prepared by both methods were found to contribute non-berry attributes to wine such as 'stalky', 'earthy' and 'cigar/tobacco'. Wines considered to be of high quality were rated higher in these non-berry-type attributes than their low quality counterparts. From these results it may be deduced that glycosidic hydrolysates contain aroma compounds that make an important contribution to the aroma of high quality Shiraz wine.

4.2 INTRODUCTION

Before undertaking a chemical analysis of the volatile aglycones derived from the pool of glycosides in Shiraz fruit, there is a need to determine the contribution made by these deconjugated volatiles to the sensory properties of Shiraz. Arising

from such a study is the opportunity to validate the premise on which the research is based, i.e. that samples from vineyards traditionally yielding fruit of a given quality can objectively be distinguished from samples sourced from other vineyards yielding fruit of a lower or higher quality. These aims necessitate the application of sensory descriptive analysis (Noble 1988) to base wines spiked with the enzyme- and acid-catalysed hydrolysates. The aims also require comparison of the sensory properties of the spiked samples with those of wines made from the grapes from which the glycosidic fractions were derived. This is the first study of the flavour properties of precursor compounds for a black grape variety.

4.2.1 Sensory analysis

Sensory analysis is a scientific discipline which is used in both the food and beverage industries to 'evoke, measure, analyse and interpret human responses to the properties of foods and other materials, which are perceived through the senses' (Civille 1986). Formal sensory analysis commenced in the 1940s for the evaluation of foods being sent to the armed forces to determine if any difference in quality could be perceived when certain foods were made with fewer or cheaper ingredients (Stone and Sidel 1985). Since this time, sensory analysis has been used extensively for quality control and market research by difference and preference testing and for research and development by the use of formal descriptive analysis (Kluba 1986).

Difference testing involves assessing the difference between two products by duo-trio, triangle or paired comparison tests. For both duo-trio and triangle

tests three samples are presented to the panel, two of which are the same, and one different. The chance of selecting the correct sample for the latter test is 50% ($p=\frac{1}{2}$), whereas for the triangle test the probability of choosing the correct sample is 33⅓% ($p=\frac{1}{3}$) (Amerine *et al.* 1965). Statistical tables for calculating the significance of both tests are available (Roessler *et al.* 1978). Paired comparisons are used to determine which sample has more or less of a specific attribute with a probability of 50% of choosing the correct response (one-tailed test as there is only one correct answer) (Noble 1988).

Preference testing has a limited use in sensory evaluation as there is great variation in individual preferences for a product. This method of testing will not allow an analyst to determine the type of differences between two samples but can give a market research company an idea of general consumer preference.

Hedonic rating of products is generally conducted using a scale of nine words ranging from 'like extremely' to 'dislike extremely' (Amerine and Roessler 1976). The scale is used widely to assess the acceptability of a product and appears to give results which are reliable and valid (Stone and Sidel 1985). Although the results from these types of analyses can and have been given numerical equivalents (Amerine and Roessler 1976), care must be taken in the statistical analysis of these results as the spread of these data does not have the same meaning as the average of the data and can lead to conclusions which are misleading and often incorrect (O'Mahoney 1986). Research into an alternative method of hedonic analysis has led to the development of a new method of ranking to obtain R-index measures of preferences. The R-index is a probability value

that determines the probability of distinguishing between two stimuli (Vie *et al.* 1991). The method of ranking uses a 4 or 5-point word scale in a manner similar to other nine-point hedonic scale but with the inclusion of a neutral position. Numerical values are assigned to each word after the assessment of the samples from which R-indices are calculated. These authors found that the relative nature of the R-index measure resisted instructional bias, a problem previously found with the nine-point scale.

Descriptive analysis is used to quantify the qualitative differences observed between two samples through difference testing. The aroma or the flavour of the samples analysed should be significantly different at least at $p<0.05$ when tested by duo-trio or triangle tests before the observed differences can be characterised by descriptive analysis. The key factor in quantitative descriptive analysis is the use of a trained panel and the application of statistical analysis to determine the outcome of the trial. A panel of at least 10-16 judges is trained to identify and score specific attributes relevant to the product being tested (O'Mahoney 1986).

Selecting a panel is dependent upon the source of potential panel members (voluntary or reimbursed), their willingness and availability to participate, ability to distinguish between samples, taste acuity, memory, good health (freedom from allergy and frequent head colds) and lastly an interest in the product (Amerine *et al.* 1965, Zook and Wessman 1977, Noble 1988, Meilgaard 1989). Screening of potential panel members by triangle difference testing enabled the sensory group at the Quaker Oats Research Laboratory to choose the most appropriate people for a variety of different sensory trials (Zook and

Wesserman 1977). Generally panellists for quantitative descriptive analysis of wine are chosen from either interested students (Noble and Shannon 1987) or members of the relevant institution. As funding is rarely provided to pay a panel, the number of people from which the panel can be chosen is limited.

When choosing a panel, even from a limited number of potential subjects, taste tests should be carried out to determine if an applicant has any form of olfactory 'blindness' or anosmia and as such unable to detect certain aromas (Altner 1978). If a panel member is partially anosmic then he or she should not be included on the panel. Judges' perception to taste and smell has been shown to vary over time (Brien and Mayo 1987) which highlights the importance of continually monitoring judge performance. As physical and mental health, diet and age contribute to olfactory performance (Kare and Tordoff 1989), it is important to instruct panel members on the basic physiology of aroma and taste. With this knowledge they can therefore immediately inform the analyst of any health aberration which may affect their ability to assess the samples.

As the identification of a specific aroma is due to a memory response triggered by electrical impulses from the nose to the brain (Dudel 1978), training or teaching a panel to recognise and reproducibly identify the attributes present in a sample is a difficult, yet possibly the most important part of descriptive analysis. The training of a panel can take weeks or months depending on the test being conducted and the familiarity of the panellists with the product being evaluated (McDaniel *et al.* 1987, Noble 1988).

4.2.2 Training for descriptive analysis

The initial part of any training program is the development of a new language suitable for describing the characteristics of the product being evaluated. The beer flavour wheel was the first formal development of a language for sensory descriptive analysis of alcoholic beverages (Meilgaard 1979). This concept was followed by the formation of a whisky flavour wheel (Shortreed *et al.* 1979) after which the American Society for Enology and Viticulture developed a wine aroma wheel (Noble *et al.* 1984a, Noble *et al.* 1987a). The wine aroma wheel developed by these authors has been reproduced in Figure 4.1 (Noble *et al.* 1987a).

All three flavour wheels have the same basic three tier structure and differ only in the descriptive terms used. In comparison to the beer and whisky flavour wheels, the wine aroma wheel relies on aroma descriptors rather than taste descriptors. The first tier terms are the general names of groups to indicate the types of flavour they contain, e.g. woody, fruity and floral. The second tier terms are the general descriptors belonging to the groups in the first tier, e.g. the woody notes in wine may be described as phenolic, resinous or burnt. The specific third tier terms relate to individual aromas for which reference standards can be made, examples of which have been given by Noble *et al.* (1987a).

The development of the flavour wheels encourages the use of universal terms in descriptive analysis and a consistency in terms used by different laboratories and different countries. These wheels, however, can only act as a guide for aroma research as the differences in background of the panel members as well as the differences in general descriptive vocabulary in each country makes the

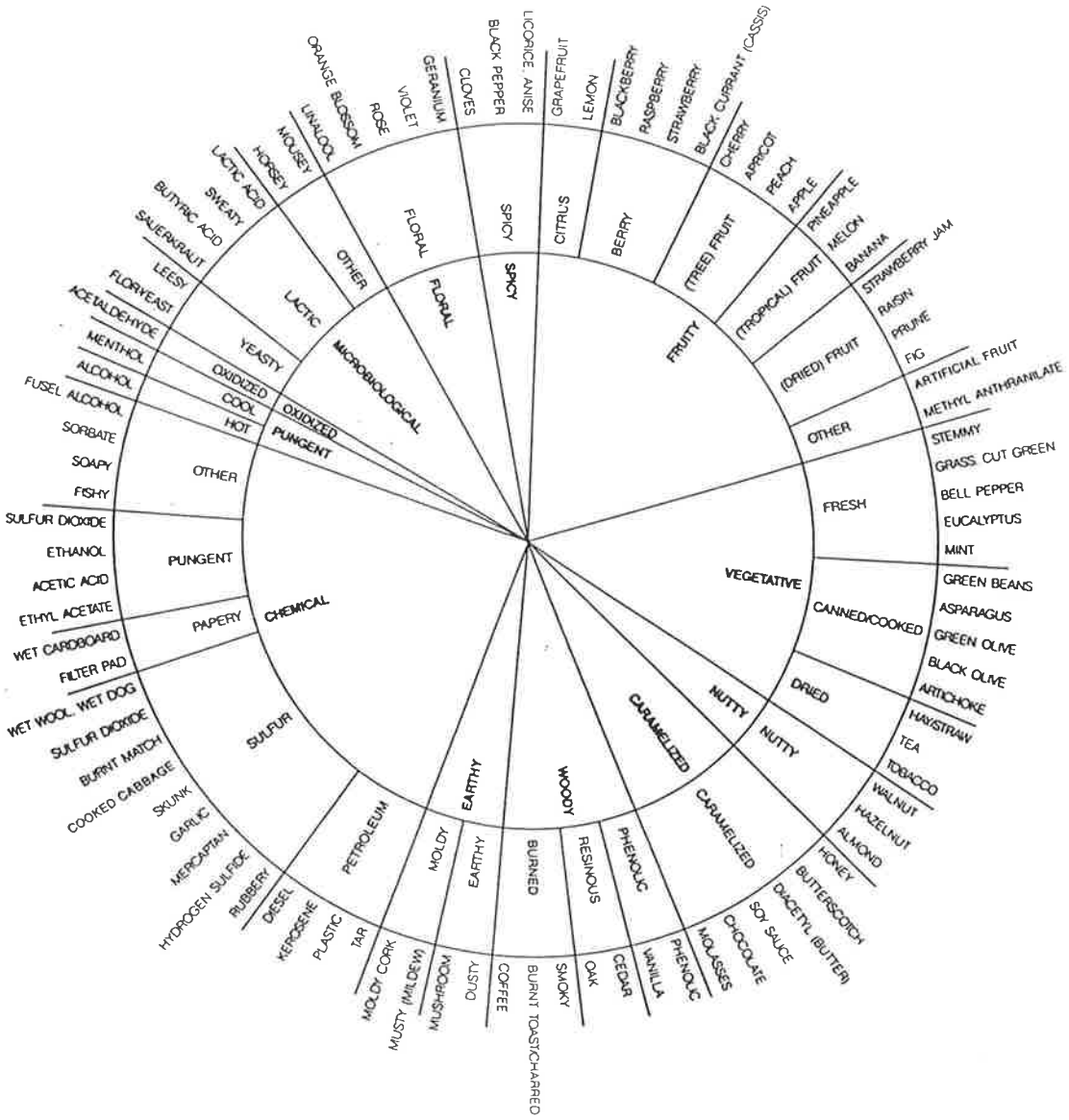


Figure 4.1 *Wine aroma wheel showing first-, second-, and third tier terms (Noble* et al. *1987)*

obligatory use of a universal aroma language untenable.

Appropriate reference standards (attributes) to describe the flavour differences amongst wine and wine-related samples are chosen initially by the general descriptive analysis of commercial samples relevant to the samples which will be analysed or else from a range of the samples themselves. A large number of

reference standards are then prepared which represent the aromas listed by the panel. The panellists are asked to choose which standards they feel adequately describe the aromas present in the samples. The aroma and intensity of the aroma of the standards are then assessed during a number of training sessions before the panellists are asked to rate the intensity of these attributes as they are perceived in the samples (McDaniel *et al.* 1987, Noble 1988).

Methods of rating the intensity of an attribute in a product includes nominal scales for naming or classifying products, ordinal scales for ordering or ranking, interval scales, and ratio scales (Stone and Sidel 1985). Wine samples are generally assessed using a nine-point interval scale (McDaniel *et al.* 1988). Interval scales assume that the distances between the points are equal and that the panel is using the entire scale. There appears to be a psychological barrier for many judges to use the full nine-point scale which leads to a 'squeezing effect', where the distribution of the data is distorted from the normal (O'Mahoney 1982). A line scale anchored at each end with terms such as high and low is claimed to alleviate some of the problems associated with the numerical scale (Noble 1988), yet the variation within judges in the evaluation of food and beverages seems to occur no matter what form of scoring is used (Amerine *et al.* 1965).

4.2.3 Application of quantitative descriptive analysis in wine research

Sensory evaluation was developed in the wine industry primarily to create a less subjective means of assessing differences amongst wines from different areas and viticultural trials. Quantitative descriptive analysis was initially used in this

industry to describe the differences between wines from different regions and countries (Kwan and Kowalski 1980, Noble *et al.* 1984b, Guinard and Cliff 1987) and as a means of creating aroma profiles for premium varieties such as Cabernet Sauvignon (Aiken and Noble 1984, Noble *et al.* 1984b, Heyman and Noble 1987), Chardonnay (Ohkubo *et al.* 1987, Noble *et al.* 1987b, Noble and Ohkubo 1989), Pinot Noir (Guinard and Cliff 1987, McDaniel *et al.* 1987), and other varieties such as Zinfandel (Noble and Shannon 1987), Gewürztraminer (McDaniel *et al.* 1988) and Seyval Blanc (Andrews *et al.* 1990). Descriptive analysis has also been used to quantify the off-flavour often found after a white or sparkling wine has been exposed to sunlight or fluorescent light (Dozon and Noble 1989) and, finally, to assess the contribution of different bacterial strains on the aroma of wine from Pinot Noir (McDaniel *et al.* 1987).

More specifically, sensory analysis has been used to determine the contribution of different secondary metabolites to the flavour of wine. Duo-trio difference tests demonstrated that the major hydroxycinnamates found in wine did not play any direct role in the bitterness and astringency of wines (Verette *et al.* 1988). Duo-trio tests were also used to determine the threshold values of five sulfur compounds present in wine. Descriptive analysis was performed on two of these compounds, dimethyl sulfide (described as asparagus, corn and molasses aromas) and ethanethiol, whose aroma has been described as onion and rubber (Goniak and Noble 1987).

A study of the sensory properties of non-volatile flavour precursors in Muscat of Alexandria and Chardonnay wines by duo-trio difference testing verified the

tasteless properties of these wine glycosides (Noble *et al.* 1987b). Previous research into the sensory properties of glycosidically bound volatile compounds, from one floral white variety and three non-floral white varieties, has been briefly discussed in only two publications (Noble *et al.* 1987b, Williams,P.J. *et al.* 1989). These studies demonstrated that only the acid-released volatiles for the non-floral varieties could be detected by duo-trio difference tests when back-added to a base wine (Williams, P.J. *et al.* 1989). The enzyme hydrolysates released from the glycosidic fraction of the floral variety could only be detected at twice the original aglycone strength (Noble *et al.* 1987b).

4.2.4 Statistical analysis of sensory data

There are several forms in which sensory data can be presented and analysed. Comparative aroma profiles and bar graphs are useful means of visualising differences between the intensities of certain attributes as rated by a panel of assessors. As well as providing a description of the product being evaluated, data from quantitative descriptive analysis can be subjected to varying forms of multivariate statistical analysis to analyse the underlying relationships in the samples and among the variables (Heyman and Noble 1989). Of the multivariate analyses available, some of those that have been used for the assessment of descriptive data are: principal component analysis (PCA), canonical variate analysis (CVA), multivariate analysis of variance (AOV), procrustes analysis and multiple regression analysis (MRA).

Principal component analysis (PCA) is a form of analysis used to simplify and interpret any trends in the descriptive data. Principal components are linear

combinations of the original variables which explain the maximum amount of variation in the data. The main use of PCA is in reducing the dimensionality of the data in order to simplify later analyses by grouping sets of data or eliminating outliers (Chatfield and Collins 1980). The results consist of two parts; a principal component diagram and a vector diagram which are generally superimposed. The axes represent the principal components and describe the degree of variability of the data while the vectors describe the relative positions and loadings of the attributes, and indicate how each attribute was used by the panel. Clustering of the vectors, i.e. vectors with a small angle between them, indicate that certain attributes are highly correlated to each other. Although PCA is a mathematical method of analysis, it gives a pictorial representation of the data from which statistical differences cannot be determined.

Canonical variate analysis (CVA) on the other hand can be used to determine the significant differences amongst samples. In CVA, linear combinations of the original variables are selected to maximise the ratio of the between-sample to the within-sample variance. Heyman and Noble (1989) compared the use of PCA and CVA for the analysis of quantitative descriptive analysis data and found that there were advantages and disadvantages with both systems of analysis. PCA was better suited for illustrating the overall relationship amongst samples, however, unlike CVA, the significant differences amongst these samples could not be determined.

Analysis of variance compares the means from several samples and tests, whether they are all the same within the experimental error, or whether one or more of the

samples are significantly different (O'Mahoney 1986). AOV will show a significant difference, however, if the variances of the samples are different and may give misleading results that are not due to real differences in the samples being analysed (O'Mahoney 1982). The statistical models for parametric tests such as AOV assume that: (a) the variances of the sets of data to be compared are equal (exhibit homoscedasticity) and (b) that all the numbers are sampled from a population that is normally distributed. This latter assumption requires that the numbers are equally distributed on an interval or ratio scale (O'Mahoney 1982). The use of these scales, however, invariably leads to a distortion of the data away from normal, hence not adhering to the requirements of the data for AOV (O'Mahoney 1982). This author concluded that the use of AOV for the analysis of descriptive data can only give approximate results and cannot be used to give exact levels of significance, as the exact probabilities are not known (O'Mahoney 1982, 1986). Another study of the use of AOV in analysing sensory attribute scores found that AOV was appropriate only for certain experiments and the validity of the results was dependent upon judge reliability (Brien and Mayo 1987).

Procrustes analysis is the least used in the analysis of descriptive data, yet, in many respects it is the most appropriate as it does not require that each judge be highly correlated with the other judges on the panel (Langron 1983). This form of analysis rationalises the ways in which people use scales and words, hence allowing the source of variation within a panel to be identified and removed before any comparisons are made (Williams, A.A. 1982). It also takes into account the range over which individual judges score the intensities of individual attributes

as they are perceived in the sample. Replicate experiments can be used to identify those judges who are inconsistent and allows for them to be removed from the analysis (Langron 1983).

Multiple regression analysis is a technique used to examine linear relationships between dependent and independent variables. The numerical ratings for each attribute and for each sample form the dependent variables. The independent variables are the variables imposed on the samples such as treatment, year and area. A design matrix or model must be established which gives the analyst control over which independent variables are to be compared as well as the freedom to add independent variables such as the ratio or the weighted sum of two variables to create another relevant variable (Korth 1982). This freedom is useful in the analysis of sensory data and enables the analyst to include the assessment of a 'carry-over' effect. The 'carry-over' effect is a possible error that may be introduced to the data set if the intensity of the aroma of one sample leads to bias of the results of another sample assessed in the same set (Veitch, pers. comm.).

The removal of judges who are inconsistent either in the rating of certain attributes or in comparison to other judges has been a matter of discussion for many analysts. Malek (1986) found that the removal of inconsistent judges from the data set led to no significant improvement in the overall panel performance, and only resulted in a decrease in the number of degrees of freedom allowed in the analysis. Due to the inconsistency in judge performance with time, Brien and Mayo (1987) suggested that when judges were unreliable and non-discriminating their data should be removed from the analysis. McDaniel *et al.* (1987) used

correlation analysis of the estimated descriptor scores for each panellist against the panel as a whole as the criterion for removing inconsistent panellists from the panel in a study of the aroma of Pinot Noir fermented by different strains of malolactic bacteria. Judges were removed if they were non-perceivers or if they did not correlate with the other judges on the panel. As these authors pointed out in a later paper, the removal of judges before analysis is not suitable if the results are to be used to draw inferences from the population of the trained judges as a whole (McDaniel *et al.* 1988). In another study, due to the inconsistency among replicates in the assessment of 'lightstruck' wines, three judges were removed from the analysis (Dozon and Noble 1989). These authors considered that the inconsistency in judge response may have been due to either inadequate training or standards which did not adequately describe the specific aroma that was being analysed in the samples.

With rigorous training and removal of judges who are anosmic, or non-perceivers of an attribute before formal profiling commences, there should be no need to remove judges' data from the analysis. The inconsistency in judge performance inherent in sensory descriptive analysis can be minimised through training and assessment of the judges' performance and ability to perceive certain aromas. As there are no set guidelines for the exclusion of data from statistical analysis of sensory data, except in extreme cases, all data should be retained in the analysis to give results that reflect the population of the judges as a whole without any bias.

4.3 EXPERIMENTAL

4.3.1 Samples

The high and low quality juice samples obtained from the Barossa Valley and Coonawarra in 1988 and 1989, for the sensory descriptive analysis, have been described in Section 2.3.2.

The four wines were prepared commercially from high and low quality grapes from the Coonawarra and the Barossa Valley regions in 1989 (*Section 2.3.2).*

4.3.2 Extraction of volatiles from juice precursor fractions

Precursor fractions isolated from Shiraz juice (500 mL) by retention on C_{18} RP silica gel were subjected to enzyme- or acid-catalysed hydrolysis as previously described (*Section 2.4.4)* with the exception that no internal standards were added. The hydrolysis products were isolated by continuous extraction with dichloromethane. The solvent was removed from these extracts by distillation through a column of Fenske's helices to give a concentrate of approximately 100 µL. The concentrate was taken up in water (10 mL) and the aqueous solution was concentrated under vacuum at room temperature for 10 min with a rotary evaporator, to remove the solvent residue. Each sample was adjusted to a volume of 20 mL with water and stored at -20°C.

4.3.3 Sensory studies

4.3.3.1 General aspects

The samples and reference standards were prepared each morning. All samples were presented to the panel in 20 mL aliquots in coded, clear, tulip-shaped

glasses. Panellists assessed the samples, by aroma only, before midday, in isolated booths under red light. The room was kept at 22°C +/- 2°C.

For both duo-trio difference tests and descriptive analyses, the precursor hydrolysates were presented to the panel in a neutral base Shiraz wine at a concentration equivalent to that at which the precursor glycosides were present in the original juice. The base wine was prepared by concentrating 300 mL of commercial bulk Shiraz wine under vacuum at room temperature for 3 h to a final alcohol strength of approximately 8%. The wine samples were taken from a fresh bottle for each replicate.

4.3.3.2 Duo-trio difference tests

These tests were conducted by the procedure described by Amerine *et al.* (1965). The neutral base wine was used as the reference in difference tests for the released volatiles, and the reference used in difference tests involving wine samples was one of the pair under evaluation.

Two sets of duo-trio tests were evaluated at each session, each sample being assessed only once by a panel of 22 to 33 persons from the members of staff of The Australian Wine Research Institute (AWRI) and Waite Institute, University of Adelaide. The significance of each duo-trio test was determined from detailed statistical tables for one tailed tests, p=1/2 (Roessler *et al.* 1978).

4.3.3.3 Descriptive analyses

Fourteen staff members from the Institute were selected to take part in the

descriptive analyses according to their availability and interest in the project. All panel members had participated in the duo-trio tests and were familiar with the samples; no further tests were made for the selection of individuals on the panel. The initial training session involved the assessment of several commercially available Shiraz wines followed by the assessment of two wines that were to be used in the experiment, and an enzyme- and acid-catalysed hydrolysate back-added to the base wine. The range of adjectives chosen by the panel to describe the latter samples is presented in Table 4.1. These aroma descriptors formed the basis for the development of a range of descriptive reference standards. A reduction of the number of standards was achieved through assessment of the frequency of use of the descriptors as well as by discussion amongst the panel members. The final twelve attributes considered by the panel as necessary to describe the sensory properties of the samples are given in Table 4.2 together with the composition of the stock mixture used for the reference standards.

The 20 samples selected for descriptive analysis were presented in a random order with no two samples being evaluated together more than once. All samples were presented in duplicate except the base wine which was randomly presented on five occasions throughout the study. At each session three samples were evaluated. The twelve reference standards were present in each booth with the samples. Panellists were asked to smell the standards individually in the order given in Table 4.2, and then rate the intensity of each aroma in the samples. Aroma intensities were rated on a ten-point scale, 0 to 9, where 0 = no attribute present and 9 = high, i.e. equal to the aroma of the standard. An example of the forms used in the descriptive analysis of the samples is given in Figure 4.2.

Table 4.1. General descriptions of wine and hydrolysate samples during early training sessions

Commercial Shiraz wine 1	Acid Hydrolysate*	Enzyme Hydrolysate*	Commercial Shiraz wine 2
Biscuit/cake	Bread	Butterscotch	Cocoa
Bready	Cardboard	Chocolate	Chocolate
Bread crumbs	Dusty	Jam	Coffee
Caramel	Caramel	Mint	Coffee beans
Chocolate	Chocolate	Animal	Honey
Coffee	Coffee	Dusty	Licorice
Dark chocolate	Honeysuckle	Cardboard	Menthol
Honey	Jammy	Cigar box	Port jelly
Jam	Licorice	Fishy/wet dog	Plum jam
Licorice bullets	Plum jam	Horsey	Dusty
Cheesy/sweaty	Soy sauce	Leather	Leather
Earthy	Tea	Toasty	Oak/wood
Hay/straw	Tobacco	Tobacco	Smokey
Smokey	Toasty	Tar	Toast
Tobacco	Blackberry	Berry	Tobacco
Cigar box	Berry essence	Blackberry	Blackberry
Berries	Citrus	Blackcurrant	Blackcurrant
Blackcurrant	Cassis	Dark cherries	Citrus
Blackberries	Cherry	Dried fruit	Cherry
Cherries	Dried fruit	Heated juice	Dried fruit
Cooked currants	Plum	Orange blossom	Orange blossom
Dried fruit	Raspberries	Olive	Port
Plum	Raisins	Plum	Plum
Port	Strawberry	Peach	Raspberry
Prune juice	Roses	Raisins	Raisins
Raspberry	Violets	Sherbert	Orange
Strawberry	Herbaceous	Strawberry jam	Floral
Strawberry jam	Hay	Floral	Rose
Wildberry	Stemmy	Rose	Violet
Cut grass	Anise	Violet	Herbaceous
Herbaceous	Black pepper	Anise	Stalky
Stalky	Cloves	Cloves	Black pepper
Allspice	Allspice	Pepper	Cinnamon
Anise	Spice/Cloves	Eucalyptus	Peppercorn
Black pepper	Mint		White pepper
Spicey	Liqueur		Estery
Asparagus			Green fruit
			Vine leaves

*Enzyme and acid hydrolysate extracts added at single strength to the base wine

Table 4.2. Descriptive attributes and composition of stock mixtures and reference standards.

Code	Attribute	Composition of stock mixture	[1]Portion used in reference standard
1	Honey/raisin	Raisins (10 g) were steeped in water (20 mL) for 48 h, combined with a 1:2 mixture of honey *(Adelaide Hills)* and water (20 mL)	2 mL
2	Berry	Blackberry jam *(Beerenberg*, 15 g*)*, blackcurrant jam *(Cottee's*, 15 g*)*, *Ribena* cordial (2 mL), blackberry juice *(John West*, 2 mL*)* added to 15% ethanol (23 mL)	5 mL
3	Strawberry	Strawberry jam *(homemade)*	4 g
4	Rose/violet	20 µg/L α-ionone ß-phenethanol (0.1 mL) in EtOH (20 mL)	0.1 mL 0.05 mL
5	Citrus	E30817 *VMane Fils* natural passionfruit flavour (0.1 ml) in EtOH (10 mL) plus 99.9% lemon juice *(Berri*, 0.2 mL)	1 mL
6	Spice	Whole cloves (20) steeped in EtOH (7.5 mL, 24 h) then liquor made up to 10 mL with EtOH Whole pimentos (10) steeped in EtOH (7 mL, 24 h) then liquor made up to 10 mL with EtOH	0.01 mL 0.2 mL
7	Pepper	Black peppercorns (10) crushed and steeped in EtOH (10 mL, 24 h) then ndiluted 1:1 with EtOH	0.2 mL
8	Stalky	Geranium stalks Freshly cut grass	1 g 200 mg
9	Earthy	Mushroom compost (1 g) in water (20 mL) Earth, bark	1 mL 500 mg
10	Cigar/tobacco	Short Panatella cigars *(Henry Winterman*, 700 mg*)* steeped in EtOH (1 mL) and water (20 mL) for 24 h Cigarette tobacco (500 mg) steeped in water (20 mL, 24 h)	4 mL 4 mL
11	Licorice	Licorice strand	4 g
12	Chocolate	Cooking chocolate *(Cadbury Bourneville*, 23.8 g*)* and cocoa *(Cadbury Bourneville*, 1 g*)* melted in water (40 mL) cooled to room temperature before use	2 mL

1. Portion of stock mixture made up in base wine (60 mL)

15.12.89 NAME:

SHIRAZ PROFILING PANEL

Please smell the standards. For each of the three coded Shiraz extracts/wines, swirl the glass, smell the aroma and rate the intensity of the aroma found in each sample. Any one descriptor will not necesarily apply to all of the samples. Please concentrate as these samples will be profiled in duplicate.

Remember: 0 = standard not present , 1 = low intensity ,
9 = high intensity, EQUAL TO THE STANDARD.

*PLEASE ASSESS THE SAMPLES IN THE ORDER GIVEN ON THE SHEET AND REST BETWEEN EACH SAMPLE TO AVOID FATIGUE.

	30	53	41
HONEY/RAISIN			
BERRY			
STRAWBERRY			
ROSE/VIOLET			
CITRUS			
SPICE			
PEPPER			
STALKY			
EARTHY			
CIGAR/TOBACCO			
LICORICE			
CHOCOLATE			

Figure 4.2 *An example of the form used for the sensory descriptive analysis of Shiraz hydrolysates and wines in this study.*

4.3.4 Statistical analyses

Statistical analyses were performed with NH Analytical software 'Statistix' (Roseville MN). The consulting services of Lindsay Veitch Inc. were employed.

No judges were removed from the panel, and the mean scores of the 14 assessors for each attribute and for each sample were used in all analyses. A design matrix was established to enable the data to be analysed as a single data set and to allow differences in quality between samples to be investigated. This design matrix was therefore constructed to include the main variables, i.e. year, y (1988 and 1989), region, r (Barossa Valley and Coonawarra), quality, q (high and low) and treatment, st 1 and st 2 (enzyme hydrolysis, acid hydrolysis and wine) and all possible interactions among them. Thus, the main effects were y, r, q, (st 1 and st 2) with degrees of freedom 1, 1, 1, and 2, respectively. Additionally, allowance was made for a possible carry-over effect, c, taking one degree of freedom. The first order interactions were y x r, y x st 1, r x q, (r x st 1 and r x st 2) and (q x st 1 and q x st 2). The term y x st 2 was omitted because it was linearly dependent on y, st 1 and y x st 1. Thus first order interactions used 1, 1, 1, 1, 2, and 2 degrees of freedom, respectively. Second order interactions were y x r x q, y x r x st 1, y x q x st 1 and (r x q x st 1 and r x q x st 2) with 1, 1, 1, and 2 degrees of freedom, respectively. The terms y x r x st 2 and y x q x st 2 were omitted because of their linear dependency on the other variables. Finally, for the third order interaction there was y x r x q x st 1 with one degree of freedom; y x r x q x st 2 was omitted because of linear dependence on the other variables. Hence, the forty observations provided 1, 5, 1, 8, 5, 1 and 19 degrees of freedom for the grand mean, main effects, possible carryover effect, first

order, second order and third order interactions and error, respectively. All significance tests used the error mean square on 19 degrees of freedom.

4.4 RESULTS AND DISCUSSION

For the purpose of comparing spiked samples and for use as a control, a neutral base wine exhibiting minimal background aroma was required. The base wine was chosen and prepared to provide a matrix as close to a red wine medium as possible.

4.4.1 Duo-trio data

The duo-trio difference test results reported in Table 4.3 demonstrate that for most samples the volatiles released from the bound fractions of Shiraz juices, either by enzyme- or acid-catalysed hydrolysis, conferred on the base wine sensory properties significantly different from those of the control. However, not all the samples could be distinguished in these tests. Most of those from Langhorne Creek were not distinguished from the control by the panel and as such these samples were excluded from further sensory analyses. Because of the almost uniformly positive difference responses given by the panel to the high and low quality hydrolysates from Barossa and Coonawarra juices, these samples were chosen for descriptive analysis. Although the 1989 low quality enzyme hydrolysis sample from Coonawarra was not significantly different from the base wine, it was included in the descriptive analyses to complete the data set.

Previous studies of the sensory properties of precursor hydrolysates from non-floral grapes have shown that only the acid hydrolysates could be detected when

Table 4.3. Results of the duo-trio difference tests for aroma of enzyme hydrolysates (EH) and acid hydrolysates (AH) compared to that of the base wine

Sample	Year	EH		AH	
		No. of correct responses	Sig	No. of correct responses	Sig
HB	1988	20/28	**	21/28	**
HB	1989	19/24	***	20/28	**
MB	1988			21/29	***
MB	1989	20/29	*	19/28	*
LB	1988	18/24	**	21/29	***
LB	1989	24/27	***	20/30	**
HC	1988	21/24	***	19/27	*
HC	1989	21/24	***	24/28	***
MC	1988	14/22	NS	19/25	**
MC	1989	21/27	***	17/29	NS
LC	1988	23/27	***	20/28	**
LC	1989	13/27	NS	19/28	*
HL	1988	22/24	***	17/30	NS
HL	1989	13/24	NS	16/26	NS
ML	1988	16/28	NS	20/25	***
LL	1988	22/27	***	10/25	NS
LL	1989	18/27	NS	17/30	NS

Sig = NS, *, **, *** indicate, no significant difference, significance at $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively
Mq Bar, sample not analysed
Refer to Chapter2: Table 2.1 for sample codes

these were back-added to a base wine (Williams, P.J. *et al.* 1989). Products given by hydrolysis of a glycosidic fraction from Chardonnay (Noble *et al.* 1987b, Williams, P.J. *et al.* 1989) and from Sauvignon Blanc and Semillon (Williams, P.J. *et al.* 1989) were not detectable when assessed at a concentration near to that at which the precursor glycosides occurred in the original wine. In contrast, most of the Shiraz samples examined gave both enzyme- and acid-catalysed hydrolysates with sensory properties detectable at single strength in a base

wine. As the previous investigations of the enzyme- and acid-catalysed hydrolysates involved only single samples for each variety this present finding for Shiraz suggests that examination of the enzyme hydrolysates from a wider range of samples of non-floral white varieties may result in the observation of positive duo-trio responses.

Duo-trio data in Table 4.4 confirm that the panel could distinguish between the volatiles released from a precursor fraction by hydrolysis with a glycosidase enzyme and those released by acid hydrolysis. Furthermore, the acid

Table 4.4. Results of duo-trio difference tests between two pairs of hydrolysates

Hydrolysate comparison	Samples	Sig
AH vs EH	HB 1988 and HB 1988	***
AH vs AH	HB 1988 and LB 1988	***

Sig = *** indicates significance at $p < 0.001$
AH = Acid hydrolysis product. EH = Enzyme hydrolysis produ
Refer to Chapter 2: Table 2.1 for sample codes

hydrolysates liberated from a high quality juice were perceived by the panel as being significantly different from those liberated from a low quality juice. The wines prepared from the high quality grape samples were significantly different from those prepared from the low quality grape samples for both the Barossa and Coonawarra regions (Table 4.5).

Table 4.5. Results of duo-trio difference tests for the aroma of high vs low quality wine samples from two regions

Region	Samples	Sig
Barossa	LB 1989 and HB 1989	**
Coonawarra	LC 1989 and HC 1989	***

Sig = **, *** indicate significance at $p < 0.01$ and at $p < 0.001$, respectively
Refer to Chapter 2: Table 2.1 for sample codes

4.4.2 Panel training

Thorough training of the panel is necessary to minimise the variability inherent in sensory descriptive analysis.

The aroma descriptors in Table 4.1 formed the basis for the development of a range of descriptive reference standards which were presented informally to the panel. The panellists were asked to evaluate these standards in terms of their relevance for describing the aroma of the samples. They were also asked to rate the suitability of the intensity of the samples. Wines and precursor hydrolysates were presented at a further twelve training sessions during which these descriptive standards were assessed and modified and further refined by consensus discussion amongst the panellists. Practice sessions were held to familiarise panellists with the use of a ten-point scale to rate the intensity of an aroma as they perceived it in the samples. Both spiked and 'real' samples were analysed during these sessions. Samples spiked with a known amount of each of the standards were presented to test the aroma acuity of the panel before the formal profiling sessions commenced.

4.4.3 Statistical analyses

The 1988 and 1989 samples were processed as one data set. The error structure for both years was expected to be the same even though there was an added treatment, i.e. wine in the second year. Consequently, the experimental design was non-orthogonal and the data was subjected to multiple regression analysis (MRA) rather than the usual analysis of variance (AOV). If AOV had been chosen as the method for statistical analysis, separate analyses would have been necessary for each of the two years, leading to difficulties in any subsequent comparisons. A further benefit of MRA was that an assessment of any carry-over effect was possible to determine if the intensity of the aroma of the wine samples biased the slightly less intense aroma of the hydrolysates. This carry over effect could not have been calculated using AOV.

Multiple regression analyses (MRA) were executed on the mean scores of the fourteen assessors for each attribute for the entire model. As a possible means of simplifying the interpretation of the data, principal component analyses (PCA) were run on the correlation matrix from the fitted values of all samples.

The first two principal components (PC1 and PC2) are described in Figure 4.3 and account for 91.2% of the total variance of the data. This principal component diagram demonstrates a separation of the hydrolysates from the wine samples along PC1 and a separation of the acid hydrolysates from the enzyme hydrolysates along PC2.

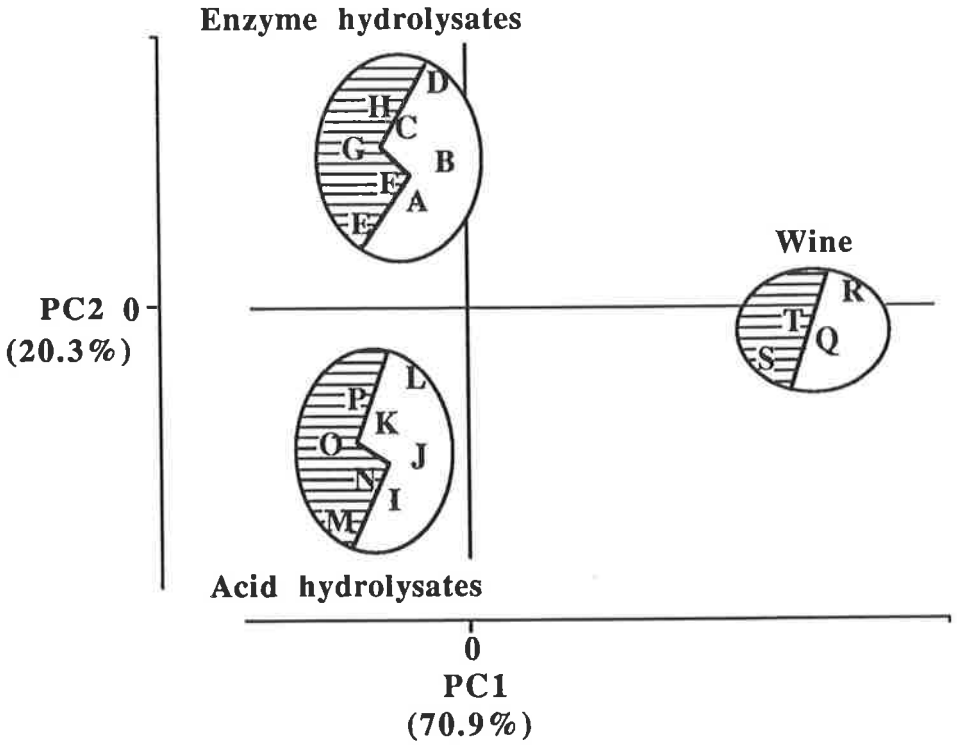


Figure 4.3 *Projection of sensory descriptive data for all samples (A to T) on principal components PC1 and PC2. The high quality samples are represented by the shaded portion.*

Codes referred to in Figure 4.3

Sample	1988 Barossa	1988 Coonawarra	1989 Barossa	1989 Coonawarra
Lq EH	A	B	C	D
Hq EH	E	F	G	H
Lq AH	I	J	K	L
Hq AH	M	N	O	P
Lq Wine			Q	R
Hq Wine			S	T

Lq EH, Hq EH = Low and high quality enzyme hydrolysis products respectively
Lq AH, Hq AH= Low and high quality acid hydrolysis products respectively

The eigenvectors calculated from the correlation matrix describe the relative positions and loadings of the twelve attributes as used by the panel and are illustrated in Figure 4.4. The vectors indicate how each attribute was used by the panel in the descriptive analysis over all the samples. The size of the vectors indicates that all the attributes were used by the panel to describe the aroma of the samples being tested. The vector diagram (Figure 4.4) corresponds to the PC diagram (Figure 4.3); superimposition of these two diagrams indicates which attributes were used by the panel to describe the aroma of the sample groups.

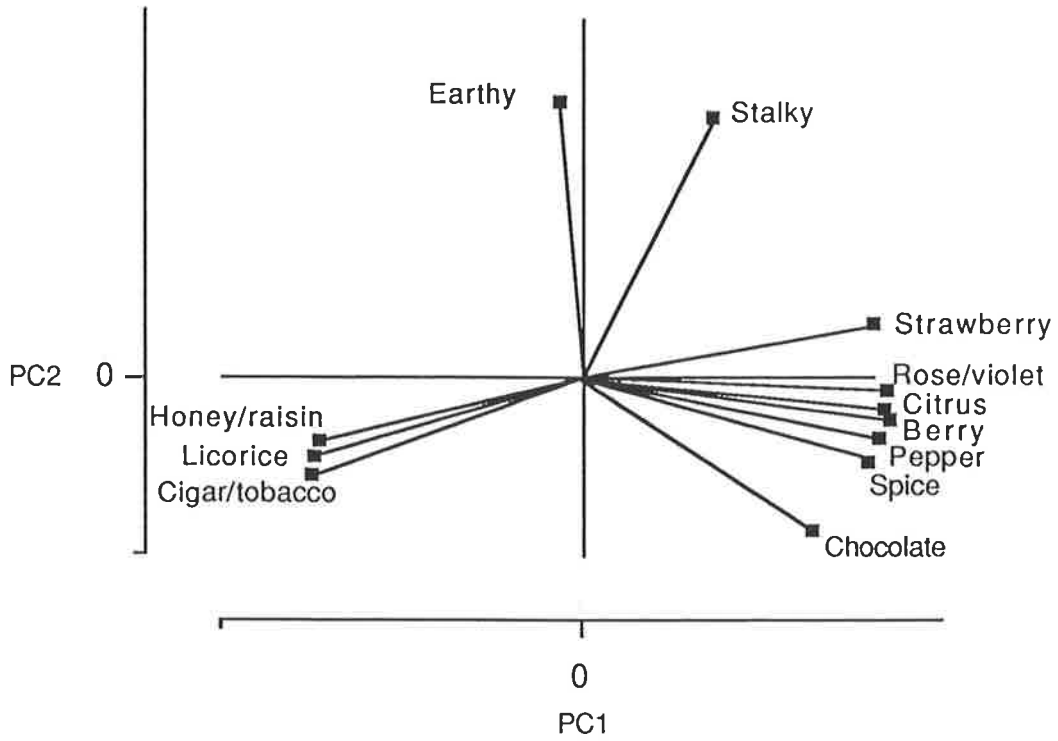


Figure 4.4 *Relative positions and loadings of the twelve attributes used in the descriptive analysis for samples A to T on principal components PC1 and PC2. Refer to Table 4.2 for description of attributes.*

The wine samples were separated from the juice hydrolysates along PC1 in terms of the attributes berry, strawberry, rose/violet, citrus and pepper which were collectively important for the aroma of the wines and the opposing earthy and cigar/tobacco attributes which were important for the hydrolysates. The separation of the hydrolysates along PC2 was accounted for mainly by variation of the attributes stalky and earthy. It is interesting to note that within both hydrolysate groups, and also within the grouped wine samples, the high quality samples are positioned on the left indicating that they are being displaced from the low quality samples by a common attribute or attributes.

4.4.4 Interactions of independent variables

The MRAs of the full model over the twelve attributes showed that many of the first and second order interactions were significant. To simplify the description of the data, it was decided to ignore any interactions which did not exhibit significance when analysed as an entire group, e.g. all first order interactions together, etc. To determine if the independent variables made a significant contribution to the overall model, F-ratios were calculated for each attribute and for each group over the 20 samples. These values are presented in Table 4.6 with their significant differences.

'Year' was a significant factor for five attributes and region for six attributes. This result was not unexpected: the two years experienced different weather patterns during grape ripening, and the two regions have different climate and soil types and also different canopy management regimes. 'Quality' was significantly different for seven of the twelve attributes studied indicating that

the panel was able to distinguish the high from the low quality samples. 'Treatment' was a significant variable for the majority of attributes over all the samples, the implication of which shall be discussed below. The first order interactions contributed significantly to the model for five of the attributes analysed - honey/raisin, strawberry, citrus, stalky and cigar/tobacco. There were no significant second order interactions and the third order interactions were barely significant for three attributes - berry, strawberry and stalky. The relative magnitudes of the F-ratios in Table 4.6 justify confining attention to the main variables and thus the significant first and third order interactions will not be discussed further.

Table 4.6. F-ratios of the significant independent variables for the twelve attributes over twenty samples with degrees of freedom (df) and significance

Attribute	Year	Region	Quality	Treatment	1st order	2nd order	3rd order
Honey/raisin	38.1 ***			15.5 ***	3.9 **		
Berry	12.5 **	6.4 *	13.1 **	8.0 **			5.7 **
Strawberry		42.0 ***	27.0 ***	10.8 ***	5.3 **		7.9 **
Rose/violet				7.9 **			
Citrus		98.0 ***	53.8 ***	26.8 ***	4.6 **		
Spice				19.6 ***			
Pepper	13.9 ***	4.5 *	26.5 ***	9.0 **			
Stalky		14.7 ***	22.8 ***	7.9 **	3.6 *		5.3 **
Earthy			13.1 **				
Cigar/tobacco	38.0 ***		71.9 ***		3.2 *		
Licorice	13.5 **			5.9 **			
Chocolate		21.2 ***					
df	1	1	1	2	8	5	1

*, **, *** indicate significance at $p < 0.05$, 0.01 and 0.001 respectively.
The residual mean square has 19 degrees of freedom

The source of the significance within each group of independent variables in Table 4.6 can be determined from the pooled means of the fitted values for the component parts of each year (1988 and 1989), region (Coonawarra and Barossa Valley), quality (high and low) and treatment (enzyme hydrolysates, acid

hydrolysates and wine) groups. The pooled means of the fitted values and their significant differences are presented in Table 4.7.

The results for the comparisons between the three treatments, i.e. enzyme hydrolysates, acid hydrolysates and wines over both regions and both years, show that the acid hydrolysates and the wines were significantly different for all

Table 4.7. Pooled means of fitted values for the significant main variables - year, region, juice and treatment- and the significant differences across the variables for the twelve aroma attributes

	Year			Region			Quality		
	1989 (n=24)	1988 (n=16)	Sig	Cw (n=20)	Bar (n=20)	Sig	Low (n=20)	High (n=20)	Sig
1 Honey/raisin	2.4	2.4	ns						
2 Berry	2.3	2.2	ns	2.3	2.2	ns	2.3	2.2	ns
3 Strawberry				1.9	1.6	**	1.9	1.7	*
4 Rose/violet									
5 Citrus				1.5	1.2	*	1.5	1.3	*
6 Spice									
7 Pepper	1.9	1.8	ns	1.9	1.8	ns	1.8	1.9	ns
8 Stalky				1.6	1.4	*	1.4	1.6	ns
9 Earthy							1.5	2.1	*
10 Cigar/tobacco	2.1	2.6	**				2.0	2.5	**
11 Licorice	1.4	1.2	*						
12 Chocolate				1.0	1.2	*			

	Treatment					
	Enzyme t1	Acid t2	Wine t3	Sig		
	(n=16)	(n=16)	(n=8)	t1/t2	t1/t3	t2/t3
1 Honey/raisin	2.3	2.7	1.8	**	**	***
2 Berry	2.1	2.2	2.8	ns	***	***
3 Strawberry	1.7	1.6	2.4	ns	***	***
4 Rose/violet	1.4	1.5	2.9	ns	***	***
5 Citrus	1.1	1.1	2.4	ns	***	***
6 Spice	1.0	1.1	1.9	ns	***	***
7 Pepper	1.7	1.8	2.3	ns	***	***
8 Stalky	1.8	1.3	1.6	***	ns	ns
9 Earthy						
10 Cigar/tobacco						
11 Licorice	1.1	1.3	1.7	**	***	**
12 Chocolate						

Cw = Coonawarra, Bar = Barossa.
Sig = ns,*, ** and *** indicate no significant difference, significance at $p < 0.1$, 0.01 and 0.001 respectively.

attributes. The enzyme hydrolysates and wines were significantly different for all attributes except stalky, and, although the enzyme- and acid-catalysed hydrolysates differed only for three attributes, honey/raisin, stalky and licorice, the level of significant difference in the three distinguishing attributes indicated that the hydrolysate types should be treated separately.

4.4.5 Differences among the samples

A two-sample t-test (O'Mahoney 1986) was used to determine the significance of the differences between individual samples (Table 4.8), hence allowing an investigation into the source of the differences shown in Table 4.7.

Table 4.8. Sensory attributes found to be significant for the comparison between high and low quality enzyme- and acid- hydrolysis products

Samples	Attributes
Enzyme hydrolysis products	
LB 1988 and HB 1988	Honey/raisin# , Pepper
LB 1989 and HB 1989	Stalky#, Earthy#
LC 1988 and HC 1988	Strawberry, Stalky#, Cigar/tobacco#
LC 1989 and HC 1989	Spicey#, Cigar/tobacco#
Acid hydrolysis products	
LB 1988 and HB 1988	NS
LB 1989 and HB 1989	Stalky
LC 1988 and HC 1988	Cigar/tobacco#
LC 1989 and HC 1989	Strawberry#, Pepper#

NS= No significant difference
# Attribute for which the high quality sample is more intense.
Refer to Chapter 2: Table 2.1 for sample identification codes.

A comparison of the high- and low-quality enzyme hydrolysates (Table 4.8), for the two regions and the two years, indicated that they were significantly different for at least one attribute for all samples except the high- and low-

quality Barossa Valley acid hydrolysates in 1988. Most of the differentiating attributes in both the enzyme- and acid-catalysed hydrolysis products were rated quantitatively higher for the high quality samples.

When the panel assessed the high- and low-quality wines, it found that only the Coonawarra pair were significantly different (Figure 4.5). The wine made from high quality Coonawarra fruit was rated higher for the non-berry-type attributes, i.e. stalky 8, earthy 9, cigar/tobacco 10 and licorice 11, while the low quality wine was rated higher in berry 2, strawberry 3 and citrus 5. The high and low quality Barossa wines (samples Q and S) could not be differentiated by descriptive analysis, even though they were found to be significantly different in the duo-trio tests (Table 4.5). Descriptive analysis data for the Barossa wines are therefore not shown.

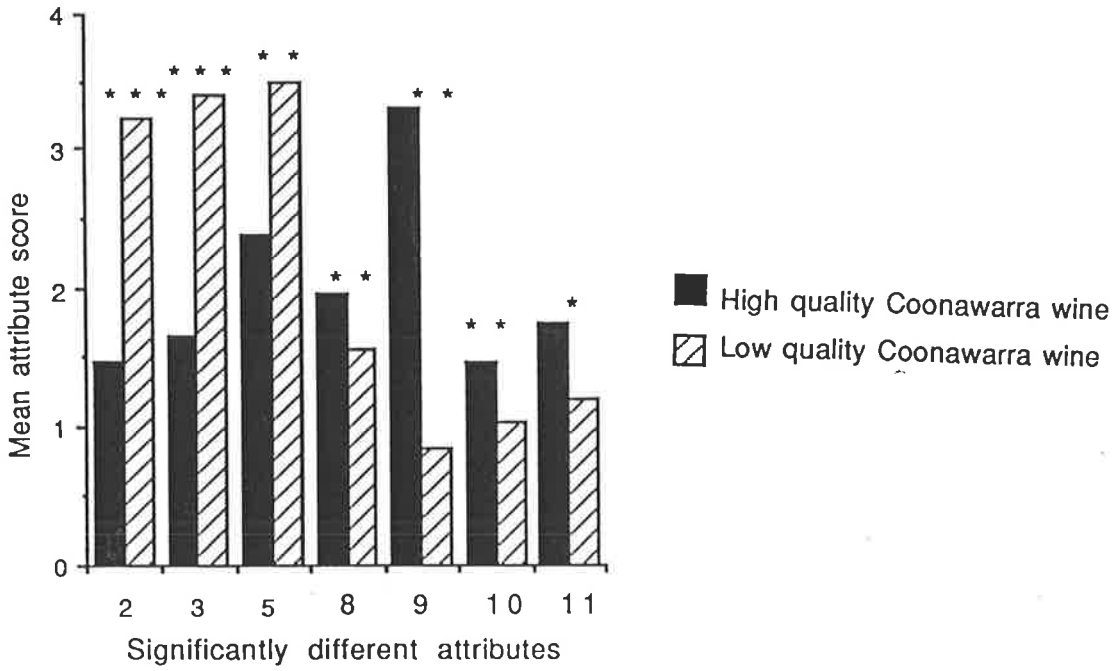


Figure 4.5 *Mean attribute scores for seven attributes showing significant differences between high- and low- quality Coonawarra wines (samples T and R). *, ** and *** indicate that differences are significant at p < 0.05, 0.01, 0.001, respectively. Refer to Table 4.2 for attribute codes.*

4.4.6 Relationships between sensory data for precursor hydrolysates and for wines

To focus on the effects of hydrolysate addition to the base wine, the mean attribute score of each of the hydrolysates was subtracted from the score of the corresponding base wine (Figure 4.6a and 4.6b). The base wine is represented in these Figures as zero on the y-axis. Histograms are shown for the attributes where either one or both of the hydrolysate pairs were significantly different from the base wine.

At least one of the attribute scores for the quality pairs of the acid hydrolysates, presented in Figure 4.6a, was rated significantly higher than the base wine for seven attributes- strawberry 3, rose/violet 4, spice 6, pepper 7, stalky 8, earthy 9 and cigar/tobacco 10. The high quality acid hydrolysates were given a higher mean score for strawberry, pepper and the quantitatively important attribute cigar/tobacco than that of their low quality counterparts.

For at least one of the enzyme hydrolysate quality pairs, five attributes were scored higher than that of the base wine (Figure 4.6b). These were rose/violet 4, citrus 5, stalky 8, earthy 9 and cigar/tobacco 10. The high and low quality enzyme hydrolysates were significantly different for the last four of these attributes. Of these, stalky, earthy and cigar/tobacco were scored significantly higher for the high quality samples. These aroma characteristics of the enzyme hydrolysates, and particularly the dominance of the non-berry-type attributes in the high quality samples were observable qualitatively in the PC and vector diagrams in Figures 4.3 and 4.4.

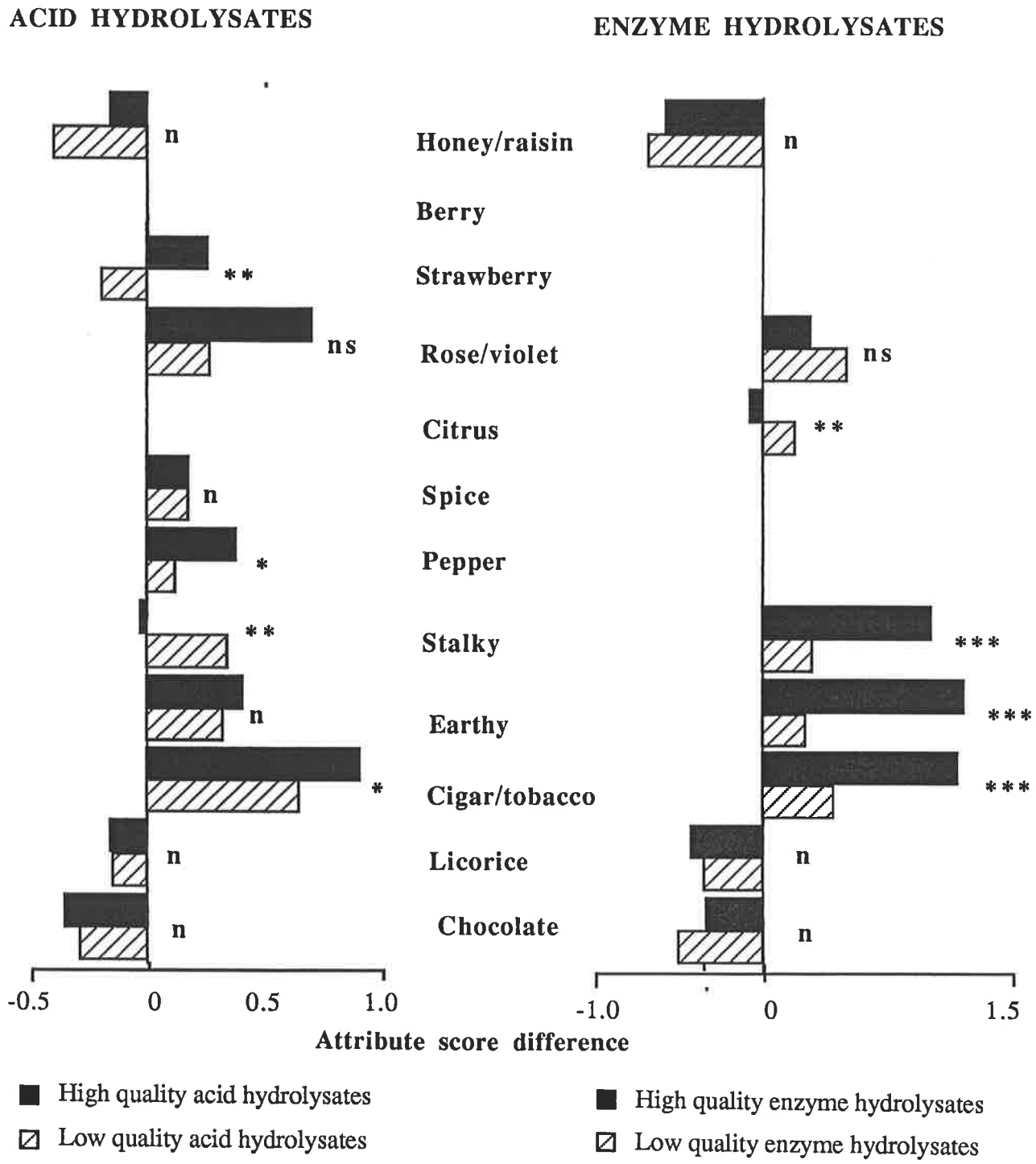


Figure 4.6 *Each histogram shows the difference between the mean attribute score for a hydrolysate and the corresponding base wine score, for those attributes in which either one or both mean scores differ significantly from zero i.e the base wine. Acid hydrolysates are shown in A and enzyme hydrolysates in B. ns, *, ** and *** indicate differences between the high and low quality hydrolysates are not significant, significant at $p < 0.05$, 0.01 and 0.001, respectively. Refer to Table 4.2 for attribute codes.*

Importantly, when the data in Figure 4.5 are interpreted in relation to those in Figures 4.6a and 4.6b it can be seen that the non-berry-type attributes, stalky, earthy and cigar/tobacco, which were quantitatively dominant in the high quality enzyme hydrolysates and, in the case of the last attribute, which was also dominant in the high quality acid hydrolysates, were the same as those characterising the high quality wine from Coonawarra. This common sensory feature of the high quality samples was also observable from the data in Table 4.6 which showed that cigar/tobacco and earthy were significant quality determinants across all treatments.

4.5 CONCLUSION

The data presented confirm that the quality differences previously assigned to the samples were distinguishable by sensory analysis, and, for one pair of wines and all but one pair of acid hydrolysates, such differences could be described and quantified. The major contributions made by both the enzyme- and acid-catalysed hydrolysates from Shiraz grapes to wines of this variety are non-berry-type sensory attributes such as 'stalky', 'earthy' and 'cigar/tobacco'. It is significant that the precursor hydrolysates from high quality grapes, particularly those prepared enzymatically, as well as the wine made from these grapes, were rated higher in non-berry attributes than the low quality samples. Accordingly, it may be deduced that the precursor hydrolysates contain aroma compounds, or a certain concentration or ratios of the same aroma compounds that are important to high quality wines of this variety.

These observations justify further studies into the chemical composition of the

glycosidically bound volatile compounds released by either enzyme- or acid-catalysed hydrolysis of extracts of Shiraz juice. It is evident that these studies should allow observation of quantitative, and possibly qualitative, differences that can be related to wine quality. It remains to be determined if the increase in non-berry sensory attributes, which are observable in Shiraz and other red wines during prolonged maturation, can be ascribed to hydrolysis and slow release of glycosidically-bound flavour compounds from the fruit.

Chapter 5

Free and glycosidically bound volatile compounds in Shiraz juice, skins and wine.

Chapter 5.

FREE AND GLYCOSIDICALLY BOUND VOLATILE COMPOUNDS IN SHIRAZ JUICE, SKINS AND WINE.

5.1 ABSTRACT

More than 200 compounds were observed as constituents of Shiraz juice, skins and wine. The free and glycosidically bound hydrolysates of Shiraz were particularly rich in substituted benzene derivatives and were also rich in norisoprenoids. This suggests that while the shikimic acid pathway is possibly dominant in Shiraz grapes, a large number of compounds are also formed as a result of the degradation of carotenoid pigments.

5.2 INTRODUCTION

The sensory descriptive analysis of acid- and enzyme-released products from high and low quality Shiraz grapes has supported the quality ratings assigned to these samples (*Chapter 4*). The same attributes were used to describe the aroma of both the high quality wine and juice hydrolysates suggesting that similar compounds were contributing to the aroma of each. A study of the volatile composition of Shiraz juice and wine samples was undertaken to determine if the differences observed by sensory descriptive analysis of high and low quality grapes were due to variation in the concentration of the same compounds or variation in the nature of the compounds present.

In order to gain an understanding of the distribution of the free and glycosidically bound compounds in the different parts of the berry, samples of Shiraz juice, skins and wine were analysed. The study was also designed to

investigate the extent of extraction of the glycosidically bound compounds from skins during vinification.

5.3 EXPERIMENTAL

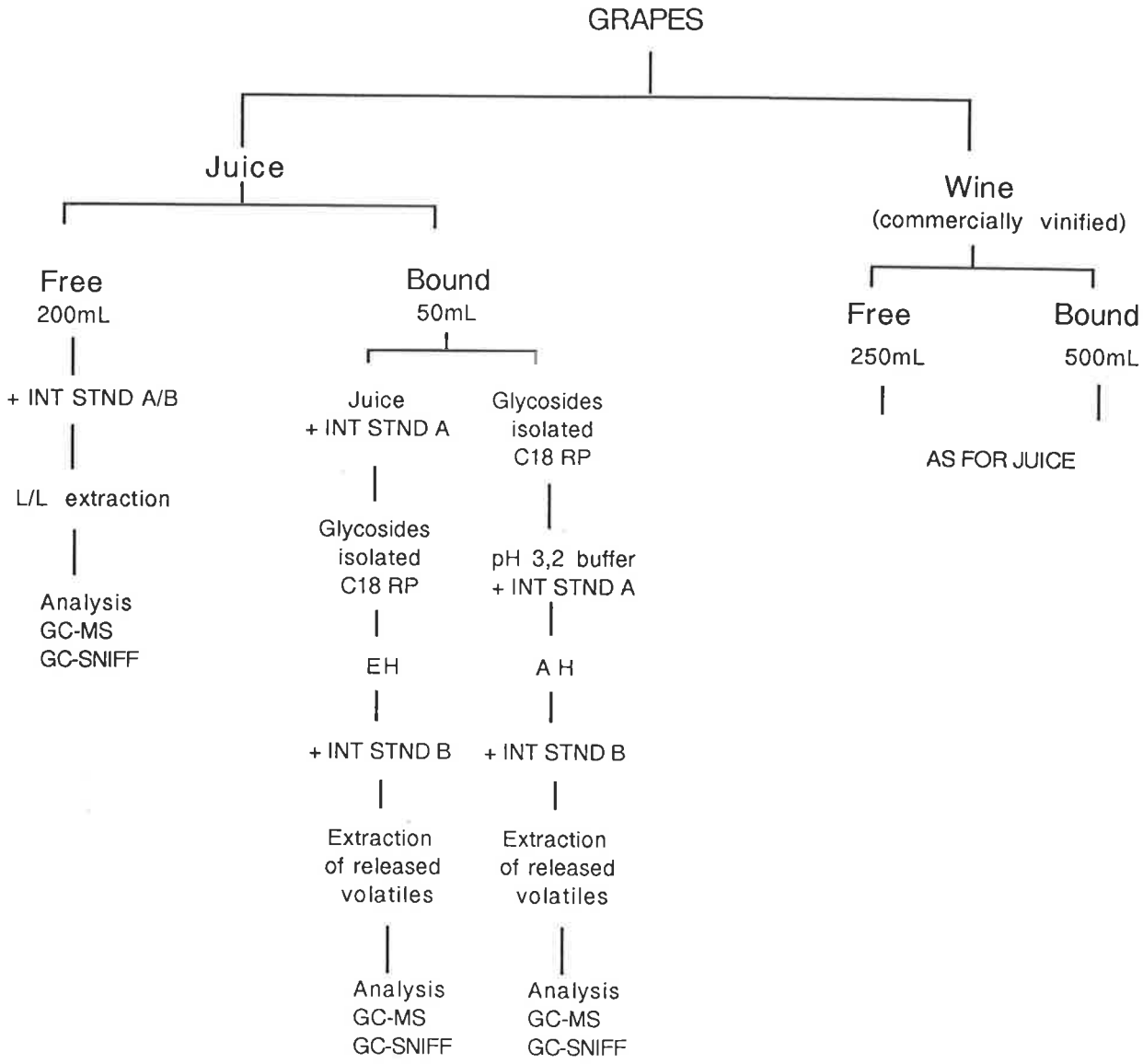
A summary of the methods described in this chapter for the analysis of the free and bound compounds present in Shiraz juice, skin and wine are presented in Fugure 5.1.

5.3.1 Chemicals and reagents

All chemicals and reagents used for the analysis of the composition of Shiraz juice, skin and wine fractions were the same as those previously described (*Section 2.3.1*).

5.3.2 Fruit and wine samples

The fruit and wine samples and the preparation of these samples were the same as those described in section 2.3.2 except that the juice samples were thawed at room temperature and centrifuged at 16 000 *g* for 25 min before the volatile and non-volatile aroma compounds were extracted. The enzyme hydrolysis products of the juice samples from the high and low quality Barossa Valley and Coonawarra vineyards in 1988, 1989 and 1990 were analysed by GC-MS. The acid hydrolysis products of juice samples from both regions in 1988 and 1989 were also analysed. The composition of the volatiles present in the skins of grapes from a medium quality Barossa Valley vineyard in 1988 (MB 1988), and the composition of wine samples made commercially from the high and low quality fruit of the 1989 vintage from the Barossa Valley and Coonawarra vineyards were also analysed.



A: n-octyl-β-D-glucopyranoside

B: butylbenzene

Figure 5.1 *Summary of the analytical procedure used to analyse the free and bound compounds present in Shiraz juice, skin and wine.*

5.3.3 Internal standards

5.3.3.1 Free volatiles

A stock solution containing two internal standards, n-octanol (1 mg/mL) and butylbenzene (1 mg/mL) was made. 20 µL of this stock solution was added to the juice and wine and 5 µL to the skin isolates before the free volatiles were isolated by continuous liquid/liquid extraction as previously described (*Section 2.4.2*).

5.3.3.2 Glycosidically bound volatiles

Aliquots of the stock solution of the standards (5 - 10 µL) were added to the juice or hydrolysis to produce a final concentration of 100 µg/mL. The stage at which the standards were added is decribed below and summarised in Figure 5.1.

5.3.3.2.1 Acid hydrolysis

Aliquots of the stock solution described in section 5.3.3.1 were added to the acid hydrolysates immediately prior to liquid/liquid extraction of the released volatiles.

5.3.3.2.2 Enzyme hydrolysis

For the quantification of the enzyme hydrolysis products, two separate stock solutions, one containing *n*-octyl-ß-D-glucopyranoside (1 mg/mL) and the other butylbenzene (1 mg/mL), were prepared. *n*-Octyl-ß-D-glucopyranoside (5 µL) was added to the juice or centrifuged skin homogenate prior to the isolation of the glycosides by C_{18} RP adsorption. To the wine C_{18} RP isolate in pH 5 buffer, *n*-octyl-ß-D-glucopyranoside (10 µL) was added prior to hydrolysis. An additional internal standard, butylbenzene (10 µL) was added to the hydrolysates from the

juice, skin and wine before continuous liquid/liquid extraction of the released volatiles.

5.3.4 Extraction of flavour compounds from juice

The free volatile fraction was isolated directly from Shiraz juice (200 mL); the glycosidically conjugated volatiles present in Shiraz juice (50 mL) were extracted using the *Sep-pak* cartridge technique as described in Chapter 2 except that internal standards were added as described above (*Section 5.3.3.1).*

5.3.5 Extraction of flavour compounds from skins

5.3.5.1 Free volatiles

Skins (20 g, equivalent to gave 50 mL juice) from medium quality Shiraz grapes from the 1988 vintage (MB) were homogenised in aqueous pH 3.2 buffer (150 mL) in a Waring blender for 90 sec. The suspension was left at room temperature for 90 min and then centrifuged to remove all solid material. The free volatile fraction was isolated directly from this extract by continuous liquid/liquid extraction as previously described in *Section 2.4.2* except that internal standards were added as described above *(Section 5.3.3.1).*

5.3.5.2 Glycosidically bound volatiles

Skins (20g) were homogenised and clarified as above *(Section 5.3.5.1)*. The clear supernatant was passed through a column of C_{18} RP adsorbent. The column was washed with water (200 mL) and the bound compounds eluted with methanol (200 mL). The methanol fraction was evaporated under vacuum to dryness and redissolved in water (20 mL). The redissolved C_{18} isolate was split into two

aliquots (10 mL). Internal standards were added as outlined above *(Sections 5.3.3.2)*. The hydrolysis procedure, isolation of the released volatiles and concentration of the solvent were all carried out as previously described (*Section 2.4.4.3)*.

In a second experiment skins (20 g) from the same vineyard were homogenised as above but in a solution made up of aqueous pH 3.2 buffer (100 mL) plus ethanol (15 mL). The suspension was held at 12°C for 17 h, brought to room temperature, centrifuged and the glycosidically bound material isolated and treated as described above.

5.3.6 Extraction of flavour compounds from wine

5.3.6.1 Free volatiles

Wine volatiles were collected according to a modification of the headspace procedure developed by Rapp and Knipser (1980) as outlined by Williams and Strauss (1978). The volatile compounds were extracted from Shiraz wine (200 mL) by passing a continuous flow of nitrogen through the wine and absorbing the entrained volatiles in Freon over a period of 20 h. The Freon headspace extract was concentrated by evaporation through Fenske's helices. Dichloromethane (2 mL) was added to the Freon concentrate to reduce the possibility of complete solvent evaporation, before storage at -20°C. The concentrate was further reduced to approximately 10 µL prior to analysis by GC-MS.

5.3.6.2 Glycosidically bound volatiles

Glycosidically bound aroma compounds were isolated from Shiraz wine (200 mL),

following the removal of the free volatiles using the headspace technique described above (*Section 5.3.6.1*). Glycosides were isolated by passing the sample, under gravity, through a column of C_{18} RP adsorbent. After washing the column with water (500 mL) the glycosidically bound volatiles were eluted with methanol (500 mL). The dried C_{18} RP isolate was treated in the same manner as the skin extracts described above.

5.3.7 GC-sniff assessment

The GC-sniff method employed was a modification of the gas-chromatographic sniffing method outlined by Christoph and Drawert (1985), with the column being connected alternatively to either the flame ionization detector (FID) or to the sniff cup, and not split as described by these authors. Each sample was assessed in duplicate by two people. The assessment was carried out by one person sniffing the effluent for 10 min and describing the names and the intensities of the aromas they perceived in the effluent. The other person recorded these details and the time of the elution of the compounds. After 10 min had elapsed, the two assessors swapped roles. The column was then connected to the FID and the chromatogram recorded before a replicate sniff run was made. Sniffing of the second run of the sample through the column was commenced half a minute earlier to ensure that no aroma active peaks had been missed during the cross-over times in the first run. The order in which the two people assessed the aroma effluent was also inverted.

5.3.8 Gas chromatography-mass spectrometry (GC-MS) analysis

Analysis of the volatiles isolated from the juice, skin and wine extracts by GC-MS proceeded as outlined in *Section 2.4.5*.

5.3.9 Quantification of free and glycosidically bound volatiles

The free and glycosidically bound volatile compounds identified by GC-MS were quantified using the Finnigan MAT quantification program with a standardised baseline for all samples. The calculated peak areas were then divided by the peak area of the two internal standards, *n*-octanol and butylbenzene, respectively. For the purpose of quantification, the response factor of the individual compounds against the internal standards for all compounds was assumed to be one.

5.4 RESULTS AND DISCUSSION

5.4.1 General remarks

The volatile secondary metabolites identified in *Vitis vinifera* Shiraz juice, skins and wine were classified into four major biogenic groups: benzene derivatives (which were mostly shikimic acid-derived metabolites, and mostly volatile phenols); norisoprenoids; monoterpenes; and aliphatic compounds, the latter being apparently derived from fatty acids. In addition to these four groups, several compounds of miscellaneous origin were also recorded.

The concentration of components identified in Shiraz juice, skin and wine extracts, as either free volatiles or volatiles released by enzyme- or acid-hydrolysis of the C_{18} RP isolate, are listed in Tables 5.1 to 5.5 under their respective biogenic groups (Table 5.1 Substituted Benzene Derivatives, Table 5.2 Norisoprenoids, Table 5.3 Monoterpenes, Table 5.4 Aliphatic Compounds and Table 5.5 Miscellaneous). Where the compounds from more than one sample were quantified, the maximum and minimum concentration are given.

Only one sample of the free volatiles, one acid hydrolysate and one aqueous ethanol extraction of the skins were analysed. The skin from the medium quality Barossa Valley fruit (MB 1988) was used for these analyses. Data for the acid-released products from the glycosidic fraction of one wine sample (HB 1989) were obtained. The data from these single analyses must be cautiously interpreted and can only be used to give an indication of the distribution of free and glycosidically bound volatiles in these Shiraz samples. Furthermore, strict comparisons cannot be made among the samples due to the different extraction techniques employed and their different coefficients of variation.

Sensory threshold data (µg/L) for the compounds for which published data exist have also been included in the tables. Evidence for the assignments has been given, together with references to compounds previously identified as grape products. The mass spectral data of the unknown compounds are given in these tables (Tables 5.1 to 5.5) in decreasing order of ion intensities. Furthermore, the Shiraz juice, skin and wine extracts contained many trace constituents that could not be confidently assigned to any of the above biogenic groups and as such have not been included in the tables.

The distribution of free and glycosidically bound volatiles in the juice, skin and wine extracts reflect the findings in the preliminary studies (*Chapter 2)*, i.e. the concentration of glycosidically bound volatiles was greater than that of the free volatiles in Shiraz berries.

Table 5.1. Substituted benzene-derived compounds identified in Shiraz juice, skin and wine extracts

No.[1]*	Substituted benzene derivatives	RT[2]	Sensory threshold data ug/L	medium	Ref	Concentration of volatiles (ug/L) JUICE EH[3] (n=24)	JUICE AH[4] (n=16)	JUICE F[5] (n=4)	SKINS EH 1[6] (n=2)	SKINS EH 2[7] (n=1)	SKINS AH (n=1)	SKINS F (n=1)	WINE HS (n=4)	WINE EH (n=4)	WINE AH (n=1)	Evidence[9]	Ref
4	Benzaldehyde	527	350	Water	e,d		2- 6										
			2000	Beer	v										62	D	hh
			6 - 500	Wine	af												
5	Phenylacetaldehyde	632	4	Water	e,d		<1 - 1										
			120	Wine	af											E	hh
6	Benzyl alcohol	764	159000	10% EtOH	q	46 - 66	3 - 13	42	309	277	+[8]	58	1- 2	92 - 404	138		
			620	Water	aa											D	hh
			100	Water/EtOH	p												
7	Phenol	789	7100	10% Spirit	o	1 - 2	0 - 1	1	28	69	[11]	1					
			5900	Water	s											A	hh
			30	Red Wine	ac												
8	Guaiacol	809	21	Water	w	0 - 2	0 - 5	<1		22	+	2					
			5 - 75	Red wine	b, ac											B,n	gg
			3	Water	e												
			3	10% Spirit	o												
9	o-Cresol	883	31	10% Spirit	o	tr											
			20 - 800	Red wine	b, ac											A	gg
			65	Water	s												
10	m-Cresol	892	68	10% Spirit	o	tr											
			5 - 380	Red wine	b, ac											A	
			85	Water	b												
11	2-Phenethanol	903	750	Water	y	19 - 64	1 - 22	21	350	423	+	247	17 - 44	57 - 926	116		
			20000	Wine	s											D	hh
			900	10% EtOH	q												
			86	Water	aa												
12	Methyl salicylate	956	71	10% EtOH	q	3 - 13	0 - 2		51	169		1					
			40	Water	e											A	dd
13	1,4-Dimethoxybenzene	1012				tr											
14	1-Methyl-2-ethylbenzene	1125					<1 - 3									B,n	
15	3-Phenylpropanol	1140				<1 - 4			8	8						E	
16	Benzoic acid	1157				tr	1 - 8	<1	50	69		1		11 - 164		D	qq
17	Ethyl salicylate	1223	115	10% EtOH	q		0 - 1									D	hh
18	cis-Cinnamic acid	1227					tr									E	
19	2,3-Dihydroxybenzoic acid methyl ester	1269				2 - 5									4	E	
20	4-Vinylguaiacol	1297	3	Water	d	6 - 24	0 - 7	3	14	79		<1		25 - 72		D	xx
			280, 800	Red wine	b, ac					41	+	11		38 -161		D	gg
21	4-Vinylphenol	1302	11	Water	aa	8 - 41	1 - 15	2	160	946	+	40				E	
			1500	Red wine	b									44 - 200	62	E	gg
22	Eugenol	1339	6	Water	e	<1 - 1										D	
			30	Water	y,aa						+	<1		17 - 221	49	D	gg
			11	10% EtOH	o												
			500, 2000	Red wine	b, ac											D	
23	Phenylacetic acid	1404				tr											
24	2-(Hydroxymethyl) benzoic acid	1539				tr	tr					12				D	hh
																E	

*Refer to page 142 for codes

Table 5.1. Substituted benzene-derived compounds identified in Shiraz juice, skin and wine extracts

No.[1]*	Substituted benzene derivatives	RT[2]	Sensory threshold data ug/L	medium	Ref	Concentration of volatiles (ug/L) JUICE EH[3] (n=24)	JUICE AH[4] (n=16)	JUICE F[5] (n=4)	SKINS EH 1[6] (n=2)	SKINS EH 2[7] (n=1)	SKINS AH (n=1)	SKINS F (n=1)	WINE HS (n=4)	WINE EH (n=4)	WINE AH (n=1)	Evidence[9]	Ref
25	Vanillin	1575	105	Water	b	0 - 6	1 - 13	6	15	"				14 - 63	18	D	xx
			65	Model wine	b												
			40	White wine	b												
			320	Red wine	b												
26	trans-Cinnamic acid	1632				tr	tr							12 - 28		A	hh
27	A dimethoxyphenol	1700	1850	Water	w	<1 - 1			65	22				45 - 30		E	
28	Methylvanillate	1716	790	Water	aa	7 - 11	<1 - 6		20	27	+	6		76 - 212	43	D	qq
29	Acetovanillone	1722	780	Water	aa	1 - 3	0 - 4		6			3		22 - 65	10	A	hh
30	4-Hydroxybenzaldehyde	1780	28	10% ETOH	q	1 - 3	<1 - 3	0	9						18	D	xx
31	Ethyl vanillate	1782				tr		1	134	164	+	5		235 - 539	110	E	
32	Tyrosol	1787				2 - 7			27		+	23		24 - 637	78	D	gg
33	Methoxytyrosol	1833				5 - 23	0 - 5	5		52	+	5				E	pp
34	4-Hydroxybenzoic acid methyl ester	1853					tr	2			+	1				A	dd
35	Butyrovanillone	1905				0 - 6	0 - 1		56	110	+	2		15 - 67	9	E	
36	2,5-Dihydroxybenzoic acid methyl ester	1920				0 - 8		2	10	6		0		29 - 96		D	xx
37	4-Hydroxybenzoicacid ethyl ester	1953					0 - <1		17	13	+	2			24	A	l
38	Ethylsyringyl ether	1978				tr										E	
39	Raspberry ketone	1985	<0.01	Water/EtOH	p	0 - 2								39		D	xx
40	Zingerone	2002				1 - 3										D	jj
41	Vanillic acid	2015	30	Water	r	10 -43	1 - 22	3	57	127	+	7		109 - 227	56	C	gg
			80000	Beer	v												
42	Dihydroconiferyl alcohol	2028				8 - 36		5	17	32	+	3		88 - 228		D	xx
43	Syringaldehyde	2065	50000	Water	v	0 - 4	3 - 19	0	45					71 - 184	35	D	xx
44	cis-Coniferyl alcohol	2070				tr										E	
45	4-Hydroxybenzoic acid	2107	40000	Water	r	0 - 1	0 - 6	0		6		3				C	hh
46	3-(4-Hydroxy-3-methoxyphenyl)-3-oxopropan-1-ol	2119				0 - 2		4	14	19				14 - 60	71	E	
47	2-(4-Hydroxy-3-methoxyphenyl)-2-oxoethanol	2112				0 - 1			16	21		1		<1 - 47	15	E	
48	Methyl syringate	2164				4 - 8	<1 - 1	2	30	57	+	5		74 - 298	59	E	xx
49	Acetosyringone	2185					0 - <1	1		14				43 - 58	15	B,m	xx
50	trans-Coniferyl alcohol	2201				14 - 67	0 - 6	1	33	23		0		33 - 384		D	xx
51	3-(4-Hydroxyphenyl)propanoic acid	2224				10 - 2								57 - 142		E	
52	Coniferaldehyde	2233				2 - 4	0 - 8		11					37		E	
53	Ethylsyringate	2252				1 - 5	0 - 4	4	73	89	+	2		204 - 891	142	E	
54	Propiosyringone	2287				0 - 7			11	15		1		23 - 73		A	hh
55	Butyrosyringone	2297				0 - 8	1 - 2		23	37		1				A	hh
56	cis -Ferulic acid	2297	660000	Beer	v	0 - 2	0 - 1									E	
57	2-(4-Hydroxy-3-methoxyphenyl)-2-oxoacetic acid	2316				0 - 1			34	61		2				E	
58	Tryptophanol	2299				0 - 2		0				79		309 - 776	52	A	gg
59	cis-4-Hydroxycinnamic acid	2385	40000	Water	r	4 - 11								21		E	
60	Syringic acid	2424				12 - 64	0 - 6		159	220				86 - 434	97	A	gg
61	trans-Ferulic acid	2501	90000	Water	r	<1 - 3	0 - 1									D	gg
62	2-(4-Hydroxy-3,5-dimethoxyphenyl)-2-oxoethanol	2517				0 - 1	tr		10	19						E	
63	trans-4-Hydroxycinnamic acid	2531	40000	Water	r	1 - 17	tr		30	70				53 - 233	47	D	gg

*Refer to page 142 for codes

Table 5.2. Norisoprenoids identified in Shiraz juice, skin and wine extracts

No.[1]*	Norisoprenoids	RT[2]	Sensory threshold data ug/L	media	Ref	Concentration of volatiles (ug/L) JUICE EH[3] (n=24)	JUICE AH[4] (n=16)	JUICE F[5] (n=4)	SKINS EH 1[6] (n=2)	SKINS EH 2[7] (n=1)	SKINS AH (n=1)	SKINS F (n=1)	WINE HS (n=4)	WINE EH (n=4)	WINE AH (n=1)	Evidence[9]	Ref
64	2,6,6-Trimethylcyclohex-2-en-1-one	658					3 - 15		II							B,m	xx
65	2,2,6,7-Tetramethylbicyclo[4.3.0]nona-4,7,9-triene	856					<1									B,f	f
66	3,5,5-Trimethylcyclohex-2-en-1-one	891					2									C	xx
67	2,6,6-Trimethylcyclohex-2-ene-1,4-dione	943				8 - 20	1 - 4							<1 - 33		D	mm
68	Vitispirane	1007				4 - 39	13 - 91		11	68	+[8]	2	1 - 2	25 - 70		D	jj
69	2,6,6-Trimethylcyclohexane-1,4-dione	1046				1 - 6										D	mm
70	Riesling acetal	1094					2 - 12									D	ab
71	1,1,6-Trimethyl-1,2-dihydronaphthalene	1186	20	Wine	kk		10 - 42				+		1 - 2	7 - 24		D	mm
72	Unknown: 157,142, 172	1296					1 - 7									D	
73	Damascenone	1326	0.009	Water	x	<1 - 5	6 - 19	1			+	1	1 - 3	<1 - 38	21	D	ff
			1600	Sweet wine	k												
			10	40% EtOH	u												
			500	Water	y												
			5 - 170		ad, ag												
74	Actinidol: isomer 1	1346				<1	17 - 58				+			10 - 50	27	D	oo
75	Actinidol: isomer 2	1354					24 -84				+			<1 - 17	47	D	oo
76	6-Hydroxy-6,7-dihydroedulan	1391				<1 - 3										E	
77	A dimethylnaphthalene	1418					1 - 4									E	
78	4-(Trimethylphenyl)-but-3-en-2-one: isomer 1	1425				<1	<1 - 2									B,w	xx
79	4-(Trimethylphenyl)-but-3-en-2-one: isomer 2	1460					1 - 4							<1 - 12	10	B,w	
80	Actinidol: isomer 3	1449					<1 - 4									D	mm
81	4-(Trimethylphenyl)-but-3-en-2-one : isomer 3	1476					<1 - 2								11	B,w	
82	Unknown: 69, 87, 99, 43, 127, 109, 43	1478				2 - 47			20			1		17 - 61			
83	8-Hydroxyedulan: isomer 1	1499					<1 - 7									B,zz	zz
84	Unknown:: 69, 83, 98, 43, 93, 109, 121, 136, 208	1505				21 - 47	5 - 10			3				50 - 218	14		
85	Actinidol: isomer 4	1511					tr									D	mm
86	Dehydro- -ionone	1543				<1 - 7	<1			13				20 - 43	69	B,w	
87	Unknown: 157, 132, 142, 172, 190, 147 : isomer 1	1552					<1				+						
88	8,9-Dehydrotheaspirone	1585				<1										D	ab
89	2-(3-Hydroxybut-1-enyl)-2,6,6-trimethylcylohex -3-en-1-one[10]	1593				<1 - 15	21 -77				+				59	D	mm
90	3-Hydroxytheaspirane : isomer 1	1601				8 - 16			19	18				37	66	D	ab
91	8-Hydroxyedulan: isomer 2	1605					<1 - 2									B,zz	zz
92	4-(Trimethylphenyl)-but-3-en-2-one : isomer 4	1610					1 - 8				+					B,w	
93	A trimethyl naphthalene	1610				<1 - 17	<1		15	65	+			16 - 72	23	E	
94	3-Hydroxytheaspirane : isomer 2	1619				5 - 15								30 - 63		D	ab
95	3-Hydroxytheaspirane : isomer 3	1646				3 - 9								21 - 48		D	ab
96	5,6-Epoxy-3-hydroxymegastigm-7-en-9-one	1664				<1 - 5										B,ss	
97	Unknown: 157, 132, 142, 172, 190, 147 : isomer 2	1745					<1 - 8										

*Refer to page 142 for codes

Table 5.2. Norisoprenoids identified in Shiraz juice, skin and wine extracts

No.[*]	Norisoprenoids	Sensory threshold data: RT[2]	ug/L	media	Ref	Concentration of volatiles (ug/L): JUICE EH[3] (n=24)	JUICE AH[4] (n=16)	JUICE F[5] (n=4)	SKINS EH 1[6] (n=2)	SKINS EH 2[7] (n=1)	SKINS AH (n=1)	SKINS F (n=1)	SKINS HS (n=4)	WINE EH (n=4)	WINE AH (n=1)	Evidence[9]	Ref
98	Unknown: 157, 132, 142, 172, 190, 147 : isomer 3	1759				<1 - 4	<1 - 10		tr					20			
99	Unknown: 157, 132, 142, 172, 190, 147 : isomer 4	1837					<1 - 1				+				22		
100	3,4-Dihydro-3-oxoactinidol : isomer1	1847				<1 - 8			25	120				94 - 279	77	D	*ab,qq*
101	trans-3-Oxo- -damascone	1850				4 - 11			24							D	*mm*
102	Megastigma-4,6,8-trien-3-one	1853				10 - 3										D	*xx*
103	3,4-Dihydro-3-oxoactinidol: isomer 2	1861				6 - 17			21	41				54	12	D	*ab,qq*
104	3,4-Dihydro-3-oxoactinidol: isomer 3	1885				<1 - 21			27	22				117	41	D	*ab,qq*
105	3-Hydroxy- -damascone	1916				1.5 - 10	3 - 58	1	20	15		2		30 - 228	83	D	*qq*
106	3,4-Dihydro-3-oxoactinidol: isomer 4	1919				<1 - 18				6				19 - 46		D	*ab,qq*
107	9-Hydroxymegastigma-5,7-dien-3-one	1930				1 - 6			11			<1				B,ab	
108	Megastigm-5-en-7-yne-3,9-diol	1942				4 - 16			21	14		<1		53 - 161	13	D	*mm*
109	3,4-Dihydro-3-hydroxyactinidol: isomer 1	1963				5 - 28	<1 - 2			12				216 - 785	157	E	
110	Megastigm-4-en-3,9-dione	1979				<1 - 7			22	17						E	
111	3,4-Dihydro-3-hydroxyactinidol: isomer 2	1985				tr										D	*ab*
112	9-Hydroxymegastigma-5,7-dien-4-one	1990				tr										B	*ab*
113	3-Oxo- -damascone	1995				48 - 120				62		<1		65 - 470		D	*mm*
114	3-Oxo- -ionol	2056				40 - 63	<1 - 4	tr	49	40		2		64 - 257	13	D	*qq*
115	9-Hydroxymegastigma-4,6-dien-3-one : isomer1	2064				<1										B,mm	*mm*
116	9-Hydroxy-megastigma-4,6,7-trien-3-one	2070				10 - 22										D	*mm*
117	3-Oxo- -ionone	2082				10 - 7	<1 - 3					<1			9	D	*mm*
118	Dehydrololiolode	2084				<1 - 1		tr	43	44		12		19 - 82		D	*mm*
119	9-Hydroxymegastigm-5-en-4-one	2123				4 - 27								<1 - 103		D	*ab*
120	9-Hydroxymegastigm-4-en-3-one	2142				5 - 12			8	24		<1		131 - 435	39	D	*mm*
121	9-Hydroxymegastigma-4,6-dien-3-one : isomer 2	2150				tr						1				D	*mm*
122	A trimethyl naphthalene	2181				0 - 19	1 - 10		51	60	+			36 - 103	23	E	
123	4,5-Dihydrovomifoliol	2315				0 - 29								99 - 407	65	D	*nn*
124	Grasshopper ketone	2343				<1 -119			68	70		1		84 - 516	65	D	*mm*
125	Unknown: 111, 126, 151, 170, 196, 208, 226	2369				8 - 25	<1- 2		15	19		<1		81 - 342	59		*ab*
126	Vomifoliol	2391				7 - 127	1 - 32	1	127	131		3		69 - 366		D	*qq*
127	Dehydrovomifoliol	2444				2 - 15	<1- 6	5	23			1		57 - 160		D	*qq*
128	Dihydrovomifoliol	2481				6 - 10			14	30				48 - 64		D	*mm*

*Refer to page 142 for codes

Table 5.3. Monoterpenes identified in Shiraz juice, skin and wine extracts

No.[1]*	Monoterpenes	RT[2]	Sensory threshold data ug/L	medium	Ref	Concentration of volatiles (ug/L) JUICE EH[3] (n=24)	JUICE AH[4] (n=16)	JUICE F[5] (n=4)	SKINS EH 1[6] (n=2)	SKINS EH 2[7] (n=1)	SKINS AH (n=1)	SKINS F (n=1)	WINE HS (n=4)	WINE EH (n=4)	WINE AH (n=1)	Evidence[9]	Ref
129	5-(2-Buten-2-yl)-2,2-dimethyltetrahydrofuran	553					1 - 3		"		+[8]					D	xx
130	Furan linalool oxide : isomer 1	665	6000	Sugar/water	uu	2 - 8	3 - 15		33	27	+	1		23 -181	32	D	ww
131	Furan linalool oxide : isomer 2	703	6000	Sugar/water	ee	7 - 29	3 - 13		30	84	+	5		43 - 472		D	ww
132	Hotrienol	780	110	Wine	//	2 - 6	2 - 7	1		11		4		9 - 18		D	ee,ww
133	Myrcenol	817					<1 - 5									D	ww
134	cis-Ociminol	882					2 - 6				+					D	ww
135	trans-Ociminol	917					2 - 10								78	D	ww
136	-Terpineol	947	280	Water	a	<1 - 1	1 - 4		9	17	+					D	ww
			350	Water	e												
			460	Sugar/water	uu												
			2000	Beer	v												
137	Pyran linalool oxide : isomer 1	959	4000	Sugar/water	uu	3 - 19	<1 - 1		24	51	+	1		<1 - 43		D	ww
138	Pyran linalool oxide : isomer 2	990	3000-5000	Sugar/water	ee	<1 - 1			51	51				<1 - 89		D	ww
139	p-Cymen-8-ol	993					<1 - 3									B,n	
140	Nerol	1031	50	Beer	v	<1 - 2			tr							D	ww
			400	Water	ee,uu												
141	(Z)-2,6-Dimethylocta-3,7-Diene-2,6-Diol	1076				<1 - 2	<1 - 2		16			<1				D	ss
142	(E)-2,6-Dimethylocta-3,7-Diene-2,6-Diol	1089				<1 - 3	<1 - 2		27			<1		<1 -49		D	ss
143	Geraniol	1090	75	Water	aa	12 - 32				54				24 -122		D	ww
			130	Sugar/water	ee,uu												
			10	Water	p												
144	2,6-Dimethyloct-7-ene-2,6-Diol	1161				1 - 3	7 - 24	10			+				78	D	r
145	1,8-Terpin	1316					3 - 24									D	ww
146	Geranic acid	1402				2 - 9		2		19				33 - 62		D	dd
147	(Z)-2,6-Dimethylocta-2,7-Diene-1,6-Diol	1450				15 - 41	<1 - 2		90	132				61	26	D	gg
148	(E)-2,6-DimetHylocta-2,7-Diene-1,6-Diol	1465				<1 - 8	<1 - 2		60					63- 429		D	

*Refer to page 142 for codes

Table 5.4. Aliphatic compounds identified in Shiraz juice, skin and wine extracts

No.[x]	Aliphatic compounds	RT[2]	Sensory threshold data ug/L	medium	Ref	Concentration of volatiles (ug/L) JUICE EH[3] (n=24)	JUICE AH[4] (n=16)	JUICE F[5] (n=4)	SKINS EH 1[6] (n=2)	SKINS EH 2[7] (n=1)	SKINS AH (n=1)	SKINS F (n=1)	WINE HS (n=4)	WINE EH (n=4)	WINE AH (n=1)	Evidence[9]	Ref
149	Butryic acid	250				[11]		2	22				48 - 129	<1 - 29	15	E	
150	Z-2-Hexenol	290	4000	Beer	*v*	0 - 3	tr			61			7 - 19	17 - 625	23	A	*gg*
151	E-3-Hexenol	343	70	Water	*e,c*	<1 - 8	tr						2 - 2	<1 - 25		A	*gg*
			13000	Beer	*v*												
152	Hexanol	354	4000	Wine	*cc*	10 - 59	0 - 12		16				4 - 10	<1 - 38	136	A	*gg*
			1080	Sweet wine	*h*												
			500	Water	*e*												
			4000	Beer	*v*												
153	E-2-Hexenol	401	17	Water	*c,j*	1 - 19	<1 - 4		55	<1						A	*gg*
			15000	Beer	*v*												
154	Pentanoic acid	684				1 -15	1 - 9	4	13	102		155	2 - 3	4 - 28		A	*gg*
155	Ethyl octanoate	807	20-32	Water	*cc,ff*	<1 - 2			65	99	+[8]	526	175 - 331		20	A	*gg*
			580	Sweet wine	*h*												
156	Hexanoic acid	871	700 - 2600	Red Wine	*af*	<1 - 5		<1	4	7		224		16 - 46	26	A	*gg*
157	4-Hydroxyhexanoic acid lactone	871						<1	16	43		81		1		A	*gg*
158	Heptanoic acid	964						<1						14 - 426		A	*gg*
159	4-Hydroxyheptanoic acid lactone	967	400	Water	*i*			1								A	*dd*
160	Ethyl nonanoate	1049	850	Sweet wine	*h*						+		1 - 3	9 - 115		E	
			1200	Beer	*v*												
161	Octanoic acid	1058	700 - 3700	Red Wine	*af*	1 - 6	3 - 14	4	16	28		211		18		A	*gg*
162	4-Hydroxyoctanoic acid lactone	1194	7	Water	*i*	<1 - 7			6		+			<1		A	
163	Nonanoic acid	1240				2 - 7	<1 - 13	1	14	11	+	54	<1 - 2	32 - 105		A	*gg*
164	Ethyl decanoate	1296	20-490	Water	*ff,ii*						+		91 - 236			A	*gg*
			510	Sweet wine	*h*												
165	4-Hydroxynonanoic acid lactone	1409	11	Water	*i*			1		tr				39	41	A	*gg*
166	Decanoic acid	1413	0 - 1600	Red Wine	*af*		<1 - 6	1		1				138 - 656	65	A	*gg*
167	4-Hydroxydecanoic acid lactone	1474				<1 - 2	<1 - 3	1		1		193		49 - 87	14	A	*gg*
168	Dodecanoic acid	1744				<1 - 3	2 - 8	3		31				<1 - 28	18	A	*gg*
169	Tridecanoic acid	1944						1								A	*gg*
170	Tetradecanoic acid	2059				tr	tr	2							17	A	*gg*
171	Linoleic acid	2594					<1 - 1	1					3 - 4	59 - 76	14	A	*gg*

*Refer to page 142 for codes

Table 5.5. Miscellaneous compounds identified in Shiraz juice, skin and wine extracts

No.[1]	Miscellaneous compounds	Sensory threshold data: RT[2]	ug/L	medium	Ref	Concentration of volatiles (ug/L): JUICE EH[3] (n=24)	JUICE AH[4] (n=16)	SKINS F[5] (n=4)	SKINS EH 1[6] (n=2)	SKINS EH 2[7] (n=1)	SKINS AH (n=1)	SKINS F (n=1)	WINE HS (n=4)	WINE EH (n=4)	WINE AH (n=1)	Evidence[9]	Ref
172	Furfural	347	tr-20000	Red wine	b, af	[11]	0 - 3									E	g
			150000	Beer	t												
			3000	Water	e												
173	Maltol	895					0 - 1									A	
174	2-Ethyl-3-methylmaleic anhydride	982					0 - 4									B,m	
175	2-Ethyl-3-methylmaleimide	1242				0 - 1	0 - 12	8				3				B,m	
176	2-Methyl-3-vinylmaleimide	1311				0 - 3	0 - 10	tr								B,m	
177	2-Azacycloheptanone	1317				tr	0 - 2				+[8]			18 - 42		B,m	
178	5-(2-Hydroxyethyl)-4-methylthiazole	1333				0 - 1		2								A	
179	5-(Hydroxymethyl)furfural	1375				1 - 3	0 - 41								13	E	
180	2-Ethylidene-3-methylsuccinimide	1531														B,m	

[1]Compound numbers referred to in text of this Chapter, [2]RT, Retention time (seconds), for GC conditions see Experimental, [3]EH, volatiles released by hydrolysis with *Rohapect C*, [4]AH, volatiles released by hydrolysis at pH 3.2, 50[0]C for 28 days, [5]F Free, unconjugated volatiles, [6]EH1, volatiles released by hydrolysis of a skin extract with *Rohapect C*, [7]EH2, volatiles released by hydrolysis of skin glycosides, (extracted from the skins in the presence of ethanol), with *Rohapect C* [8]+, consituent observed in this fraction, quantitative data not determined, [8]HS, constituents observed in the headspace analysis of wine samples. [9]Evidence; A, GC retention time and mass spectrum same as reference sample; B, mass spectrum same as published spectrum; C, mass spectrum same as reference compound; D, previously proven in laboratory; E, interpretation of mass spectrum, tentative assignment, [10]Carotenoid numbering, [11]No given data signifies that the compound was either not identified or quantified in the sample. [a]Ahmed *et al.* 1978, [b]Boidron *et al.* 1988, [c]Buttery *et al.* 1969, [d]Buttery *et al.* 1976, [e]Buttery *et al.* 1971, [f]Buttery *et al.* 1990, [g]Chatonnet *et al.* 1989, [h]deWet 1978, [i]Engel *et al.* 1988, [j]Eriksson *et al.* 1976, [k]Etievant *et al.* 1983, [l]Güntert *et al.* 1986, [m]Heller and Milne 1978, [n]Jennings and Shibamoto 1980, [o]Jounela-Erikson and Lehtohen 1981, [p]Larsen and Poll 1990, [q]Lindeman *et al.* 1982, [r]Maga 1973, [s]Maga 1978, [t]Marais 1983, [u]Masuda *et al.* 1980, [v]Meilgaard 1975, [w]Mikami *et al.* 1981, [x]Ohloff 1978a, [y]Ohloff 1978b, [z]Pabst *et al.* 1991, [aa]Pyysalo *et al.* 1977, [bb]Rankine and Pocock 1969, [cc]Rankine *et al.* 1969, [dd]Rapp 1988a, [ee]Ribéreau-Gayon, P. *et al.* 1975, [ff]Rothe *et al.* 1972, [gg]Schreier 1979, [hh]Schreier 1976, [ii]Siek *et al.* 1971, [jj]Simpson *et al.* 1977, [kk]Simpson 1978 , [ll]Simpson 1979, [mm]Sefton *et al.* 1989, [nn]Sefton *et al.* 1991 , [oo]Strauss *et al.* 1986a, [pp]Strauss *et al.* 1986b, [qq]Strauss *et al.* 1987a, [rr]Strauss *et al.* 1987b, [ss]Strauss *et al.* 1988, [tt]Takagi *et al.* 1978, [uu]Terrier *et al.* 1972, [vv]Wasserman 1966, [ww]Williams, P.J. *et al.* 1980, [xx]Williams, P.J. *et al.* 1982a, [yy]Williams, P.J. *et al.* 1989, [zz]Winter *et al.* 1979, [ab]Winterhalter *et al.* 1990, [ac] Etievant 1981, [ad] Etievant *et al.* 1983, [ae]Etievant *et al.*1989, [af]Maarse and Visscher 1989, [ag] Simpson and Miller 1984.

5.4.1.1 Free volatiles

Most of the free volatile compounds identified in Shiraz berries were either substituted benzene derivatives (up to 67% of the free volatiles in the juice extract) or aliphatic compounds which accounted for 65% of the free volatile composition of the skin extract (Table 5.6). The paucity of grape-derived compounds identified in the wine headspace, both in number and concentration, was due to the fact that the secondary metabolites observed in the juice and skins were obscured by co-eluting fermentation volatiles in the wine. Fermentation compounds, such as aliphatic alcohols and esters that were identified in the Shiraz wine extracts analysed in this study (data not given), have been previously identified in wine headspace fractions of this variety (Vernin *et al.* 1988). Since these constituents are common to wines made from all grape varieties, and not particular to Shiraz, they were neither quantified nor reported here.

5.4.1.2 Glycosidically bound volatiles

The majority of the glycosidically bound volatiles isolated from Shiraz juice, skin and wine extracts were from two main biogenic classes- substituted benzene derivatives and norisoprenoids (Tables 5.1 and 5.2, respectively). Both the number and concentration of aglycones identified in these classes were greater than those identified in the other three biogenic groups (Table 5.6). The wine, followed by the skins, was the richest source of glycosides of the three Shiraz components studied. The juice contained the lowest concentration of glycoconjugates (Table 5.6). The concentration of the enzyme-released products identified in the wine samples can be approximately accounted for by adding the

Table 5.6 Average total concentration (ug/L) of free and released volatiles in Shiraz juice, skin and wine extracts

	EH	%	AH	%	F	%
Juice						
Norisoprenoids	318	*43*	203	*48*	8	*5*
Substituted benzene derivatives	297	*40*	106	*25*	105	*67*
Monoterpenes	95	*13*	85	*20*	13	*8*
Aliphatic compounds	25	*3*	0	*0*	22	*14*
Miscellaneous	7	*1*	29	*7*	10	*6*
Total	743		423		158	

	EH 1	%	EH 2	%	F	%
Skins						
Norisoprenoids	542	*19*	897	*18*	28	*1*
Substituted benzene derivatives	1823	*65*	3307	*66*	670	*30*
Monoterpenes	315	*11*	445	*9*	10	*<0*
Aliphatic compounds	124	*4*	382	*8*	1444	*65*
Miscellaneous	0	*0*	0	*0*	81	*4*
Total	2804		5031		2233	

	EH	%	AH	%	HS	%
Wine						
Norisoprenoids	2020	*32*	1027	*35*	3	*14*
Substituted benzene derivatives	3365	*53*	1276	*43*	17	*83*
Monoterpenes	474	*8*	213	*7*	0	*0*
Aliphatic compounds	104	*2*	389	*13*	1	*5*
Miscellaneous	328	*5*	64	*2*	0	*0*
Total	6290		2969		21	

concentration of the enzyme-released products in the juice to the concentration of the enzyme-released products in the aqueous ethanolic skin extract. The concentration of norisoprenoid glycosides in the wine, however, was higher than this simple mass balance would suggest. Although the concentration of glycosidically bound volatile compounds extracted when the skins were macerated in an aqueous ethanolic solution was greater than the concentration of these compounds extracted in buffer only, neither of these model skin extraction techniques used for the study of the skin glycosides were as rigorous as would occur during vinification, where a higher fermentation temperature and prolonged skin contact time would promote the extensive extraction of compounds from grape skins. The extraction of a greater quantity of skin glycosides in the presence of alcohol is possibly due to the cell walls within the skins rupturing more readily in this medium and thus releasing the glycosides into the wine during fermentation.

The conditions under which the aqueous ethanolic skin extraction were conducted (15% alc vol/vol, 12 °C 17 h) were apparently sufficient to extract most of the substituted benzene derivatives from the skin, as determined by the concentration of these compounds in the wine. In another experiment the skins were macerated in buffer (pH 3.2) and stored for one week at room temperature. Under these conditions the skins had begun to ferment before the glycosidically bound volatile compounds were isolated. The concentration of the norisoprenoids identified after enzyme-hydrolysis of this extract were an order of magnitude higher (data not shown) than the data in Table 5.2, supporting the hypothesis that more rigorous conditions are required to extract these compounds from the

skin of Shiraz berries. Vinification conditions could therefore be manipulated to extract a lesser or greater concentration of particular glycosidic compounds from the skins.

5.4.2 GC-sniff analysis

The odour comments recorded during the GC-sniff assessment of the free volatiles and the acid- and enzyme-released products are listed above the relevant areas of each chromatogram (Figure 5.2a, b and c). Aromas were perceived continuously during each sample run through the GC column. The range of adjectives used to describe these volatile extracts demonstrates the complexity of the aroma of Shiraz berries. Some of these descriptors are clearly of relevance to the varietal characteristics of Shiraz, e.g. spice, currant and fruity, whilst others such as cigar box, caramel and wood, indicate a relationship between natural grape aroma and aroma attributed to oak. Many of the descriptors used to describe these aroma extracts correspond to the attributes selected by the panel for the sensory descriptive analysis of the high and low quality Shiraz juice and wine samples (*Chapter 4, Table 4.2*).

The aroma profile of the free volatiles and the acid- and enzyme-released products differed from each other, although several similar terms were used for describing the aroma effluent of each chromatogram. The most frequently used

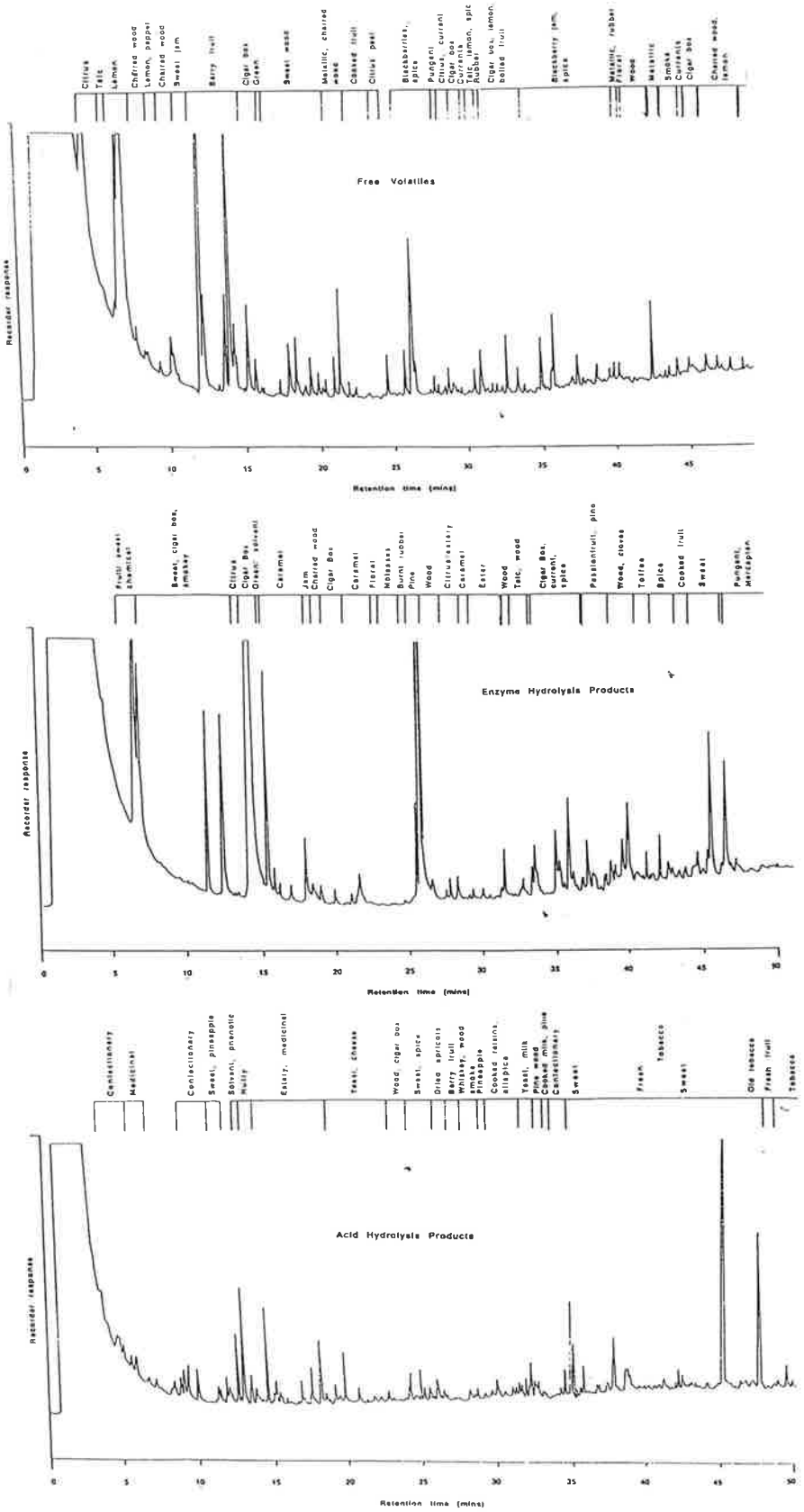


Figure 5.2 *GC- sniff assessment of the a) free volatile extract, b) enzyme-released products and c) acid-released products from a medium quality Barossa Valley juice sample.*

aroma terms used for describing the free volatiles were citrus, currants and cigar box. Both the acid- and enzyme-hydrolysis products had an aroma more complex than that of the free volatiles. Caramel, spice and cigar box were the most frequent aroma terms used to describe the enzyme-released products, whereas tobacco, estery and pimento were used to describe the acid-released products. The aroma of this latter extract provides some indication and understanding of the flavour characteristics that would be expected upon maturation of a Shiraz wine made from these grapes.

5.4.3 Secondary metabolites of Shiraz juice, skin and wine components

The structures of the free and glycosidically bound volatile compounds observed in Shiraz juice, skin and wine extracts are presented in Figures 5.3 to 5.6 at the end of this chapter.

5.4.3.1 Substituted benzene derivatives (Table 5.1, Figure 5.3)

This group of secondary metabolites were found in the highest concentration and were the most numerous volatiles observed as enzyme- and acid-released compounds in the wine extracts and as enzyme-released compounds from the skins (Table 5.6). The substituted benzene derivatives were also present at a high concentration in the juice extracts, accounting for 40% of the volatile compounds released by enzyme hydrolysis and 25% of those released by acid hydrolysis. The aroma threshold value of many of these volatile phenols is known (Table 5.1), and it is evident that most are present at a concentration below that of their sensory threshold. Nevertheless, sensory studies have indicated that the additive aroma characteristics of these compounds make an important contribution to the aroma

of red wine (Etiévant 1981).

The major benzene derivatives identified in extracts of *Vitis vinifera* Shiraz were benzyl alcohol **(6)**, ß-phenethanol **(11)**, 4-vinylphenol **(21)**, vanillic acid **(41)** and syringic acid **(60)**, which were present at a high concentration in all extracts studied. The concentration of a second group of benzene derivatives including benzoic acid **(16)**, ethyl vanillate **(31)**, butyrovanillone **(35)** and ethylsyringate **(53)**, was high in the enzyme-released products of the skin extracts and in both the acid- and enzyme-released products of the wine extracts. *trans*-Coniferyl alcohol **(50)** and 4-vinylguaiacol **(20)** were abundant in the enzyme-released juice products. Of those compounds for which sensory threshold data in wine are available, i.e. compounds **(11)**, **(20)** and **(21)**, none was present at a concentration above its threshold in the three fractions studied.

Raspberry ketone **(39)** was the only substituted benzene derivative present in Shiraz grapes at a concentration above its sensory threshold. The concentration of this compound was also significantly high in the wine extracts and may contribute to the berry aroma often associated with Shiraz grapes and wines. Raspberry ketone may also have been contributing to the berry aroma identified by the panel during the sensory descriptive analysis of the acid- and enzyme-hydrolysates and wine samples (*Section 4.4.5)*, where the berry attribute was found to be significantly high for the low quality hydrolysates and wines.

5.4.3.2 Norisoprenoids (Table 5.2, Figure 5.4)

The norisoprenoids were the most abundant secondary metabolites in the juice

samples and accounted for 43% of the volatile compounds released by enzyme hydrolysis and 48% of those released by acid hydrolysis. They were also present in a high concentration in the wine and accounted for approximately 30% for both acid- and enzyme-released products.

The majority of the norisoprenoids identified as constituents of Shiraz were 13 carbon compounds. These compounds accounted for 96% of the total concentration of norisoprenoids present in the Shiraz extracts. This dominance supports the hypothesis that these compounds are generated from site-specific enzyme catabolism of the carotenoids present in this variety. There are six ketones which can be formed from the primary cleavage at the 9,10 and 9',10' double bonds of the grape carotenoids. These are 3-hydroxy-ß-ionone, grasshopper ketone, α-ionone, 5,6-epoxy-3-hydroxymegastigm-7-en-9-one, ß-ionone and 3-hydroxy-α-ionone. Of these six ketones, only 3-hydroxy-α-ionone has not been identified as a natural grape product. Sequential oxidation and reduction of the volatiles formed from these initial parent ketones leads to the formation of a large range of volatile compounds with varying structures. The structure of these volatiles cannot be linked by simple degradation reactions or acid-catalysed rearrangements, supporting the contention that the norisoprenoids observed in *Vitis vinifera* are genuine metabolites of the fruit (Sefton *et al.* 1989, Williams, P.J. *et al.* 1991).

Several of the norisoprenoids that have been identified in the enzyme hydrolysates of Shiraz juice, skin and wine may be artefacts and not natural grape metabolites of Shiraz. The first set of possible artefacts may arise from

extraneous acid hydrolysis of the aglycones, presumably during the isolation of the volatiles by liquid/liquid extraction in dichloromethane. Model reactions in aqueous acid of the megastigmatriols, megastigm-5,7-diene-3,4,9-triol, megastigm-4-ene-3,6,9-triol and megastigm-4,7-diene-3,6,9-triol, have demonstrated that several products can be generated from these compounds (Dimitriadis *et al.* 1985, Strauss *et al.* 1986a). Of the volatiles so derived, vitispirane **(68)**, actinidol: isomer 1 **(74)** and 2-(3-hydroxybut-1-enyl)-2,6,6-trimethylcyclohex-3-en-1-one **(89)** have been identified as both enzyme and acid released products of Shiraz juice C_{18} RP isolates. Although the exact biogenetic degradation of the carotenoids in Shiraz grapes is not known, it cannot be assumed that the aglycones **(68)**, **(74)** and **(89)** observed as enzyme hydrolysis products are present in Shiraz juice as glycoconjugates.

A second set of compounds has been recently identified as artefacts arising from the use of a high concentration of fungal enzyme for the complete hydrolysis of the glycosides. A high concentration of *Rohapect C* is now known to generate oxidative transformation products from some aglycones (Sefton and Williams 1991). Of the aglycones identified in Shiraz juice, skin and wine extracts, 3-oxo-ß-damascone **(101)**, 3-oxo-α-damascone **(113)** and 9-hydroxymegastigma-4,6,7-trien-3-one **(116)** could be regarded as oxidative artefacts of 3-hydroxy-ß-damascone and megastigm-5-en-7-yne-3,9-diol (Sefton and Williams 1991). Most other aglycones identified as enzyme hydrolysis products in the Shiraz components studied can be regarded as genuine metabolites of the fruit.

Although the flavour properties of many norisoprenoids are known, the sensory threshold values of only 1,1,6-trimethyl-1,2-dihydronaphthalene **(71)** and damascenone **(73)** have been determined. Both of these compounds are present in the extracts studied at a concentration above their known threshold values and, as such, may contribute to the aroma of Shiraz grapes and wines. The high concentration of most of the norisoprenoids identified in the Shiraz skin and wine samples suggests that this group of compounds may contribute significantly to the aroma of the resultant wine. These compounds may be responsible for the significantly high intensity of the cigar/tobacco attribute identified by the panel as an important aroma attribute of high quality Shiraz hydrolysates and wine (*Section 4.4.4.6*).

The megastigmane C_{13} norisoprenoids were predominantly functionalised at C3 (megastigmane numbering) with two exceptions: 9-hydroxymegastigma-5,7-dien-4-one **(112)** and 9-hydroxymegastigm-5-en-4-one **(119)**.

The only C_{11} norisoprenoid present in Shiraz, dehydrololiolide **(118)**, was identified on the basis of published spectra and work previously completed in the laboratory (Sefton *et al.* 1989). This compound is known to be rapidly formed by oxidation of 3,4-dihydro-3-oxoactinidol (of which four isomers have been observed, **(100)**, **(103)**, **(104)** and **(106)** in these extracts), at room temperature, in the presence of air (Uegaki *et al.* 1979) and as such its presence should be treated with caution.

The four C_9 norisoprenoids identified in this study are all known constituents of tobacco (Enzell 1981, 1985) and grapes (Williams P.J. *et al.* 1982b, Sefton *et al.* 1989). The trimethylcyclohex-2-en-1-ones **(64)** and **(66)** and 2,6,6-trimethylcyclohexane-1,4-dione **(69)** were identified as constituents of Shiraz juice, whereas 2,6,6-trimethylcyclohex-2-ene-1,4-dione **(67)** was only identified in wine. The cyclohexenone **(66)** has been postulated as a derivative of ß-damascol **(A)**, via the hydroperoxide **(B)** (Figure 5.7) (Schulte-Elte *et al.* 1971). 3,5,5-Cyclohexenone **(66)** and the

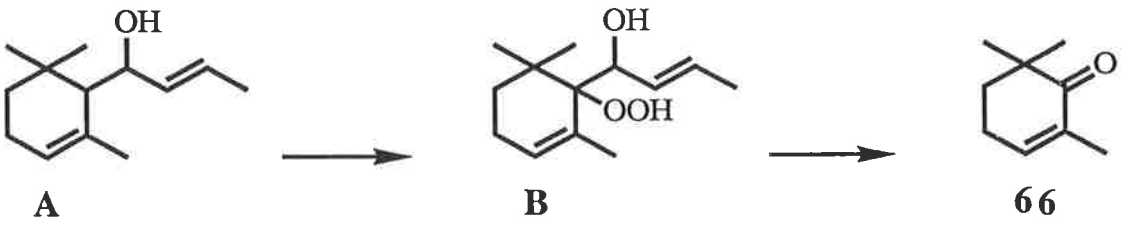


Figure 5.7 *Formation of 3,5,5-cyclohehenone (Schulte-Elte et al. 1971).*

diones **(67)** and **(69)** have been observed as acid-catalysed degradation products of 3-oxo-α-ionol **(114)** (Strauss *et al.* 1987a). However, only minor amounts of **(66)**, **(67)** and **(69)** were formed at pH 1 from the degradation of 3-oxo-α-ionol **(114)**, and the acid hydrolysates in this study were liberated at pH 3.2 and all extractions were conducted in a mildly acidic medium. 2,2,6-Trimethylcyclohex-2-ene-1,4-dione **(67)** has been previously identified in saffron and appears to be the most potent aroma compound of the C_9 norisoprenoids identified to date (Enzell 1985). The aroma of this compound has been described as `dried leaves or straw-like' and the compound has been used to improve the flavour of infusions and fermented drinks (Ohloff 1978c). However, no data on the threshold value of this

compound are available.

5.4.3.3 Monoterpenes (Table 5.3, Figure 5.5)

The level of monoterpenes identified in Shiraz accounted for only 0-20% of the total volatile composition depending upon the sample studied (Table 5.6) and supports the assignment of Shiraz as a non-floral variety. The monoterpenes were present at concentrations below that of their known sensory threshold for all identified components, except geraniol **(143)** which was detected at a concentration above that of its threshold in the enzyme-released products of the skin extract. As was observed for the norisoprenoid group, the concentration of monoterpenes is higher in their conjugated form than as free volatiles.

5.4.3.4 Aliphatic and miscellaneous compounds (Table 5.4 and Table 5.5, Figure 5.6)

The concentration of aliphatic compounds found in Shiraz juice, skins and wines was less than that of any one of the three classes of compounds described above for all extracts except the free volatiles of the skins and juice, and the acid hydrolysates of the wine extracts (Table 5.6). Of the compounds identified in this class only ethyl octanoate **(155)** and ethyl decanoate **(164)** were present above threshold. Most of the aliphatic compounds observed in Shiraz have been reported as constituents of other grape varieties (Schreier *et al.* 1976a), and, although this group of compounds may contribute to the general vinous aroma of Shiraz wines, their importance for characterising the aroma of this variety appears limited.

The C_5 to C_9 γ-lactones identified in these extracts may contribute markedly to the aroma of Shiraz wines as many of these compounds have known potent flavour properties (Ohloff 1978b).

Furfural **(172)**, which has a caramel-like flavour, forms during the aging of wine via carbohydrate degradation (Rapp 1988). Other miscellaneous compounds identified in Shiraz, such as 2-ethyl-3-methylmaleic anhydride **(174)** and 2-ethyl-3-methylmaleimide **(175)**, have previously been identified in tobacco (Wahlberg *et al.* 1987). 2-Ethyl-3-methylmaleic anhydride **(174)** was identified as an acid hydrolysis product of Shiraz juice, and 2-ethyl-3-methylmaleimide **(175)** was found predominantly in the free fraction, with smaller amounts observed in the enzyme- and acid- hydrolysis products. 2-Methyl-3-vinylmaleimide **(176)** was also identified as an enzyme- and acid-released product in Shiraz and in trace quantities in the free fraction.

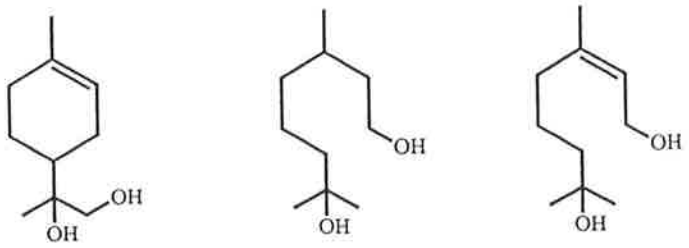
The aroma of 2-ethyl-3-methylmaleic anhydride **(174)** has been described as leathery (Hasagawa 1983), an attribute often used to described aged Shiraz wines. This compound has also been identified as a trace component of elderberry juice where its aroma was described as 'meaty' (Poll and Lewis 1986). The maleimide **(175)** has been previously identified in cigarette smoke (Schumacher *et al.* 1977), tobacco essence and essential oil (Lloyd *et al.* 1976) and has been described as sweet, adding body and a flue-cured note to the extract (Lloyd *et al.* 1976). The other maleimide identified in Shiraz grapes, **(176)**, has a sweet, light, caramel aroma (Lloyd *et al.* 1976).

5.5 CONCLUSION

Substituted benzene derivatives, as free volatiles in the juice extracts and both free and glycosidically bound volatiles in the skin and wine, were numerous and were present at a concentration higher than that of the compounds from the other biogenic groups. Although most of these compounds were present below their individual sensory threshold levels, they may be important aroma constituents for *Vitis vinifera* Shiraz due to their additive and synergistic properties.

The norisoprenoid glycosides accounted for the second most abundant group of flavour compounds in the skin and wine samples and were the most abundant compounds identified in the juice. Many norisoprenoids are important flavourants in nature and in industry and have been reported as glycoside conjugates in many other fruits, *e.g.* apricot, plum, peach, raspberries and tomatoes (for references see *Chapter 1, Table 1.2)* and as such may contribute to the aroma of wines made from this variety.

The paucity of the free volatiles and the identification of potent flavour compounds amongst the acid- and enzyme-released volatile products suggests that the key to understanding the differences in aroma of different grape varieties may be found not in the unconjugated volatiles but in the potential flavour compounds glycosidically bound in the juice and skin of the berry.



1 2 3

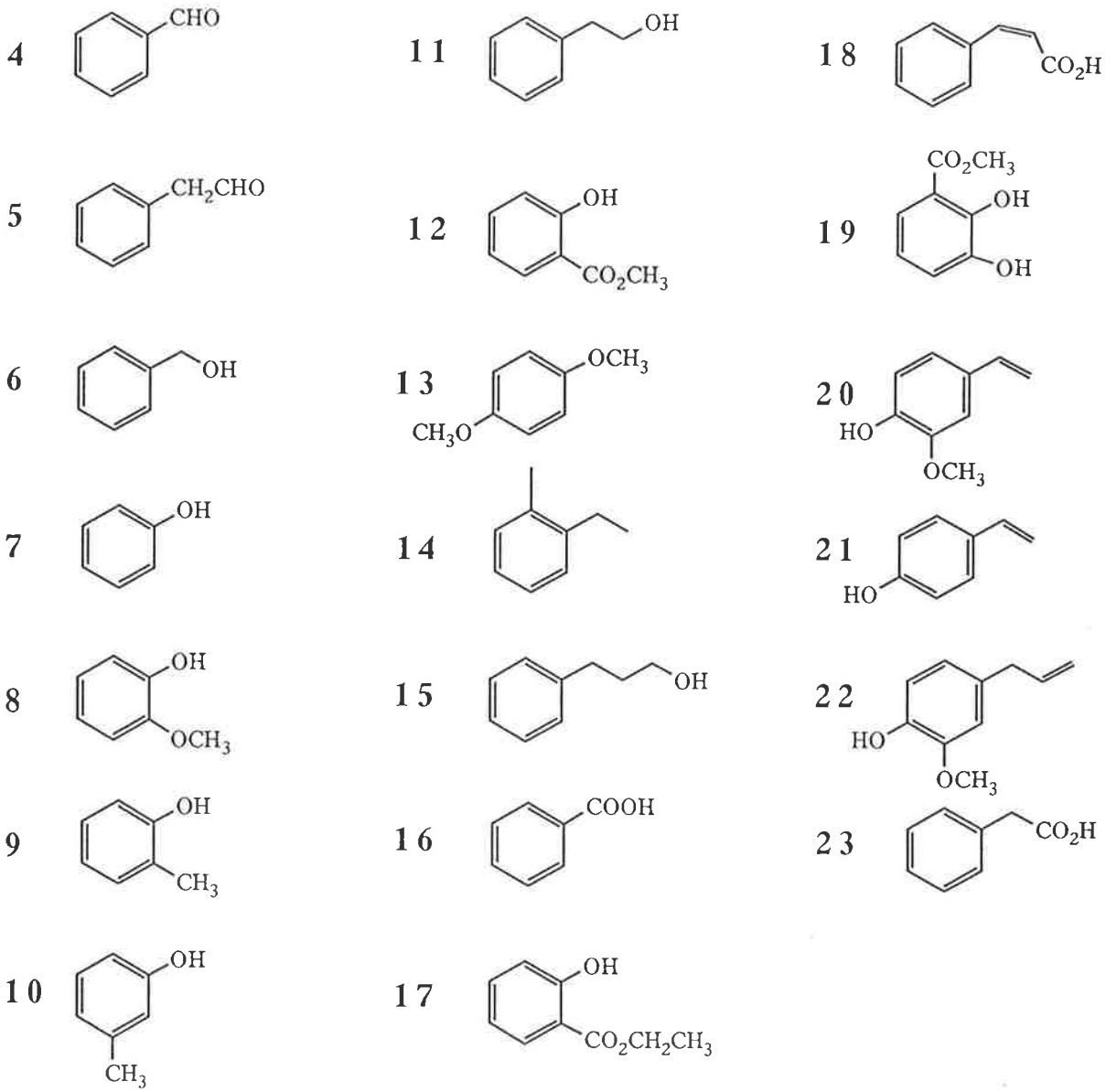


Figure 5.3 *Substituted benzene derivatives identified in Shiraz juice, skin and wine extracts. The numbers refer to the compounds in Table 5.1.*

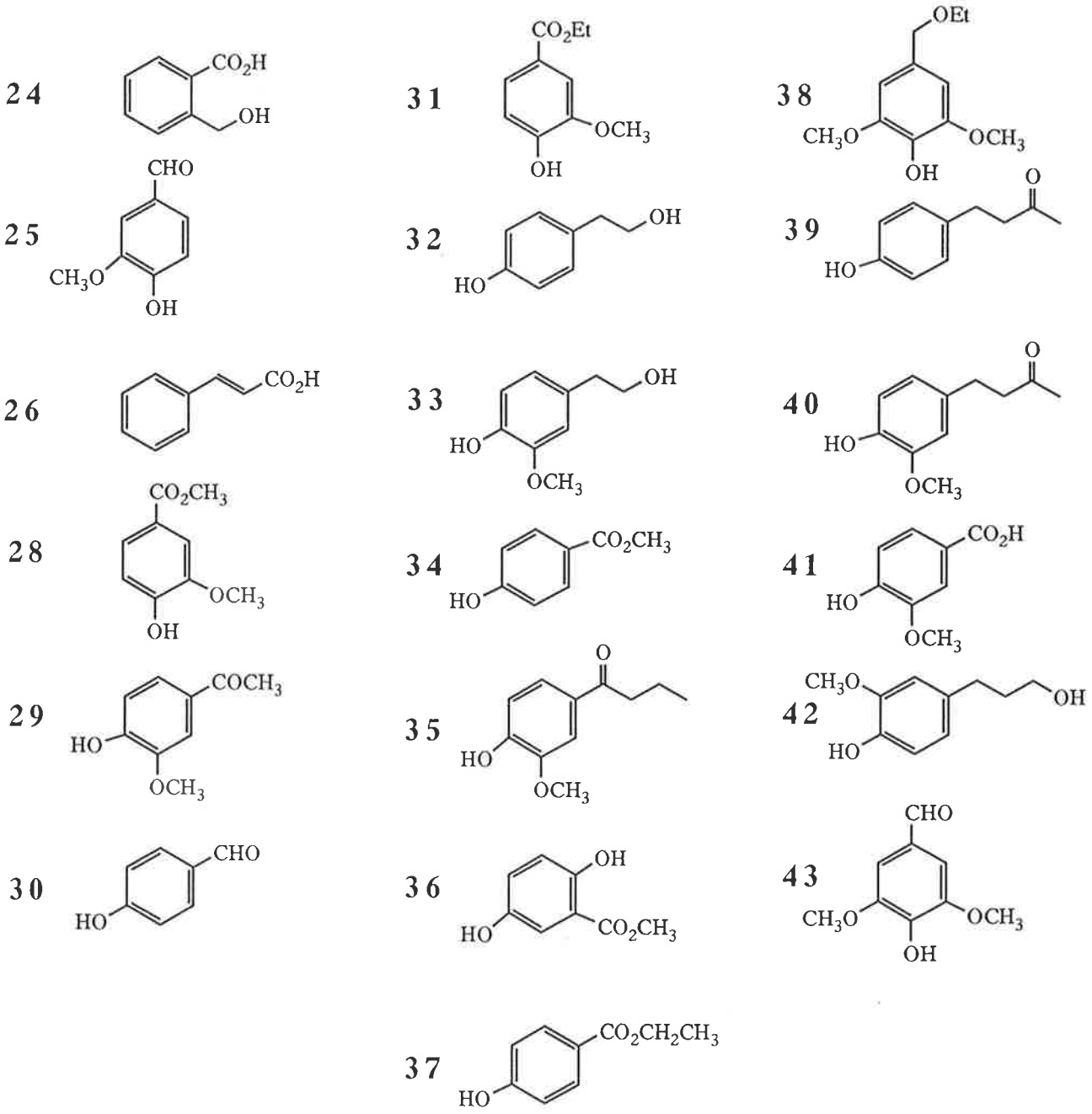


Figure 5.3 continued

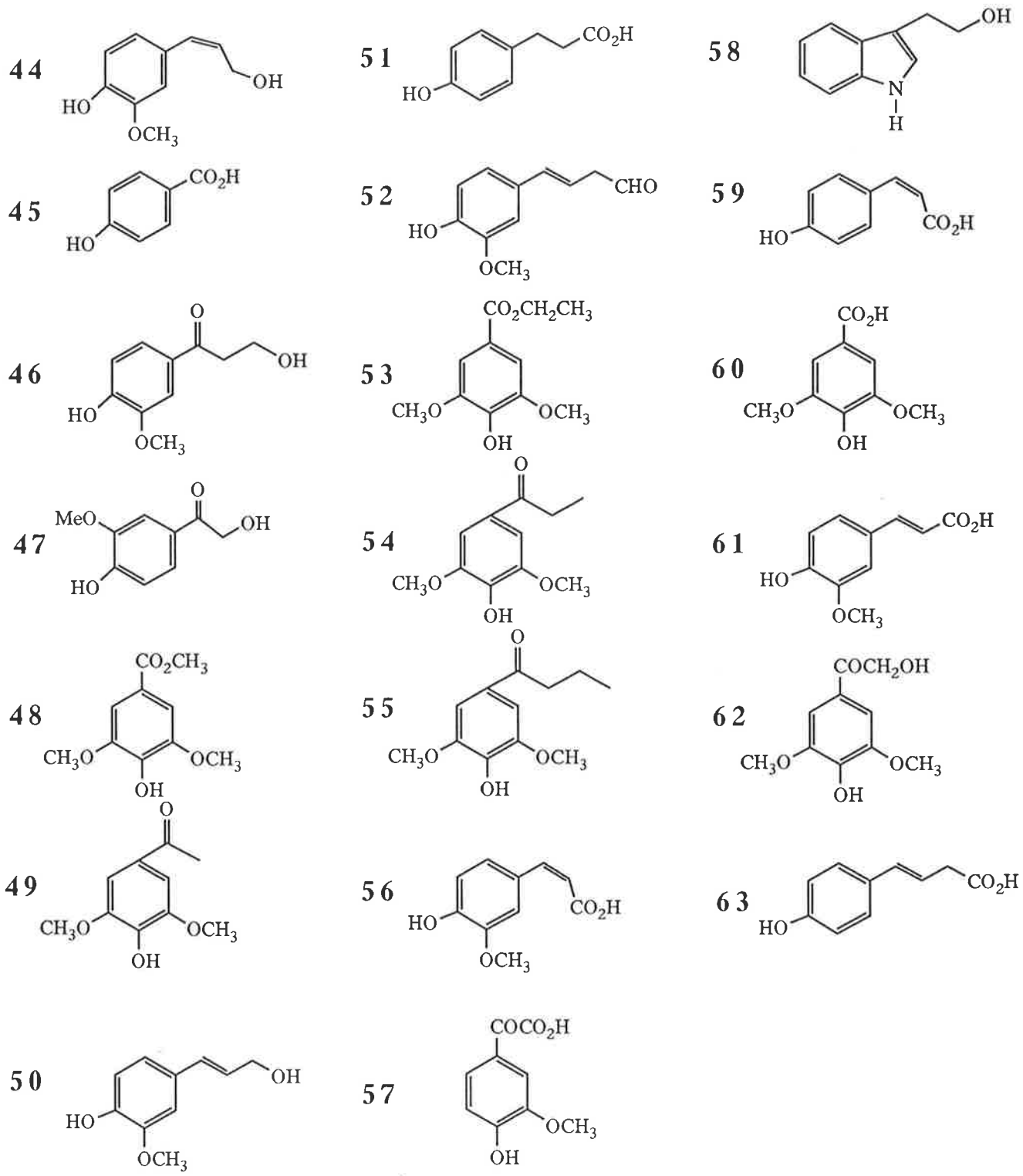


Figure 5.3 continued

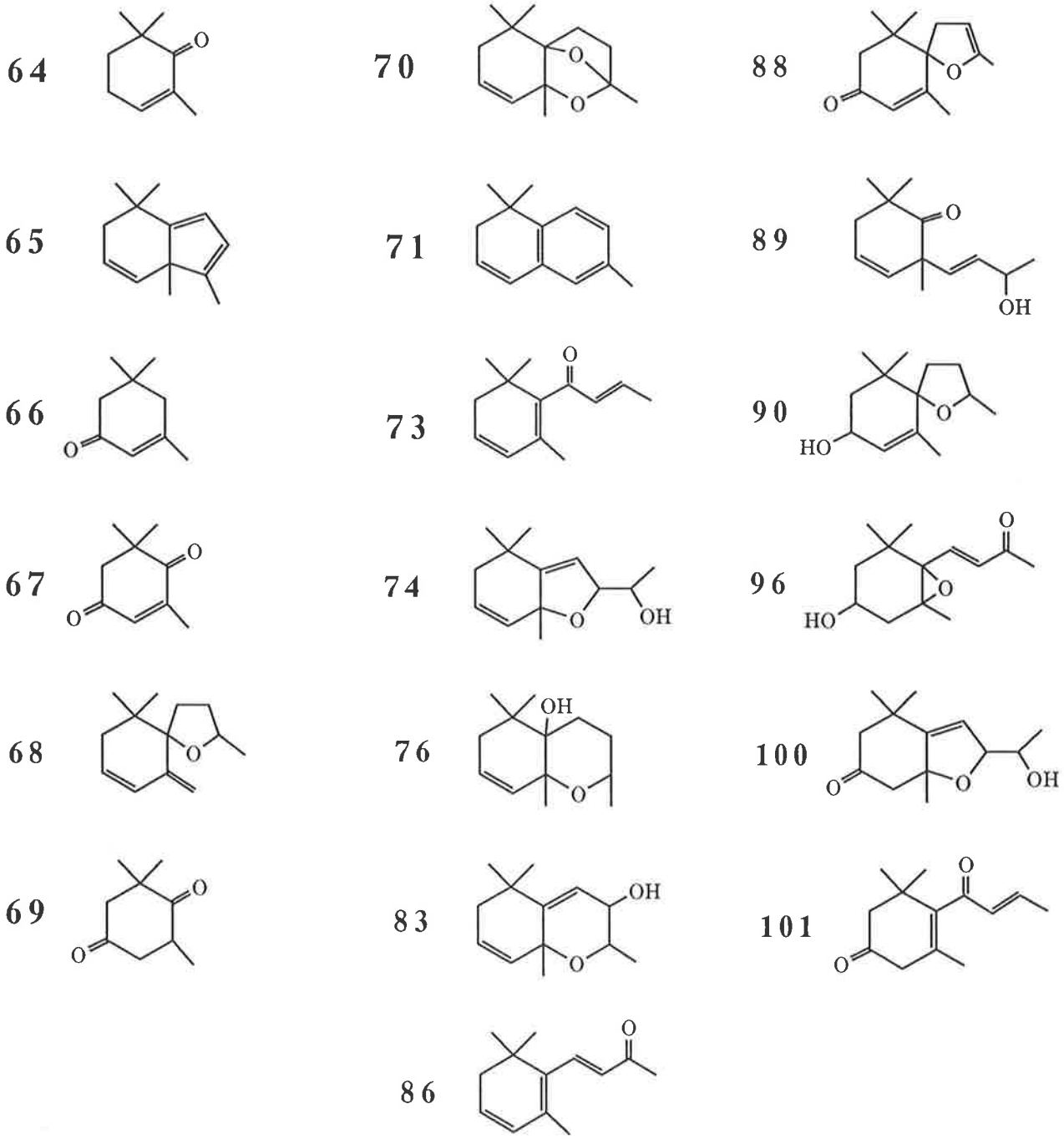


Figure 5.4 *Norisoprenoids identified in Shiraz juice, skin and wine extracts. The numbers refer to the compounds in Table 5.2.*

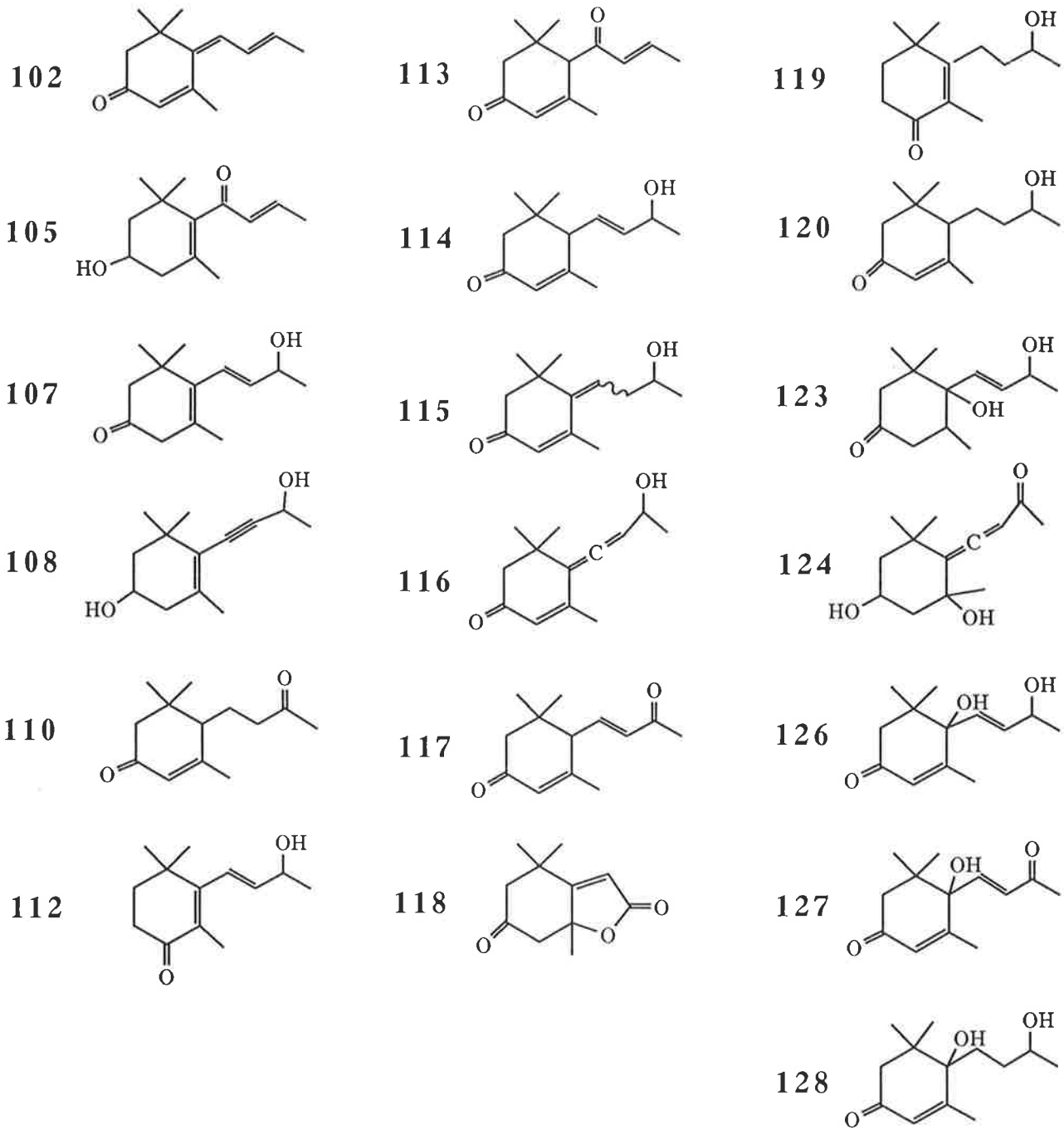


Figure 5.4 continued.

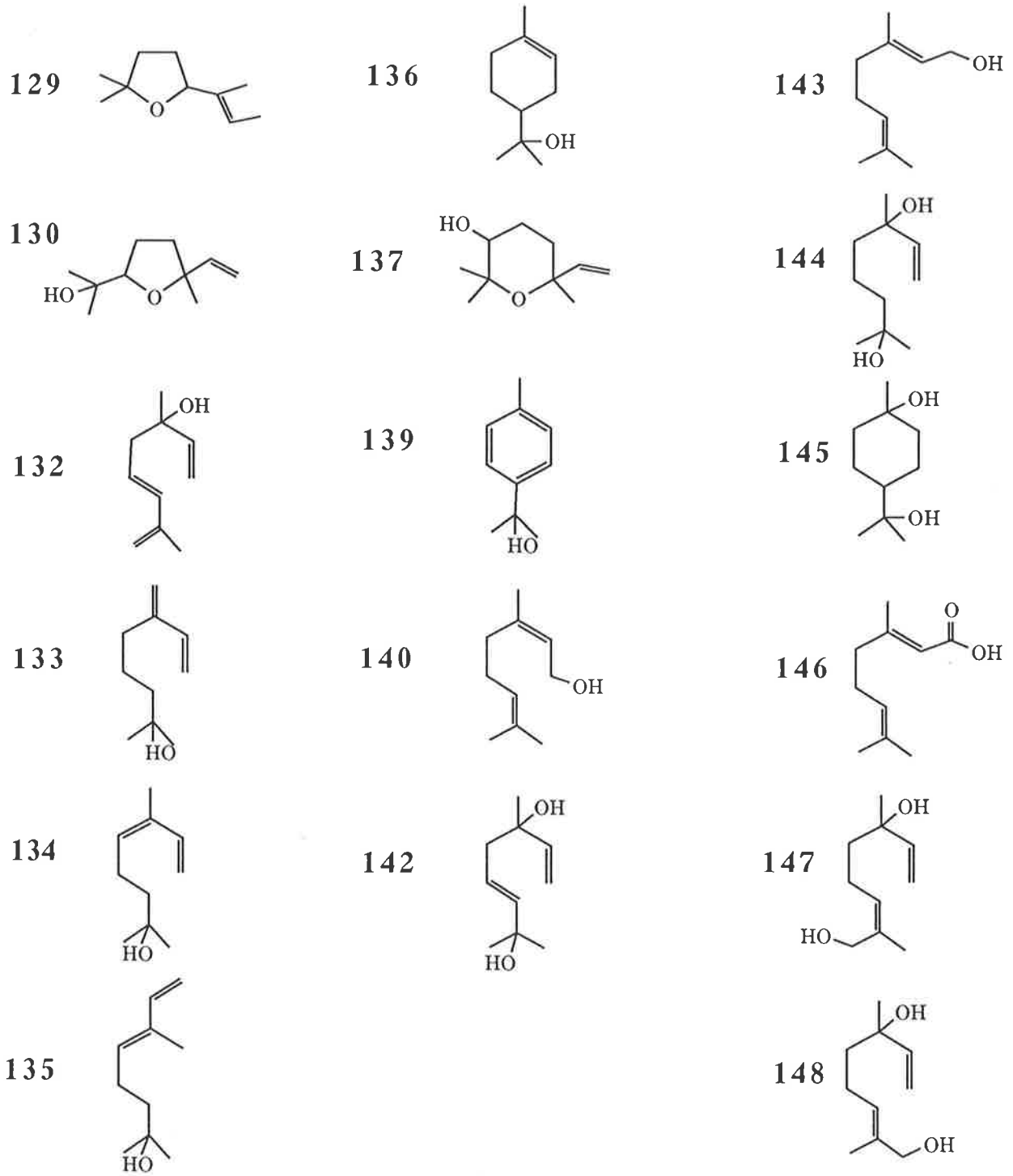


Figure *5.5 Monoterpenes identified in Shiraz juice, skin and wine extracts. The numbers refer to the compounds in Table 5.3.*

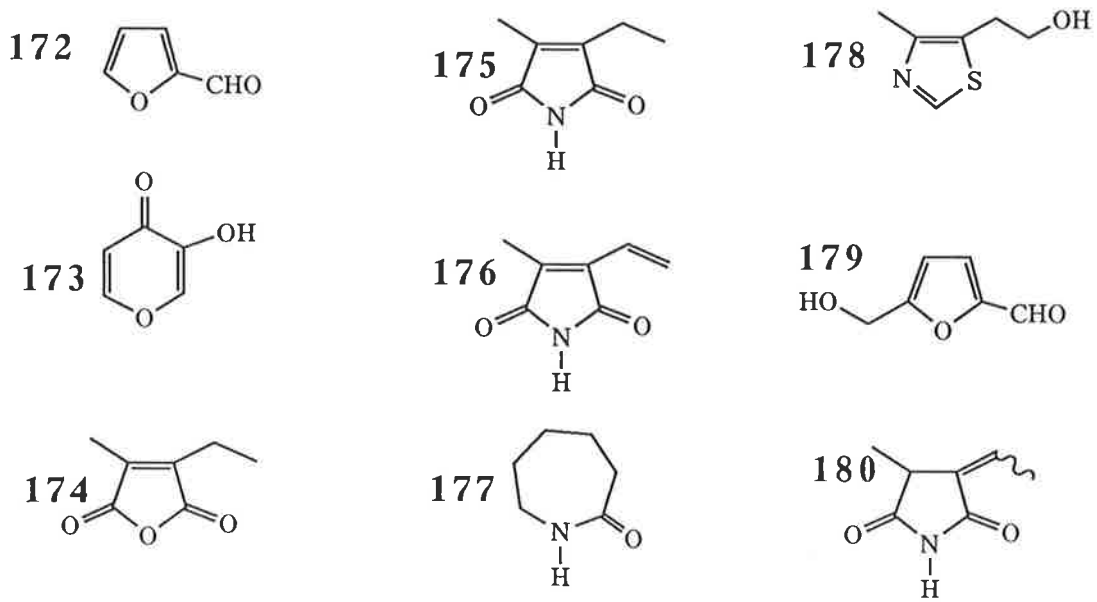


Figure 5.6 *Miscellaneous compounds identified in Shiraz juice, skin and wine extracts. The numbers refer to the compounds in Table 5.5*

Chapter 6

QUANTITATIVE ANALYSIS OF ACID- AND ENZYME-RELEASED VOLATILE COMPOUNDS FROM HIGH AND LOW QUALITY SHIRAZ JUICE.

Chapter 6.

QUANTITATIVE ANALYSIS OF ACID- AND ENZYME-RELEASED VOLATILE COMPOUNDS FROM HIGH AND LOW QUALITY SHIRAZ JUICE.

6.1 ABSTRACT

Quantitative analysis of both the enzyme- and acid-released volatiles from Shiraz juice C_{18} RP isolates demonstrated that there was a greater total concentration of volatiles in the high quality juice samples than in the low quality juice samples. The volatiles released by acid hydrolysis of the C_{18} RP isolates from high and low quality Shiraz juice were quantified by GC-MS and the data subjected to multivariate analysis of variance. Although seven compounds were present at a significantly higher concentration in the high quality samples, no conclusion can be drawn as to their contribution to grape quality until a greater number of samples are tested.

6.2 INTRODUCTION

Most volatile aroma extracts from fruits and fruit products contain hundreds of compounds. The probability that one or more compounds will be consistently present in the high quality samples at a concentration higher than that in the low quality samples is therefore a matter of random chance rather than certitude. To conduct comparative quantitative analysis on volatile aroma extracts from different samples, the techniques used for the isolation, extraction, concentration and identification of the compounds must be reproducible both between and within the samples being analysed. A large number of samples, as well as replicates of each sample, must be analysed to give a statistically

meaningful result.

The number of variables (compounds) should be less than the number of samples analysed to allow statistical interpretation of the data (O'Mahoney 1982). In addition, GC-MS analysis of grape extracts generally yield a large data set consisting of a data point for the concentration of each compound present in the extract. The data set must therefore be reduced to ensure that it is of a manageable size for statistical analysis.

Reduction in the size of the data set can be achieved by principal component analysis (Moret *et al.* 1986), stepwise discriminant analysis (Liardon *et al.* 1984) or by arbitrary methods whereby the data set can be sorted according to parameters set by the researcher, *e.g.* inclusion of compounds present in all the chromatograms or the exclusion of compounds which co-elute with another compound and are therefore difficult to quantify.

The reduced data set can then be analysed by several methods of multivariate analysis such as: stepwise regression analysis (Liardon *et al.* 1984); correspondence analysis (McRae *et al.* 1990); PCAIV (Schlich *et al.* 1987) and analysis of variance (Callo and Rius 1988). This allows the researcher to determine if there is a single compound, or a set of compounds, for which significant differences in concentration can be used as parameters of fruit quality.

This chapter presents the results of the multivariate analysis of variance

performed on the volatiles released by acid-hydrolysis, as well as the qualitative comparison between the total volatile concentration of both the enzyme- and the acid-released products from the C_{18} RP isolate of Shiraz juice samples of high and low quality.

6.3 EXPERIMENTAL

6.3.1 Grape samples

High and low quality grape samples from the Barossa Valley and Coonawarra from 1988 and 1989 were used for the quantitative analysis (Table 6.1). The experimental detail for the quality assignment and fruit processing has been previously described (*Sections 2.3 and 2.4*).

Table 6.1 Samples used for the quantitative analysis of Shiraz juice components

	1988		1989		1990	
Sample	AH	EH	AH	EH	AH	EH
HB	x	x	x	x		x
LB	x	x	x	x		x
HC	x	x	x	x		x
LC	x	x	x	x		x

Refer to Tables 2.1 and 2.2 for sample identification codes

6.3.2 Isolation and release of the glycosidically bound volatiles from Shiraz juice

The glycosidically bound volatiles from the juice samples were isolated and the volatile components released by either acid- or enzyme-hydrolysis of the C_{18} RP isolate as previously described (*Section 2.4.4*). All samples were analysed in duplicate.

6.3.3 Quantification

The volatiles released by either enzyme- or acid-catalysed hydrolysis of the C_{18} RP isolate were quantified as outlined in *Section 5.3.9.*

6.4 RESULTS AND DISCUSSION

The qualitative composition of Shiraz juice, skin and wine extracts was found to be similar (*Chapter 5).* Only one of these fractions needed to be studied, therefore, to allow comparative quantitative analysis of high and low quality Shiraz samples. In chapter 5 it was demonstrated that Shiraz glycosides rather than the free volatiles may be useful for characterising grape samples of high and low quality. For the purpose of the comparative study, glycoconjugates were isolated directly from Shiraz juice samples with C_{18} RP adsorbent, and the acid- and enzyme-released volatiles analysed by GC-MS. Juice samples were chosen in preference to skins because of the variation (solvent, temperature and fermentation during extraction) associated with the extraction of glycosides from the skins, prior to their isolation from the extraction medium with C_{18} RP adsorbent.

6.4.1 Differentiation of high and low quality grape samples by analysis of total volatile concentration

Quantitatively, the high quality samples had a greater total concentration of both acid- and enzyme-released volatiles than that of their low quality counterparts, except in 1989 for the Barossa Valley samples when the opposite was observed (Table 6.2, Table 6.3).

Table 6.2. Total concentration (ug/L) of volatiles released by acid hydrolysis of Shiraz C18 RP isolates

Sample	1988 Mean	1988 *Std dev*	1988 CV%	1989 Mean	1989 *Std dev*	1989 CV%
HB	1128	226	20	622	48	8
LB	815	65	8	761	81	11
HC	662	1	0	884	134	15
LC	379	67	18	665	36	5

Refer to Table 2.1 for sample identification codes

Table 6.3 Total concentration (ug/L) of volatiles released by enzyme hydrolysis of Shiraz C 18 RP isolates

Sample	1988 Mean	1988 *Std dev*	1988 CV%	1989 Mean	1989 *Std dev*	1989 CV%	1990 Mean	1990 *Std dev*	1990 CV%
HB	856	*34*	4	748	*37*	5	974	*10*	1
LB	674	*7*	1	842	*76*	9	250	*10*	4
HC	1039	*155*	15	786	*63*	8	657	*13*	2
LC	660	*46*	7	658	*7*	1	619	*19*	3

Refer to Table 2.1 for sample identification codes

The data from the enzyme-released products from the Barossa Valley samples are reinterpreted in Figure 6.1 which shows the relationship between the volatile concentration of the enzyme-released products and Value Index (VI)[1] for these samples. Only the data from the Barossa Valley samples could be treated in this way as data for correlating the complex costing of grape growing and the final value of the wine made was not available from the winery in Coonawarra.

[1] *The Value Index derives from the classification by winemakers of young wines to a range of premiums which express the anticipated value in the wine market of each grape parcel.*

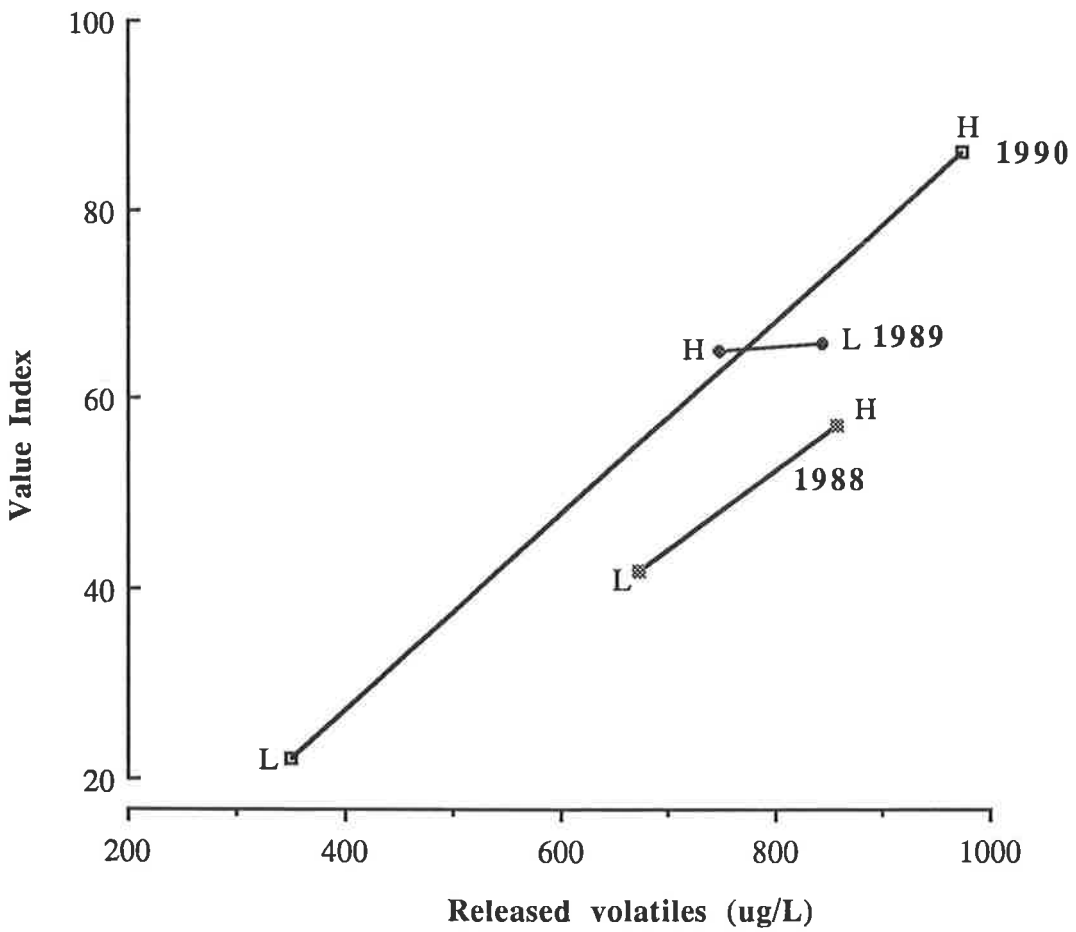


Figure 6.1 *Relationship between the concentration of enzyme-released volatile secondary metabolites (ug/L) and the grape Value Index for three sets of high and low quality samples over three years.*

Interpretation of the acid-released volatiles in the same manner gave similar results and therefore only one set of data are discussed below.

The graph of VI against concentration of released volatiles (Figure 6.1) demonstrates that for the 1988 and 1990 samples the young wines made from 'low' quality grapes were assessed as having both a low concentration of volatiles and a low VI, while the volatile concentration and the VI were both higher in the 'high' quality samples. As can be observed from this figure there was no difference between VI values from the vineyards traditionally rated as high and

low quality in 1989 and only a small difference in volatile concentration was observed (Figure 6.1). The lack of a difference in quality in the Barossa Valley in 1989 was thought to be due to the abnormal climatic conditions during ripening. Excessively hot weather caused the unirrigated, high quality vineyard to undergo severe water stress, causing partial defoliation and delayed ripening, and which may in turn have interfered with the vines' secondary metabolism. Irrigation of the vines from the block designated as producing lower quality fruit prevented water stress and, as a consequence, they were able to continue accumulating secondary metabolites throughout the ripening period. After the 1989 vintage the quality of the wine from the high quality vineyard was subsequently re-classified to slightly below that of the low quality wine (W. Allen pers. comm.).

6.4.2 Differences among the concentration of individual acid-released compounds

Quantification of the individual acid-released compounds was found to be more reproducible than quantification of the volatiles released by enzyme hydrolysis of the C_{18} RP isolates (*Section 2.5.5.3).* It was therefore decided to apply multivariate analysis of variance only to the data obtained from the quantification of the individual acid-released products in juice samples from the 1988 and 1989 vintage.

The 1990 samples were not included in the statistical analysis, because they were analysed on a different GC-MS instrument to the 1988 and 1989 samples and would therefore have imposed an additional variable to the analysis. The data from the samples from each region, Coonawarra and the Barossa Valley, were processed separately as the vines were grown on different soils, experienced different

weather patterns and had different canopy structures. The statistical test, therefore, was weak as it had only 9 degrees of freedom, less than that required to give a conclusive statistical result. However, practical considerations precluded analysis of a greater number of replicates for each sample.

Sixty-four acid-released compounds were present in both the high and low quality Shiraz juice samples from the Barossa Valley and Coonawarra. Those compounds that shouldered another peak, co-eluted with a neighbouring compound or were not reproducibly analysable (*Section 2.5.5.3)* were removed from the data set. Forty compounds were therefore subjected to multivariate analysis of variance. The quantification data for these compounds are given in Appendix B.

Of the forty compounds present in these samples, two were aliphatic compounds, seven were monoterpenes, thirteen were norisoprenoids and ten were substituted benzene derivatives. The remaining eight compounds were of unknown origin. Results from the analysis of variance of these forty acid-released products from the Barossa and Coonawarra samples are given in Table 6.4 and Table 6.5, respectively.

Analysis of variance demonstrated that the concentration of only seven of the forty compounds analysed was available for describing the differences between the high and low quality grapes from the Barossa Valley: two monoterpenes, three norisoprenoids and two substituted benzene derivatives (Table 6.4). The concentration of three of these compounds: an isomer of furan linalool oxide, α-terpineol and methylvanillate were positively correlated with the high quality

Table 6.4 Mean concentration (ug/L) of the acid hydrolysis products from high and low quality juice samples from the Barossa Valley. Significant differences determined by ANOVA

Compound	RT (sec)	1989 Hq / Lq	1988 Hq / Lq	Significant differences: Quality	Year	Y x Q
Monoterpenes						
Furan linalool oxide: isomer 1	664	5.2	10.7	**		
		9.2	9.4			
-Terpineol	946	1.2	3.5			**
		2.7	2.2			
Norisoprenoids						
Actinidol: isomer 1	1344	27.4	51.7		**	
		39.5	54.9			
Actinidol: Isomer 2	1351	39.0	76.2			*
		57.6	78.6			
2-(3-Hydroxybut-1-enyl)-2,6,6-trimethylcyclohex-3-en-1-one	1593	45.3	64.2		*	
		49.7	70.4			
Substituted benzene derivatives						
1,4-Dimethoxybenzene	1094	3.6	9.5	**		
		9.2	13.9			
Methylvanillate	1714	0.5	4.9			*
		0.5	2.3			

No asterisks, * and ** denotes not significant and significant at $p<0.05$ and $p<0.01$ respectively.

samples for 1988.

The differences between the high and low quality Coonawarra samples could be described by the concentration of only five compounds: one monoterpene, two norisoprenoids and two substituted benzene derivatives (Table 6.5). The high quality Coonawarra samples from both years were characterised by a higher concentration of four compounds: α-terpineol, an unknown norisoprenoid with

Table 6.5 Mean concentration (ug/L) of the acid hydrolysis products from high and low quality juice samples from Coonawarra. Significant differences determined by ANOVA.

Compound	RT (sec)	1989 Hq / Lq	1988 Hq / Lq	Significant differences: Quality	Year	Y x Q
Monoterpenes						
-Terpineol	946	1.47 / <1	1.92 / 1.08	*		
Norisoprenoids						
4-Trimethylphenyl-but-3-en-2-one: isomer 4	1610	6.50 / 3.91	3.57 / 1.06	*		
157, 132, 172, 190	1836	8.10 / 5.10	5.60 / 5.20		**	
Substituted benzene derivatives						
Vanillin	1575	7.11 / 10.08	8.74 / 7.45			*
Methyl syringate	2162	9.02 / 2.63	9.12 / 2.99	*		

No asterisks, * and ** denotes not significant and significant at $p<0.05$ and $p<0.01$ respectively.

mass spectra *m/z* 157, 142, 172,190, an isomer of 4-(trimethylphenyl)-but-3-en-2-one and methyl syringate. The concentration of a fifth compound, vanillin was positively correlated with the high quality 1988 samples but negatively correlated with the 1989 samples.

The concentration of only one compound, α-terpineol, was found to be significantly higher in the high quality samples for both regions. This compound, however, is present at a concentration below its known sensory threshold value, and therefore is unlikely to contribute significantly to the flavour of high quality Shiraz grapes or wine.

6.5 CONCLUSION

The quantitative analysis of Shiraz hydrolysates has demonstrated that there is a difference in the total concentration of both the acid- and enzyme-released products, with the high quality samples containing a concentration of volatile compounds higher than that of their low quality counterparts. Differences between these high and low quality grape extracts were also demonstrated by sensory descriptive analysis. However, statistical analysis of the individual acid-released components in the high and low quality Shiraz juice extracts has indicated that the differences observed in the total concentration of volatiles were not correlated with the aroma or concentration of individual compounds present in these extracts.

These results suggest that a simple method of quantifying the total volatile concentration of juice may in fact be more appropriate for the development of a fruit quality index, rather than analysing individual compounds by GC-MS.

An easier and more reproducible method than GC-MS was therefore sought for the quantification of the total concentration of secondary metabolites of Shiraz grapes in an effort to determine a means of statistically differentiating amongst samples of high and low quality. The simple method, which is described in the following chapter, allows the researcher to analyse a large number of samples and replicates of each sample to give statistically meaningful results.

Chapter 7

ASSAY FOR THE QUANTIFICATION OF RELEASED GLUCOSE FROM FRUIT GLYCOSIDES AS A MEASURE OF GRAPE AND WINE QUALITY

Chapter 7

ASSAY FOR THE QUANTIFICATION OF RELEASED GLUCOSE FROM FRUIT GLYCOSIDES AS A MEASURE OF GRAPE AND WINE QUALITY

7.1 ABSTRACT

A three-part assay was developed to measure the concentration of glycosyl-glucose (G-G) involving (a) isolation of juice glycosides, (b) release of glucose with a glycosidase enzyme and (c) quantification of the released glucose with a hexokinase/glucose dehydrogenase system. This assay was applied to samples of defined quality. The results support the hypothesis that high quality grapes have a higher concentration of glycosidically-bound secondary metabolites, previously indicated by the GC-MS analysis of their total volatile concentration. This relatively simple assay promises to offer an objective method of measuring total concentration of grape flavour and therefore potential wine quality.

7.2 INTRODUCTION

Gas chromatography-mass spectrometry (GC-MS) was found to be inadequate for the differentiation of grape samples of differing quality due to the large number of compounds present in the aroma extracts and also the prohibitive number of samples, or replicates of each sample, that must be analysed before statistics can be applied in a meaningful way to the quantitative data obtained (*Chapter 6*).

A common feature of the glycosylated secondary metabolites present in fruit in general is that they are glucosides, in which the glucose moiety may or may not be further substituted (Williams 1991). Enzyme- and acid-catalysed hydrolysis of plant glycosides should therefore yield equimolar proportions of an aglycone and D-glucose. Minor amounts of arabinose, rhamnose and apiose will be given depending on the presence and nature of the disaccharides. Assuming this is the case for all glycosides, a determination of the glycosyl-glucose (G-G) will permit an estimation of the total concentration of glycosylated secondary metabolites present in fruit. A comparative analysis of the volatile composition of Shiraz grapes of assigned high and low quality has indicated that there was a greater concentration of volatile secondary metabolites present in high quality grapes (*Section 6.4.1*). Thus, a measure of the concentration of G-G should provide a simple measure of potential fruit flavour, and therefore potential wine quality.

7.2.1 Assays for the quantification of glucose present in plant glycosides

The analysis of glucose as an end product of enzymic hydrolysis has been used, *inter alia*, for the quantification of stevioside in *Stevia rebaudiana* (Mizukami et al. 1982) and glucosinolate in rapeseed (Mailer and Wratten 1985). Both assays consisted of a colorimetric method which involved a glucose oxidase/peroxidase system. However, naturally-occurring phenolic compounds within certain plant extracts were found to inhibit the peroxidase activity in this assay (Mizukami *et al.* 1982, Mailer and Vonarx 1989).

The glucosinolate concentration in rapeseed has also been indirectly quantified through a measure of enzymatically-released glucose with a hexokinase/glucose-6-phosphate dehydrogenase system (Lein and Schön 1969). A comparison of the peroxidase assay and the hexokinase assay by these authors demonstrated that the hexokinase assay was not susceptible to interferences by phenolic compounds present in rapeseed or any other material in the matrix. Other methods used for the quantification of glucose, such as gas chromatography of a trimethylsilyl derivative, paper chromatography, enzyme and chemical breakdown of the glucosinolate followed by enzymatic quantification of D-glucose, have been outlined in a review on the quantification methods of glucosinolate in rapeseed (McGregor *et al.* 1983).

Quantification of glucose has many further applications and is used extensively for routine medical analysis and research, quality control of food and beverages, horticultural research and many others which shall not be outlined here.

7.3 EXPERIMENTAL

7.3.1 Chemicals and reagents

p-Nitrophenyl-ß-D-glucopyranoside, *p*-nitrophenyl-α-L-rhamnopyranoside and *p*-nitrophenyl-α-L-arabinofuranoside were purchased from Sigma Chemical Co. (St Louis, MO, USA).

Novoferm 12 was donated by Novo Ferment AG, (Neumatt, CH-4243 Dittigen). *Rohapect C* was donated by Enzymes Australia from Röhm (Darmstadt, Germany).

The Boehringer Mannheim kit (No. 716251) containing triethanolamine buffer pH 7.6 with NADP (110 mg), ATP (260 mg), magnesium sulfate, hexokinase and glucose-6-phosphate dehydrogenase was purchased from Sigma Chemical Co., Australia.

Assay buffer: pH 5 citrate/phosphate buffer contained aqueous citric acid solution (48 mL, 0.1M) and aqueous sodium hydrogen orthophosphate solution (53 mL, 0.2M).

The *Sep-pak* cartridges, solvents and water used were as previously descibed (*Section 2.3.1)*

7.3.2 Enzyme activities

The glycosidase activities of a solution of *Novoferm 12* (1 mL *Novoferm 12* diluted to 24 mL with pH 5 assay buffer) were measured by the method of Leung and Bewley (1981) (except that a 0.3M sodium azide solution was added at 0.02% vol/vol to inhibit microbial activity) using the appropriate *p*-nitrophenolglycosides, listed above (*Section 7.3.1)*, as substrates.

7.3.2.1 Effect of temperature on the rate of hydrolysis

The glucosidase activity was assayed at 37, 45, 50, 55 and 60°C and the rhamnosidase and arabinosidase activities were assayed at 55°C as previously described (*Section 2.4.4.1.1).*

All assays were conducted with two blanks: deactivated enzyme (heated at 90°C, 10 min) buffer and substrate, and active enzyme solution without substrate.

7.3.2.2 Effect of sodium azide on enzyme activity

The activity of the enzyme towards *p*-nitrophenol-ß-D-glucopyranoside was determined in assay buffer and in buffer with sodium azide according to the method outlined above.

Shiraz C_{18} RP isolates (50 mL) were extracted from Shiraz juice as previously

described (*Section 2.4.3.2*). Sodium azide was added to two C_{18} RP isolates resuspended in assay buffer prior to hydrolysis. Two further C_{18} RP isolates were hydrolysed in assay buffer without sodium azide. One pair of extracts (with and without azide) was hydrolysed at 55°C for 3 h whilst the other pair of extracts was hydrolysed at 37°C for 16 h. Both pairs of extracts were assayed for G-G as outlined below.

After the pair of C_{18} RP isolates (with and without sodium azide) hydrolysed at 55°C for 3 h, had been assayed for the concentration of G-G, the remaining solutions were pooled and the resultant volume measured. The required volume of an octanol solution (1 mg/mL) was added to this measured volume to give a final concentration of 100 µg/L octanol. The volatile aroma compounds were extracted by continuous liquid/liquid extraction with dichloromethane, concentrated and analysed by GC-MS as outlined in *Sections 2.4.4.3 and 2.4.5.*

7.3.2.3 Thermal stability of Novoferm 12

Replicate solutions of *Novoferm 12* (1 mL/24 mL buffer) were heated at 55°C for 219 h. Aliquots (1 mL) were removed from both solutions at hourly intervals for the first 6 h and subsequently at 24 h intervals. The glucosidase activity of the aliquots was determined according to the method described in *Section 7.3.2.*

7.3.3 Fruit and wine samples

Fruit samples sourced from high and low quality vineyards in the Barossa Valley and Coonawarra in 1988, 1989 and 1990 were assayed for their G-G concentration. The quality assignments and methods of fruit processing have been previously described (*Sections 2.3.2 and 5.3.2).* One wine sample, a high quality Coonawarra wine from the 1989 vintage was also analysed.

7.3.4 Assay for the quantification of glycosyl-glucose (G-G) in Shiraz juice and wine samples

Figures 7.1, 7.2 and 7.3 outline the steps involved in the G-G assay.

7.3.4.1 Isolation of intact glycosides from Shiraz juice and wine

Sep-pak cartridges were activated with methanol (50 mL) and washed with water (50 mL) before use. A 50 mL glass syringe was used both to prepare the C_{18} RP adsorbent and to pass the juice through the cartridges.

Shiraz juice (50 mL) was passed twice through the same prepared cartridge (Figure 7.1a, 7.1b and 7.1c), the cartridge was washed with water (50 mL) (Figure 7.1d), and the glycosidically bound material eluted with methanol (50 mL) (Figure 7.1e). The methanol eluate was evaporated to dryness under vacuum, and the C_{18} RP isolate was resuspended in assay buffer (pH 5, 25 mL).

Glycosidically bound volatiles were isolated from a high quality Coonawarra wine as previously described (*Section 5.3.6.2*) except that the dried methanol extract was resuspended in assay buffer (pH 5, 100 mL).

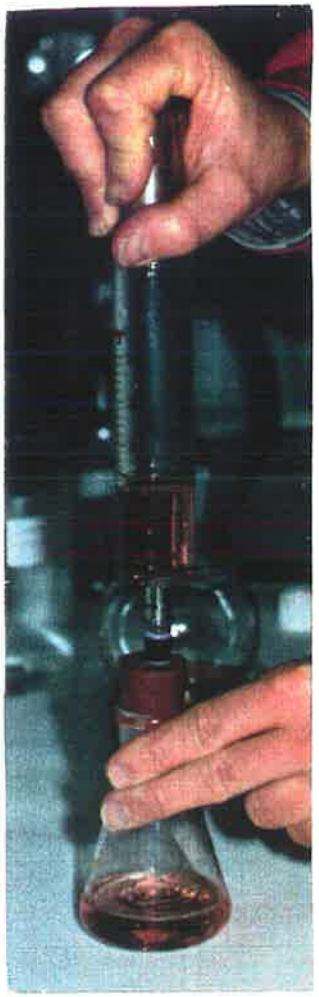
7.3.4.2 Release of G-G from the intact glycosides

Aliquots (5 mL) of the C_{18} RP isolate resuspended in buffer (pH 5) were pipetted into five screw-top vials (Figure 7.2a). *Novoferm 12* enzyme solution (250 µL of a solution containing 1 mL enzyme in 24 mL buffer) was added to each vial (Figure 7.2b). The vials were sealed and placed in a water bath, preheated to 55°C, for 3 h. The vials were then removed and the solution was allowed to return to room temperature.

7.3.4.3 Quantification of G-G

The concentration of G-G in each of the five sample vials was quantified in triplicate as specified by the instructions for use of Boehringer Mannheim kit for D-glucose. This gave fifteen results per extract (Figure 7.3).

b



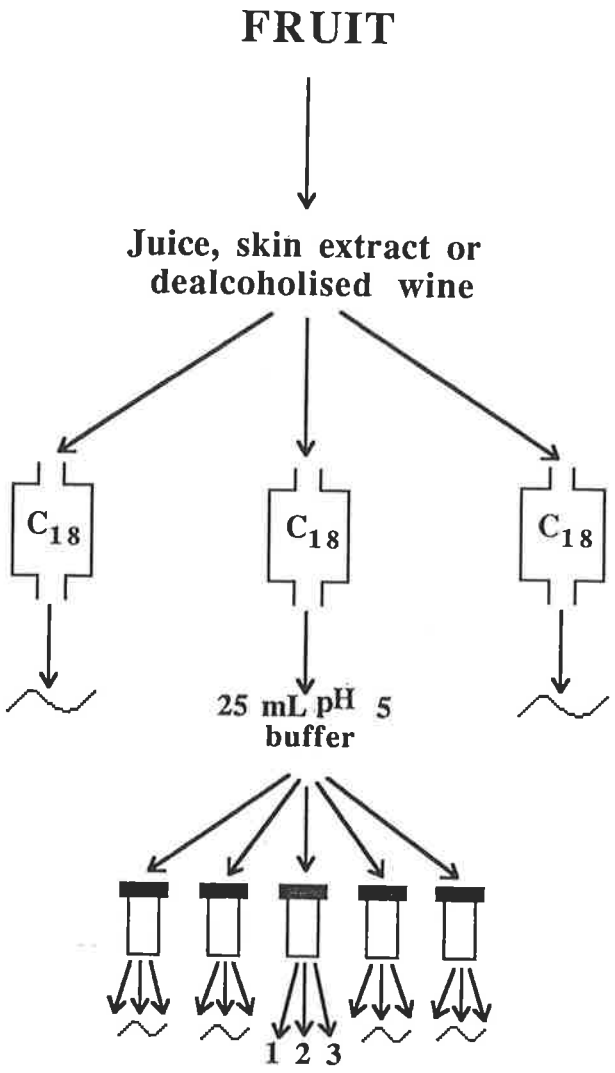
c

d

e

a

b



		CV %
STEP 1	Extraction of glycosides	4.12
STEP 2	Hydrolysis: release of GBG	2.18
STEP 3	Quantification of released GBG	3.88

Figure 7.3 *Summary of the GBG assay and the corresponding coefficients of variation for each step.*

7.3.5 Composition of volatiles hydrolytically released with different enzyme preparations

The C_{18} RP isolate was obtained from two aliquots of Shiraz juice (50 mL) as described above. The dry C_{18} RP isolate was redissolved in either assay buffer (40 mL), and *Novoferm 12* solution (40 µL) added, or assay buffer (39 mL), and *Rohapect C* solution (2 mg/mL) of which 1 mL was added.

7.3.6 Investigation of interferences to the assay

7.3.6.1 Phenolic interference

7.3.6.1.1 Removal of polyphenolics with polyvinylpolypyrrolidone (PVPP)

PVPP (15 g) was added to Shiraz juice (100 mL), agitated and then filtered through a Buchner filter to yield approximately 75 mL of treated juice. PVPP-treated juice (50 mL) and non PVPP-treated juice (50 mL) were assayed for G-G every 30 min for the first hour and then every hour for a further four hours.

7.3.6.1.2 Addition of a phenolic glycoside to the substrate

Phloridzin (80.1 mg) was dissolved in assay buffer (45 mL) and added to a C_{18} RP extract (10 mL, equivalent to 26.25 mL juice). *Novoferm 12* enzyme solution (2 mL) was added and the solution incubated at 55°C for 3 h. The concentration of glucose released from a mixture of phloridzin and juice glycosides was quantified by the assay for G-G as described above.

7.3.6.2 Possible sources of extraneous glucose

7.3.6.2.1 Free/endogenous glucose in the Novoferm 12 *and* Rohapect C *enzyme preparations*

A solution of the assay buffer (20 mL), without substrate but with the addition of either *Novoferm 12* enzyme solution (200 µL) or *Rohapect C* solution (2 g *Rohapect C*/100 mL buffer) (200 µL) was incubated overnight at 37°C. The glucose present in the heated enzyme solutions was quantified using the G-G assay as described above.

7.3.6.2.2 Anthocyanin glycosides

The C_{18} RP isolate was obtained from four aliquots of Shiraz juice (50 mL) as

previously described except that the methanol eluate was redissolved in a solution of methanol (20 mL). An aliquot (0.6 mL) of this methanol solution was added to hydrochloric acid (1M, 3.6 mL) left for 2 h and the absorbance spectrum read from 200 to 720 nm.

7.4 RESULTS AND DISCUSSION

7.4.1 Experiments to optimise the assay protocol

Reproducibility of the *p*-nitrophenolglucoside assay was found to be greatest ($p<0.001$, Figure 7.4) if, after addition of Na_2CO_3, the colour was allowed to develop for 30 min.

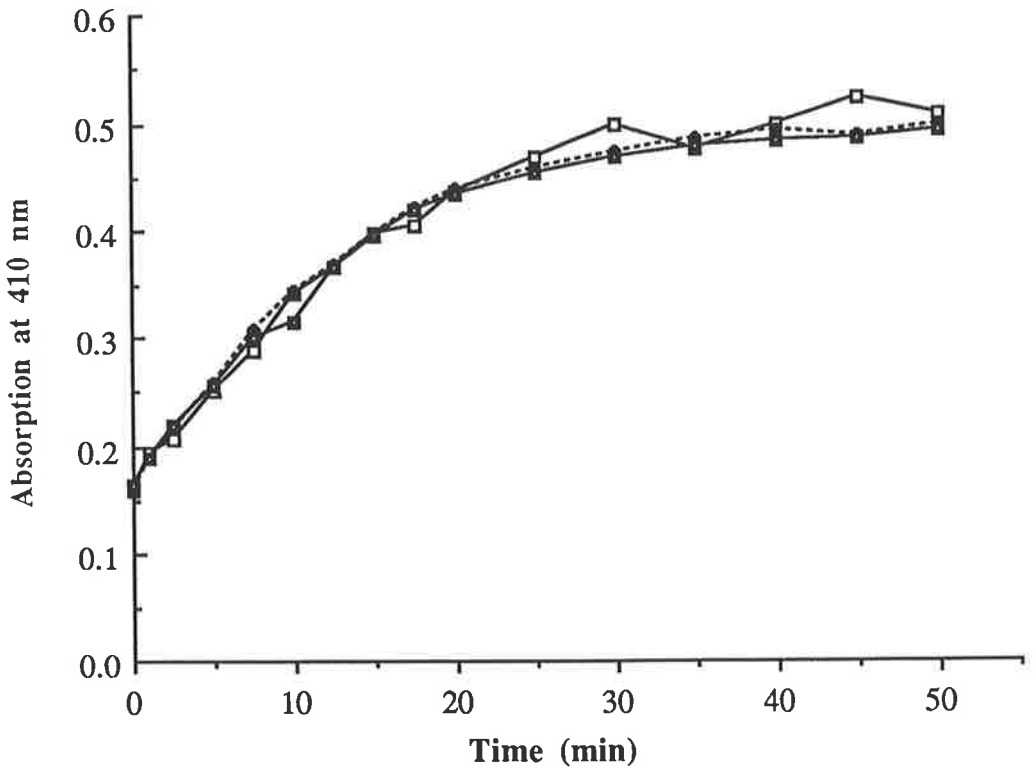


Figure 7.4 *Glucosidase activity, expressed as ΔA_{410}, read at 0 (□), 25 (◊) and 175 (■) minutes after the addition of Na_2CO_3.*

7.4.1.1 Rate of hydrolysis

30 µL *Novoferm 12* (containing 0.8 mg protein as determined by the Bradford assay (Bradford 1976)) was required to hydrolyse 995.7 µM *p*-nitrophenolglucoside in 25 min.

Glucose liberation from *p*-nitrophenolglucoside by *Novoferm 12* was faster at 55°C than at 37°C and reached a maximum within 25 min when heated at 55°C (Figure 7.5). The flattening of the curve at a maximum glucose level indicated that, under these conditions, there was no glucose oxidase activity in the preparation that may interfere with the assay. Furthermore, both the α-arabinosidase and the α-rhamnosidase functions were also active at 55°C (*Figure 7.11*).

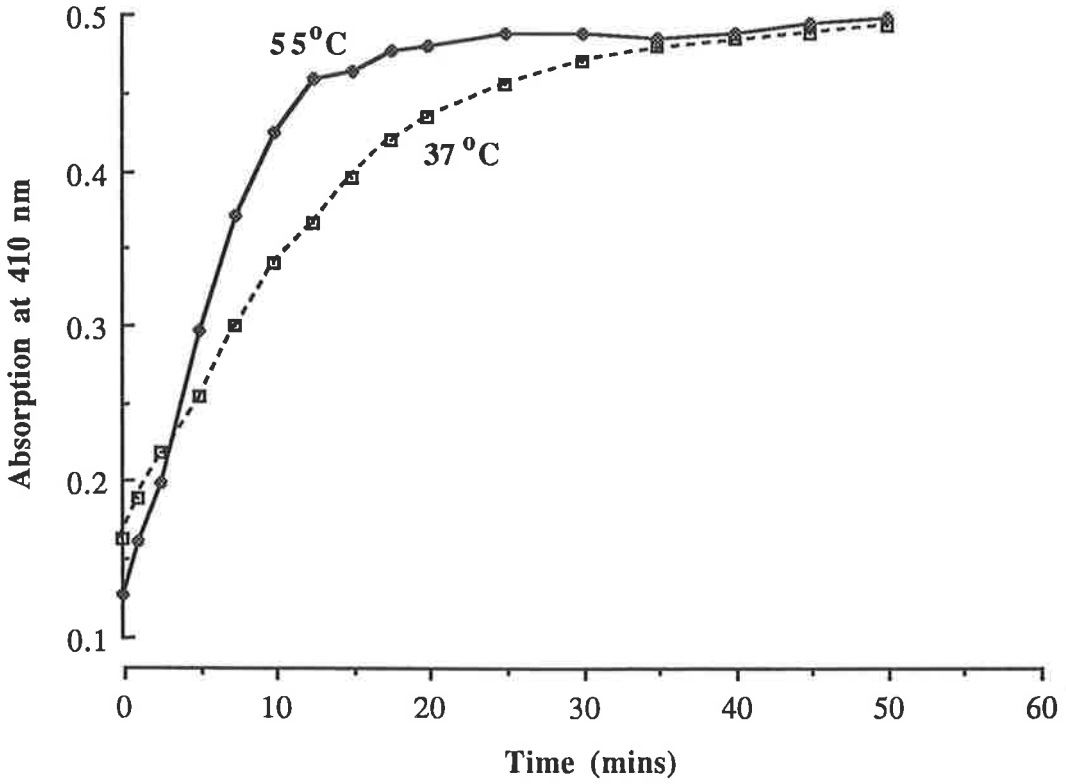


Figure 7.5 *Rate of hydrolysis of p-nitrophenylglucoside by Novoferm 12 at 37°C and 55°C measured by ΔA_{410} due to the p-nitrophenylate anion from the aglycone released by enzyme hydrolysis.*

The rates of glycoside hydrolysis for a range of temperatures at which the assay was tested are shown in Figure 7.6. It was evident that temperatures above 55°C did not afford any advantage to the rate of hydrolysis. The glucosidase activity of *Novoferm 12* was maintained at 55°C for 190 h for both replicate solutions analysed, only after which time it lost some of its activity (Figure 7.7). The data

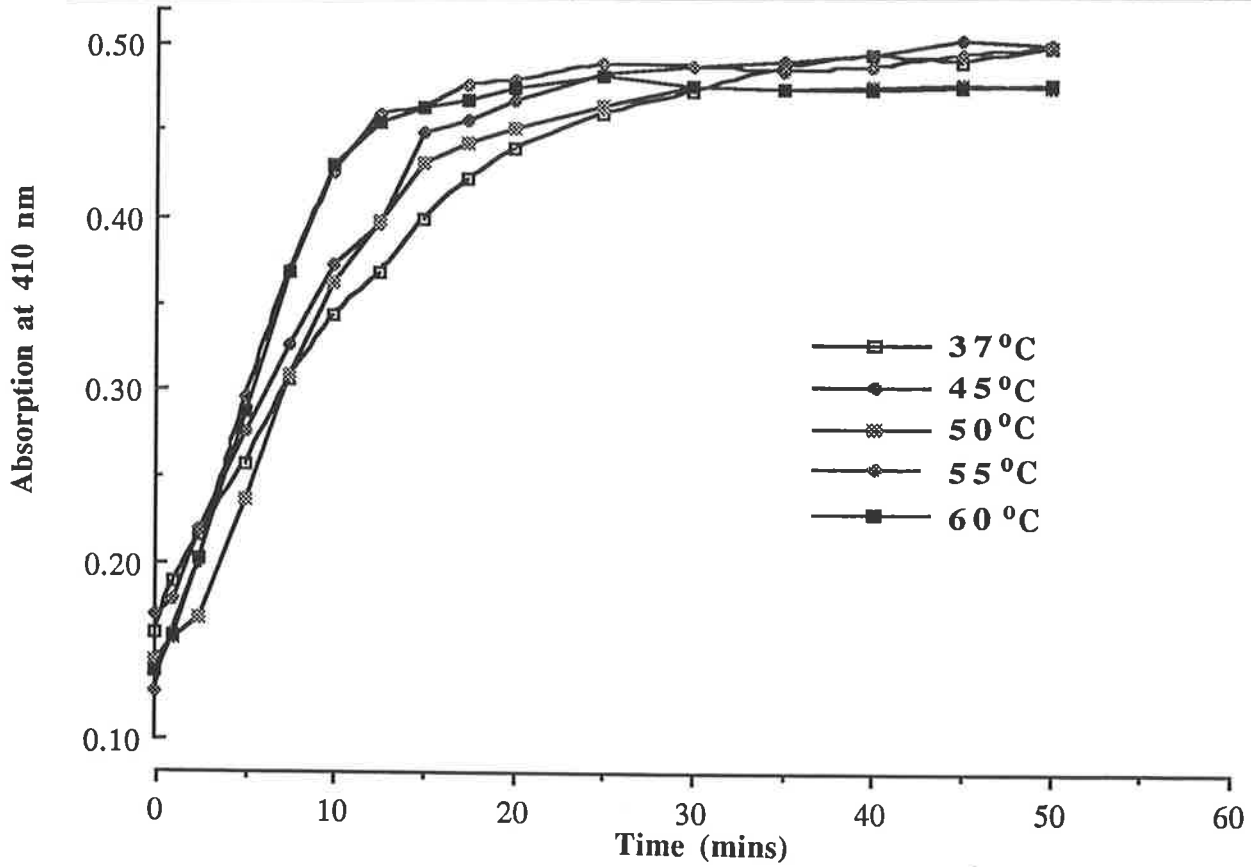


Figure 7.6 *Effect of temperature on hydrolysis of p-nitrophenylglucoside by Novoferm 12*

shown in Figure 7.7 represents the average of the data from the two solutions assayed over the same time span. This result demonstrated that the enzyme preparation could be employed for the assay at 55°C without risking loss of the glucosidase activity during the course of the normal 3 h hydrolysis period.

7.4.1.2 Inhibition of microbial activity

Hydrolysis of *p*-nitrophenylglucoside with *Novoferm 12* in pH 5 buffer with, and without sodium azide demonstrated that the glucosidase activity of *Novoferm 12* was not inhibited by the addition of this antimicrobial agent (Figure 7.8). This diagram, in fact, indicates that the activity of *Novoferm 12* was enhanced by the addition of sodium azide. This effect may have been due to an altered concentration of cations in the buffered solution and as a result, enzyme activity

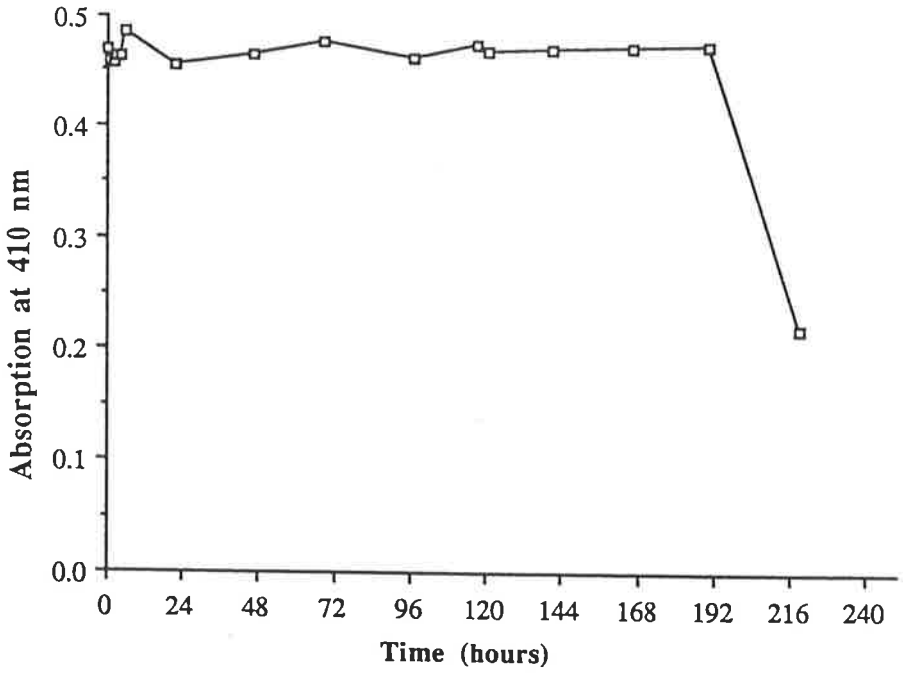


Figure 7.7 *Effect of time of incubation on the glucosidase activity of* Novoferm 12 *at 55°C*

may have been affected. Hydrolysis of two Shiraz C_{18} RP isolates in buffer at 55°C for 3 h, one with and the other without the addition of azide, showed a 15% decrease in the released glucose concentration measured in the latter solution. Under less rigorous conditions, 37°C for 16h, the concentration of released glucose from the hydrolysis of a C_{18} RP isolate in buffer without sodium azide was only 1.1% of the concentration of released glucose from the hydrolysis of a C_{18} RP isolate in buffer with azide. It was apparent therefore that the inclusion of azide inhibited microbial activity and stopped the accompanying loss of glucose. Sodium azide was included in the assay buffer in all subsequent experiments.

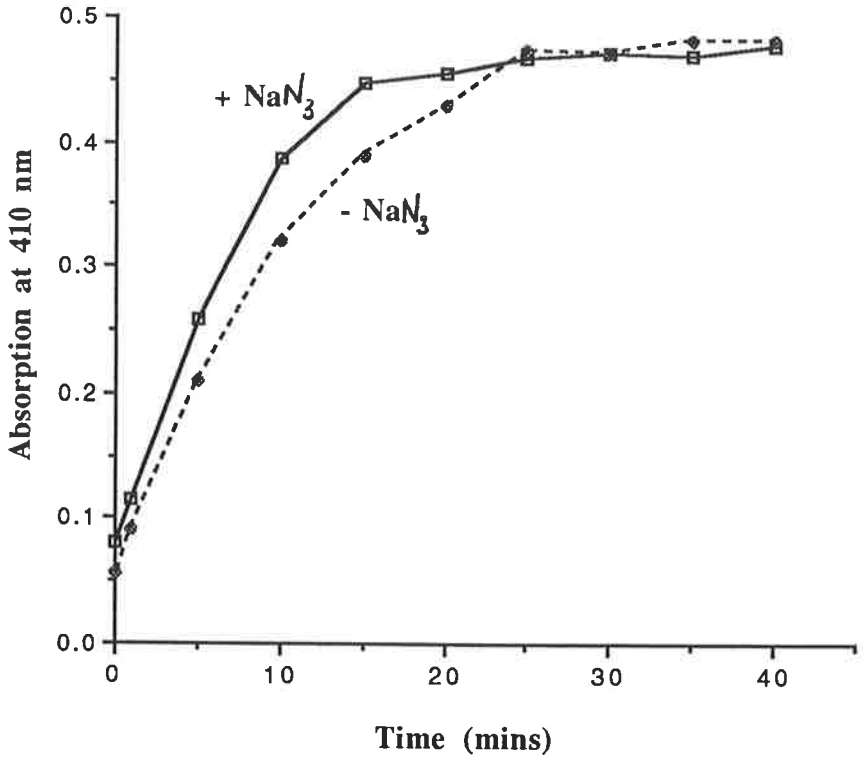


Figure 7.8 *Glucosidase activity of* Novoferm 12 *on p-nitrophenylglucoside in the presence of sodium azide.*

The volatile composition, analysed by GC-MS (data not shown) was compared for a juice glycosidic extract hydrolysed by *Novoferm 12*, at 55°C for 3 h, with and without sodium azide to determine if there was any modification of the released volatiles in the presence of azide. Neither the quantitative nor qualitative analyses of the samples were significantly different, therefore, it was concluded that sodium azide neither interfers in the *Novoferm 12* hydrolysis of the glycosides nor causes any chemical rearrangement of the structure of the released compounds.

7.4.1.3 Phenolic compounds

The PVPP-treated juice did not show enhanced glucose concentration as would have been expected if fruit phenolics were interfering with the assay (Figure

7.9). In fact, treatment with PVPP led to a decrease in glucose concentration of between 6 and 10% indicating that the PVPP may have removed some phenolic glycosides (Figure 7.9). Furthermore, addition of a phenolic glycoside, phloridzin (data not shown), to the C_{18} RP isolate before hydrolysis did not inhibit the glucose assay. Both of these results support the findings of Lein and Schön (1969) that the hexokinase assay, used in step four for the quantification of G-G (*Figure 7.3*), was not susceptible to inhibition from the phenolics present in the assay medium.

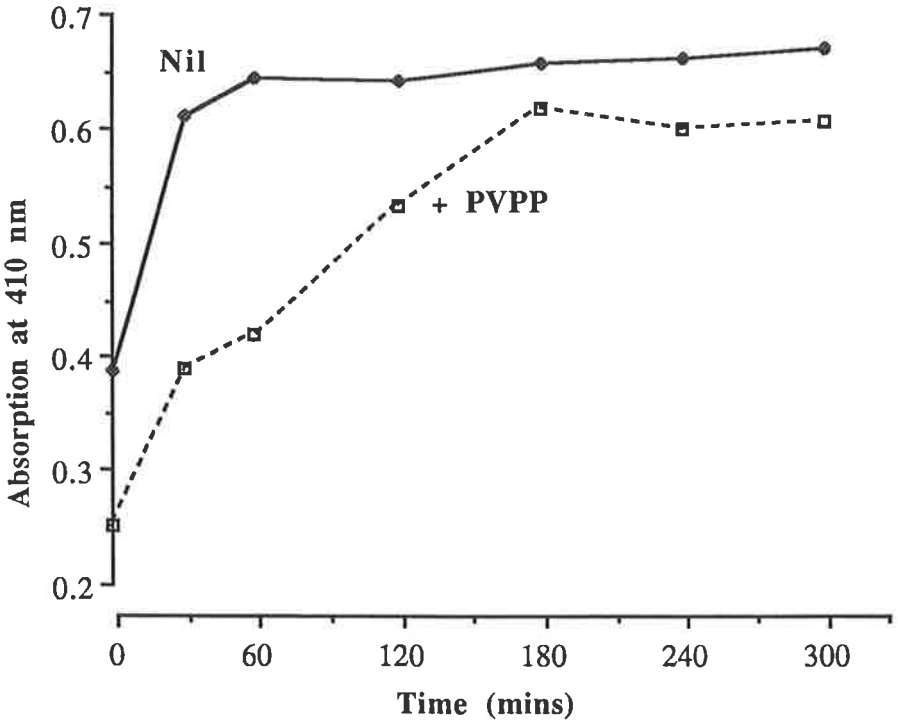


Figure 7.9 *Concentration of glycosyl-glucose (G-G) in PVPP (polyvinylpolypyrrolidone)- and non-PVPP-treated juice*

7.4.1.3.1 Liberation of additional glucose from anthocyanins

The concentration of G-G identified in Shiraz C_{18} RP isolates was greater than that expected from glycosylated volatile aglycones, which accounted for only 5% of quantified G-G. Due to the discrepancy between the expected and observed

concentration of G-G other secondary metabolites known to exist in a glycosylated form were investigated. The concentration of anthocyanins was measured by UV spectrophotometry according to the methods of Somers and Ziemelis (1985). It was assumed that the majority of the anthocyanins present in the C_{18} RP isolate were molecules of malvidin-3-glucoside from which the molar equivalent of glucose was determined (Table 7.1). The concentration of total G-G, and that present as glucose equivalents of malvidin-3-glucoside for the high and low quality Barossa and Coonawarra grape samples are presented in Table 7.1. Anthocyanins accounted for 3-7% of the total G-G observed in these samples. The high quality samples both had an anthocyanin concentration higher than that of their low quality counterparts, supporting the hypothesis that the high quality fruit is rich in glycosylated secondary metabolites. The origin, however, of the majority of the G-G measured by this assay has not as yet been determined.

Table 7.1. Measure of anthocyanins in Shiraz C 18 RP isolates and their glucose equivalents calculated on the basis that malvidin-3-glucoside is the major anthocyanin present in the juice extracts.

Sample	Total G-G (uM)	Anthocyanins (mg/L)	Glucose equivalents from anthocyanin (uM)	% of total G-G
HB 1988	677	16.1	34.5	5.1
LB 1988	248	7.8	16.8	6.8
HC 1988	452	9.4	34.5	4.5
LC 1988	233	3.5	20.1	3.3

Refer to Chapter 2: Table 2.1 for sample identification codes

7.4.2 Assay for the quantification of glycosyl-glucose (G-G) in Shiraz juice and wine

The assay for the quantification of G-G in Shiraz juice and wine samples was executed in three parts: (a) isolation of the intact glycosides from a juice or wine sample; (b) release of G-G with a glycosidase enzyme; and (c) quantification of the released G-G with a hexokinase/glucose-6-phosphate dehydrogenase system.

7.4.2.1 Isolation of intact glycosides from Shiraz juice and wine

The glycosides were isolated from Shiraz juice by adsorption onto a C_{18} RP adsorbent contained in a commercial *Sep-pak* cartridge. The glycosides from wine were isolated by passing the wine through a large packed column of C_{18} RP adsorbent. Although the column was used for the extraction of wine glycosides in this instance, the *Sep-pak* cartridges could also be used.

7.4.2.1.1 Efficiency of the retention of intact glycosides onto a C_{18} RP cartridge

The efficiency of the retention of the glycosides on the C_{18} RP cartridge has been discussed previously (*Section 2.4.3.2*).

7.4.2.1.2 Efficiency of extraction of the intact glycosides

The use of *Sep-pak* cartridges for the isolation of glycosides as a first step in the assay was found to be more time-efficient than using a manually packed column. The use of the cartridge enabled the extraction of several 50 mL aliquots in an hour (approximately 1 complete extraction per 10 min). They are also relatively inexpensive and can be used many times, with careful washing, without any loss in efficiency. However, for these experiments they were recycled only once.

7.4.2.2 Release of G-G with a glycosidase enzyme: choice of a suitable enzyme preparation

Enzyme rather than acid-catalysed hydrolysis was employed for the release of G-G in this assay for several reasons. The conditions required to release the majority of the acid hydrolysis products from the glycosidic fraction (*Section 2.4.4.2)* were too long for application of an assay that should find wide application for the analysis of fruit glycosides. Furthermore, the temperature that would be required to increase the rate of reaction to less than a few hours would possibly lead to degradation of the released glucose. On the other hand, the release of G-G by enzyme hydrolysis appeared to be complete in less than 1 h (Figure 7.9).

Three enzyme preparations were tested for use in the second part of the assay, almond emulsin (a commercial plant-derived enzyme), *Rohapect C* and *Novoferm 12* (fungal-derived enzymes).

Quantification of the glucose liberated by hydrolysis of the C_{18} RP isolate with almond emulsin demonstrated that this enzyme released only 15% of the glucose liberated by *Rohapect C.* This supports the findings of previous researchers that almond emulsin does not completely hydrolyse all glycosides present in the glycosidic fraction (Günata *et al.* 1985b, Aryan *et al.* 1987). The enzyme solutions prepared and quantified under assay conditions but without substrate showed that a high background concentration of glucose was contributed by *Rohapect C* (0.12 mM), however, no glucose was contributed by the *Novoferm 12* preparation.

There was no significant difference in the glycosidase activities between the two batches of *Novoferm 12* donated for this study, although there was a slight drop in the glucosidase activity of the first batch after the solution had been open for two months (Figure 7.10).

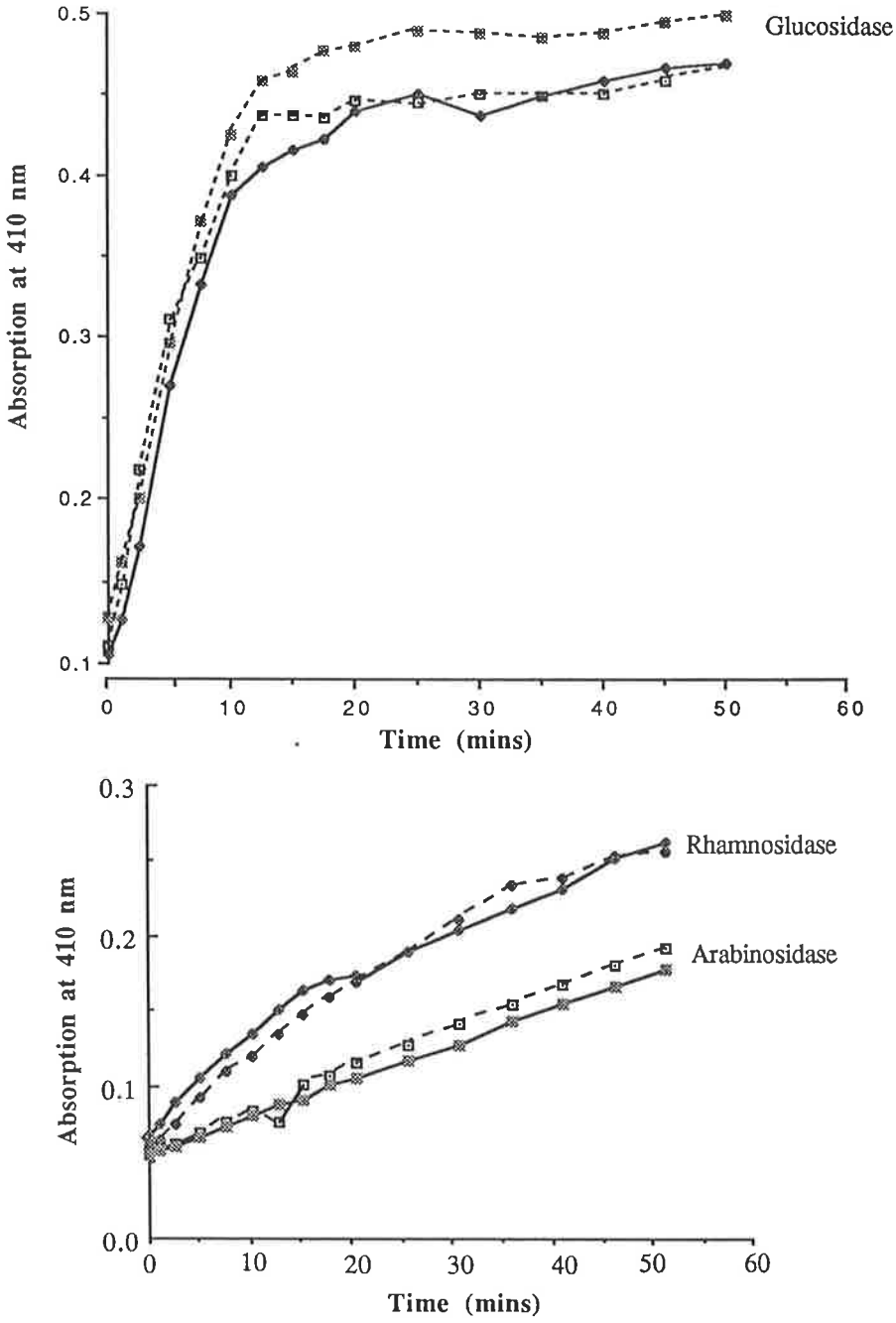


Figure 7.10 *Glycosidase activities measured in two batches of* Novoferm 12; *batch 1 acquired 11/12/89 and tested on 17/12/90 (■), and 11/2/91 (□) and batch 2 acquired 7/12/90 and tested on 11/2/91 (♦)*

7.4.2.2.1 Volatile aglycones released by hydrolysis with Rohapect C *and* Novoferm 12

Novoferm 12 has not previously been used in flavour research for the separation of volatile aglycones and sugar units from grape glycosides. The array and concentration of volatiles liberated by this enzyme were examined, therefore, by GC-MS and compared to the volatiles liberated by hydrolysis with *Rohapect C*.

The qualitative composition of the volatiles liberated by *Rohapect C* and *Novoferm 12* enzyme preparations was the same. Quantitatively, there was only a minor difference in the concentration of liberated volatile compounds in one area of the chromatogram for three compounds, vitispirane (presumably an artefact of the isolation procedure), nerol and an unknown compound (Figure 7.11). From these results it can be concluded that *Novoferm 12* has essentially the same activities as *Rohapect C* and is suitable for the analysis of the composition of grape volatiles.

Novoferm 12 was chosen as the most suitable enzyme for the hydrolysis of the C_{18} RP isolates of Shiraz juice as it contains no endogenous glucose that might interfere in the assay, and appeared to effect complete hydrolysis of the glycosidic extract at 55°C in 3 h (Figure 7.9). Furthermore, *Novoferm 12* has the required glycosidic activities to cleave the disaccharides present in Shiraz juice extracts.

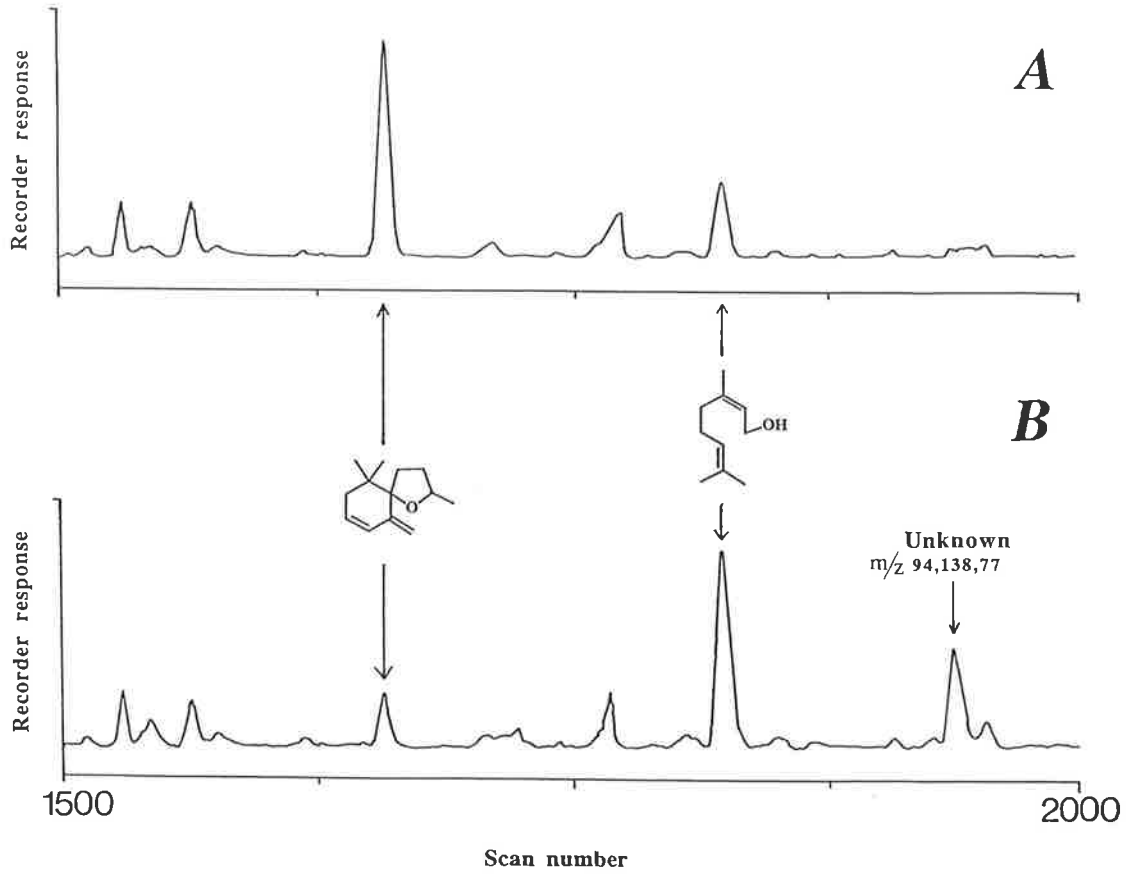


Figure 7.11 *Gas chromatograms of the volatiles liberated by enzyme hydrolysis of a Shiraz juice C18 RP isolate with (a)* Novoferm 12 *and (b)* Rohapect C. *The areas of the chromatogram where quantitative differences in the compounds was observed are indicated.*

7.4.3 Quantification of G-G

The concentration of glycosyl-glucose released by enzyme hydrolysis was quantified by a hexokinase/glucose-6-phosphate dehydrogenase system, which measured the amount of enzymatically-released glucose by a colour change at 340

nm due to the stoichiometric formation of NADPH. This enzyme system was chosen for its specificity for D-glucose, a property important for the precise quantification of D-glucose with no interference from other sugars released during hydrolysis of the glycosides. Furthermore, the hexokinase assay was not susceptible to interference from the high phenolic concentration shown to be present in Shiraz (Somers 1966).

7.4.3 Applications of the assay

7.4.3.1 Ripening trial

The assay was applied to Shiraz berries sampled between veraison and harvest from the planting distance trial of the SA Department of Agriculture (*Section 8.3.1)* to observe the changes in concentration of the glycosides in relation to free sugar accumulation. The results are presented in Chapter 8 with the other analyses relating to this trial.

7.4.3.2 Juice samples of established high and low quality

Analyses of the G-G liberated by enzyme hydrolysis of juice extracts of Shiraz fruit of known quality (*Section 2.3.2)* are presented in Table 7.2. *(The raw data have been given in Appendix C.)* All sample pairs were significantly different ($p < 0.05$) with a C.V. < 7.5%. There was a direct correlation between the glucose concentration and fruit quality assignment for all except one sample pair, i.e. the 1989 high and low quality juices from the Barossa Valley. Reinterpretation of these data in Figure 7.12 demonstrates that the relationship between the juice concentration of G-G and Value Index (VI) was the same as that observed for the relationship between the total concentration of volatile secondary metabolites and

Table 7.2. Concentration of glycosidically bound glucose (uM) in juices of high and low quality Shiraz grapes

	1990				1989				1988			
Sample	Mean	*Std dev*	CV%	Sig	Mean	*Std dev*	CV%	Sig	Mean	*Std dev*	CV%	Sig
HB	411	*16*	4	***	314	*26*	8	**	677	*53*	8	***
LB	234	*9*	4		463	*35*	8		248	*8*	3	
HC	389	*10*	3	**	241	*10*	4	***	452	*2*	4	**
LC	311	*6*	2		128	*0*	0		233	*9*	4	

Sig: ** and *** indicates significant differences between the high and low quality samples at $p < 0.05$ and $p < 0.001$ respectively

VI (*Section 6.4.1*). The graph of VI against G-G (Figure 7.12) shows that the young wines made from 'low' quality grapes in 1988 and 1990 were assessed as having both a low G-G and a low VI while the G-G and the VI were both higher in the 'high' quality samples.

The graph of G-G against volatiles (Figure 7.13) shows that in general terms these two parameters are positively correlated for all samples except for the 1989 high and low quality Barossa Valley samples. This graph indicates that region, year and quality, as well as other viticultural parameters that will be discussed in the following chapter, all contribute to the concentration of secondary metabolites present in the grape berry.

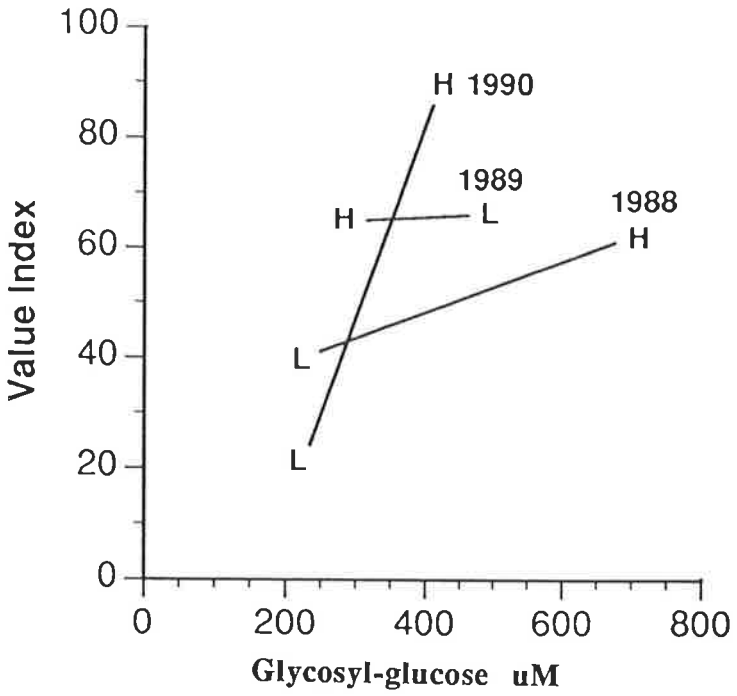


Figure 7.12 *Relationship between released glycosyl-glucose (uM) (G-G) and the grape Value Index for three sets of high and low quality samples over three years.*

7.4.3.3 Application to wine samples

The application of this assay to wine samples is possible as the glycosidically bound compounds can be isolated from diluted or dealcoholised wine. Preliminary investigations into the concentration of released glucose from a C_{18} RP isolate from wine (single experiment only, data not shown) indicated that 30% of the compounds bound in the juice are released during fermentation. This loss may be due to the combination of glycosylated polyphenolics with proteins which subsequently precipitate out of solution or are removed with fining and filtration of the wine, or it may be due to the release of aglycones from the glycosidically bound flavour compounds during vinification.

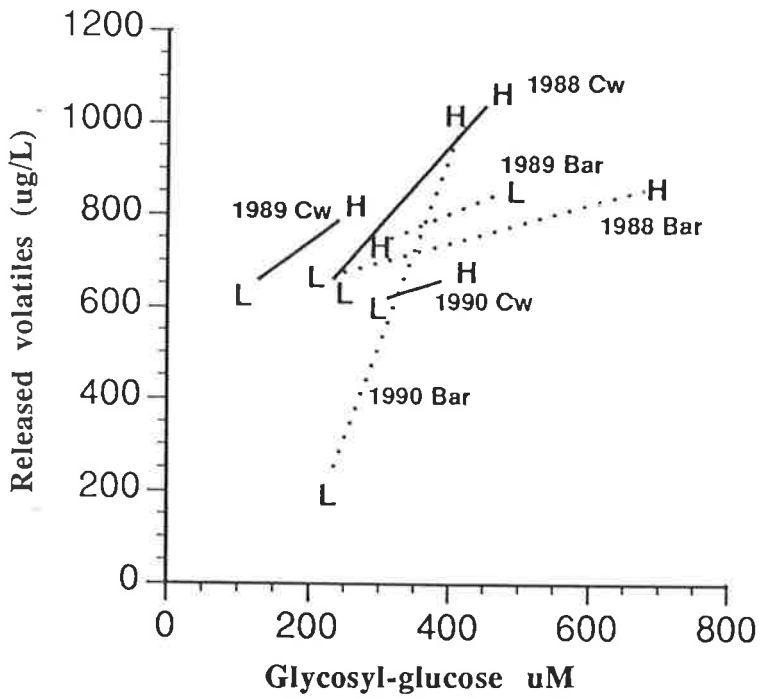


Figure 7.13 *Relationship between released glycosyl-glucose (uM) (G-G) and concentration of total enzyme-released volatiles for the Barossa Valley (·····) and Coonawarra (——) sets of high and low quality samples over three years.*

7.5 CONCLUSION

The analysis of glucose liberated by the enzyme hydrolysis of glycosides in grapes has been developed as an objective measure of the concentration of glycosidically bound secondary metabolites including potential flavour compounds in the berry.

Due to the minimum number of data points presented it is difficult to draw any conclusions from the graphs of G-G versus VI, and G-G versus the concentration of enzyme-released volatiles, however, the suggestion of a positive slope from the low to high quality samples is encouraging. Many more samples need to be

analysed and correlated against a quality rating before a definitive statement about the relationship between G-G concentration and quality can be made. It is interesting that of those samples for which the concentration of G-G, and the concentration of volatiles released by acid hydrolysis and also by enzyme hydrolysis were available, only one showed a reversal of values for the high and low quality samples, i.e. the 1989 Barossa pair. The reversed values were observed for all three quantitative measures.

The assay of G-G in the wine sample studied suggests that this assay may offer a useful means of monitoring changes in glycosidically bound constituents throughout fermentation and maturation. It also suggests that glycosidically bound volatile compounds may be hydrolysed during fermentation to release volatile secondary metabolites which could contribute to the aroma of wine.

Chapter 8

APPLICATION OF FLAVOUR AND QUALITY PARAMETERS TO A VINE PLANTING DENSITY TRIAL

Chapter 8.

APPLICATION OF FLAVOUR AND QUALITY PARAMETERS TO A VINE PLANTING DENSITY TRIAL

8.1 ABSTRACT

The methods developed in this project for analysing fruit flavour and potential wine quality were applied to grape samples sourced from a SA Department of Agriculture vine density trial in Coonawarra, South Australia. The results from these measurements were compared to general viticultural measures: yield (tonne/ha and kg/vine, bunch weight and berry weight); measures of fruit composition (pH, titratable acidity potassium and °Brix); and the tasting score of the resultant wine.

The results have indicated that bunch thinning before veraison has a greater effect on both viticultural and must parameters than root ripping for both the high and low density vines. The interaction between root ripping and fruit thinning was significant only for the low density vines for three parameters: bunch weight, glycosyl-glucose (G-G) and released volatiles. The measure of the concentration of fruit secondary metabolites as glycosyl-glucose was found to be proportional to the released volatiles across all treatments at both vine spacings. Such a relationship is encouraging for the use of the simple assay developed during this project to measure potential fruit flavour and therefore wine quality and also as a means of monitoring the changes in fruit flavour during viticultural trials.

8.2 INTRODUCTION

The limited number of samples used for the chemical/sensory investigations reported in the preceding chapters needed supplementation with additional and different samples. It was considered that these should be from a Shiraz field experiment employing treatments aimed at producing wines of different qualities and with measurements made of vine performance, juice composition and sensory assessment of the resultant wines.

Such an opportunity was provided in a planting density trial established in the vineyards of Wynns (part of the Penfolds group) at Coonawarra, South-East South Australia, by M.G. McCarthy, South Australian Department of Agriculture. The aim of this experiment and the initial results have been described by McCarthy (1988, 1990); briefly the aims were to assess the effects of narrow-row planting (1.75 m) on yield and quality in a comparison with that from a wide-row planting (3.75 m), and to assess the management problems presented by close spacing. The normal plantings of this vineyard (wide-row) are acknowledged as producing wine of an acceptable quality.

Matching experiments were superimposed on the high and low density plantings with the aim of producing fruit of differing quality. The treatments imposed were a factorial combination of fruit thinning and deep ripping of roots.

Compositional changes were measured during ripening within each of the treatments. Small-lot winemaking and sensory assessment of the wines made

from the fruit samples at commercial maturity was organised by Mr A.J.W. Ewart at The University of Adelaide, Roseworthy Campus. The concentration of glycoside products in the same juice samples was measured and the results compared with viticultural treatment effects and wine scores.

A brief summary of some published results on the effects of vine density, fruit thinning and root ripping is provided as a background for the experiment.

8.2.1 Effect of planting density

Close spacing is normal practice in many old-world vineyards while, in contrast, wide-spaced rows have been more common in the new world. The reasons for this have been explained by Winkler *et al.* (1974), but there is now renewed interest in the effects of spacing on vine performance and wine quality. It has been repeatedly demonstrated that increasing the vine density increases yield per hectare even though yield per vine is decreased (Shaulis and Kimball 1955, Shaulis *et al.* 1966, Turkington *et al.* 1980, Fisher and Pool 1988, Archer and Strauss 1991). This effect refers to the mature state of the vineyard and not the initial effect of close spacing during the establishment years during which the dense planting promotes vigour by the mutual protection that is provided (a widely observed phenomenon in planted crops).

Although it is disputed as to how vine density affects eventual shoot vigour (i.e. after the initial effect of spacing has been passed), there is wide agreement that crowded canopies have a negative effect on resultant fruit quality because of increased disease likelihood and lessened exposure of the

fruit to light. Some workers have found that close spacing devigorates high-vigour varieties (Morris and Cawthorn 1981, Hedberg and Raison 1982, Intrieri 1987) but others have found the opposite (see Smart and Robinson 1991 re 'the big vine theory'). The latter authors state that vines that are inherently vigorous need a large number of shoots per vine otherwise shoots will be excessively vigorous; pruning and training need adjustment to achieve a large number of well-spaced shoots of moderate vigour.

Clearly the root contribution to each vine is of central importance in the shoot vigour/vine size relationship. Archer *et al.* (1988) showed that high vine density increased root density per hectare and root depth penetration; the size of the root system was correlated with the size of the vine canopy. On this basis, decreasing vine spacing of vigorous varieties should devigorate shoots, a principal which accords with their results.

In spite of the importance of the topic, there is a poor understanding of the effect of vine density on winegrape quality; partly, this is due to the difficulty of such experiments. Archer and Strauss (1991) have done some of the most detailed work and have found that the best wine quality resulted from an 'intermediate' planting density of 1 x 2 m. Under their conditions, closer and wider spacings gave a lessened quality and the 'intermediate' spacing produced less dense canopies but a larger leaf area/fruit mass ratio.

8.2.2 Effect of fruit thinning

In Europe, crop load per vine is regarded as a major determinant of winegrape

quality. It is an empirical judgment that has become enshrined by legislation in the setting of production limits per hectare for prescribed regions. Such a system does not operate in Australia, but there is interest in the possible effect of yield, and in particular thinning, on wine quality (McCarthy 1985).

Unlike planting density, fruit thinning effects are not confounded by canopy effects. Some authors have observed little or no change in measurable parameters of fruit composition or wine quality as a result of thinning (Bravdo *et al.* 1985, Brown and Grey 1985). In contrast Bravdo *et al.* (1984) found that by decreasing yield to less than 12 kg fruit per kg of prunings there was a concomitant increase in quality.

8.2.3 Effect of root ripping

The size of the canopy of a vine (vine capacity) reflects the size of the root system or, rather, what the root system contributes to the vine via xylem flow, i.e. water, mineral nutrients and organic metabolites (e.g. reduced nitrogen compounds and hormones) synthesised in the root tips. A vine with a large capacity but a small number of shoots will grow vigorous shoots and crowded, shady canopies which are not conducive to wine quality. For the same root contribution, the larger the number of well spaced shoots the lower their vigour and hence a resultant better light penetration to the fruit, with less likelihood of disease, and improved wine quality (Geisler and Feree 1984).

Thus it is possible that lessened root contribution may improve fruit quality in vineyards with crowded canopies. This lessening could be achieved by several

methods: limiting soil water or minerals; by reducing roots by some physical constraint; or by cutting roots (Van Zyl 1988). Root ripping was therefore included as a treatment.

8.3 EXPERIMENTAL

8.3.1 Planting density trial

The trial consisted of 2 Ha of clonal Shiraz vines planted 0.75 m apart in rows 1.5 m wide (high density planting, 8888 vines per hectare) and another 2 Ha planted 1.75 m apart in rows 3.75 m wide (low density planting, 1632 vines per hectare) in 1985. The viticultural management of the experiment and preliminary vine density and yield results for the first three years after planting have been outlined (McCarthy 1990). Some of these results are included in *Appendix D.1.*

Four treatments were imposed on the high and low density plantings in 1988 in parallel, randomised block experiments, each with four replicates. Each plot consisted of 120 (high density) or 18 (low density) vines per plot and all plots were surrounded by barrier vines (Figure 8.1).

The four treatments, as outlined in Table 8.1 were as follows. The control plots were left to yield a normal crop for both planting densities. Treatment 2 involved thinning the crop to one bunch per shoot (before veraison, on 16/1/90). Treatment 3, ripping, was implemented by deep-ripping of the roots in the inter-row space, twice during the growing season (on 6/2/90 and 8/3/90). Treatment 4 was both bunch-thinned and the soil-ripped.

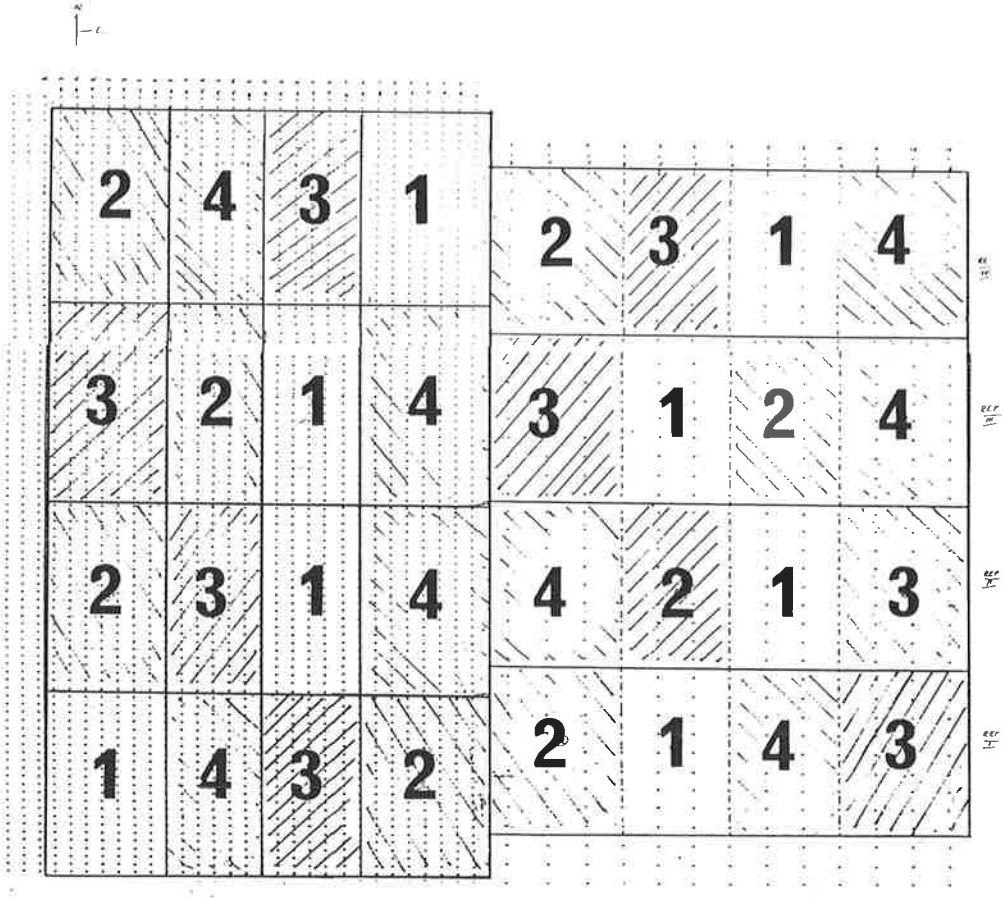


***Figure 8.1** Map of the high and low density plantings showing the randomisation of treatments within each block. Codes: 1) control; 2) ripped; 3) thinned; and 4) thinned plus ripped*

Table 8.1. Experimental treatments imposed within the commercial narrow row and conventional spaced vineyard in Coonawarra (four replicates per spacing).

Treatment	Experimental details
Control	Normal crop at either space
Thinning	Crop thinned to one bunch per shoot
Root pruning (ripping)	Roots pruned by deep ripping during growing season
Thinning and root pruning	Crop thinning and root pruning

Table 8.1 adapted from M.G. McCarthy 1989 with permission.

8.3.2 Fruit samples

Fruit was harvested from 18 vines from the high density plots and 5 vines from the low density plots for the compositional, viticultural and winemaking experiments. The fruit from these vines was pooled to create four field replicates per treatment. Grapes were harvested as near as possible to the same level of maturity for each plot. Harvest date was determined by measuring the concentration of soluble solids (°Brix) in the must of a random 50-berry sample taken every ten days. Fruit from the thinned plots of the high density rows were harvested on 20/3/90 and the remaining high density plots, (control, ripped, thinned and ripped) were harvested on 27/3/90. All low density plots were harvested on 26/3/90. Grape samples (15 kg for the berry analyses and 30 kg for small-lot winemaking) were harvested at random from each of the four replicates of the four treatments for both the high and low density plots (32 lots in total) on the dates specified above. The fruit was kept in a cool room overnight and processed as previously described (*Sectioin 2.3.2)* at the Roseworthy Campus experimental winery the following day.

8.3.3 Wine processing and analysis

The 32 harvested lots of grapes from the high and low density plantings were processed using standard winemaking procedures at the Roseworthy Campus by Mr. J. Sitters. Sensory evaluation of the wines was conducted by a panel of eight experienced wine judges using the Australian 20 point system (a maximum of three points for colour, seven for aroma and ten for taste). The 32 wines were split into two lots of 16 and presented twice to the judges over two occasions to provide estimates of judge performance. Wines made from field

replicates I and II, for all four treatments in both vineyard spacings, were assessed on the 23-24/10/90 whereas the wines made from replicates III and IV were assessed on the 31/10/90 and 1/11/90.

8.3.4 General fruit analyses

Measures of pH and titratable acidity were made before the juice was frozen. Titratable acidity was measured by titrating a juice aliquot (10 mL) against NaOH buffer (0.1N) to an end point of pH 8.20. The potassium concentration was determined by atomic absorption spectrometry.

8.3.5 Analysis of glycosyl-glucose (G-G) and released volatile aroma compounds

The concentration of G-G was determined in Shiraz juice (50 mL) by the assay described in Chapter 7. The results of these analyses are given in *Appendix D.2.* The average concentration of the G-G present in the four field replicates for each treatment was used for all statistical analyses.

After removal of 100 µL aliquots from each of the five, 5 mL vials for the analysis of G-G, the remaining solutions were pooled, the volume measured and the required volume of a 1 mg/mL *n*-octanol solution added to give a final concentration of 100 µg/L *n*-octanol. The volatile aroma compounds were extracted by continuous liquid/liquid extraction with dichloromethane, concentrated and analysed by GC-MS as outlined in *Section 2.4.5.* The concentration of the individual compounds identified in these extracts is given in *Appendix D.3.* The results reported in this Chapter and used for the

statistical analysis represent the collective weights of volatiles in the juice extracts.

8.3.6 Statistical analysis of data

8.3.6.1 General fruit analyses

The data from each of the high and low density plots were treated separately for all analyses as the high and low density plots were not randomised together. Analysis of variance (AOV) was performed on the mean values of the berry and must parameters to determine if the four treatments imposed upon the high and low density plots had a significant effect on the fruit composition.

8.3.6.2 Wine evaluation

Analysis of variance was performed on each judges' score for each wine. To determine if either aroma or taste were making a greater contribution to the final score, analyses of variance were also performed on these individual component parts of the total score. Colour was not analysed separately as the scores did not vary sufficiently for a meaningful analysis to be performed on these data.

The data were analysed in two ways to account for the two dates on which the analyses were performed. The first analysis of variance was performed on the complete data set, i.e. the scores for all judges and all wines assessed on both occasions. The second analysis treated the data sets from the first and second assessment dates separately (*Appendix D.4*). Since these analyses agree substantially, only the overall results are presented here.

8.4 RESULTS AND DISCUSSION

The means of the measurements from the plots and samples from the four treatments are shown in Table 8.2 together with the results from the analysis of variance. These will be discussed first as to their viticultural significance and second from the viewpoint of the main objective of testing the relationship between wine quality and the glycosyl-glucose assay. It is necessary, however, to consider the compositional changes during ripening of the fruit. Since degree of ripeness is held to have such a large effect on the quality of grapes for wine, it was proposed to harvest each plot as near as possible to the same °Brix and so avoid this complicating factor.

8.4.1 Changes with ripening

The sugar (°Brix) content of the 50-berry samples taken from the experimental vines is shown in Figure 8.2a (the glycosyl-glucose data in Figure 8.2b are discussed below under 8.4.3); the Brix values show the typical steady rise during the last month of ripening, with little difference between the high and low density plantings. Spot checks of the individual treatments led to a decision to harvest the thinned plots of the high density vines on 20/03/90, followed by all low density plots on 26/03/90, and the other three treatments of the high density vines on 27/03/90. The actual Brix values of the eight treatments, shown in Table 8.2, reveal that this decision was in the right direction but that differences in sugar content at harvest nevertheless covered 2.5 °Brix: thinning without ripping (the value at lower left of each group of four in the table) had been judged to be ripening faster than the other three treatments, but the harvest of this treatment in the low density

Table 8.2. Average (4-replicates) of measures from plots in two adjacent Shiraz vineyards with a low and high density (1524 and 8889 vines per ha.). Statistical analysis by ANOVA were treated separately (see footnotes).

Measure	LOW DENSITY			HIGH DENSITY		
	Nil Thinned	Ripped Th. + Rip.		Nil Thinned	Ripped Th. + Rip.	
Yield (t/ha)	14.4	17.1		17.3	15.6	
	10.8	9.3	**-	11.6	10.6	**-
Yield (kg/vine)	9.5	11.2		2..0	1.8	
	7.9	6.1	**-	1.3	1.2	**-
Bunches / vine	100.0	104.0		27.0	26.0	
	71.0	67.0	**-	17.0	16.0	***-
Bunch weight (g)	95.0	108.0		73.0	69.0	
	111.0	91.0		74.0	73.0	
Berry weight (g)	1.2	1.2		1.1	1.1	
	1.3	1.4		1.2	1.2	
		* -				
°Brix	24.1	22.9		24.3	24.0	
	25.4	23.5		23.0	23.4	*-
Titratable acidity	5.5	6.0		5.5	5.4	
	4.9	6.0		6.2	6.1	*+
K+ (g/L)	1.2	1.2		1.7	1.8	
	1.5	1.4	**+	1.5	1.4	
					* -	
pH	3.6	3.7		3.8	3.7	
	3.8	3.7	*+	3.6	3.5	***-
Volatiles (ppb)	1197	774		740	1122	
	1141	1770		758	1206	
G-G (uM)	401	327		382	412	
	362	457		351	438	
Tasting score	15.0	15.0		15.1	15.0	
	15.7	15.2	*+	14.2	14.5	***-

1. Asterisks at lower right of the four values denotes a significant difference between the thinned and unthinned treatments. Similarly, asterisks above the top right value shows the significance of the ripping effect. 2. The plus or minus signs show the direction of the effect compared with unthinned or unripped 3. no asterisks, *, ** and *** denotes not significant, significant at $p<0.05$, $p<0.01$ and $p<0.001$ respectively. 4. X denotes a significant ($p<0.05$) interaction between thinning and ripping

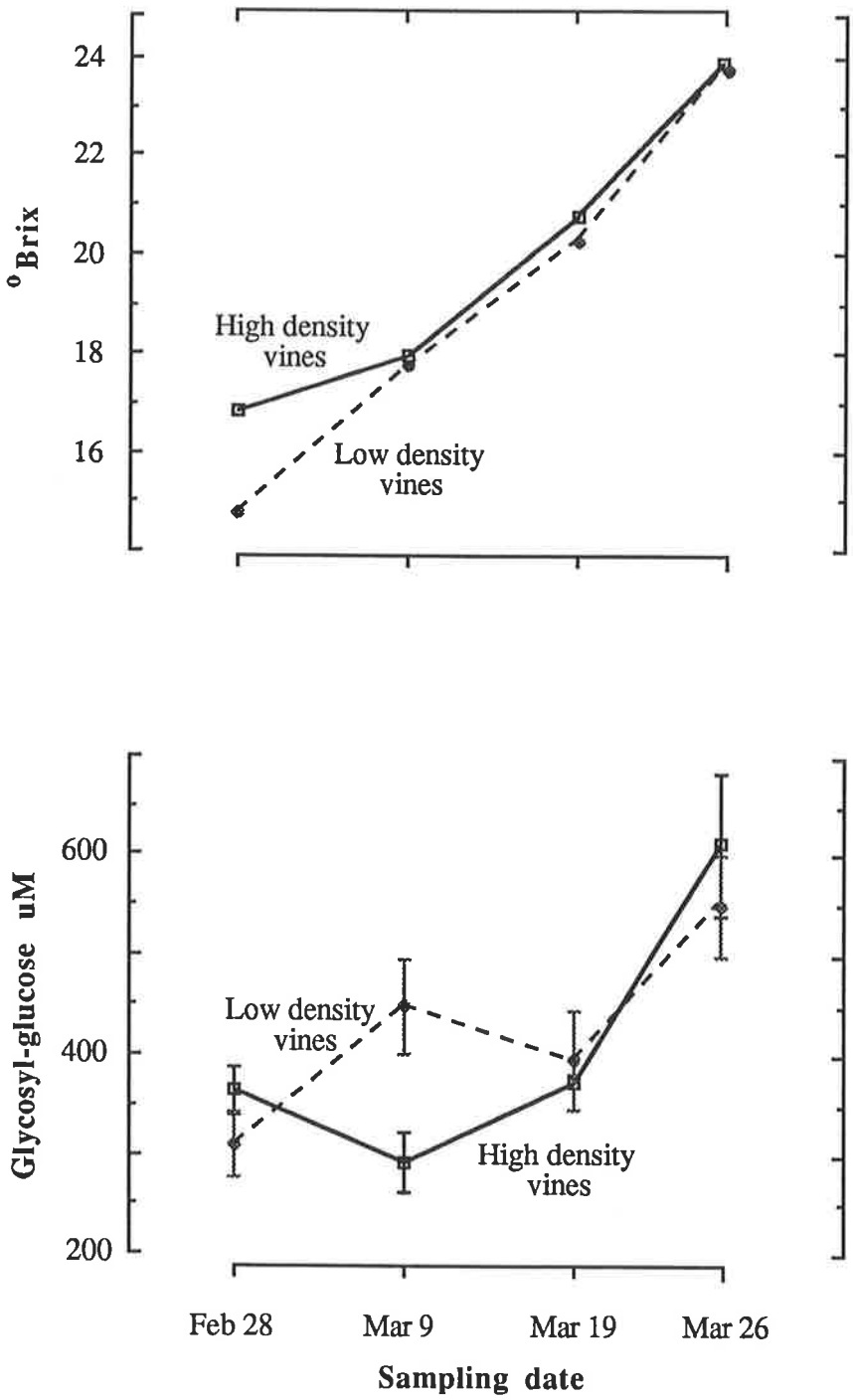


Figure 8.2 *Accummulation of (a) sugar (^{0}Brix) and (b) secondary metabolites (G-G) during berry ripening from the high and low density control vines.*

vines was slightly late. The range of sugar content in the fruit from the other seven treatments was 1.4 °Brix. These differences are larger than was planned and, as will be seen below, have confounded interpretation of the effects of treatment.

8.4.2 Effect of viticultural treatments

Statistical comparisons of the results from the low versus high density plantings is not possible because the treatments were not randomised across this variable. Nevertheless, the trials were adjacent, and in similar soils (Figure 8.3), justifying some cautious comparison. Thinned and unthinned plots had a similar yield per hectare in both planting densities (Table 8.2). The 5.8-fold difference in numbers of vines per hectare was matched by a 5.5-fold reduction in yield per vine in the high density planting. This reduction was mainly due to a 4-fold reduction in bunches per vine, augmented by a reduction in bunch weight (40%) and in berry weight (11%). At both densities, vine yields - per hectare and per vine - were significantly lowered by bunch thinning, as also was bunch number per vine; in all cases the reduction was about two thirds, which was expected. Ripping had no effect on these measures. Bunch and berry weights within each density group were not significantly altered by any of the treatments.

Differences in juice composition were somewhat confusing partly due to the above-mentioned Brix differences; the direction of change in most of these components is usually linked with increase in Brix. In low density vines Brix was lowered in the ripping plots. No other measure showed any effect due to

8.5*a*

Figure 8.3 *(a) High and (b) low density plantings at Coonawarra demonstrating the soil type and the vine canopy structure in each block. Photos were taken in February 1990.*

8.5b

ripping. Thinning, however, caused an increase in potassium levels and pH, a not uncommon linkage.

The lowering of Brix following the ripping of low density vines calls for comment. The reduction of about 7% was significant at p=5% and, since all of these plots were sampled on the same day, represents a clear effect due to the treatment. This result was the only significant effect due to ripping in this trial and is without precedence. It is difficult to explain, but may have been due to the root distribution in the low density plantings. The roots at this spacing may have been more widely distributed in the upper layers of the soil, as previously observed (Champagnol 1984, Archer and Strauss 1985), and ripping would have therefore destroyed a greater proportion of each vine's roots than in the dense vines in which the roots are normally situated deeper and more directly below the vines (Archer 1987). Root ripping of the low density vines might therefore be expected to cause a greater proportional decrease in supply of water and minerals which would in turn influence leaf photosynthetic efficiency (Iland pers. comm.) with a consequent lowering of sugar production.

8.4.3 Effect of treatment on glucosides

The concentration of secondary metabolites in grapes from each of the four field replicates of the four treatments imposed on the high and low density plantings was measured by both GC-MS and the G-G assay. The trends observed in the concentration of volatile secondary metabolites were the same as those for glycosyl-glucose and reflect the results shown for the high and

low quality grape samples in Chapters 6 and 7 (Tables 6.3, 7.2).

The exploratory test of the changes in G-G during ripening is shown in Figure 8.2b (data given in Appendix 4a and 4b) where they can be compared with changes in Brix.

The measures demonstrate the rapidity of accumulation of secondary metabolites in the latter stages of ripening for both planting densities (Figure 8.2b). A small change in °Brix can therefore correspond to a relatively large change in glycosyl-glucose, indicative of secondary metabolites. This highlights the necessity of harvesting grapes as close to the same levels of maturity as possible when conducting viticultural trials of this nature. The problems associated with the stage of ripeness are difficult to overcome in a field experiment due to asynchronous ripening patterns common in most grapes (Coombe and Iland 1987).

The effect of ripping and thinning in the two vine densities on enzyme-released glucose (G-G) and the aglycones (released flavour volatiles) are shown in Table 8.2. There is an encouraging positive correlation between the two measurements (Figure 8.4); the line for high density vines has somewhat higher G-G for the level of released volatiles in the low density vines. This result adds credence to the idea that measurement of glycosyl-glucose may be a useful indicator of the concentration of bound flavour compounds and hence of the potential quality of juice for winemaking.

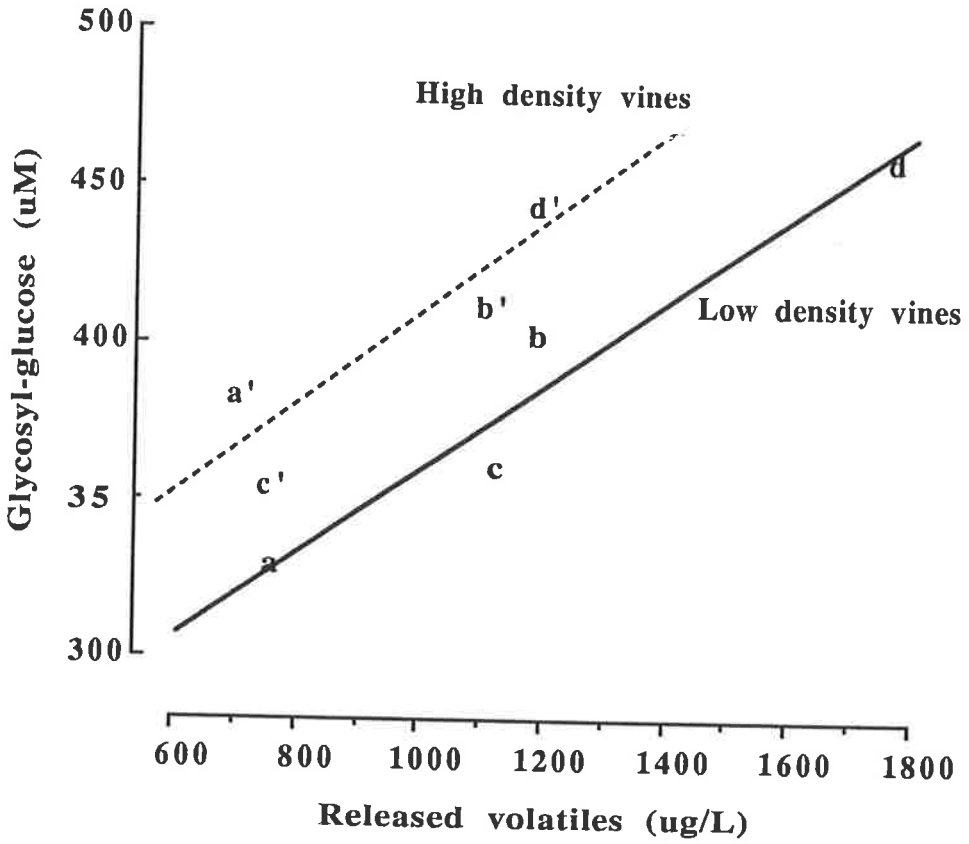


Figure 8.4 *Relationship between glycosyl glucose and concentration of released volatiles for the control (a', a), ripped (b', b), thinned (c', c), and thinned plus ripped (d', d) plots for the high and low density vines, respectively. The linear regression of the four plots for the high density planting is* $r^2 = 0.844$ *and* $r^2 = 0.945$ *for the low density planting.*

It may be noteworthy that the combination treatment, ripping plus thinning, resulted in the highest values of G-G and released volatiles in both density lots. This effect was the major contributing factor to the significant interaction shown in the low density vines. This may have been due to the better leaf area to fruit weight ratio achieved by fruit thinning as when only root-ripping was applied there may not have been sufficient photosynthate capacity in the

canopy to ripen the berries thus inhibiting accumulation of secondary metabolites. This result may have importance in future studies on the role of the roots in the control of berry composition and deserves further investigation.

8.4.4 Relationship of measures to wine taste scores

The purpose of this experiment was to test the hypothesis that the G-G level was an indicator of the quality of wine grapes. The means and statistical analysis of the tests of the young red wines are presented in table 8.2 and can be compared with all other measurements.

The treatments had some significant effects on tasting score. In particular, thinning raised the score in low density vines but lowered it in high density vines. In seeking an explanation of this incongruous result it was noticed that there was a direct correlation between tasting score and °Brix. Such a result is not uncommon in dry red wines made from Shiraz grapes. This result is graphed in Figure 8.5. The correlation coefficient is at a high level for the six points close to the line; the two outliers from this line are the two ripped treatments of the low density vines which had higher scores than the Brix levels might indicate. The role of roots may therefore be among the factors controlling grape quality and should be given more attention. There was a further positive correlation between tasting score and pH, a result that is contrary to industry perception.

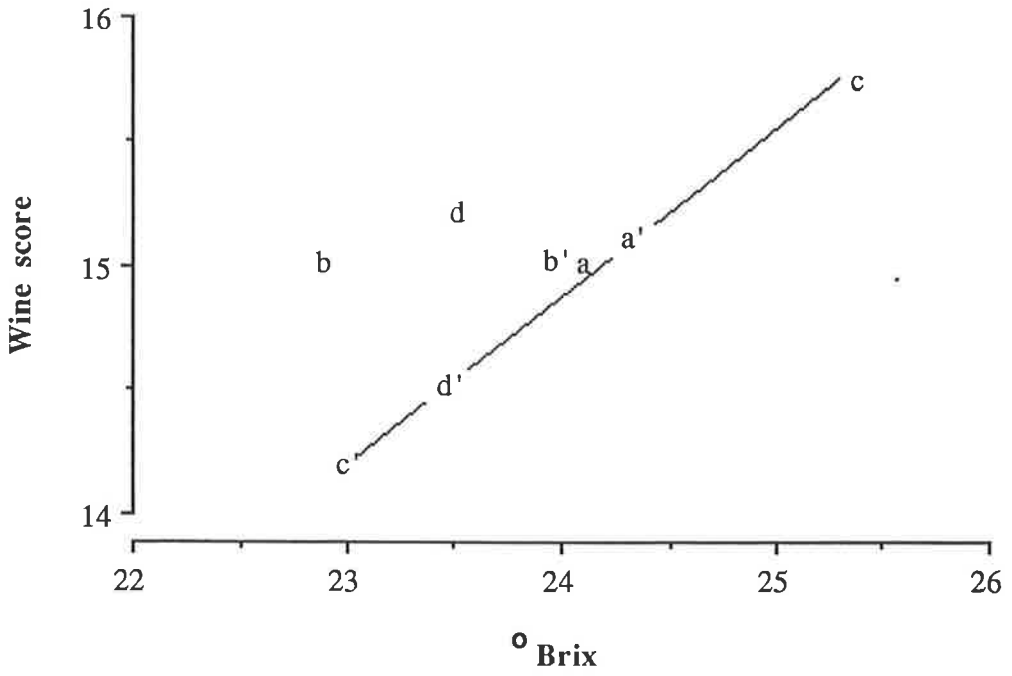


Figure 8.5 *Relationship between juice °Brix and wine scores for the control (a', a), ripped (b', b), thinned (c', c), and thinned plus ripped (d', d) plots for the high and low density vines respectively. The linear regression of the four plots excluding b and d has* $r^2 = 0.983$

No other factors showed significant correlations with tasting score, including glycosyl-glucose levels. In future tests, attention should be given to the possible confounding effect of differences in ripeness since G-G levels increase so rapidly late in the ripening of the berries.

8.5 CONCLUSION

Application of the G-G assay, described in Chapter 7, to grape samples sourced from a SA Department of Agriculture vine density trial demonstrated that the concentration of glycosyl-glucose (G-G) was proportional to that of the released volatiles across all treatments and at both vine spacings, thus

confirming the value of measuring G-G as means of monitoring changes in flavour during viticultural trials.

The concentration of G-G in the fruit from the high density planting was higher than that of the low density planting over all treatments. This result suggests that the high density planting is able to produce higher yields per hectare of higher quality fruit than that produced by the low density vines.

Ripping of roots in low density vines caused a significant reduction of juice Brix compared with the non-ripped plots harvested on the same day. This runs counter to the argument developed in the introduction where it was surmised that ripping effects might be more prominent in vines with crowded canopies i.e. high density vines. Nevertheless, all of the above-mentioned effects attributable to the ripping of roots raise interesting questions on the role of roots in the ripening of winegrapes.

The thinning treatment imposed on the high density vines and the ripping treatment imposed on the low density vines led to a significant decrease in °Brix and a decrease (although not significant) in the concentration of G-G.

What appears to be an anomalous effect of thinning on the wine score of low and high density plantings is better explained as differences in wine score associated with fruit ripeness. This highlights the problems associated with sensory analysis as a means of assessing wine quality as unless all grape samples are picked at exactly the same level of ripeness, the final alcohol

content, and therefore mouth-feel, in the wines will differ.

8.6 ACKNOWLEDGMENTS

I would like to acknowledge the assistance of Miss Lynne Giles, Department of Biometry, The University of Adelaide, Waite Campus for her help with and explanation of the statistical analysis of this data; Mr M.G. McCarthy, Department of Agriculture, Nuriootpa, South Australia for the grape samples and viticultural data and Mr J. Sitters and Mr A.J.W. Ewart, University of Adelaide, Roseworthy Campus, who made and analysed the wines for this trial. I would also like to acknowledge the assistance of Mr. P.G. Iland for his help with the interpretation of the viticultural measurements.

GENERAL CONCLUSION

The complexity of the composition of conjugated volatiles of Shiraz, which was demonstrated in this study, has rendered difficult the identification of a unique compound or a group of similar compounds as an index of quality. However, GC-MS analysis of the enzymatically and acid- catalysed hydrolysis products from high and low quality Shiraz juice indicated that the high quality grapes had a higher concentration of volatile secondary metabolites than their low quality counterparts. Sensory descriptive analysis of the hydrolysates demonstrated that these compounds also contributed significantly to the aroma of high and low quality Shiraz grapes and wine. A simple method of quantifying the total concentration of glycosidically bound volatiles in the juice was therefore considered more appropriate for the development of a Shiraz grape quality index rather than analysing individual compounds by GC-MS.

Development of an assay for the quantification of the glycosyl-glucose present in Shiraz juice involved (a) isolation of the intact glycosides, (b) release of the glycosyl-glucose (G-G) by enzyme hydrolysis and (c) quantification of the released G-G. As high quality grapes, upon hydrolysis, were found to furnish a higher concentration of volatile secondary metabolites than their low quality counterparts, it was assumed that these samples should also yield an equally high concentration of G-G.

Application of this assay to grapes of assigned quality demonstrated that the

concentration of G-G was proportional to the concentration of volatile secondary metabolites, thus confirming the hypothesis on which the assay was based. This result indicates that quantification of the G-G concentration is an efficient means of measuring potential flavour of Shiraz grapes. Furthermore, application of this assay to a vine planting density experiment highlighted the value of measuring the concentration of G-G as a means of monitoring changes in fruit flavour from viticultural trials.

Measurement of G-G provides a means by which the potential fruit flavour, and therefore potential wine quality, can be determined without having to rely on the inherently variable techniques of pilot-scale vinification and subjective sensory assessment of the resultant wine. This assay therefore has laid the ground work for the development of an objective index of grape and potential wine quality.

REFERENCES

Abbott, N.A.; Coombe, B.G.; Sefton, M.A.; Williams, P.J. The composition of Shiraz grapes in relation to the quality of table wines. 4éme Symposium International d'Oenologie: Actualites oenologiques; 15-17 Juin, **1989**; Bordeaux, France, 94-99.

Ahmed, E.M.; Dennison, R.A.; Dougherty, R.H.; Shaw, P.E. Flavour and odour thresholds in water of selected orange juice components. *J. Agric. Food Chem.* **1978**, *26*, 187-191.

Aiken, A.W; Noble, A.C. Comparison of the aromas of oak and glass-aged wines. *Am. J. Enol. Vitic.* **1984**, *35*, 196-198.

Altner, H. Physiology of olfaction. In: *Fundamentals of Sensory Physiology.* Schmidt, R.F. Ed. Springer-Verlag, New York, **1978**, 228-236.

Amerine, M.A.; Pangborn, R.M.; Roessler, E.B. *Principles of Sensory Evaluation of Food.* Academic Press, New York, **1965**.

Amerine, M.A.; Roessler, E.B. *Wines: their sensory evaluation.* Freeman, San Fransisco, **1976**.

Andrews, J.T.; Heymann, H.; Ellersieck, M. Sensory and chemical analysis of Missouri Seyval blanc wines. *Am. J. Enol. Vitic.* **1990**, *41*, 116-120.

Archer, E. Effect of plant spacing on root distribution and some qualitative parameters of vines. In: *Proceedings of the Sixth Australian Wine Industry Technical Conference.* T.H. Lee Ed., 14-17 July, 1986, Adelaide, South Australia, Australian Industrial Publishers, Adelaide, **1987**, 55-58.

Archer, E.; Strauss, H.C. Effect of plant density on root distribution of three year old grafted 99 Richter grapevines. *S. Afr. J. Enol. Vitic.* **1985**, *6*, 25-29.

Archer, E.; Strauss, H.C. The effect of vine spacing on the vegetative and reproductive performance of *Vitis vinifera* L. (cv. Pinot noir). *S. Afr. J. Enol. Vitic.* **1991**, *12*, 70-76.

Archer, E.; Swanepoel, J.J.; Strauss, H.C. Effect of vine spacing and trellising systems on grapevine root distribution. In: *The grapevine root and its environment.* J.J Van Zyl Ed., *Vitic. Oenol. Res. Inst. Stellenbosch* **1988**,

Aryan, A.P.; Wilson, B.; Strauss, C.R.; Williams, P.J. The properties of glycosidases of *Vitis vinifera* and a comparison of their ß-glucosidase activity with that of exogenous enzymes. An assessment of possible applications in enology. *Am. J. Enol. Vitic.* **1987**, *38*, 182-188.

Barron, D.; Etiévant, P.X. The volatile constituents of strawberry jam. *Z. Lebens. Unters. Forsch.* **1990,** *191*, 279-285.

Baumes, R.; Cordonnier, R. Nitz, S.; Drawert, F. Identification and determination of volatile constituents in wines from different cultivars. *J. Sci. Food Agric.* **1986,** *37*, 927-943.

Bertuccioli, M.; Montedoro, G. Concentration of headspace volatiles above wine for direct chromatographic analysis. *J. Sci. Food Agric.* **1974,** *25*, 675-687.

Bitteur, S.; Günata, Z.; Brillouet, J-M.; Bayonove, C.; Cordonnier, R. GC and HPLC of grape monoterpenyl glycosides. *J. Sci. Food Agric.* **1989,** *47*, 341-352.

Blanche G.P.; Reglero, G. Herraiz, M.; Tabera, J. A comparison of different extraction methods for the volatile components of grape juice. *J. Chrom. Sci.* **1991,** *29*, 11-15.

Boidron, J.N.; Chatonnet, P. Pons, M. Influence du bois sur certaines substances odorantes des vins. *Conn. Vigne Vin* **1988,** *22*, 4, 275-294.

Boniface, C.; Vernin, G.; Metzger, J.; Doang, D.; Blondot, V.; Fraisse, D. Evolution de quelques composes volatils de bais de clones de Syrah au cours de la maturation. *Rev. Fr. Oenol.* **1987,** *110,* 28-37.

Bu'Lock, *Biosynthesis of natural products.* McGraw-Hill, Berkshire, England, **1965.**

Bradford, M.M. A rapid and sensitive method for the quantification of microgram quantities of proteins using the principle of protein dye binding. *Anal. Biochem.* **1976,** *72*, 248-254.

Bravdo, B.; Hepner, Y,; Loinger, C.; Cohen, S.; Tabacman, H. Effect of crop level on growth, yield and wine quality of a high yielding Carignane vineyard. *Am. J. Enol. Vitic.* **1984,** *35*, 247-252.

Bravdo, B.; Hepner, Y,; Loinger, C.; Cohen, S.; Tabacman, H. Effect of crop level and crop load on growth, yield, must and wine composition and quality of Cabernet Sauvignon. *Am. J. Enol. Vitic.* **1985,** *36*, 125-131.

Brien, C.J.; Mayo, O. Analysis of judge performance in wine-quality evaluations. *J. Food Sci.* **1987,** *57*, 1273-1279.

Brown, N.; Gray, M. Gisborne trials show little link between high yields and °Brix. *Southern Hort. Grapegrower and Winemaker.* **1985,** *2*, 26-27.

Buttery, R.G.; Seifert, R.M.; Lundin, R.E.; Guadagni, D.G.; Ling, L.C.

Characterisation of an important aroma component of bell peppers. *Chem. Ind.* **1969**, 490-491.

Buttery, R.G.; Guadagni, D.G.; Ling, L.C.; Seifert, R.M.; Lipton, W. Additional volatile components of cabbage, broccoli, and cauliflower. *J. Agric. Food Chem.* **1976,** *24*, 829-832.

Buttery, R.G.; Seifert, R.M.; Guadagni, D.G.; Ling, L.C. Characterisation of additional volatile components of tomato. *J. Agric. Food Chem.* **1971**, *19*, 524-529.

Buttery, R.G.; Takeoka, G.; Teranishi, R.; Ling, L.C. Tomato aroma components: Identification of glycoside hydrolysis volatiles. *J. Agric. Food Chem.* **1990**, *38*, 2050-2053.

Cabibel, M.; Nicolas, J. Lipoxygenation from tomato fruit (*Lycopersicon esculentum L.)* Partial purification, some properties and *in vitro* cooxidation of some carotenoid pigments. *Sci. Aliment.* **1991,** *11*, 277-290.

Callo, M.P.; Rius, G.F.X. Study of the aroma profile of Tarragona wines. II Quantitative analysis. *Anal. Bromatol.* **1988,** *40*, 229-235.

Canal Llaubères, R-M. Utilisation des enzymes dans les procédés d'extraction en oenologie. *Rev. Fr. d'Oenol.* **1990**, *30*, 28-33.

Chamblee, T.S.; Clark, B.C.; Brewster, G.B.; Radford, T.; Iacobucci, G.A. Quantitative analysis of the volatile constituents of lemon peel oil. Effects of silica gel chromatography on the composition of its hydrocarbon and oxygenated fractions. *J. Agric. Food Chem.* **1991,** *39,* 162-169.

Champagnol, F. Physiological state of the vine and quality of the harvest. In: *Quality of the Vintage*. Proceedings of the International Symposium. 14-21 February, 1977; Cape Town, South Africa. Stellenbosch, South Africa: OIV; **1977**, 107-116.

Champagnol F. *Elements de la Physiologie de la Vigne et de Viticulture Generale.* Dehan, Montpellier, **1984,** 233-244.

Chatfield, C.; Collins, A.J. *Introduction to multivariate analysis.* Chapman and Hall. London, **1980,** 57-79.

Chatonnet, P.; Boidron, J.N.; Pons, M. Incidence du traitment thermique du bois de chêne sur sa composition chimique. 2e Partie: Évolution de certains composés en fonction de l'intensité de brulage. *Conn. Vigne Vin* **1989**, *23*, 223-250.

Christoph, N.; Drawert, F. Olfactory thresholds of odour stimuli determined by gas chromatographic sniffing technique; structure-activity relationships. Berger, R.G.; Nitz, S.; Schreier, P. Eds., *Topics in flavour research.* H.

Eichhorn; Marzling-Hangeham, West Germany, **1985**.

Cirami, R.M.; McCarthy, M.G.; Glenn, T. Comparison of the effects of rootstock on crop, juice and wine composition in a replanted nematode-infested Barossa Valley vineyard. *Aust. J. Exp. Agric. Anim. Husb.* **1984,** *24*, 283-289.

Cirami, R.M.; McCarthy, M.G.; Furkaliev, D.G.; The interaction between minimal pruning and rootstock- effects on yield, fruit and wine composition. In: *Proceedings of the Sixth Australian Wine Industry Technical Conference.* Lee T.H., Ed., 14-17 July, 1986, Adelaide, South Australia, Australian Industrial Publishers, Adelaide, South Australia, **1987**, 214-217.

Civille, G.V. The technical and business roles of sensory evaluation in industry. In: *Proceedings of the Twentieth Annual Sensory Evaluation Symposium*; 21 November, 1985, Downing D.L. Ed., New York State Agricultural Experimental Station, Cornell University, Ithaca, **1986**, 1-2.

Clingeleffer, P.R.; Kerridge, G.H.; Possingham, J.V. Effect of variety on wine quality. In:*Proceedings of the Sixth Australian Wine Industry Technical Conference.* Lee T.H., Ed., 14-17 July, 1986, Adelaide, South Australia, Australian Industrial Publishers, Adelaide, South Australia, **1987**, 78-81.

Coombe, B.G.; Dundon, R.J.; Short, A.W.S. Indices of sugar-acidity as ripeness criteria for winegrapes. *J. Sci. Food Agric.* **1980,** *31*, 495-502.

Coombe, B.G.; Iland, P.G. Grape berry development. In: *Proceedings of the Sixth Australian Wine Industry Technical Conference.* Lee T.H., Ed., 14-17 July, 1986, Adelaide, South Australia, Australian Industrial Publishers, Adelaide, South Australia, **1987**, 50-54.

Cootes, R.L; Wall, P.J. Nettlebeck, R. Grape quality assessment. In: *Grape quality assessment from vineyard to juice preparation:* Lee, T.H., Ed.; Proceedings of a seminar, 25 August 1981, Melbourne, Victoria. Adelaide, SA.: Australian Society of Viticulture and Oenology, **1981,** 39-56.

Cordonnier, R.; Bayonove, C. Biochimie appliquée.- Mise en évidence dans la baie de raisin, variété Muscat d'Alexandrie, de monoterpènes liés révélables par une ou plusieurs enzymes de fruit. *C.R. Acad. Sc.* Paris, t. 278, Série D, **1974**, 3387-3390.

Cordonnier, R.E; Günata, Y.Z.; Baumes, R.L.; Bayonove, C.L. Recherche d'un matériel enzymatique adapté a l'hydrolyse des précurseurs d'arôme de nature glycosidique du raisin. *Conn. Vigne Vin* **1989**, *23*(1), 7-23.

Dimitriadis, E.; Williams, P.J. The development and use of a rapid analytical technique for estimation of free and potentially volatile monoterpene flavourants of grapes. *Am. J. Enol. Vitic.* **1984,** *35*, 66-71.

Dimitriadis, E.; Strauss, C.R.; Wilson, B.; Williams, P.J. The actinidols: nor-isoprenoid compounds in grapes, wines and spirits. *Phytochem.* **1985,** *24*, 767-770.

Douillard, C.; Guichard, E. The aroma of strawberry (*Fragiaria ananassa*): characterisation of some cultivars and influence of freezing. *J. Sci. Food Agric.* **1990**, *50*, 517-531.

Dozon, N.M.; Noble, A.C. Sensory study on the effect of fluorescent light on sparkling wine and its base wine. *Am. J. Enol. Vitic.* **1989,** *40*, 265-271.

Dry, P; Smart, R.E. Closer row spacing - is the excitement justified? *Aust. Grapegrower and Winemaker* **1985**, *260*, 12.

Dubois, P. Volatile phenols in wines. In: *Phenolic, sulphur and nitrogen compounds in food flavours.* Charalambous, G. Ed.; ACS Symposium Series 26, American Chemical Society: Washington, DC, **1976**, 110-119.

Dubois, P.; Dekempe, J. Constituents volatils odorants des vins de Bourgogne élevés en fûts de chêne neufs. *Rev. Fr. Oenol.* **1982**, *88*, 51-53.

Dudel, J. General sensory physiology, psychophysics. In: *Fundamentals of Sensory Physiology.* Schmidt, R.F. Ed.; Springer-Verlag, New York, **1978,** 2-29.

Du Plessis, C.S. Grape maturity and wine quality. *S. Afr. J. Enol. Vitic.* **1982,** *3*, 41-45.

Eggenberger, W. Malolactic fermentation of wines in cool climates. In: *Proceedings of the Second International Symposium for Cool Climate Viticulture and Oenology*; Smart, R.; Thornton, R.; Rodriguez, S.; Young, J. Eds., 11-15 January, 1988, Auckland, New Zealand. New Zealand Society of Viticulture and Oenology, Auckland, New Zealand, **1988**, 232-237.

Engel, K-H.; Tressl, R. Formation of aroma components from non-volatile precursors in passion fruit. *J. Agric. Food Chem.* **1983,** *31*, 998-1002.

Engel, K-H.; Flath, R.A.; Buttery, R.G.; Mon, T.R.; Ramming, D.W.; Teranishi, R. Investigation of volatile constituents in nectarines. 1. Analytical sensory characterisation of aroma components in some nectarine cultivars. *J. Agric. Food Chem.* **1988**, *36*, 349-353.

Enzell, C.R. Influence of curing on the formation of tobacco flavour. In: *Bioflavour '81. Proceedings of the Third Weurman Symposium* , Schreier, P. Ed., 28-30 April, 1981, Munich; Walter de Gruyter, Berlin, Germany, **1981**, 255-273.

Enzell, C.R. Biodegradation of carotenoids - an important route to aroma compounds. *Pure Appl. Chem.* **1985,** *57*, 693-700.

Eriksson, C.E.; Lundgren, B.; Vallentin, K. Odor detectability of aldehydes and alcohols originating from lipid oxidation. *Chem. Senses Flavor* **1976**, *2*, 3-15.

Etiévant, P. Volatile phenol determination in wine. *J. Agric. Food Chem.* **1981**, *29*, 65-67.

Etiévant, P. Wine. In *Volatile compounds in foods and beverages.* Maarse, H. Ed., M. Dekker, New York, **1991**, 483-546.

Etiévant, P.X.; Issanchou, S.N.; Bayonove, C. The flavour of Muscat wine: the sensory contribution of some volatile compounds. *J. Sci. Food Agric.* **1983**, *34*, 497-504.

Etiévant, P.X.; Maarse, H.; van der Berg, F. Wine analysis: study and comparison of techniques developed for the study of volatile constituents. *Chromatographia,* **1986**, *21*, 379-386.

Etiévant, P.X.; Issanchou, S.N.; Marie, S. Ducruet, V. Flanzy, C. Sensory impact of volatile phenols on red wine aroma: influence of carbonic maceration and time of storage. *Sci. Aliment.* **1989**, *9*, 19-33.

Eugster, C.H.; Märki-Fischer, E. The chemistry of rose pigments. *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 654-672.

Fischer, N.; Nitz, S.; Drawert, F. Uber gebundene aromastoffe in pflanzen. 2.Mitteilung frei und gebundene aromastoffe in grunden und schwarzen tee (*Camellia sinensis*) *Z. Lebens. Unters. Forsch.* **1987**, *185*, 195-201.

Fischer, H.; Pool, R.M. Interaction between morphology and row spacing effects on growth and yield of two grapevine cultivars. In: *Proceedings of the Second International Cool Climate Viticulture and Oenology Symposium.* Smart, R.; Thornton, R.; Rodriguez, S.; Young, J. Eds., 11-15 January, 1988, Auckland, New Zealand, New Zealand Society for Viticulture and Oenology, Auckland, New Zealand, **1988**, 169-171.

Freeman, B.M.; Lee, T.H.; Turkington, C.R. Interaction of irrigation and pruning level on growth and yield of Shiraz vines. *Am. J. Enol. Vitic.* **1979**, *30*, 218-223.

Freeman, B.M.; Lee, T.H.; Turkington, C.R. Interaction of irrigation and pruning level on grape and wine quality of Shiraz vines. *Am. J. Enol. Vitic.* **1980**, *31*, 124-135.

Geisler, D.; Ferree, D.C. Response of plants to root pruning. *Hortic. Rev.* **1984**, *6*, 155-188.

Glories, Y. Anthocyanin and tannins from wine, organoleptic properties. In: *Plant flavanoids in biology and medicine*, Second Symposium International,

Strasbourg, **1987,** 1-12.

Goniak, O.J.; Noble, A.C. Sensory study of selected volatile sulfur compounds in white wine. *Am. J. Enol. Vitic.* **1987,** *38*, 223-227.

Gross, J. *Pigments in fruits*: Academic press : London **1987.**

Guichard, E.A.; Ducruet, V.J. Quantitative study of volatiles in a model system by a headspace technique. *J. Agric. Food Chem.* **1984,** *32*, 838-840.

Guinard, J-X; Cliff, M. Descriptive analysis of Pinot Noir wines from Caneros, Napa and Sonoma. *Am. J. Enol. Vitic.* **1987**, *38*, 211-215.

Günata, Y.Z.; Bayonove, C.L.; Baumes, R.L.; Cordonnier, R.E. The aroma of grapes. Localisation and evolution of free and bound fractions of some grape components c.v. Muscat during development and maturation. *J. Sci. Food Agric.* **1985a**, *36*, 857-862.

Günata, Y.Z.; Bayonove, C.L.; Baumes, R.L.; Cordonnier, R.E. The aroma of grapes. 1. Extraction and determination of free and glycosidically bound fractions of some grape aroma components. *J. Chromatogr.* **1985b**, *331*, 83-90.

Günata, Y.Z.; Bayonove, C.L.; Baumes, R.L.; Cordonnier, R.E. Stability of bound fractions of some aroma components of grapes cv. Muscat during the wine processing: preliminary results. *Am. J. Enol. Vitic.* **1986**, *37*, 112-114.

Günata, Y.Z.; Bitteur, S.; Brillouet, J-M.; Bayonove, C.L.; Cordonnier, R.E. Sequential enzymic hydrolysis of potentially aromatic glycosides from grape. *Carbohydrate Research* **1988**, *184*, 139-149.

Günata, Y.Z.; Bayonove, C.L.; Tapeiro, C.; Cordonnier, R.E. Hydrolysis of grape monoterpenyl ß-D-glucosidases by various ß-glucosides. *J. Agric. Food Chem.* **1990**, *38*, 1232-1236.

Güntert, M.; Rapp, A.; Takeoka, G.R.; Jennings, W. HGRC and HGRC-MS applied to wine constituents of low volatility. *Z. Lebensm. Unters. Forsch.* **1986**, *182*, 200-204.

Hasagawa, T. Co Ltd; 2-Ethyl-3-methylmaleic anhydride as a flavour and fragrant agent. Japanese patent 58096013 **1983,** (Chem Abst. *99*: 93528k).

Haslam, E. *Natural proanthocyanidins in the flavanoids.* J.B. Harbonne; T.H. Marby; H. Marby Eds., **1975,** 505-559.

Hedberg, P.R.; Raison, J. The effect of vine spacing and trellising on yield and fruit quality of Shiraz grape vines. *Am. J. Enol. Vitic.* **1982,** *33*, 20-30.

Heller, S.K.; Milne, G.W.A. EPA/NIH Mass spectral data base vol 1. US. Dept.

Commer. Natl. Bur. Stand., Washington D.C., **1978**.

Henschke, P.M.; Young, D.; Maggs, J.; Iland, P.; Gawel, R. The effect of canopy management on grape and wine composition and style: experiences at Henschke's Mount Edelstone vineyard. *Austr. N.Z. Wine. Ind. J.* **1990**, *5*, 309-314.

Hepner, Y.; Bravdo, B. Effect of crop level and drip irrigation scheduling on the potassium status of Cabernet Sauvignon and Carignane vines and its influence on must and wine composition and quality. *Am. J. Enol. Vitic.* **1985**, *36*, 140-147.

Heymann, H.; Noble, A.C. Descriptive analysis of commercial Cabernet Sauvignon wines from California. *Am. J. Enol. Vitic.* **1987**, *38*, 41-44.

Heymann, H.; Noble, A.C. Comparison of canonical variate and principal component analyses of wine descriptive analysis data. *J. Food Sci.* **1989**, *54*, 1355-1358.

Hoare, R. Winegrape quality and winegrape pricing: Review of some systems. In: *Proceedings of the Second International Symposium for Cool Climate Viticulture and Oenology*; Smart, R.; Thornton, R.; Rodriguez, S.; Young, J. Eds., 11-15 January, 1988, Auckland, New Zealand. New Zealand Society Viticulture and Oenology, Auckland, New Zealand, **1988**, 188-190.

Huglin, P. Influence of cultivation practices on the quality of the harvest in temperate regions. In: *Quality of the Vintage.* Proceedings of the international symposium. 14-21 February; Cape Town, South Africa. Stellenbosch, South Africa: OIV; **1977**, 359-367.

Iland P.G.; Marquis, N. Pinot Noir - viticultural directions for improving fruit quality. In: *Proceedings of the Seventh Australian Wine Industry Technical Conference.* 13-17 August, 1989, Williams, P.J.; Davidson, D.M.; Lee, T.H. Eds., Australian Industrial Publishers, Adelaide, SA; **1990**, 233.

Intrieri, C. Experiences on the effect of vine spacing and trellis system on canopy micro-climate, vine performance and grape quality. *Acta Hort.* **1987**, *206*, 69-86.

Jackson, G.; Timberlake, C.F.; Bridle, P.; Vallis, L. Red wine quality. Correlations between colour, aroma and flavour and pigment and other parameters of young Beaujolais. *J.Sci. Food Agric.* **1978**, *29*, 715-727.

Jackisch, P. *Modern winemaking.* Cornell University Press, Ithica, New York, **1985**.

Jellinek, G. *Sensory evaluation of food. Theory and practice.* Ellis Horwood, Chichester, England, **1985**.

Jennings, W.G.; Wohleb, R.H.; Lewis, M.J. Gas chromatographic analysis of headspace volatiles of alcoholic beverages. *J. Food Sci.* **1972,** *37,* 69-71.

Jennings, W.; Shibamoto, T. *Qualitative analysis of flavour and fragrance volatiles by gas capillary gas chromatography.* Academic Press: London, **1980.**

Jounela-Erikson, P.; Lehtonen, M. Phenols in the aroma of distilled beverages. In: *The Quality of Foods and Beverages Volume 1.* Charalambous, G. and Inglett, G. Eds., Academic Press, **1981,** 167-181.

Kare, M.R.; Tordoff, M.G. Myths and realities of the sense of taste. In: *Proceedings of the European Brewing Convention,* Zurich, **1989,** 13-36.

Kinsella, J.E.; Patton, S.; Dimick, P.S. Chromatographic separation of lactone precursors and tentative identification of the γ-lactones of 4-hydroxyoctanoic and 4-hydroxynonanoic acids in butter fat. *J. Am. Oil Chem. Soc.* **1967,** *48,* 532-538.

Kliewer, W.M. Sugar and organic acids of *Vitis vinifera. Plant Phys.* **1966,** *41,* 923-931

Kliewer, W.M. Influence of temperature, solar radiation and nitrogen on coloration of Emperor grapes. *Am. J. Enol. Vitic.* **1977,** *28,* 96-103.

Kluba, R. M. Sensory evaluation of alcoholic beverages. In: *Proceedings of the Twentieth Annual Sensory Evaluation Symposium*; 21 November, 1985, Downing D.L. Ed., New York State Agricultural Experimental Station, Cornell University, Ithaca, **1986,** 25-31.

Korth, B. Use of regression in sensory evaluation. *Food Technol.* **1982,** 91-95.

Krammer, G.; Winterhalter, P.; Schwab, M.; Schreier, P. Glycosidically bound aroma compounds in the fruits of *Prunus* species: Apricot (P. *armeniaca,* L.), peach (P. *persica,* L.), yellow plum (P. *domestica,* L. ssp. Syriaca). *J. Agric. Food Chem.* **1991,** *39,* 778-781.

Kwan, W.; Kowalski, B.R.; Skogerboe, R. Pattern recognition analysis of elemental data. Wines of *Vitis vinifera* cv. Pinot Noir from France and the United States. *J. Agric. Food Chem.* **1979,** *28*: 356-359.

Kwan, W.; Kowalski, B.R. Correlation of objective chemical measurements and subjective sensory evaluations: wines of *Vitis vinifera* variety Pinot Noir from France and the United States. *Analyt. Chim. Acta* **1980,** *122,* 215-222.

Langron, S.P. The application of Procrustes statistics to sensory profiling. In: *Sensory quality of food and beverages.* Williams, A.A.; Atkins, R.K. Eds., Ellis Horwood, **1983,** 89-95.

Larsen, M.; Poll, L. Odour thresholds of some important aroma compounds in

raspberries. *Z. Lebensm. Unters. Forsch.* **1990**, *191*, 129-131.

Lea, A.G.H. Bitterness and astringency, the procyanidins of fermented apple ciders. In: *Bitterness in foods and beverages.* Rouseff, A. Ed., Elsevier, Amsterdam, **1990,** 145-148.

Lein, K-A.; Schön, N.J. Quantitative glucosinolatbestimmung aus halbkörnern von *Brassica-Arten. Angew. Botanik* **1969**, *43*, 87-92.

Leung, D.W.M.; Bewley, J.D. Immediate phytochrome action in inducing α-galactosidase in lettuce seeds. *Nature* **1981**, *289*, 587-588.

Liardon, R.; Ott, U.; Daget, N. Analysis of coffee headspace profiles by multivariate statistics. In: *Analysis of volatiles, methods of application.* Schreier, P. Ed.; **1984,** 447-459.

Lichine, A. *Encyclopedia of wines and spirits.* Cassel Ltd, Massachusettes, USA, **1979.**

Lloyd, R.A.; Miller, C.W.; Roberts, D.L.; Giles, J.A.; Dickerson, J.P.; Nelson, N.H.; Rix, C.E.; Ayers, P.H. Flue-cured tobacco flavour. 1. Essence and essential oil components. *Tob. Sci.* **1976**, *20*, 40-48.

Maarse, H.; Visscher, C.A. Volatile compounds in alcoholic beverages-quantitative and qualitative data, TNO-CIVO Food analysis Institute, Zeist, The Netherlands, 1989.

Maga, J.A.; Lorenz, K. Taste threshold values for phenolic acids which can influence flavour properties of certain flours, grains and oilseeds. *Cereal Sci. Today* **1973**, *18*, 326.

Maga, J.A. Lactones in food. *CRC Crit. Rev. Food Sci.* **1976**, *8*, 1.

Maga, J.A. Simple phenol and phenolic compounds in food. In: *CRC Crit. Rev. Food Sci.* **1978,** 323-372.

Mailer, R.J.; Wratten, N. Comparison and estimation of glucosinolate levels in Australian rapeseed cultivars. *Aust. J. Exp. Agric.* **1985**, *25*, 932-938.

Mailer, R.J.; Vonarx, M.M. Errors in the determination of glucosinolate in rapeseed using glucose oxidase-peroxidase. *Analyst* **1989**, *114*, 1507-1508.

Malek, D.M.; Munroe, J.H.; Schmitt, D.J. Statistical evaluation of sensory judges. *Am. Soc. Brew. Chem.* **1986**, *44*, 23-27.

Mann, J. *Secondary Metabolism.* Atkins, P.W.; Holker, J.S.E.; Holliday, A.K. Eds., Oxford Chemistry Series, Oxford University Press, Oxford, England, **1987**.

Marais, J. Terpenes in the aroma of grapes and wines. *S. Afr. J. Enol.* **1983**, *4*, 49-60.

Masuda, M.; Okawa, E.; Nishimura, K.; Yunome, H. Occurrence and formation oftrans-2,6,6-trimethyl-1-crotonyl-cyclohexa-1,7-diene,inalcoholicbeverages. *J. Food Sci.* **1980**, *45*, 396-397.

McCarthy, M.G. Vine and fruit response to vertical shoot training. In : *Coonawarra Viticulture.* Lester, D.C.; Lee, T.H. Eds,, Proceedings of a seminar, 8 June 1983, Coonawarra, South Australia, Australian Society of Viticulture and Oenology; **1983**, 37-43.

McCarthy, M.G. The effect of irrigation on grape quality. In: *Irrigation, salinity and grape quality.* Proceedings of a seminar. Lester, D.C.; Lee, T.H. Eds., 2 November, 1984, Loxton, South Australia, Australian Society of Viticulture and Oenology, Adelaide, South Australia, **1985,** 35-49.

McCarthy, M.G. Evaluation of high density vineyard planting. In: *Proceedings of the Second International Symposium for Cool Climate Viticulture and Oenology*; Smart, R.; Thornton, R.; Rodriguez, S.; Young, J. Eds., 11-15 January, 1988, Auckland, New Zealand. New Zealand Society Viticulture and Oenology, Auckland, New Zealand, **1988**, 172-173.

McCarthy, M.G. Planting density, irrigation and rootstock effects on the plant and soil. In: *Proceedings of the Seventh Australian Wine Industry Technical Conference*; 13-17 August, 1989, Williams, P.J.; Davidson, D.M.; Lee, T.H. Eds., Australian Industrial Publishers, Adelaide, SA; **1990**, 66-68.

McCarthy, M.G.; Staniford, A.J. Response of Shiraz vines in the Barossa Valley to drip irrigation. In: *Proceedings of the Fifth Australian Wine Industry Technical Conference; Advances in Viticulture for Economic Gain.* Lee, T.H.; Somers, T.C., Eds., 29 November - 1 December, 1983, Perth, Western Australia: The Australian Wine Research Institute, Adelaide, South Australia, **1983**, 81-106.

McDaniel, M.R.; Henderson, L.A.; Watson, B.T.; Heatherbell, D.A. Sensory panel training and screening for descriptive analysis of the aroma of Pinot Noir fermented by different strains of malolactic bacteria. *J. Sensory Studies.* **1987**, *2*, 149-167.

McDaniel, M.R.; Henderson, L.A.; Watson, B.T.; Heatherbell, D.A. Sensory panel training and descriptive analysis: Gewurztraminer clonal wines. In: *Proceedings of the Second International Cool Climate Viticulture and Oenology Symposium*; 11-15 January 1988; Auckland, New Zealand. New Zealand society for viticulture and oenology, Auckland, New Zealand; **1988**, 346-349.

McGregor, D.I.; Mullin, W.J.; Fenwick, G.R. Review of analysis of glucosinolates. Analytical methodology for determining glucosinolate composition and content. *J. Assoc. Off. Anal. Chem.* **1983**, *66*, 825-849.

McRae, K.B.; Lidster, P.D.; DeMarco, A.C.; Dick, A.J. Comparison of polyphenol profiles of apple fruit cultivars by correspondence analysis. *J. Sci. Food Agric.* **1990,** 329-342

Meilgaard, M.C. Flavour chemistry of beer: Part II: Flavour and threshold of 239 aroma volatiles. *Tech. Q. Master Brew. Assoc. Am.* **1975**, *12*, 151-168.

Meilgaard, M.C. Use of the taste panel in flavour stability studies: Organisation, threshold, synergism, antagonism. *Louvain Brew. Lett.* **1989**, *2*, 3-11.

Meyer, B.J.; Hernandez, R. Seed tannin extraction in Cabernet Sauvignon. *Am. J. Enol. Vitic.* **1970,** *21*, 184-188.

Mikami, Y.; Fukunaga, Y.; Arita, M.; Obi, Y.; Kisaki, T. Preparation of aroma compounds by microbial transformation of isophorone with *Aspergillus niger*. *Agric. Biol. Chem.* **1981**, *45*, 791-793.

Mizukami, H.; Shiiba, K.; Ohashi, H. Enzymatic determination of stevioside in *Stevia rebaudiana*. *Phytochem.* **1982**, *21*, 1927-1930.

Moret, I.; Capodaglio, G.; Scarponi, G.; Romanazzi, M. Statistical evaluation of the group structures of five Venetian wines from chemical measurements. *Anal. Chim. Acta* **1986,** *191,* 331-350.

Morris, J.R.; Cawthorn, D.L. Effect of soil depth and in-row vine spacing on yield and juice quality in a mature Concord vineyard. *Proc. Amer. Hort. Sci.* **1981**, *106*, 318-320.

Muller, C.J.; Kepner, R.E.; Webb, A.D. Lactones in wines - a review. *Am. J. Enol. Vitic.* **1973,** *24*, 5-9.

Nijssen, L.J.; Maarse, H. Volatile compounds in blackcurrant products. *Flav. Fragrance* **1986**, *1*, 143-148.

Nitz, S.; Fischer, N.; Drawert, F. Uber gebundene aromastiffe in planzen I. Mitt.: Fluchtige terpenverbindungen aus gebundenen vorstufen in Marjorana *Hortensis Moech. Chem. Mikrobiol. Technol. Lebensm.* **1985**, *9*, 87-94.

Noble, A.C. Analysis of wine sensory properties. In: *Modern Methods of Plant Analysis.Vol 6, Wine Analysis.* Jackson, J.F.; Linskens, H.F. Eds., Springer-Verlag, Berlin; **1988**, 9-28.

Noble, A.C.; Murakami, A.A.; Coope, G.F. Reproducibility of headspace analysis of wines. *J. Agric. Food Chem.* **1979,** *27*, 450-452.

Noble, A.C.; Arnold, R.A.; Masuda, B.M.; Pecore, S.D.; Schmidt, J.O.; Stern, P.M. Progress towards a standardized system of wine aroma terminology.

Am. J. Enol. Vitic. **1984a**, *35*, 107-109.

Noble, A.C.; Williams, A.A.; Langron, S.P. Descriptive analysis and quality ratings of 1976 wines from four Bordeaux communes. *J. Sci. Food Agric.* **1984b**, *35*, 88-98.

Noble, A.C.; Shannon, M. Profiling Zinfandel wines by sensory and chemical analysis. *Am. J. Enol. Vitic.* **1987**, *38*, 1-5.

Noble, A.C.; Arnold, R.A.; Buechsenstein, J.; Leach, E.J.; Schmidt, J.O.; Stern, P.M. Modification of a standardised system of wine aroma terminology. *Am. J. Enol. Vitic.* **1987a**, *38*, 143-145.

Noble, A.C.; Strauss, C.R.; Williams, P.J.; Wilson, B. Sensory evaluation of non-volatile flavor precursors in wine. In: *Flavour Science and Technology: Proceedings of the Fifth Weurman Flavour Research Symposium.* Martens, M.; Dalen, G.A.; Russwurm Jr., H. Eds., John Wiley and Sons, New York, **1987b**, 383-390.

Noble, A.C.; Ohkubo, T. Evaluation of flavour of Californian Chardonnay wines. In: *Proceedings of the International Symposium on the Aromatic Substances in Grapes and Wines.* Scienza, A.; Versini, G. Eds., 25-27 June 1987, S. Michele all'Adige, Italy; **1989**, 361-370.

Ohkubo, T.; Noble, A.C.; Ough, C.S. Evaluation of Californian Chardonnay wines by sensory and chemical analysis. *Sci. Aliment.* **1987**, *7*, 573-587.

Ohloff, G. Importance of minor components in flavors and fragrances. *Perfumer Flavorist* **1978a**, *3*, 11-22.

Ohloff, G. Recent developments in the field of naturally-occurring aroma components. *Fortsch. Chem. Org. Naturst.* **1978b**, *35*, 431-527.

Ohloff, G. In: *Progress in the chemistry of organic natural products, vol 35.* Herz, W; Grisebach, H.; Kirby, G.W. Eds., Springer-Verlag, New York, **1978c.**

O'Mahoney, M. Some assumptions and difficulties with common statistics for sensory analysis. *Food Technol.* **1982**, 75-82.

O'Mahoney, M. *Sensory evaluation of food. Statistical methods and procedures.* Tannenbaum, S.R.; Walstra, P. Eds., Marcel Dekker Inc., New York, **1986.**

Ough, C.S.; Singleton, V.L. Wine quality prediction from juice °Brix/acid ratio and associated compositional changes for White Riesling and Cabernet Sauvignon. *Am. J. Enol. Vitic.* **1968**, 129-138.

Ough, C.S.; Alley, C.J. Effect of Thompson Seedless grape maturity on wine composition and quality. *Am. J. Enol. Vitic.* **1970**, *21*, 78-84.

Pabst, A.; Barron, D.; Etiévant, P.; Schreier, P. Studies on the enzymatic hydrolysis of bound aroma constituents from raspberry fruit pulp. *J. Agric. Food Chem.* **1991**, *39*, 173-175.

Peynaud, E. *Knowing and making wine.* John Wiley and Sons, New York, New York, **1984.**

Peynaud, E. *The taste of wine. The art and science of wine appreciation.* Macdonald & Co., London, England, **1987.**

Pigott, S. German wines: Class Distinction. *Decanter* **1988**, *August*, 45-47.

Poll, L.; Lewis, M.J. Volatile components of elderberry juice. *L. Wiss. Unters. Technol.* **1986**, *3*, 258-262.

Pongracz, D.P. *Practical Viticulture.* David Philip, Publisher; Claremont, Cape Provence; South Africa, **1978.**

Pyysalo, T.; Suihko, M.; Honkanen, E. Odour thresholds of the major volatiles identified in cloudberry *(Rubus chamaemorus L.)* and artic bramble *(Rubus Articus L.). L. Wiss. Unters. Technol.* **1977**, *10*, 36-39.

Ramaswami, S.K.; Briscese, P.; Garguillo, R.J.; von Geldern, T. Sesquiterpene hydrocarbons: from mass confusion to orderly line-up. In: *Flavours and Fragrances: A World Perspective.* Proceedings of the 10th International Congress of Essential Oils, Fragrances and Flavours, Lawrence, B.M.; Mookherjee, B.D.; Willis, B.J. Eds., 16-20 November 1986, Washington, DC, USA, **1986,** 951-980.

Rankine, B.C.; Fornachon, J.C.M.; Bridson, D.A. Diacetyl in Australian dry red wines and its significance to wine quality. *Vitis* **1969**, *8*, 129-134.

Rankine, B. *Making good wine.* Macmillan, Melbourne, Australia, **1989.**

Rankine, B.C.; Pocock, K.F. ß-Phenethanol and n-hexanol in wines: influence of yeast strain, grape variety and other factors; and taste thresholds. *Vitis* **1969**, *8*, 23-37.

Rapp, A. Wine aroma substances from gas chromatographic analysis. In: *Modern methods of plant analysis. Wine analysis.* Linskens, H.F.; Jackson, J.F. Eds., Springer-Verlag, Berlin, Vol. 6, **1988a**, 29-66.

Rapp, A. Studies on terpene compounds in wines. In: *Frontiers of Flavour, Proceedings of the Fifth International Flavour Conference.* Charalambous, G. Ed., 1-3 July, 1987, Porto Karras, Chalkidiki, Greece; Elsevier Science, Amsterdam, **1988b**, 799-813.

Rapp, A.; Knipser, W. A new method for the enrichment of headspace components using wine as an example. *Chromatographia,* **1980,** *13,* 698-702.

Rapp, A.; Knipser, W.; Engel, W. Identifizierung von 3,7-dimethyl-okta-1,7-dien-3,6-diol im trauben und weinaroma von muscatsorten. *Vitis* **1980,** *19,* 226-229.

Rapp, A.; Knipser, W.; Hastrich, H.; Engel, W. Possibilities of characterizing wine quality and vine varieties by means of capillary chromatography. In: *Proceedings of the Grape and Wine Centennial Symposium,* Webb, A.D. Ed., 18-21 June,1980, Davis CA, University of California, **1982,** 304-316.

Rapp, A.; Mandery, H.; Ullemeyer, H. 3,7-Dimethyl-1,7-octandiol-eine neue Terpen-komponente des trauben-und wein aromas. *Vitis* **1983,** *22,* 225-230.

Rapp, A.; Mandery, H.; Güntert, M. Terpene compounds in wine. In : *Proceedings of the Alko Symposium on Flavour Research of Alcoholic Beverages,* Nykänen, L.; Lehtonen, P. Eds., Helsinki, 1984, Foundation for Biochemical and Industrial research, **1984,** *3,* 255-274.

Razungles, A.; Bayonove C.L.; Cordonnier, R.E.; Baumes, R.L. Etude des caroténoides du raisin à maturité. *Vitis* **1987,** *26,* 183-191.

Razungles, A.; Bayonove C.L.; Cordonnier, R.E.; Sapis, J.C. Grape carotenoids: changes during the maturation period and localization in mature berries. *Am. J. Enol. Vitic.* **1988,** *39,* 44-48.

Ribéreau-Gayon, P.; Boidron, J.N.; Terrier, A. Aroma of Muscat grape varieties. *J. Agric. Food Chem.* **1975,** *23,* 1042-1047.

Ribéreau-Gayon, J.; Peynaud, E.; Ribéreau-Gayon, P.; Sudraud, P. *Traité d'oenologie: Sciences et techniques du vin, tome 2, Caractères des vins, Maturation du Raisin, Levures et Bactéries.* Bordas, Paris, **1975.**

Ribéreau-Gayon, P.; Glories, Y. Structure of condensed phenolic compounds in *vinifera* grapes and wine. Influence of ripening and infection by *Botrytis cinerea* on phenolic content. Grape and wine centennial: symposium proceedings; 18-21 June 1980; Univerisity of California, Davis. California, Davis: University of California; **1982**

Ribéreau-Gayon, P.; Glories, Y. Phenolics in grapes. In: *Proceedings of the Sixth Australian Wine Industry Technical Conference.* Lee T.H., Ed., 14-17 July, 1986, Adelaide, South Australia, Australian Industrial Publishers, Adelaide, South Australia, **1987,** 247-256.

Robichaud, J.L.; Noble, A.C. Astringency and bitterness of selected phenolics in wine. *J. Sci. Food Agric.* **1990,** *53,* 343-353.

Roessler, E.B.; Pangborn, R.M.; Sidel, J.L.; Stone, H. Expanded statistical tables for estimating significance in paired-preference, paired-difference, duo-trio and triangle tests. *J. Food Sci.* **1978,** *43*, 940-947.

Rothe, M.; Wolm, G.; Tunger, L.; Siebert, H.J. Schwellenkonzentrationen von aromastoffen und ihre nutzung sur auswertung von aromaanalysen. *Nahrung* **1972,** *16*, 483-495.

Salles, C.; Essaied, H.; Chalier, P.; Jallageas, J.C.; Crouzet, J. Evidence and characterisation of glycosidically bound volatile components in fruits. In: *Bioflavour 87;* Schreier, P. Ed., de Gruyter: Berlin, Germany, **1988,** 145-160.

Schlich, P.; Issanchou, S.; Guichard, E.; Etiévant, P.; Adda, J. RV coefficient: A new approach to select variables in PCA and to get correlations between sensory and instrumental data. In: *Flavour Science and Technology.* Proceedings of the 5th Weurman Flavour Research Symposium, Oslo, 23-25 March, 1987, Martens, M.; Dalen, G.A.; Russwurm, H.Jr. Eds., John Wiley and Sons, **1987**, 469-474.

Schreier, P.; Drawert, F. Kerenyi, Z.; Junker, A. Gas chromatographic-mass spectrometric study of the volatile components of wines. VI. Aroma compounds of Tokaj Aszu wines. a). Neutral compounds. *Z. Lebensm. Unters. Forsch.* **1976,** *161*, 249-258.

Schreier, P. Flavour composition of wines: A review. *CRC Crit. Rev. Sci. Nutr.* **1979**, 59-111.

Schreier, P.; Drawert, F.; Junker, A. Identification of volatile constituents from grapes. *J. Agric. Food Chem.* **1976a,** *24*, 331-336.

Schreier, P.; Drawert, F.; Junker, A. Sesquiterpen-kohlenwasserstoffe in trauben. *Z. Lebensm. Unters.-Forsch.* **1976b,** *160*, 271-274.

Schulte-Elte, K.H.; Müller, B.L.; Ohloff, G. Die farbstoffsensibilisierte photo-oxygenierung von ß-damascol. Ein einfaches verfahren zur darstellung von ß-damascenon. *Helv. Chim. Acta* **1971**, *54*, 1899-1910.

Schumacher, J.N.; Green, C.R.; Best, F.B.; Newell, M.P. Smoke composition. An extensive investigation of the water-soluble portion of cigarette smoke. *J. Agric. Food Chem.* **1977**, *25*, 310-319.

Schwab, W; Schreier, P. Glycosidic conjugates of aliphatic alcohols from apple fruit (*Malus sylvestris* Mill cv. Jonathan). *J. Agric. Food Chem.* **1990a**, *38*, 757-763.

Schwab, W; Schreier, P. Vomifoliol 1-0-ß-D-xylopyranosyl-6-0-ß-D-glucopyranoside: a disaccharide glycoside from apple fruit. *Phytochem.* **1990b**, *29*, 161-164.

Sefton, M.A.; Skouroumounis, G.K.; Massy-Westropp, R.A.; Williams, P.J. Norisoprenoids in *Vitis vinifera* white wine grapes and the identification of a precursor of damascenone in these fruits. *Aust J. Chem.* **1989**, *42*, 2071-2084.

Sefton, M.A.; Williams, P.J. The generation of oxidation artefacts during the hydrolysis of norisoprenoid glycosides by fungal enzyme preparations. *J. Agric. Food Chem.* **1991**, *39*, 1994-1997.

Sefton, M.A.; Francis, I.L.; Williams, P.J. The volatile composition of Chardonnay juices and their hydrolysates. Am. J. Enol. Vitic. **1991**, (submitted).

Shaulis, N.; Kimball, K. Effect of plant spacing on growth and yield of Concord grapes. *Proc. Amer. Hort. Sci.* **1955**, *66*, 192-200.

Shaulis, N.; Amberg, H.; Crowe, D. Response of Concord grapes to light, exposure and Geneva Double curtain training. *Proc. Amer. Hort. Sci.* **1966**, *89*, 268-280.

Shaw, G.J.; Allen, J.M.; Yates, M.K. Volatile flavour constituents of Feijoa (*Feijoa sellowiana*) - analysis of fruit flesh. *J. Sci. Food Agric.* **1990**, *50*, 357-361.

Shortreed, G.W.; Rickard, P.; Swan, J.S.; Burtles, S.M. The flavour terminology of Scotch Whiskey, *Brewer's Guardian*, **1979**, *11*, 55-62.

Siegel, O.; Tatter, I. Spectralanalytische interschung von traubenmost und boden. *Vitis* **1961**, *2*, 283-287.

Siek, T.J.; Albin, I.A.; Sather, L.A.; Lindsay, R.C. Comparison of flavour thresholds of aliphatic lactones with those of fatty acids, esters, aldehydes, alcohols and ketones. *J. Dairy Sci.* **1971**, *54*, 1-4.

Simpson, R.F. 1,1,6-Trimethyl-1,2-dihydronaphthalene: an important contributor to the bottle aged bouquet of wine. *Chem. Ind.* **1978**, *1*, 37.

Simpson, R.F. Aroma composition of bottle aged wine. *Vitis* **1979**, *18*, 149-154.

Simpson, R.F.; Miller, G.C. Aroma composition of aged Riesling wine. *Vitis* **1983**, *22*, 51-63.

Simpson, R.F.; Miller, G.C. Aroma composition of Chardonnay wine. *Vitis* **1984**, *23*, 143-158.

Simpson, R.F.; Strauss, C.R.; Williams, P.J. Vitispirane: A C_{13} spiro-ether in the aroma volatiles of grape juice, wines and distilled grape spirits. *Chem. Ind.* **1977**, 663-664.

Singleton, V.L. Grape and wine phenolics, background and prospects. In:

Proceedings of the Grape and Wine Centennial Symposium, Webb, A.D. Ed., 18-21 June,1980, Davis CA, University of California, **1982,** 47-70.

Singleton, V.L. Oxygen with phenols and related reactions in musts, wines, and model systems: observations and practical implications. *Am. J. Enol. Vitic.* **1987,** *38*, 69-77

Singleton, V.L.; Noble, A.C. Wine flavour and phenolic substances. In: *Phenolics, sulphur and nitrogen compounds in food flavours*. a symposium of the American Chemical Society, ACS symposium series 26, **1976,** 49-70.

Sinton, T.H.; Ough, C.S.; Kissler, J.J.; Kasimatis, A.N. Grape juice indicators for prediction of potential wine quality. 1. Relationship between crop level, juice and wine composition and wine sensory ratings and scores. *Am. J. Enol. Vitic.* **1978,** *29*, 267-271.

Smart, R.; Robinson, M. *Sunlight into wine: A handbook for winegrape canopy management*. Ministery of Agriculture and Fisheries, New Zealand, Winetitles, Australian Industrial Publishers, South Australia, Australia, **1991.**

Smart, R.E.; Robinson, J.B.; Due, G.R.; Brien, C.J. Canopy microclimate modification for the cultivar Shiraz. 1. Definition of canopy microclimate. *Vitis* **1985a**, *24*, 17-31.

Smart, R.E.; Robinson, J.B.; Due, G.R.; Brien, C.J. Canopy microclimate modification for the cultivar Shiraz. 2. Effects on must and wine composition. *Vitis* **1985b**, *24*, 119-128.

Somers, T.C. Grape phenolics: the anthocyanins of *Vitis vinifera*, variety Shiraz. *J. Sci. Food Agric.* **1966**, *17*, 215-219.

Somers, T.C. Pigments and tannins of wine. *Aust. Wine Brew. Spirit Rev.* **1967,** *85*, 38-40.

Somers, T.C. The polymeric nature of wine pigments. *Phytochem.* **1971**, *10,* 2175-2186.

Somers, T.C. In search of quality for red wines. *Food Technol. Aust.* **1975,** *27,* 49-56.

Somers, T.C. Anatomy of a young red wine. In: *Proceedings of the Third Australian Wine Industry Technical Conference*, Albury; 9-11 August 1977. Adelaide SA: The Australian Wine Research Institute. **1977**, 56-59.

Somers, T.C.; Evans, M.E. Wine quality: correlations with colour density and anthocyanin equilibria in a group of young red wines. *J. Sci. Food Agric.* **1974,** *25*, 1369-1379.

Somers, T.C.; Evans, M.E. Spectral evaluation of young red wines: Anthocyanin equilibria, total phenolics, free and molecular SO_2, "Chemical age". *J. Sci. Food. Agric.* **1977,** *28*, 279-287.

Somers, T.C.; Verette, E. Phenolic composition of natural wine types. Linskens, H.F.; Jackson, J.F. Eds.; Wine Analysis. Berlin: Springer-Verlag; **1988**, 267-272.

Somers, T.C.; Ziemelis, G. Spectral evaluation of total phenolic components in *Vitis vinifera*: grapes and wine. *J. Sci. Food Agric.* **1985**, *146*, 1275-1284.

Stahl, W.H. Ed. *Compilation of odor and taste threshold values data.* Mc Cormick and Co., Baltimore, **1973.**

Stone, H.; Sidel, J.L. *Sensory evaluation practices.* Academic Press, Orlando, Florida, **1985.**

Strauss, C.R.; Williams, P.J.; Wilson, B.; Dimitriadis, E. Formation and identification of aroma compounds from non-volatile precursors in grapes and wine. In: *Flavour research of alcoholic beverages. Proceedings of the Alko symposium.* Nykänen,L.; Lehtonen, P. Eds., Helsinki, 1984, Foundation for Biotechnical and Industrial Fermentation Research **1984,** *3,* 51-60.

Strauss, C.R.; Dimitriadis, E.; Wilson, B.; Williams, P.J. Studies on the hydrolysis of two megastigma-3,6,9-triols rationalizing the origins of some volatile C13 norisoprenoids of *Vitis vinifera* grapes. *J. Agric. Food Chem.* **1986a**, *34*, 145-149.

Strauss, C.R.; Wilson, B.; Gooley, P.R.; Williams, P.J. Role of monoterpenes in grape and wine flavour. In: *Biogeneration of aromas,* a symposium sponsored by the Division of Agriculture and Food Chemistry at the 190th meeting of the American Chemical Society; Parliment, T.H.; Croteau, R. Eds., 8-13 September 1985; Chicago, Illinois, ACS symposium series 317, American Chemical Society, Washington, DC, **1986b**, 222-242.

Strauss, C.R.; Wilson, B.; Anderson, R.; Williams, P.J. Development of precursors of C_{13} nor-isoprenoid flavorants in Riesling grapes. *Am. J. Enol. Vitic.* **1987a**, *38*, 23-27.

Strauss, C.R.; Gooley, P.R.; Wilson, B.; Williams, P.J. Application of droplet countercurrent chromatography to the analysis of conjugated forms of terpenoids, phenols, and other constituents of grape juice. *J. Agric. Food Chem.* **1987b**, *35*, 519-524.

Strauss, C.R.; Wilson, B.; Williams, P.J. Novel monoterpene diols and diol glycosides in *Vitis vinifera* grapes. *J. Agric. Food Chem.* **1988**, *36*, 569-573.

Symonds, P.; Cantagrel, R. Application d'analyse discriminante à la

différentiation des vins. *Ann. Fals. Exp. Chim.* **1982**, *75*, 63-74.

Takagi, Y.; Fujimori, T.; Kaneko, H.; Fukuzumi, T.; Noguchi, M. Isolation of a new tobacco constituent, (3S, 5R, 6S, 9ϵ)-3-Hydroxy-5,6-epoxy-ß-ionol, from Japanese domestic SUIFU tobacco. *Agric. Biol. Chem.* **1978**, *42*, 1785-1787.

Tang, J.; Zhang, Y.; Hartmen, T.G.; Rosen, R.T.; Ho, C-T. Free and glycosidically bound volatile compounds in fresh celery (*Apium graveolens* L.). *J. Agric. Food Chem* **1990**, *36*, 1937-1940.

Terrier, A.; Boidron, J.N.; Ribéreau-Gayon, P. Teneurs en composés terpéniques dans les raisins de *Vitis vinifera*. C.R. Acad. Sci. Ser. D. **1972**, *275*, 941-944.

Timberlake, C.F.; Bridle,P.; Jackson, M.G.; Vallis, L. Correlations between quality and pigment parameters in young Beaujolais red wines. *Ann. Nutr. Aliment.* **1978**, *32*, 1095-1101.

Turkington, C.R.; Peterson, J.R.; Evans, J.C. A spacing, trellising, and pruning experiment with Muscat Gordo Blanco grapevines. *Am. J. Enol. Vitic.* **1980**, *31*, 298-302.

Uegaki, R.; Fujimori, T.; Kaneko, H.; Kato, K.; Noguchi, M. Isolation of dehydrololiolide and 3-oxo-actinidol from *Nicotiana tabacum*. *Agric. Biol. Chem.* **1979**, *43*, 1149-1150.

Vérette, E.; Noble, A.C.; Somers, T.C. Hydroxycinnamates of *Vitis vinifera*: Sensory assessment in relation to bitterness in white wines. *J. Sci. Food Agric.* **1988**, *45*, 267-272.

Vernin, G.; Boniface, C.; Metzger, J.; Fraisse, D.; Doan, D.; Alamercery, S. Aromas of Syrah wines: identification of volatile compounds by GC-MS spectra data bank and classification by statistical methods. In: *Proceedings of the Fifth International Flavour Conference,* Charalambous, G.; Ed., 1-3 July, 1987, Chalkidiki, Greece, Elsevier Science, Amsterdam, The Netherlands, **1988**, 655-685.

Vie, A.; Gulli, O.; O'Mahoney, M. Alternative hedonic measures. *J. Food Sci.* **1991**, *56*, 1-5.

Voirin, S.G.; Baumes, R.L.; Bitteur, S.M., Günata, Z.; Bayonove, C.L. Novel monoterpene disaccharide glycosides of *Vitis vinifera* grapes. *J. Agric. Food Chem.* **1990**, *38*, 1373-1378.

Voirin, S.G. Connaissance de l'arome du raisin: analyses et syntheses de precurseurs heterosidiques. University of Montpellier, Montpellier, France, **1990**.

Wahlberg, I.; Karlson, K.; Austin, D.J.; Junker, N.; Roeraade, J.; Enzell,

C.R.; Johnson, W.H. Effects of flue-curing and aging on the volatile, neutral and acidic constituents of Virginia tobacco. *Phytochem.* **1977**, *16*, 1217-1231.

Wahlberg, I.; Enzell, C.R. Tobacco isoprenoids. *Natural Product Reports* **1987**, 237-276.

Wall, P. Yalumba's grape quality assessment system in raising wine quality. *Aust. Grapegrower Winemaker* **1986**, 20-22.

Wasserman, A.E. Organoleptic evaluation of three phenols present in woodsmoke. *J. Food Sci.* **1966**, *31*, 1005-1010.

Weeks, W.W. Carotenoids: a source of flavour and aroma. In: *Biogeneration of aromas,* a symposium sponsored by the Division of Agriculture and Food Chemistry at the 190th meeting of the American Chemical Society; Parliment, T.H.; Croteau, R. Eds., 8-13 September 1985; Chicago, Illinois. ACS symposium series 317; American Chemical Society: Washington, DC, **1986**, 157-166.

de Wet, P. Odour thresholds and their application to wine flavour studies. Proceedings of the 2nd congress of the South African Society of Enology and Viticulture, Cape Town, October 3-4, **1978**, 28-42.

Williams, A.A. Recent developments in the field of wine flavour research. *J. Inst. Brew.* **1982**, *88*, 43-53.

Williams, D.H.; Stone, M.J.; Hauck, P.R.; Rahman, S. K. Why are secondary metabolites (natural products) biosynthesized? *J. Nat. Prod.* **1989**, *52*, 1189-1208.

Williams, P.J.; Strauss, C.R. Apparatus and procedure for reproducible, high-resolution gas chromatographic analysis of alcoholic beverage headspace volatiles. *J. Inst. Brew.* **1977**, *83*, 213-219.

Williams, P.J.; Strauss, C.R. The influence of film yeast activity on the aroma volatiles of flor sherries - a study of volatiles isolated by headspace sampling. *J. Inst. Brew.* **1978**, *84*, 148-152.

Williams, P.J.; Strauss, C.R.; Wilson, B. Hydroxylated linalool derivatives as precursors of volatile monoterpenes of Muscat grapes. *J. Agric. Food Chem.* **1980**, *28*, 766-771.

Williams, P.J.; Strauss, C.R.; Wilson, B; Massy-Westropp, R.A. Novel monoterpene disaccharide glycosides of *Vitis vinifera* grapes and wines. *Phytochem.* **1982a**, *21*, 2013-2020.

Williams, P.J.; Strauss, C.R.; Wilson, B; Massy-Westropp, R.A. Use of C_{18} reversed-phase liquid chromatography for the isolation of monoterpene glycosides and norisoprenoid precursors from grape juice and wines. *J. Chromatog.* **1982b**,

235, 471-480.

Williams, P.J.; Strauss, C.R.; Wilson, B.; Massy-Westropp, R.A. Glycosides of 2-phenethanol and benzyl alcohol in *Vitis vinifera* grapes. *Phytochem.* **1983**, *22*, 2039-2041.

Williams, P.J.; Strauss, C.R.; Wilson, B.; Dimitriadis, E. Recent studies into grape terpene glycosides. In: *Proceedings of the Fourth Weurman Flavour Research Symposium, Progress in Flavour Research,* Adda, J. Ed.; 9-11 May 1984, Dourdan, France; Elsevier Science: Amsterdam, **1985,** 349-357.

Williams, P.J.; Strauss, C.R.; Aryan, A.P; Wilson, B. Grape flavour- a review of some pre and post harvest influences. In: *Proceedings of the Sixth Australian Wine Industry Technical Conference.* Lee. T.H. Ed., 14-17 July, 1986, Adelaide, South Australia, Australian Industrial Publishers, Adelaide, **1987**, 111-116.

Williams, P.J.; Sefton, M.A.; Wilson, B. Nonvolatile conjugates of secondary metabolites as precursors of varietal grape flavor components. In: *Flavor Chemistry: Trends and Developments.* Teranishi, R.; Buttery, R.G.; Schahidi, F. Eds., ACS symposium series 388. American Chemical Society, Washington, DC; **1989**, 35-38.

Williams P.J. Hydrolytic flavour release from non-volatile flavour precursors. In: *Flavour Research Workshop.* Acree, T. Ed.; ACS Professional Reference Book Series; **1991**, in press.

Williams, P.J; Sefton, M.A.; Francis, I.L Glycosidic precursors of varietal grape and wine flavour. In: *Thermal and Enzymatic Conversions of Precursors to Flavour Compounds.* ACS Symposium series, **1991,** in press.

Wilson, B.; Strauss, C.R.; Williams, P.J. Changes in free and glycosidically bound monoterpenes in developing Muscat grapes. *J. Agric. Food Chem.* **1984**, *32*, 919-924.

Winkler, A.J.; Cook, J.A., Kliewer, W.M.; Lider, L.A. *General Viticulture.* University of California Press, Los Angeles, California, **1974.**

Winter, M.; Schulte-Elte, K.H.; Velluz, A.; Limacher, J.; Pickenhagen, W.; Ohloff, G. Aromastoffe der roten passionsfrucht. Zwei neue edulanderivate. *Helv. Chim. Acta* **1979**, *62*, 131-134.

Winterhalter, P.; Schreier, P. Free and bound norisoprenoids in Quince (*Cydonia oblonga* Mill.) *J. Agric. Food Chem.* **1988a**, *36*, 1251-1256.

Winterhalter, P.; Schreier, P. Studies on C_{13} nor-isoprenoid precursors. In: *Bioflavour '87.* Walter de Gruyter, Berlin, Germany, **1988b**, 255-273.

Winterhalter, P. Bound terpenoids in the juice of the purple passion fruit

(*Passiflora edulis* Sims.) *J. Agric. Food Chem.* **1990,** *38*, 452-455.

Winterhalter, P; Sefton, M.A.; Williams, P.J. Two-dimensional GC-DCCC analysis of the glycoconjugates of monoterpenes, norisoprenoids and shikimate derived metabolites from Riesling wine. *J. Agric. Food Chem.* **1990**, *38*, 1041-1048.

Wu, P.; Kuo, M-C.; Ho, C-T. Glycosidically bound aroma compounds in ginger (*Zingiber officinale Roscoe*). *J. Agric. Food Chem.* **1990,** *38*, 1553-1555.

Wu, P.; Kuo, M-C.; Hartmen, T.G.; Rosen, R.T.; Ho, C-T. Free and glycosidically bound aroma compounds in pineapple (*Ananas comosus* L. Merr.). *J. Agric. Food Chem.* **1991,** *39*, 170-172.

Zook, K.; Wessman, C. The selection and use of judges for descriptive panels. *Food Technol.* **1977**, 56-61.

APPENDIX

Appendix A.1 Concentration (ug/L) of the enzyme-released products used to measure the reproducibility of the analysis

No*	RT	Rep 1	Rep 2	Rep 3	Mean	*Stdev*	CV %
	962	44.0	44.0	44.0	**44.0**	*0.0*	**0.0**
5	1304	21.9	20.8	22.2	**21.6**	*0.8*	**3.5**
179	1413	1.1	1.1	1.2	**1.2**	*0.1*	**4.6**
154	1937	5.7	5.7	6.1	**5.8**	*0.3*	**4.6**
	3157	8.3	8.6	9.1	**8.6**	*0.4*	**4.9**
19	1975	5.6	5.3	5.9	**5.6**	*0.3*	**5.3**
	4306	4.4	4.2	4.7	**4.4**	*0.2*	**5.3**
	3080	1.6	1.5	1.6	**1.6**	*0.1*	**5.6**
20	2024	40.4	36.5	40.9	**39.3**	*2.4*	**6.2**
12	1397	11.5	12.7	11.3	**11.8**	*0.8*	**6.7**
131	943	14.0	15.5	13.3	**14.3**	*1.1*	**7.8**
36	3188	9.0	7.8	8.0	**8.3**	*0.7*	**7.9**
6	1059	47.7	48.3	41.5	**45.8**	*3.8*	**8.2**
	1784	1.0	0.9	0.9	**0.9**	*0.1*	**10.5**
	339	185.9	220.0	180.7	**195.5**	*21.3*	**10.9**
30	2318	20.4	16.2	19.6	**18.7**	*2.2*	**11.8**
161	2879	3.0	3.9	3.8	**3.6**	*0.5*	**14.1**
130	873	3.0	4.0	3.8	**3.6**	*0.5*	**14.3**
93	2612	2.7	2.0	2.8	**2.5**	*0.4*	**16.4**
33	3024	28.6	26.0	20.4	**25.0**	*4.2*	**16.8**
28	2809	25.2	20.9	29.4	**25.2**	*4.2*	**16.8**
42	3387	54.1	48.2	67.3	**56.6**	*9.8*	**17.3**
113	4222	10.4	7.3	8.9	**8.8**	*1.6*	**17.6**
46	3274	7.4	5.6	8.0	**7.0**	*1.3*	**17.9**
51	4342	5.7	8.3	7.9	**7.3**	*1.4*	**18.7**
	712	98.4	111.7	75.2	**95.1**	*18.5*	**19.4**
21	2269	32.4	22.9	33.2	**29.5**	*5.7*	**19.5**
	2048	55.6	36.7	49.1	**47.1**	*9.6*	**20.3**
	2423	13.4	8.9	12.5	**11.6**	*2.4*	**20.8**
	4145	31.3	20.4	27.1	**26.3**	*5.5*	**20.9**
	2625	4.1	4.4	6.0	**4.9**	*1.0*	**20.9**
	3405	13.9	9.0	12.4	**11.8**	*2.5*	**21.6**
	3798	7.2	4.5	6.0	**5.9**	*1.3*	**22.8**
	3642	20.3	19.4	29.7	**23.1**	*5.7*	**24.6**
	2943	6.6	4.0	6.1	**5.6**	*1.4*	**24.7**
	1095	0.6	1.0	0.9	**0.8**	*0.2*	**26.4**
	2777	2.6	1.5	1.7	**1.9**	*0.6*	**28.8**
	2710	5.4	7.1	9.7	**7.4**	*2.1*	**28.8**
	1133	0.7	1.2	0.9	**0.9**	*0.3*	**28.9**
	3264	8.7	4.7	6.9	**6.8**	*2.0*	**29.6**
	3460	12.7	7.7	15.1	**11.9**	*3.8*	**31.9**
	2236	1.5	2.9	3.0	**2.5**	*0.8*	**32.9**
	3232	11.7	6.1	13.6	**10.4**	*3.9*	**37.2**
	1656	26.7	11.6	15.6	**17.9**	*7.8*	**43.7**
	3543	2.2	2.6	4.8	**3.2**	*1.4*	**44.1**
	1083	1.2	3.2	2.9	**2.5**	*1.1*	**44.7**
	4154	5.8	2.5	3.2	**3.8**	*1.7*	**45.4**
	4116	27.5	19.0	9.5	**18.7**	*9.0*	**48.3**
	2678	0.6	2.1	2.8	**1.8**	*1.2*	**63.8**
	3179	8.8	1.6	14.7	**8.4**	*6.6*	**78.8**
	1485	1.1	1.4	5.5	**2.7**	*2.5*	**92.4**
	2521	12.4	1.2	1.5	**5.0**	*6.4*	**127.4**
	#REF!						
		908.4	**827.4**	**864.3**	**866.7**	*40.5*	**4.7**

*Number referred to in Chapter 5, Tables 1 to 5

Appendix A.2 Concentration (ug/L) of the acid-released products used to measure the reproducibility of the analysis

		Concentration (ppb)					
No.*	**RT**	Rep 1	Rep 2	Rep 3	**Mean**	***Stdev***	**CV%**
80	2216	3.4		3.4	**3.4**	***0.01***	**0.24**
144	1738	59.8	61.1	59.6	**60.2**	***0.83***	**1.38**
136	1349	3.5		3.5	**3.5**	***0.05***	**1.49**
75	2059	89.4	90.7	87.2	**89.1**	***1.75***	**1.96**
93	3488	18.1		18.8	**18.4**	***0.52***	**2.83**
82	2332	13.3	13.4	14.1	**13.6**	***0.42***	**3.06**
89	2475	81.1	76.1	78.5	**78.6**	***2.50***	**3.19**
25	2447	18.9	20.3	20.1	**19.8**	***0.76***	**3.83**
98	2749	23.8	25.1	**23.1**	**24.0**	***1.04***	**4.33**
175	1892	11.1	10.1	11.4	**10.9**	***0.66***	**6.07**
163	1905	7.8	7.2		**7.5**	***0.46***	**6.12**
138	1374	3.8		3.4	**3.6**	***0.28***	**7.67**
121	2505	7.0	7.8		**7.4**	***0.57***	**7.72**
31	2809	24.3	21.1	21.5	**22.3**	***1.78***	**7.97**
105	3013	9.2	11.0	10.4	**10.2**	***0.90***	**8.83**
108	3158	9.1	7.6	8.8	**8.5**	***0.79***	**9.29**
	1596	5.5	4.6	5.3	**5.1**	***0.48***	**9.38**
			23.6	27.0	**25.3**	***2.44***	**9.66**
135	1300	5.0	6.0	5.9	**5.6**	***0.57***	**10.16**
99	2879	64.2	50.1	54.3	**56.2**	***7.24***	**12.89**
74	2046	67.3	53.6	54.2	**58.4**	***7.75***	**13.27**
179	2133	46.0	60.4	51.4	**52.6**	***7.24***	**13.77**
20	1960	43.1	57.4	50.0	**50.2**	***7.13***	**14.22**
	2833	8.6	8.5	6.6	**7.9**	***1.13***	**14.31**
85	2306	10.0	7.5	9.8	**9.1**	***1.41***	**15.53**
	2076	2.8	3.2	3.8	**3.3**	***0.51***	**15.75**
48	3433	6.2	8.4	8.2	**7.6**	***1.21***	**15.94**
130	858	15.2	10.7	13.0	**13.0**	***2.24***	**17.24**
62	3357	6.3		8.2	**7.3**	***1.31***	**17.97**
134	1242	8.7	6.0	8.5	**7.7**	***1.46***	**18.94**
68	1443	55.0	36.1	48.7	**46.6**	***9.63***	**20.66**
28	2675	5.0	7.6	7.5	**6.7**	***1.44***	**21.46**
6	1043	6.9	7.9	5.1	**6.6**	***1.43***	**21.60**
21	1996	39.0	62.7	56.1	**52.6**	***12.24***	**23.28**
141	1608	3.6	5.6	3.9	**4.4**	***1.10***	**25.25**
55	2994	4.5	7.7	6.2	**6.1**	***1.64***	**26.65**
131	923	9.9	17.0	16.9	**14.6**	***4.10***	**28.09**
11	1276	11.8	6.5	9.9	**9.4**	***2.70***	**28.70**
73	2002	11.0	17.7	20.0	**16.2**	***4.70***	**28.96**
92	2486	2.9	5.2	6.4	**4.8**	***1.78***	**36.72**
67	1338	7.5	4.2	9.5	**7.1**	***2.63***	**37.31**
	2140	14.6	31.2	20.3	**22.1**	***8.41***	**38.14**
8	1114	3.7	6.6		**5.2**	***2.01***	**39.04**
	2941	2.8	7.4	6.3	**5.5**	***2.37***	**43.07**
132	1062	2.3		5.3	**3.8**	***2.13***	**55.87**
147	2185	5.7		2.5	**4.1**	***2.30***	**56.02**
154	940	29.3		9.1	**19.2**	***14.29***	**74.44**
43	3275	174.9	62.0	43.8	**93.6**	***71.00***	**75.88**
41	3247	28.4	169.3		**98.8**	***99.65***	**100.83**
		1091.5	1106.3	947.4	**1048.4**	***87.74***	**8.37**

*Number referred to in Chapter 5, Tables 1 to 5

Appendix B. Concentration (ug/L) of the acid hydrolysis products used for the statistical analysis, Chapter 6.

	Hq Bar 89		Hq Bar 88		Lq Bar 89		Lq Bar 88		Lq Cw 88		Lq Cw 89		Hq Cw 88		Hq Cw 89	
	#1	#2	#1	#2	#1	#2	#1	#2	#1	#2	#1	#2	#1	#2	#1	#2
No.*																
Acids																
157	35.02	43.58	41.12	29.99	34.12	23.95	35.65	37.79	13.34	20.01	149.47	23.05	24.71	34.79	9.48	149.5
168	1.08	0.72	3.99	2.22	1.29	1.47	1.72	1.79	2.34	1.02	3.79		2.34	4.83	6.01	3.79
Monoterpenes																
129	1.14	0.72	1.02	2.1	2.28	2.77	1.73	1.64	0.95	0.98			1.02	1.05	1.58	2.67
130	5.82	4.48	10.4	10.95	9.9	8.44	9.06	9.69	5.06	4.5	4.55	3	7.52	5.93	15.44	6.44
131	3.37	3.47	8.29	7.29	5.2	6.06	5.87	4.72	3.02	3.54	5.78	3.55	2.79	4.87	12.64	5.78
132	3.7	2.19	4.85	5.16	4.04	4	5.36	7	5.26	4.89	2.13	1.95	4.36	3.97		4.88
	1.58	1.04	2.01	3.73	1.74	1.89	3.83	3.43	3.03	4.23			1.83	1.8	6.56	1.66
134	2.62	1.75	4.48	5.34	5.96	6	4	5.69	2.03	2.67	3.21	2.74	3.14	2.79	6.19	2.17
135	4.15	3.32	5.79	7.45	7.82	7.93	6.05	9.08	2.64	1.87	1.54	1.76	7.46	4.05	9.92	3.97
136	1.29	1.06	3.45	3.61	2.69	2.79	2.01	2.32	1.27	0.89	0.32		2.14	1.7		1.25
141	31.29	33.29	52	51.2	52.38	44.66	58.66	37.16	22.03	15.23	25.11	30.29	28	37.47	13.47	49.26
Norisoprenoids																
69	1.87	1.93	2.62	3.69	3.78	3.77	3.7	2.21	1.45	3.78			1.91	1.81	3.02	2.06
70	34.15	25.64	58.99	90.98	47.09	50.4	38.77	39.56	18.25	15.22	11.33	12.54	17.89	16.93	35.18	23.29
71	22.92	17.14	32.3	42.21	39.11	41.39	22.91	21.92	7.63	6.37	5.31	12.36	10.06	9.47	24.87	20.85
73	14.75	13.06	14.27	15.84	16.44	16.18	18.86	14.52	6.05	4.99	16.21	7.15	10.58	12.8	14.85	14.1
74	29.42	25.36	51.41	51.9	41.08	37.9	57.96	51.86	16.61	15	20.36	25.16	23.36	26.47	28.11	44.89
75	42.05	35.98	75.95	76.47	61.52	53.67	84.08	73.2	24.41	16.67	55.22	39.52	32.24	38.32	34.52	63.13
78	0.81	0.58	1.04	1	1.42	1.32	0.84	0.96	0.51	1.25			0.44	0.73	1.43	1.79
79	1.18	0.96	1.46	4.26	1.98	3.8		1.41	0.5	0.79			1.23	2.65	1.72	2.45
89	42.78	47.87	70.76	57.67	55.11	44.32	77.05	63.73	20.9	25.67	51.2	48.35	31.6	37.4	23.53	70.77
92	3.1	2.45	4.6	1.75	5.15	6.71	3.35	2.17	1.03	1.1			2.81	4.34	7.58	5.42
	2.21	1.68	5.09	14.01	2.35	2.08	2.94	2.2	0.74	2.56	2.33	3.59			1.33	2.65
	3.57	2.58	7.04	0.23	3.55	3.29			1.89	1.5	4.52	1.26	4.44	3.71	6.09	12.99
97	7.35	8.31	10.62	4.68	9.4	9.04	7.41	6.6	3.04	7.33	6.01	4.17	7.32	3.94	5.19	11.06
105	7.68	4.76	11.19	21.99	5.32	6.57	17.95	6.71	2.82	2.3	29.31	21.46	5.2	7.76	15.03	58.24
121	5.89	5.32	3.82	4.58	8.31	7.99	3.86	1.92	2.52	4.5	4.56	3.18	1.26	7.43	9.71	4.95
123	15.03	15.52		15.5	16.53	13.25	24.31	14.74			22.31	17.34	9.33	7.72	1.65	24.38
Phenolics																
4	3.01	2.2	2.6	5.01	5.66	5.72	6.03	1.78	2.51	2.29	4.56	4.02	5.11	2.51	15.27	4.4
6	2.7	2.52	5.8	10.98	5.34	5.34	7.84	6.75	3.17	3.05	9.89	7.4	6.18	5.07	13.37	10.32
8	1.24	0.85	2.86	4.83	2.61	2.85	0.87	1.03	0.46	0.55			1.62	1.24	2.4	
11	1.73	1.36	6.57	15.73	3.48	3.95	6.72	5.04	2.2	2.02	7.88		4.55	6.77	22.16	8.99
13	4.16	3.08	7.48	11.48	8.53	9.84			1.48	1.56	3.74	1.67	1.83	2.44	3.53	4.27
14	0.91	0.38	1.77	2.17	1.64	2.74	1.37	1.52	0.62	0.44	0.55		0.8		2.27	1.11
21	1.35	1.53	3.08	7.47	1.5	1.38	3.76	2.09	3.29	3.89	6.51	2.61	2.5	4.48	6.73	14.64
25	9.9	12.68	10.44	8.69	12.85	10.29	9.53	5.44	6.04	9.05	8.57		6	11.48	4.24	9.97
28	0.25	0.75	4.13	5.58	0.54	0.47	2.6	1.89	0.62	1.24	3.33	2.54	1.21	1.51	4.84	10.23
29	0.44		2.61	0.5	0.75	0.75	3.84	3.08	0.78	0.99			1.2	1.78	1.9	3.03
30	5.04	6.37	10.28	0.45	8.54	7.52	6.11	2.6	1.44	2.54	3.02	3.1	3.96	8.51	4.26	3.4
43	17.83	21.56	15.97	15.02	18.63	16.06	12.26	7.6	7.48	5.06	10.25	8.54	3.4	19.45		12.05
Unknown																
101	1.65	1.3	2.31	2.26	2.47	2.51	5.56	1.37	2.33	5.49	2.98		3.3	3.77	1.8	2.89
19	4.86	4.85	6.66	4.45	6.46	7.03	8.72	8.4	8.58	6.34	16.52	12.19	7.69	8.49	7.6	16.05
20	3.32	2.35	4.63	4.05	5.03	4.77	8.61	2.64	2.67	5.55	4.44		4.54	3.8	3.23	4.51
37	3.56	3.47	2.56	3.45	7.61	7.6	7.43	1.88	1.18	1.02	1.78	1.66	4.33	5.21	7.35	3.03
39	1.16	0.31	2.87	2.65	0.78	1.24	2.04	0.54	0.88	5.21	0.55		0.69	1.53	1.09	0.7
40	7.71	6.54	14.8	14.67	12.32	11.9	9.41	7.15	3.56	3.44	6.54		7.92	11.57	5.72	5.49
48	15.47	22.68	36.35	18.68	10.18	9.11	11.78	7.91	5.39	1.2	10.36	203.74	13.5	12.08	1.76	11.67
53	9.67	11.38	22.33	1.18			11.78	8.29	5.43	10.5	15.96	11.82	14.14	14.37	9.53	37.7
67	11.61	12.44	12.69	9.16	6.8	7.66	9.55	6.06	8.76	9.99	8.65	9.07	8.96	9.48	8.34	14.76
68	5.59	3.74	2.62	4.51	3.36	2.9	5.34	3.29	3.13	4.51			3.66	3.94	4.76	7.75
70	10.31	14.95	46.72	15.64	8.07	8.29	46.32	50.37	14.59	19.87	6.89	5.23	55.33	28.25	35.36	38.76
71	16.91	12.63	23.33	15.51	10.91	9.85	19.86	13.73	14.05	20.56	26.12	11.49	25.76	11.09	21.93	27.8

*Compound number referred to in Chapter 5, Tables 1 to 5

Appendix C1 Concentration of glycosyl-glucose (G-G) in Barossa Valley juice samples

	Rep	A1	A2	A2-A1 G-G mM									
Hq Bar 90	1	0.182	0.376	0.088	Hq Bar 89	1	0.189	0.331	0.064	Hq Bar 88	1	0.242	0.527	0.129
		0.189	0.369	0.082			0.195	0.355	0.073			0.248	0.537	0.131
		0.188	0.373	0.084			0.201	0.352	0.068			0.243	0.544	0.136
	2	0.194	0.372	0.081		2	0.202	0.333	0.059		2	0.236	0.541	0.138
		0.191	0.370	0.081			0.197	0.355	0.072			0.246	0.543	0.135
		0.199	0.385	0.084			0.202	0.345	0.065			0.249	0.549	0.136
	3	0.193	0.371	0.081		3	0.199	0.341	0.064		3	0.245	0.561	0.143
		0.198	0.394	0.089			0.199	0.326	0.058			0.243	0.546	0.137
		0.189	0.378	0.086			0.203	0.351	0.067			0.245	0.537	0.132
	4	0.198	0.379	0.082		4	0.196	0.327	0.059		4	0.251	0.554	0.137
		0.193	0.372	0.081			0.205	0.352	0.067			0.262	0.567	0.138
		0.196	0.381	0.084			0.203	0.342	0.063			0.253	0.554	0.136
	5	0.195	0.381	0.084		5	0.198	0.328	0.059		5	0.255	0.566	0.141
		0.194	0.372	0.081			0.199	0.346	0.067			0.255	0.567	0.141
		0.193	0.372	0.081			0.201	0.334	0.060			0.245	0.539	0.133
			Mean	**0.419**				**Mean**	**0.314**				**Mean**	**0.714**
			Stdev	***0.015***				***Stdev***	***0.026***				***Stdev***	***0.022***
Hq Bar 90	1	0.209	0.386	0.080						Hq Bar 88	1	0.243	0.472	0.104
		0.210	0.387	0.080								0.246	0.513	0.121
		0.218	0.387	0.077								0.256	0.537	0.127
	2	0.212	0.389	0.080							2	0.249	0.547	0.135
		0.217	0.388	0.078								0.255	0.538	0.128
		0.212	0.384	0.078								0.248	0.531	0.128
	3	0.213	0.385	0.078							3	0.248	0.538	0.131
		0.216	0.386	0.077								0.250	0.537	0.130
		0.220	0.395	0.079								0.247	0.532	0.129
	4	0.192	0.364	0.078							4	0.251	0.537	0.130
		0.189	0.361	0.078								0.245	0.544	0.136
		0.189	0.358	0.077								0.244	0.536	0.132
	5	0.209	0.380	0.078							5	0.241	0.507	0.121
		0.202	0.379	0.080								0.247	0.529	0.128
		0.208	0.384	0.080										
			Mean	**0.392**									**Mean**	**0.663**
			Stdev	***0.008***									***Stdev***	***0.044***
Hq Bar 90	1	0.182	0.379	0.089						Hq Bar 88		0.199	0.494	0.134
		0.189	0.379	0.086							1	0.204	0.521	0.144
	2	0.205	0.401	0.089								0.201	0.485	0.129
		0.189	0.378	0.086								0.210	0.530	0.145
	3	0.207	0.286	0.036							2	0.206	0.540	0.151
		0.193	0.364	0.078								0.203	0.525	0.146
	4	0.201	0.417	0.098							3	0.210	0.547	0.153
		0.195	0.382	0.085								0.205	0.535	0.150
	5	0.199	0.419	0.100								0.204	0.498	0.133
		0.195	0.396	0.091							4	0.213	0.542	0.149
			Mean	**0.421**								0.210	0.533	0.146
			Stdev	***0.100***								0.206	0.511	0.138
											5	0.209	0.546	0.153
												0.208	0.514	0.139
													Mean	**0.754**
													Stdev	***0.043***

*((A2-A1)*0.8637*0.525 g/L)-BLANK g/L in 25mL juice*

*(((A2-A1)*0.8637*0.525 g/L)-BLANK)*5.5506 mM in 25mL juice*

Appendix C1 Concentration of glycosyl-glucose (G-G) in Barossa Valley juice samples

	Rep	A1	A2	A2-A1 G-G mM		Rep	((A2-A1)*0.8637*0.525 g/L)-BLANK g/L in 25mL juice (((A2-A1)*0.8637*0.525 g/L)-BLANK)*5.5506 mM in 25mL juice				Rep			
Lq Bar 90	1	0.210	0.311	0.046	Lq Bar 89	1	0.209	0.403	0.088	Lq Bar 88		0.182	0.292	0.050
		0.211	0.317	0.048			0.212	0.444	0.105		1	0.183	0.295	0.051
		0.212	0.321	0.049			0.209	0.401	0.087			0.181	0.294	0.051
	2	0.214	0.325	0.050		2	0.213	0.407	0.088			0.186	0.297	0.050
		0.213	0.325	0.051			0.217	0.411	0.088		2	0.187	0.301	0.052
		0.214	0.326	0.051			0.210	0.407	0.089			0.182	0.299	0.053
	3	0.210	0.321	0.050		3	0.228	0.427	0.090			0.186	0.303	0.053
		0.218	0.331	0.051			0.215	0.403	0.085		3	0.188	0.304	0.053
		0.216	0.334	0.054			0.220	0.415	0.088			0.186	0.304	0.054
	4	0.218	0.330	0.051		4	0.225	0.410	0.084			0.191	0.313	0.055
		0.219	0.329	0.050			0.224	0.385	0.073		4	0.185	0.301	0.053
		0.213	0.322	0.049			0.223	0.389	0.075			0.187	0.305	0.054
	5	0.212	0.322	0.050		5	0.221	0.409	0.085			0.190	0.307	0.053
		0.223	0.333	0.050			0.232	0.422	0.086		5	0.194	0.312	0.054
		0.214	0.325	0.050			0.222	0.413	0.087			0.190	0.309	0.054
			Mean	**0.234**				**Mean**	**0.438**				**Mean**	**0.248**
			Stdev	***0.009***				***Stdev***	***0.039***				***Stdev***	***0.008***
					Lq Bar 89	1	0.251	0.448	0.089	Lq Bar 88	1	0.278	0.437	0.072
							0.262	0.479	0.098			0.275	0.446	0.078
							0.257	0.459	0.092			0.277	0.453	0.080
						2	0.266	0.480	0.097		2	0.279	0.450	0.078
							0.263	0.477	0.097			0.280	0.467	0.085
							0.266	0.483	0.098			0.274	0.416	0.064
						3	0.263	0.482	0.099		3	0.272	0.422	0.068
							0.264	0.483	0.099			0.286	0.504	0.099
							0.275	0.485	0.095			0.283	0.504	0.100
						4	0.267	0.475	0.094		4	0.274	0.468	0.088
							0.271	0.466	0.088			0.288	0.505	0.098
							0.270	0.482	0.096			0.280	0.472	0.087
						5	0.269	0.483	0.097		5	0.266	0.392	0.057
							0.264	0.483	0.099			0.284	0.477	0.088
							0.260	0.467	0.094			0.284	0.473	0.086
								Mean	**0.488**				**Mean**	**0.411**
								Stdev	***0.020***				***Stdev***	***0.071***

Appendix C2 Concentration of glycosyl-glucose (G-G) in Coonawarra juice samples

	Rep	A1	A2	A2-A1 G-G mM			*((A2-A1)*0.8637*.525 g/L)-BLANK g/L in 25mL juice* *(((A2-A1)*0.8637*.525 g/L)-BLANK)*5.5506 mM in 25mL juice*							
Hq Cw 90	1	0.211	0.377	0.075	Hq Cw 89	1	0.173	0.276	0.047	Hq Cw 88	1	0.180	0.383	0.092
		0.202	0.380	0.081			0.176	0.285	0.049			0.180	0.378	0.090
		0.201	0.373	0.078			0.176	0.276	0.045			0.185	0.395	0.095
	2	0.201	0.370	0.077		2	0.175	0.272	0.044		2	0.189	0.396	0.094
		0.203	0.377	0.079			0.173	0.283	0.050			0.191	0.407	0.098
		0.200	0.371	0.078			0.177	0.288	0.050			0.190	0.379	0.086
	3	0.196	0.363	0.076		3	0.178	0.286	0.049		3	0.194	0.399	0.093
		0.203	0.377	0.079			0.178	0.288	0.050			0.194	0.400	0.093
		0.198	0.366	0.076			0.176	0.287	0.050			0.194	0.396	0.092
	4	0.186	0.357	0.078		4	0.173	0.282	0.049		4	0.193	0.395	0.092
		0.184	0.354	0.077			0.175	0.292	0.053			0.189	0.386	0.089
		0.184	0.356	0.078			0.177	0.295	0.054		5	0.195	0.396	0.091
	5	0.194	0.373	0.081		5	0.180	0.305	0.057			0.192	0.384	0.087
		0.195	0.371	0.080			0.179	0.296	0.053					
			Mean	**0.416**				**Mean**	**0.270**				**Mean**	**0.492**
			Stdev	***0.010***				***Stdev***	***0.015***				***Stdev***	***0.020***
					Hq Cw 89	1	0.169	0.319	0.068	Hq Cw 88	1	0.192	0.375	0.083
							0.171	0.282	0.050			0.193	0.408	0.097
						2	0.172	0.275	0.047		2	0.201	0.371	0.077
							0.173	0.288	0.052			0.199	0.391	0.087
						3	0.177	0.281	0.047		3	0.200	0.370	0.077
							0.183	0.295	0.051			0.199	0.361	0.073
						4	0.180	0.287	0.049		4	0.203	0.448	0.111
							0.179	0.286	0.049			0.204	0.411	0.094
						5	0.178	0.284	0.048		5	0.198	0.361	0.074
							0.189	0.290	0.046			0.197	0.359	0.073
								Mean	**0.248**				**Mean**	**0.611**
								Stdev	***0.036***				***Stdev***	***0.070***
Lq Cw 90	1	0.193	0.328	0.061	Lq Cw 89	1	0.180	0.246	0.030	Lq Cw 88	1	0.176	0.276	0.045
		0.197	0.338	0.064			0.185	0.253	0.031			0.170	0.274	0.047
		0.200	0.342	0.064			0.182	0.250	0.031			0.177	0.290	0.051
	2	0.199	0.340	0.064		2	0.182	0.252	0.032		2	0.177	0.286	0.049
		0.196	0.338	0.064			0.176	0.247	0.032			0.188	0.294	0.048
		0.197	0.340	0.065			0.183	0.251	0.031			0.176	0.288	0.051
	3	0.200	0.341	0.064		3	0.183	0.249	0.030		3	0.180	0.282	0.046
		0.195	0.337	0.064			0.181	0.251	0.032			0.182	0.290	0.049
		0.193	0.335	0.064			0.179	0.247	0.031			0.178	0.285	0.049
	4	0.195	0.336	0.064		4	0.176	0.245	0.031		4	0.177	0.278	0.046
		0.192	0.331	0.063			0.180	0.247	0.030			0.177	0.280	0.047
		0.194	0.335	0.064			0.182	0.250	0.031			0.178	0.294	0.053
	5	0.192	0.336	0.065		5	0.175	0.243	0.031		5	0.189	0.295	0.048
		0.193	0.333	0.063			0.183	0.249	0.030			0.185	0.297	0.051
		0.199	0.335	0.062			0.177	0.248	0.032			0.183	0.293	0.050
			Mean	**0.329**				**Mean**	**0.151**				**Mean**	**0.254**
			Stdev	***0.006***				***Stdev***	***0.004***				***Stdev***	***0.012***

Appendix C2 Concentration of glycosyl-glucose (G-G) in Coonawarra juice samples

	Rep	A1	A2	A2-A1 G-G mM			*((A2-A1)*0.8637*.525 g/L)-BLANK g/L in 25mL juice* *(((A2-A1)*0.8637*.525 g/L)-BLANK)*5.5506 mM in 25mL juice*						
					Lq Cw 89	1	0.164	0.235	0.032	Lq Cw 88	1	0.176	0.265	0.040
							0.169	0.222	0.024			0.177	0.267	0.041
						2	0.169	0.218	0.022		2	0.177	0.278	0.046
							0.164	0.221	0.026			0.178	0.278	0.045
						3	0.170	0.232	0.028		3	0.182	0.282	0.045
							0.168	0.245	0.035			0.183	0.277	0.043
						4	0.164	0.234	0.032		4	0.181	0.300	0.054
							0.170	0.226	0.025			0.187	0.285	0.044
						5	0.170	0.235	0.029		5	0.195	0.377	0.083
							0.169	0.248	0.036			0.186	0.293	0.049
								Mean	**0.146**				**Mean**	**0.066**
								Stdev	***0.027***				***Stdev***	***0.079***

Appendix D.1

Table 1. Experimental treatments imposed within the commercial narrow row and conventional spaced vineyard in Coónawarra (Four replicates for each spacing).

Treatment	Experimental details
Control	Normal crop at either spacing
Thinning	Crop thinned to one bunch per shoot
Root pruning	Roots pruned by deep ripping during growing season
Thinning +root pruning	Crop thinned + root pruning

Each experimental plot is sufficiently large to yield at least 30 kg of fruit.

Table 2. Vine spacing, planting density and rootstocks used in a replicated trial on the Nuriootpa Viticultural Centre.

	Narrow	Intermediate	Wide
Row width (m)	1.5	2.5	3.5
Vine spacing (m)	0.75	1.25	1.75
Vines / ha	8890	3200	1630
Rootstock	420 A	420 A	420 A
	Own roots	Own roots	Own roots
	K 51-40	K 51-40	K 51-40

Table 3. Yield (tonnes per hectare) of narrow and conventional row width plantings in Coonawarra for harvest years 1987 to 1989.

	Narrow	Conventional
Planted October 1985		
1987	1	0
1988	12	1
1989	16	11

Table 4. Yield and its components for 1989 harvest for narrow and conventional row width plantings in Coonawarra.

	Narrow	Conventional
Fruit wt (kg / vine)	1.8	6.9
Bunches	31	63
Bunch wt (kg)	60	111
Berry wt (g)	1.23	1.29
Berries per bunch	49	86

Table 5. Effect of fruit thinning on yield and maturity components of fruit on narrow and conventional row widths in Coonawarra in 1989.

	Narrow		Conventional	
	Control	Thinned(*)	Control	Thinned(*)
Fruit wt (kg / vine)	1.8	1.4	6.9	5.4
Bunches	31	17	63	45
Bunch wt (g)	59.6	83.2	110.6	120.4
Brix	25.2	25.9	23.4	26.5
pH	3.47	3.55	3.45	3.55
Titratable Acid	5.3	6.2	4.7	5.7

(*) Thinned plots were harvested one week before control plots

Table 6. Shoots grown and 1989 pruning weight for vines at narrow and conventional row widths at Coonawarra.

	Narrow	Conventional
Shoots grown per vine	21	37
Shoots per hectare	187,000	60,000
Shoots per metre	28	21
Weight of prunings (kg / vine)	0.61	1.89
Weight of prunings (kg / metre)	0.82	1.07
Buds retained per Ha	204,000	93,000
Buds retained per metre	31	33

Table 7. Yield, weight of prunings and buds retained for vines planted at three spacings and three rootstocks in the Barossa Valley

	Narrow	Intermediate	Wide
Vines / ha	8890	3200	1630
Yield (kg per vine)			
Rootstock			
420 A	1.6 (14.0)	1.5 (4.8)	3.4 (5.5)
Own roots	1.8 (16.4)	2.8 (9.1)	2.5 (4.1)
K 51-40	1.4 (12.3)	2.8 (9.1)	4.1 (6.7)
Pruning weight (kg per vine)			
Rootstock			
420 A	0.14	0.15	0.20
Own roots	0.33	0.26	0.37
K 51-40	0.22	0.42	0.45
Buds retained per Ha	267,000	96,000	49,000

( ) = Calculated tonnes per hectare

(McCarthy 1990)

Appendix D2 Concentration of glycosyl-glucose (uM) from the enzymic hydrolysis of a juice glycosidic extract from the Department of Agriculture vine density planting trial Coonawarra, 1990

		High Density Vines					Low Density Vines				
	Rep	Mean	Stdev	Mean	*Stdev*	*CV%*	Mean	Stdev	Mean	*Stdev*	*CV%*
Control	1	320	11	382	*44*	***12***	382	11	401	*66*	***17***
	2	418	10				486	10			
	3	379	8				407	11			
	4	409	16				328	7			
Ripped	1	485	16	412	*112*	***27***	404	11	327	*54*	***17***
	2	396	16				280	11			
	3	506	16				301	12			
	4	260	13				324	12			
Thinned	1	420	13	351	*65*	***19***	326	11	362	*76*	***21***
	2	277	24				280	9			
	3	318	11				456	23			
	4	390	12				387	10			
Thinned	1	531	21	438	*87*	***20***	379	7	457	*63*	***14***
and	2	327	8				456	13			
Ripped	3	418	31				532	11			
	4	475	10				462	7			

Appendix D.3a Concentration (mM) of glycosyl-glucose (G-G) from the Coonawara Ripening Trial: High density planting

Harvest date		Feb 28					Mar 9					Mar 19					Mar 20		
1A	1	0.273	0.410	0.062	2A	1	0.244	0.359	0.052	3A	1	0.281	0.438	0.071	4A	1	0.311	0.535	0.102
		0.272	0.415	0.065			0.247	0.369	0.055			0.285	0.448	0.074			0.319	0.552	0.106
		0.275	0.426	0.068			0.245	0.360	0.052			0.288	0.451	0.074			0.320	0.556	0.107
	2	0.288	0.434	0.066		2	0.252	0.369	0.053		2	0.288	0.454	0.075		2	0.319	0.552	0.106
		0.278	0.430	0.069			0.257	0.387	0.059			0.290	0.464	0.079			0.314	0.548	0.106
		0.280	0.440	0.073			0.252	0.365	0.051			0.303	0.480	0.080			0.316	0.554	0.108
	3	0.275	0.424	0.068		3	0.256	0.373	0.053		3	0.292	0.458	0.075		3	0.317	0.553	0.107
		0.278	0.430	0.069			0.254	0.377	0.056			0.293	0.464	0.078			0.314	0.550	0.107
		0.270	0.420	0.068			0.260	0.414	0.070			0.292	0.456	0.074			0.320	0.568	0.112
	4	0.272	0.433	0.073		4	0.258	0.383	0.057		4	0.295	0.476	0.082		4	0.313	0.551	0.108
		0.277	0.430	0.069			0.260	0.379	0.054			0.296	0.465	0.077			0.316	0.556	0.109
		0.280	0.437	0.071			0.254	0.373	0.054			0.292	0.462	0.077			0.312	0.546	0.106
	5	0.279	0.428	0.068		5	0.256	0.374	0.054		5	0.294	0.464	0.077		5	0.306	0.536	0.104
		0.276	0.440	0.074			0.251	0.370	0.054			0.297	0.465	0.076			0.323	0.560	0.107
		0.274	0.432	0.072			0.259	0.383	0.056			0.291	0.459	0.076			0.311	0.545	0.106
			Mean	**0.340**				**Mean**	**0.264**				**Mean**	**0.381**				**Mean**	**0.549**
			Stdev	**0.018**				**Stdev**	**0.025**				**Stdev**	**0.015**				**Stdev**	**0.013**
1B	1	0.194	0.350	0.071	2B	1	0.224	0.358	0.061	3B	1	0.196	0.365	0.077	4B	1	0.320	0.587	0.121
		0.192	0.356	0.074			0.228	0.366	0.063			0.198	0.378	0.082			0.319	0.595	0.125
		0.195	0.363	0.076			0.230	0.371	0.064			0.197	0.376	0.081			0.321	0.595	0.124
	2	0.182	0.342	0.073		2	0.228	0.376	0.067		2	0.203	0.362	0.072		2	0.329	0.614	0.129
		0.181	0.346	0.075			0.228	0.376	0.067			0.205	0.363	0.072			0.323	0.608	0.129
		0.186	0.351	0.075			0.233	0.379	0.066			0.204	0.360	0.071			0.315	0.603	0.131
	3	0.191	0.356	0.075		3	0.233	0.381	0.067		3	0.217	0.375	0.072		3	0.310	0.592	0.128
		0.188	0.351	0.074			0.232	0.379	0.067			0.214	0.373	0.072			0.313	0.602	0.131
		0.184	0.338	0.070			0.231	0.378	0.067			0.223	0.383	0.073			0.310	0.594	0.129
	4	0.189	0.352	0.074		4	0.236	0.383	0.067		4	0.229	0.390	0.073		4	0.307	0.584	0.126
		0.195	0.356	0.073			0.234	0.384	0.068			0.229	0.390	0.073			0.305	0.590	0.129
		0.196	0.362	0.075			0.230	0.377	0.067			0.227	0.390	0.074			0.310	0.594	0.129
	5	0.204	0.367	0.074		5	0.239	0.389	0.068		5	0.238	0.397	0.072		5	0.204	0.484	0.127
		0.206	0.373	0.076			0.239	0.387	0.067			0.239	0.402	0.074			0.201	0.475	0.124
		0.206	0.371	0.075			0.243	0.391	0.067			0.236	0.395	0.072			0.203	0.481	0.126
			Mean	**0.367**				**Mean**	**0.324**				**Mean**	**0.367**				**Mean**	**0.663**
			Stdev	**0.010**				**Stdev**	**0.011**				**Stdev**	**0.019**				**Stdev**	**0.015**
1C	1	0.238	0.393	0.070	2C	1	0.181	0.300	0.054	3C	1	0.213	0.369	0.071	4C	1	0.244	0.526	0.128
		0.231	0.399	0.076			0.185	0.299	0.052			0.212	0.374	0.073			0.253	0.548	0.134
		0.238	0.408	0.077			0.178	0.303	0.057			0.212	0.376	0.074			0.254	0.544	0.131
	2	0.235	0.398	0.074		2	0.186	0.306	0.054		2	0.230	0.395	0.075		2	0.258	0.550	0.132
		0.236	0.407	0.078			0.190	0.317	0.058			0.234	0.400	0.075			0.267	0.569	0.137
		0.239	0.408	0.077			0.195	0.313	0.054			0.240	0.410	0.077			0.263	0.561	0.135
	3	0.246	0.416	0.077		3	0.194	0.323	0.058		3	0.240	0.409	0.077		3	0.265	0.560	0.134
		0.237	0.417	0.082			0.191	0.326	0.061			0.240	0.408	0.076			0.268	0.568	0.136
		0.243	0.418	0.079			0.197	0.330	0.060			0.240	0.412	0.078			0.265	0.568	0.137
	4	0.252	0.422	0.077		4	0.210	0.333	0.056		4	0.251	0.419	0.076		4	0.280	0.579	0.136
		0.259	0.435	0.080			0.204	0.331	0.058			0.250	0.420	0.077			0.279	0.577	0.135
		0.244	0.432	0.085			0.202	0.336	0.061			0.248	0.417	0.077			0.273	0.590	0.144
	5	0.252	0.431	0.081		5	0.207	0.333	0.057		5	0.257	0.426	0.077		5	0.280	0.575	0.134
		0.254	0.428	0.079			0.214	0.337	0.056			0.257	0.426	0.077			0.279	0.582	0.137
		0.249	0.426	0.080			0.213	0.335	0.055			0.262	0.434	0.078			0.284	0.589	0.138
			Mean	**0.390**				**Mean**	**0.271**				**Mean**	**0.378**				**Mean**	**0.707**
			Stdev	**0.019**				**Stdev**	**0.015**				**Stdev**	**0.011**				**Stdev**	**0.020**

Appendix D.3b Concentration (mM) of glycosyl-glucose (G-G) from the Coonawara Ripening Trial: Low density planting

Harvest date			Feb 28				Mar 9				Mar 19				Mar 20		
1A	1	0.261	0.373	0.051	2A	1	0.264	0.470	0.093	3A	1	0.262	0.432	0.077	4A	1	0.294	0.498	0.093
		0.275	0.398	0.056			0.267	0.483	0.098			0.271	0.461	0.086			0.293	0.497	0.093
		0.276	0.402	0.057			0.266	0.485	0.099			0.278	0.461	0.083			0.277	0.471	0.088
	2	0.277	0.403	0.057		2	0.269	0.485	0.098		2	0.290	0.475	0.084		2	0.327	0.536	0.095
		0.271	0.393	0.055			0.271	0.493	0.101			0.300	0.450	0.068			0.330	0.539	0.095
		0.273	0.398	0.057			0.270	0.490	0.100			0.298	0.482	0.083			0.332	0.539	0.094
	3	0.271	0.390	0.054		3	0.269	0.493	0.102		3	0.300	0.481	0.082		3	0.342	0.553	0.096
		0.276	0.402	0.057			0.276	0.504	0.103			0.303	0.489	0.084			0.336	0.543	0.094
		0.270	0.388	0.054			0.268	0.488	0.100			0.305	0.500	0.088			0.341	0.556	0.097
	4	0.277	0.404	0.058		4	0.266	0.489	0.101		4	0.306	0.495	0.086		4	0.342	0.557	0.097
		0.274	0.395	0.055			0.267	0.491	0.102			0.305	0.495	0.086			0.342	0.550	0.094
		0.274	0.398	0.056			0.276	0.514	0.108			0.309	0.498	0.086			0.345	0.554	0.095
	5	0.270	0.390	0.054		5	0.266	0.486	0.100		5	0.312	**0.497**	0.084		5	0.353	0.565	0.096
		0.288	0.417	0.058			0.265	0.489	0.102			0.310	0.497	0.085			0.357	0.580	0.101
		0.275	0.399	0.056			0.272	0.499	0.103			0.313	0.498	0.084			0.356	0.572	0.098
			Mean	**0.266**				**Mean**	**0.515**				**Mean**	**0.418**				**Mean**	**0.484**
			Stdev	**0.011**				**Stdev**	**0.018**				**Stdev**	**0.027**				**Stdev**	**0.017**
1B	1	0.278	0.416	0.063	2B	1	0.237	0.390	0.069	3B	1	0.234	0.375	0.064	4B	1	0.273	0.509	0.107
		0.256	0.402	0.066			0.256	0.431	0.079			0.240	0.386	0.066			0.271	0.504	0.106
		0.264	0.415	0.068			0.248	0.426	0.081			0.243	0.373	0.059			0.261	0.506	0.111
	2	0.264	0.411	0.067		2	0.246	0.429	0.083		2	0.254	0.405	0.068		2	0.254	0.491	0.107
		0.261	0.407	0.066			0.247	0.431	0.083			0.258	0.410	0.069			0.252	0.487	0.107
		0.267	0.415	0.067			0.252	0.436	0.083			0.253	0.405	0.069			0.254	0.495	0.109
	3	0.270	0.427	0.071		3	0.247	0.431	0.083		3	0.262	0.412	0.068		3	0.246	0.488	0.110
		0.272	0.427	0.070			0.246	0.432	0.084			0.261	0.415	0.070			0.242	0.474	0.105
		0.267	0.423	0.071			0.244	0.425	0.082			0.261	0.418	0.071			0.243	0.477	0.106
	4	0.271	0.434	0.074		4	0.249	0.436	0.085		4	0.263	0.417	0.070		4	0.231	0.463	0.105
		0.273	0.437	0.074			0.240	0.423	0.083			0.268	0.427	0.072			0.229	0.463	0.106
		0.272	0.428	0.071			0.250	0.436	0.084			0.267	0.422	0.070			0.236	0.472	0.107
	5	0.269	0.420	0.068		5	0.245	0.432	0.085		5	0.262	0.414	0.069		5	0.222	0.458	0.107
		0.268	0.423	0.070			0.246	0.430	0.083			0.261	0.416	0.070			0.220	0.458	0.108
		0.268	0.417	0.068			0.247	0.437	0.086			0.261	0.414	0.069			0.218	0.445	0.103
			Mean	**0.340**				**Mean**	**0.414**				**Mean**	**0.336**				**Mean**	**0.550**
			Stdev	**0.017**				**Stdev**	**0.022**				**Stdev**	**0.018**				**Stdev**	**0.011**
1C	1	0.176	0.307	0.059	2C	1	0.242	0.426	0.083	3C	1	0.286	0.486	0.091	4C	1	0.250	0.425	0.079
		0.183	0.322	0.063			0.248	0.439	0.087			0.285	0.484	0.090			0.254	0.438	0.083
		0.174	0.318	0.065			0.250	0.445	0.088			0.290	0.494	0.093			0.260	0.449	0.086
	2	0.176	0.319	0.065		2	0.257	0.457	0.091		2	0.269	0.460	0.087		2	0.279	0.470	0.087
		0.183	0.324	0.064			0.260	0.455	0.088			0.276	0.477	0.091			0.278	0.470	0.087
		0.180	0.323	0.065			0.252	0.445	0.088			0.263	0.450	0.085			0.276	0.461	0.084
	3	0.196	0.337	0.064		3	0.256	0.455	0.090		3	0.258	0.416	0.072		3	0.292	0.483	0.087
		0.192	0.332	0.063			0.259	0.456	0.089			0.300	0.513	0.097			0.290	0.480	0.086
		0.196	0.337	0.064			0.258	0.455	0.089			0.297	0.506	0.095			0.298	0.500	0.092
	4	0.205	0.345	0.063		4	0.255	0.442	0.085		4	0.290	0.484	0.088		4	0.303	0.495	0.087
		0.200	0.340	0.063			0.262	0.459	0.089			0.260	0.436	0.080			0.301	0.494	0.088
		0.201	0.344	0.065			0.261	0.459	0.090			0.262	0.427	0.075			0.296	0.489	0.088
	5	0.211	0.352	0.064		5	0.251	0.450	0.090		5	0.281	0.483	0.092		5	0.299	0.493	0.088
		0.211	0.350	0.063			0.264	0.462	0.090			0.295	0.496	0.091			0.308	0.503	0.088
		0.212	0.353	0.064			0.257	0.450	0.088			0.301	0.507	0.093			0.300	0.498	0.090
			Mean	**0.310**				**Mean**	**0.447**				**Mean**	**0.444**				**Mean**	**0.437**
			Stdev	**0.008**				**Stdev**	**0.012**				**Stdev**	**0.040**				**Stdev**	**0.016**

Appendix D.4a Concentration (ug/L) of compounds identified as enzyme-released products from the SA Department of Agriculture vine density trial, Coonawarra 1990: High density vines

Major mass spectra ions	Compound name	Appr. RT	T1R1	T1R2	T1R3	T1R4	ave	*mean*	T2R1	T2R2	T2R3	T2R4	ave	*mean*	T3R1	T3R2	T3R3	T3R4	ave	*mean*	T4R1	T4R2	T4R3	T4R4	ave	*mean*
91, 106						3	3																			
60, 73	Butyric acid									15			15													
56, 69, 82, 43	cis-2-hexenol	324		70	59		64	*32*		325tr																
105, 120, 77					8		8	*2*	92				92	*23*												
41, 67, 82	Z-3-hexenol	425	39			17	28	*14*	31	67	25	53	44	*44*	55	52	53	31	48	*48*	99	30	79	20	57	*57*
59,69, 84, 56	Hexenol	446	15			76	45	*23*			87	284	186	*93*	78	71	76	259	121	*121*	99	64	119	63	86	*86*
91, 120						376tr				225			225	*56*							441tr	626tr	410tr			
105, 120, 84			20	4		3	9	*7*	8	14	12	7	10	*10*	2	2	4	3	3	*3*	1		9	2	4	*3*
57, 67, 71, 82	trans-2-hexenol		8		12	6	9	*7*		528tr							1		1	*0*						
77, 105, 106	benzaldehyde	775	7			2	4	*2*																		
	OCTANOL		100	100	100	100	100	*100*	100	100	100	100	100	*100*	100	100	100	100	100	*100*	100	100	100	100	100	*100*
60, 73	Pentanoic acid	1075	23	64	35		41	*31*	41	64	15	24	36	*36*	23	1	6	12	11	*11*	964tr	26	8	14	16	*12*
105, 77, 120, 136																								1	1	*0*
79, 77, 91, 108, 107	benzyl alcohol	1226	162	191	172	87	153	*153*	109	315	130	215	192	*192*	100	160	98	75	108	*108*	87	95	92	82	89	*89*
71, 67, 82	Hotrienol	1258	3			2	2	*1*		7	1		4	*2*	2	2		1	2	*1*	3		5		4	*2*
94, 66	Phenol	1275		8		1	5	*2*		6	1	1029tr	3	*2*	2	4	1		2	*2*	2		3	1	2	*2*
109, 124, 81	Guaiacol	1312	2	1			1	*1*		4			4	*1*		1			1	*0*	11				11	*3*
60, 73	hexanoic acid	1430	7	15	6	4	8	*8*	5	11	6	7	7	*7*	4	5	4	4	4	*4*	3	6	7	8	6	*6*
85,56, 70, 114	hydroxyhexanoic acid lactone	1430	5			1	3	*2*	1179tr	6	3		4	*2*		1180tr	1144tr	1	1	*0*	1		1	1210tr	1	*0*
108, 107, 90, 79, 77	Cresol	1453							1183tr	1		1192tr	1	*0*	1166tr	1215tr	1180tr	1281tr								
91, 122	2-Phenethanol	1491	76	75	65	34	63	*63*	54	97	43	110	76	*76*	41	64	40	31	44	*44*	40	60	42	33	44	*44*
120, 92, 152	Methyl Salicylate	1592	3	28	7	3	10	*10*	3	10	18	8	10	*10*	4	5	4	4	4	*4*	3	7	2	12	6	*6*
68, 94, 59, 155	Pyran linalool oxide #1	1599		1		2	1	*1*	3			2	2	*1*		1333tr	1		1	*0*	2		1306tr	1	1	*1*
108, 107	Cresol									1286tr					1288tr	1341tr	0		0	*0*				0	0	*0*
107, 122, 150	a dimethyl or diethyl phenol	1613																				1739tr				
60, 73, 95, 112	Heptanoic acid									6			6	*1*		12		1460tr	12	*3*		36	21	1	19	*14*
85, 95, 103	lactone																	1460tr				1774tr		0	0	*0*
135, 150, 91	p-cymen-8-ol	1663																								
192, 177, 93, 121, 136, 149	Vitispirane	1690	22				22	*5*		3			3	*1*	1				1	*0*	1				1	*0*
123, 138	1,4-dimethoxybenzene					0	0	*0*		1370tr					1365tr		1378tr	1494tr					1441tr			
69, 41, 93, 121, 126, 154	Nerol	1736				1	1	*0*								9	1410tr		9	*2*	0			1	1	*0*
88, 101, 115, 73	ethyl octanoate	1769	2			0	1	*1*																		
60, 73, 84, 101, 115	octanoic acid	1786	16	23	19	10	17	*17*	18	27	15	24	21	*21*	12		10	12	11	*9*	10			17	14	*7*
67, 71, 82	z-2,6-dimethylocta-3,7-diene-2,6-diol	1820		12		6	9	*5*	13	16		17	15	*11*	11	11	10	8	10	*10*	4	46		10	20	*15*
67, 71, 82	e-2,6-dimethyloct-3,7-ene-2,6-diol	1846				17		*4*													9		16.1, 19.65(21(		9	*2*
105, 77, 122	Benzoic Acid	1975	2	9		3	4	*3*	1624tr		4	1	3	*1*		1	1	1	1	*1*	2			2	2	*1*
71, 81, 96, 121, 139	2,6-dimethyloct-7-ene-2,6-diol	1982	2				2	*0*	1637tr			3	3	*1*	1634tr	1			1	*0*						
60, 73, 98, 115, 129	Nonanoic acid	2133	9	29	10	11	15	*15*	26	40	13	20	25	*25*	13	18	8	19	15	*15*	7	76	22	16	30	*30*
85,	Unknown					1	1	*0*										1	1	*0*				1	1	*0*
136, 168	2,3-Dihydroxybenzoic acid methylester	2189	2				2	*0*		5	2	3	3	*3*	2	3	2	2	2	*2*	3	14	1	1	5	*5*
157, 142, 172	trimethyl naphthalene #2	2240													1800tr						1918tr					
135, 150, 107	4-vinylguaiacol	2241	9		21	9	13	*10*		37	18	25	26	*20*	13	13	14	15	14	*14*	14	67	17	15	28	*28*
120, 91	1 4-vinylphenol	2252				4	4	*1*			4	6	5	*2*				13	13	*3*	17	47			32	*16*
69, 105, 121, 175, 190	Damascenone	2298													2000tr											
164, 149, 137	Eugenol	2322														1965tr	1907tr					2285tr				
175, 190, 157, 147, 142, 131	Unknown	2445								2223tr																
71, 67, 110, 119, 137	E-2,6-dimethylocta-2,7-diene-1,6-diol	2561	32				32	*8*																		
105, 77, 134, 152	2-hydroxymethyl benzoic acid								2196tr																	
151, 123, 95, 180, 77	Propiovanillone					64	64	*16*	63	100	60	100	81	*81*	62	104	46	48	65	*65*	38	295	141	59	133	*133*
154, 166	3-hydroxytheaspirane #1	2820				3	3	*1*		5	3	3	4	*3*	5	4	3		4	*3*	2492tr	12	8	5	8	*6*
170, 155	trimethyl naphthalene	2839				2	2	*1*	2	5	2	3	3	*3*	2				2	*1*	2	9	1	1	3	*3*
154, 166	3-hydroxytheaspirane #2	2856										5	5	*1*	1	2470tr	2404tr		1	*0*						
154, 166	3-hydroxytheaspirane #3	2907								1			1	*0*		3	2	2	2	*2*	2			2	2	*1*
154, 93, 65, 137, 122	Dimethoxyphenol	3010				2	2	*0*		6	3		4	*2*	2				2	*1*		16			16	*4*
151, 182	Methylvanillate	3040																			2621tr		2445tr	6	6	*2*
151, 166	Acetovanillone	3052	3			1	2	*1*							2500tr	3	2592tr	3	3	*2*	3	14	5	3	6	*6*
151, 123, 194	Unknown					1	1	*0*															2	1	1	*1*
60, 73, 129, 143	dodecanoic acid		2			1	2	*1*	3	3	1	1	2	*2*	1	2	1	2	1	*1*	3	3078tr	3	3	3	*2*
157, 132, 172, 190, 147	Unknown	3121																			1				1	*0*
121, 122	Hydroxybenzaldehyde	3162	2			1	2	*1*	2	4	1	2	2	*2*	1	1	1	1	1	*1*		6	1	1	3	*2*

Appendix D.4a Concentration (ug/L) of compounds identified as enzyme-released products from the SA Department of Agriculture vine density trial, Coonawarra 1990: High density vines

Major mass spectra ions	Compound name	Appr. RT	T1R1	T1R2	T1R3	T1R4	ave	*mean*	T2R1	T2R2	T2R3	T2R4	ave	*mean*	T3R1	T3R2	T3R3	T3R4	ave	*mean*	T4R1	T4R2	T4R3	T4R4	ave	*mean*
151, 181, 196	ethyl vanillate	3165		0			**0**	***0***													1				**1**	***0***
107, 138	Tyrosol	3176		4		1	**3**	***1***	2	2617tr			**2**	***0***	1	4		1	**2**	***1***	1	6		0	**3**	***2***
149, 177	phthalate	3218	28	15	21	6	**17**	***17***	27	31	7	10	**19**	***19***	10	10	6	7	**8**	***8***	7	191	10	9	**54**	***54***
151, 196, 168, 123, 181	Unknown									2672tr						2768tr		2840tr				3218tr				
137, 168	Methoxytyrosol	3263	41	35	60	15	**38**	***38***	35	31	18	27	**28**	***28***	17	30	11	19	**19**	***19***	16	89	21	11	**34**	***34***
95, 123, 179	3,4-dihydro-3-oxoactinidol #1	3289				3	**3**	***1***	3	8	0	1	**3**	***3***	1	1	0	1	**1**	***1***	1	6	1	0	**2**	***2***
95, 123, 179	3,4-dihydro-3-oxoactinidol#2	3317							5		3		**4**	***2***			3	1	**2**	***1***	3	11	3	3	**5**	***5***
95, 123, 179	3,4-dihydro-3-oxoactinidol#3	3362	5				**5**	***1***	5			7	**6**	***3***	5	3		2	**3**	***2***						
151, 123, 95, 208	Unknown					1	**1**	***0***		1			**1**	***0***		1	1		**1**	***0***						
151, 123, 108, 194	Butyrovanillone	3400	3			3	**3**	***1***	2	15	5	9	**8**	***8***	5	2	4	3	**4**	***4***	4	10	5	6	**6**	***6***
69, 121, 149, 175, 193, 208	3-hydroxy- -damascone	3420	21		21	9	**17**	***13***	17	31	11	26	**21**	***21***	18	20	13	15	**16**	***16***	16	104	25	19	**41**	***41***
136, 168, 108, 208	2,5-Dihydroxybenzoic acid methylester	3428	3	5		2	**3**	***2***	5	7	3	5	**5**	***5***	3	5	3	3	**4**	***4***	2	16	4		**7**	***6***
193, 175, 208	megastigm-5-en-7-yne-3,9-diol	3470		9	11	5	**8**	***6***	7	17		12	**12**	***9***	9	8	6	6	**7**	***7***	8	43	11		**20**	***15***
121, 69, 138, 168, 93	4-hydroxybenzoicacid ethyl ester	3492				1	**1**	***0***																		
85, 57, 97, 125, 163, 181	Hydroxyactinidol #1	3511										6	**6**	***1***	2				**2**	***0***						
107, 133, 164, 210	Raspberry ketone	3552										1	**1**	***0***												
155, 170, 127, 112, 222	Unknown Norisoprenoid	3567														3022tr										
168, 153	Vanillic acid	3610	45	17	61	8	**33**	***33***	4	77	6	35	**31**	***31***	34	3043tr	16	18	**23**	***17***			30	10	**20**	***10***
137, 182	Dihydroconiferyl alcohol	3634	24	14	29	11	**20**	***20***	20	33	9	25	**22**	***22***	20	53	11	20	**26**	***26***	19	158	19	13	**52**	***52***
198, 183	isomer of syringic acid	3647	17	12	16	7	**13**	***13***	13	24	8	16	**15**	***15***	11	12	8	9	**10**	***10***	9	57	14	7	**22**	***22***
108, 69, 152, 123, 208	3-oxo-a -ionol	3687	11	5	11	5	**8**	***8***	13	18	5	14	**12**	***12***	3009tr	11	6	4	**7**	***5***	3	46	6	3	**14**	***14***
182, 181	Syringaldehyde	3705																7	**7**	***2***	7	39	9	6	**15**	***15***
60, 73, 129, 168, 185, 207	tetradecanoic acid																					3512tr				
147, 162, 206	Demoles Ketone	3715																						3215tr		
137, 180	cis Coniferyl alcohol	3715																					2		**2**	***1***
149, 164	9-hydroxymegastigma-4,6-dien-3-one #1					4	**4**	***1***																		
151, 110, 123, 194, 209	Dehydrololiolode	3740			1	2	**1**	***1***	3125tr		1	2	**1**	***1***			0		**0**	***0***						
151, 123, 167	Unknown					1	**1**	***0***																		
151, 123, 182	2-(4-hydroxy-3-methoxyphenyl)2-oxoetha	3794			18		**18**	***5***			2	3	**3**	***1***	2				**2**	***0***						
109, 121, 137, 165, 193, 208	9-hydroxymegastigm-5-en-4-one	3815				1	**1**	***0***																		
135, 123, 108, 150, 177, 195, 210	Blumenol C	3852			5	2	**3**	***2***	3	6	2	8	**5**	***5***	3		3		**3**	***2***			3		**3**	***1***
181, 212	Methyl syringate	3893				2	**2**	***0***	10	6	2	5	**6**	***6***				5	**5**	***1***		29	6		**18**	***9***
170, 155	trimethyl naphthalene	3925	2		5	2	**3**	***2***	3	6	2	4	**4**	***4***	4	6	2	4	**4**	***4***		18	4		**11**	***6***
137, 180	trans-coniferyl alcohol	3963	23		55	17	**32**	***24***	35	65	21	57	**44**	***44***	28	62	16	34	**35**	***35***	18	249	50	16	**83**	***83***
151, 123, 168, 180	Unknown					1	**1**	***0***		11			**11**	***3***												
178, 135, 147, 163, 194, 209	Coniferaldehyde	4025			5	1	**3**	***1***	2	4		3	**3**	***2***	2	7	1	2	**3**	***3***		25			**25**	***6***
181, 198, 226	ethylsyringate	4060																			2		3	2	**2**	***2***
181, 194, 224	butyrosyringone	4147	3				**3**	***1***							3	3	2	3	**3**	***3***		13	4	1	**6**	***5***
194, 179	cis -ferulic acid	4147			5		**5**	***1***																		
130, 161	Tryptophanol	4151			7	3	**5**	***3***	3	9	4	5	**5**	***5***	3				**3**	***1***				1	**1**	***0***
85, 71, 109, 124, 208	Unknown 17	4181							3500tr		1	3	**2**	***1***	3413tr	1		1	**1**	***1***			1		**1**	***0***
43, 123, 163, 209	Grasshopper Ketone	4234				32	**32**	***8***	67	92	22	73	**64**	***64***	54	58	40	44	**49**	***49***	24	327		29	**127**	***95***
111, 126, 151, 170, 196, 208	Unknown norisoprenoid	4284				3	**3**	***1***	1		3		**2**	***1***	3500tr	3654tr	3553tr	3729tr			3660tr	45	3411tr		**45**	***11***
151, 196, 123, 108	carboxyvanillate	4182	6	1	10		**6**	***4***	4	10		9	**7**	***6***	4	5	3	4	**4**	***4***	4	26	6	3	**10**	***10***
124, 168, 206	Vomifoliol	4326	19	22	21	7	**17**	***17***	14	26	8	23	**18**	***18***	14	17	11	13	**14**	***14***	9	70	18	9	**26**	***26***
198, 183	Syringic acid	4389	39	34	63	27	**41**	***41***		33	3468tr	25	**29**	***14***	34	9	11	26	**20**	***20***	27	115	29	12	**46**	***46***
168, 212	unknown shikimate	4414	13	8	15		**12**	***9***	8	13	6	9	**9**	***9***	6	9	4	6	**6**	***6***	5	45	7	4	**15**	***15***
124, 166, 210	Dehydrovomifoliol	4427							2	6		5	**4**	***3***		4	3	3	**3**	***2***	2	17	1	2	**5**	***5***
110, 152, 170	Dihydrovomifoliol	4498	4	4		2	**3**	***3***	3	7	2	5	**4**	***4***	2	5	2	2	**3**	***3***	2	19	3	1	**6**	***6***
194, 179	trans ferulic Acid	4535	2	2	5	1	**3**	***3***		4	2	3	**3**	***2***	1			3922tr	**1**	***0***			3589tr	0	**0**	***0***
181, 154, 212	2-(4-hydroxy-3,5-methylphenyl)-2-oxoeth	4565		1	2	0	**1**	***1***		2	3630tr		**2**	***1***		3865tr	0	3942tr	**0**	***0***		4531tr				
164, 163, 147, 212	trans-4-hydroxycinnamic acid	4593	1	1	4	2	**2**	***2***		3	1		**2**	***1***	1				**1**	***0***	2			0	**1**	***1***
151, 124, 164, 182	unkown shikimate	4627	3	4	9	3	**5**	***5***		12	5	7	**8**	***6***	4	4	2	4	**3**	***3***	3	17	3	4	**7**	***7***
73, 60	octadecadienoic acid					3	**3**	***1***																		
181, 226						1	**1**	***0***		5			**5**	***1***		2		2	**2**	***1***			3	2	**2**	***1***
	TOTAL CONCENTRATION		788	724	877	571	**1044**	***740***	787	1711	626	1363	**1560**	***1122***	737	904	572	817	**830**	***758***	660	2717	892	555	**1413**	***1206***

Appendix D.4b Concentration (ug/L) of compounds identified as nzyme-released products from the SA Department of Agriculture vine density trial, Coonawarra 1990: Low density vines

Major mass spectra ions	Compound name	Appr. RT	T1R1	T1R2	T1R3	T1R4	ave	*mean*	T2R1	T2R2	T2R3	T2R4	ave	*mean*	T3R1	T3R2	T3R3	T3R4	ave	*mean*	T4R1	T4R2	T4R3	T4R4	ave	*mean*
91, 106									3	8		5	**6**	***4***		4	20	22	**15**	***12***	58					***15***
56, 69, 82, 43	cis-2-hexenol	324		82			**82**	***20***	244	150		140	**178**	***133***	101	119		120	**113**	***85***		164	134		**149**	***75***
41, 67, 82	Z-3-hexenol	425	29	22	21	29	**25**	***25***	357tr	356tr	21	19	**20**	***10***	10	18	16	22	**17**	***17***	281					***70***
59,69, 84, 56	Hexenol	446	118		105	136	**120**	***90***									271		**271**	***68***				198	**198**	***49***
91, 120	Unknown		433tr						387tr	1			**1**	***0***	391tr		2	2	**2**	***1***	5					***1***
105, 120, 84	Unknown		2	436tr	428tr	427tr	**2**	***0***	1	4	2	5	**3**	***3***	2	1	12	13	**7**	***7***	42	12	1	1	**14**	***14***
77, 105, 106	benzaldehyde	775		810tr						794tr																
	OCTANOL		100	100	100	100	**100**	***100***	100	100	100	100	**100**	***100***	100	100	100	100	**100**	***100***	100	100	100	100	**100**	***100***
60, 73	Pentanoic acid	1075	10	59	20	37	**32**	***32***	1		2	2	**2**	***1***		8			**8**	***2***			20		**20**	***5***
105, 77, 120, 136	Unknown				3		**3**	***1***	1		2		**1**	***1***	2				**2**	***0***						
79, 77, 91, 108, 107	benzyl alcohol	1226	71	124	132	98	**106**	***106***	113	130	145	82	**117**	***117***	57	75	87	68	**72**	***72***	166	177	169	106	**155**	***155***
71, 67, 82	Hotrienol	1258		6		5	**5**	***3***		5	1054tr		**5**	***1***	1	4	1	2	**2**	***2***	7	9	3	5	**6**	***6***
94, 66	Phenol	1275			1032tr	2	**2**	***1***	1	1	1	1	**1**	***1***	1	1			**1**	***0***			1041tr	1	**1**	***0***
109, 124, 81	Guaiacol	1312				2	**2**	***0***				5	**5**	***1***								7		9	**8**	***4***
60, 73	hexanoic acid	1430	3	9	4	6	**5**	***5***	4	4	5	7	**5**	***5***	3	4	3	2	**3**	***3***	10	7	4	5	**7**	***7***
85,56, 70, 114	hydroxyhexanoic acid lactone	1430	1	6	1176tr	1195tr	**4**	***2***	1	1201tr	1	2	**1**	***1***	1219tr	2	3	1219tr	**2**	***1***	11	3		3	**6**	***4***
108, 107, 90, 79, 77	Cresol	1453	1246tr		1188tr	1203tr																				
91, 122	2-Phenethanol	1491	38	75	55	57	**56**	***56***	52	50	56	40	**49**	***49***	31	40	44	41	**39**	***39***	87	72	88	44	**73**	***73***
120, 92, 152	Methyl Salicylate	1592	4		4	3	**4**	***3***	4	5	16	9	**9**	***9***	3	3	4	2	**3**	***3***	31	58		17	**35**	***27***
68, 94, 59, 155	Pyran linalool oxide #1	1599	1		4		**3**	***1***	3	3	2		**3**	***2***	2	2	4	1334tr	**3**	***2***	1353tr	1291tr				
108, 107	Cresol		1	1351tr	1310tr	1330tr	**1**	***0***	1	1327tr	1	1	**1**	***0***	1347tr	1338tr						1297tr	30		**30**	***7***
107, 122, 150	a dimethyl or diethyl phenol	1613													1366tr								1			***0***
60, 73, 95, 112	Heptanoic acid			1408tr												1348tr								2	**2**	***0***
135, 150, 91	p-cymen-8-ol	1663		1494tr																						
192, 177, 93, 121, 136, 149	Vitispirane	1690								1			**1**	***0***								26	18		**22**	***11***
123, 138	1,4-dimethoxybenzene										1438tr				1425tr	1415tr		1435		***359***						
69, 41, 93, 121, 126, 154	Nerol	1736		1491tr					2	1436tr		1	**1**	***1***		1446tr	1475tr	1465tr			1477tr					
60, 73, 84, 101, 115	octanoic acid	1786	9	21	13	13	**14**	***14***	9	10	11	15	**11**	***11***	6	11	9	5	**8**	***8***	25	19		16	**20**	***15***
67, 71, 82	z-2,6-dimethylocta-3,7-diene-2,6-diol	1820	7	22			**15**	***7***	10	1482tr		7	**9**	***4***	8	1492tr	9	1513tr	**9**	***4***	21	1452tr	11	14	**16**	***12***
67, 71, 82	e-2,6-dimethyloct-3,7-ene-2,6-diol	1846								10			**10**	***2***		9		8	**9**	***4***		14	1		**7**	***4***
69, 121, 136, 154	Geraniol	1848								4			**4**	***1***	4			4	**4**	***2***						
105, 77, 122	Benzoic Acid	1975	2	5		5	**4**	***3***		3			**3**	***1***									2	1694tr	**2**	***0***
71, 81, 96, 121, 139	2,6-dimethyloct-7-ene-2,6-diol	1982							3				**3**	***1***												
60, 73, 98, 115, 129	Nonanoic acid	2133	7	21	20	7	**14**	***14***	11	15	12	16	**13**	***13***	5	12	5	4	**7**	***7***	32	20	18	18	**22**	***22***
85,	Unknown		1		3	1843tr	**2**	***1***	1		1	1889tr	**1**	***0***	1	1	3		**2**	***1***		1801tr		1871tr		
136, 168	2,3-Dihydroxybenzoic acid methylester	2189	1	1853tr	3	3	**2**	***2***	1	3	2		**2**	***2***	1	1	1880tr	1855tr	**1**	***0***	1881tr	5	18	18	**13**	***10***
135, 150, 107	4-vinylguaiacol	2241	17	18	20	23	**20**	***20***	9	12	12	10	**11**	***11***	7	14	15	11	**12**	***12***	16	82	83	78	**65**	***65***
120, 91	4-vinylphenol	2252		7			**7**	***2***														163	97	115	**125**	***94***
121, 150	Unknown			5	3			***2***	2	3		3	**3**	***2***	3	2	3	2	**2**	***2***	7	5	7	3	**5**	***5***
137, 66	2-methyl-3vinylmaleimide	2268																				1880tr				
69, 105, 121, 175, 190	Damascenone	2298															2048tr					1880tr				
164, 149, 137	Eugenol	2322													2293tr	2278tr					2330tr					
126, 111, 85, 84, 70, 67, 177,	6-hydroxy-6,7-dihydroedulan	2421																					2		**2**	***0***
73 ,60, 83, 115, 129, 143, 172	Decanoic acid	2462																					4	3	**3**	***2***
173, 188	4-(2,3,6-trimethylphenyl)-but-3-en-2-one #1	2486																								
71, 67, 110, 119, 137	Z-2,6-dimethylocta-2,7-diene-1,6-diol	2533		27				***7***	11	18			**14**	***7***			31	10	**21**	***10***	29		22		**26**	***13***
71, 67, 110, 119, 137	E-2,6-dimethylocta-2,7-diene-1,6-diol	2561		67				***17***	23	26			**24**	***12***				26	**26**	***7***	53		57		**55**	***27***
175, 190, 157, 147, 133, 91, 1	dehydro-b-ionone/	2711																				2	7	2312tr	**5**	***2***
157, 132, 172, 190, 147	Unknown	2728									2235tr															
151, 123, 95, 180, 77	Propiovanillone		45	127	69	90	**83**	***83***	43	40	55	52	**47**	***47***	52	49	40	32	**43**	***43***	148	120	89	99	**114**	***114***
154, 166	3-hydroxytheaspirane #1	2820	4	10	4	8	**6**	***6***	5	5	3	4	**4**	***4***	2	4	6	1	**3**	***3***	2438tr	15	8	5	**9**	***7***
170, 155	trimethyl naphthalene	2839	3	6	4	2	**4**	***4***	2	2	1	1	**2**	***2***	1	2	3	2	**2**	***2***	4					***1***
154, 166	3-hydroxytheaspirane #2	2856									2		**2**	***0***								10	9			***5***
147, 148, 154	trans-cinnamic acid	2880																				2345tr				
154, 166	3-hydroxytheaspirane #3	2907										2	**2**	***1***								15	12		**13**	***7***
154, 93, 65, 137, 122	Dimethoxyphenol	3010	2	6	4	3	**4**	***4***	2	3	3		**3**	***2***	2	3	2	2	**2**	***2***	4					***1***
151, 182	Methylvanillate	3040	9	15			**12**	***6***	8	11	13	8	**10**	***10***	6	7	9	6	**7**	***7***		36	33	27	**32**	***24***
151, 166	Acetovanillone	3052	3	15	4	4	**7**	***7***	2	3	3	3	**3**	***3***	2	4	4	2	**3**	***3***	2651tr	10	14	6	**10**	***8***
60, 73, 129, 143	dodecanoic acid					2	**2**	***0***	3	4	3	4	**4**	***4***	2	3			**2**	***1***		10	7	5	**7**	***6***

Appendix D.4b Concentration (ug/L) of compounds identified as nzyme-released products from the SA Department of Agriculture vine density trial, Coonawarra 1990: Low density vines

Major mass spectra ions	Compound name	Appr. RT	T1R1	T1R2	T1R3	T1R4	ave	*mean*	T2R1	T2R2	T2R3	T2R4	ave	*mean*	T3R1	T3R2	T3R3	T3R4	ave	*mean*	T4R1	T4R2	T4R3	T4R4	ave	*mean*
157, 132, 190, 172, 147	Unknown	3096																				2554tr	3			*1*
157, 132, 172, 190, 147	Unknown	3121																				10	7		**9**	*4*
121, 122	Hydroxybenzaldehyde	3162	2688tr	2702tr	2	2647tr	**2**	*1*	1	1	1	1	**1**	*1*	1	1	2755tr	2739tr	**1**	*0*	2759tr	2630tr	2725tr			
107, 138	Tyrosol	3176	2698tr								4		**4**	*1*												
149, 177	phthalate	3218	7	12	9	7	**9**	*9*	3	10	4	4	**5**	*5*	5	6	6	2	**5**	*5*	12	14	13	11	**13**	*13*
151, 196, 168, 123, 181	Unknown		14	40				*14*	12			8	**10**	*5*			20	2802tr		*5*	2824tr		2711tr			
137, 168	Methoxytyrosol	3263	24	78	32	32	**41**	*41*	22	20	18	26	**22**	*22*	17	27	37	18	**25**	*25*	44	171	121	94	**108**	*108*
95, 123, 179	3,4-dihydro-3-oxoactinidol #1	3289	1	2792tr	2722tr	2	**1**	*1*		1		2	**2**	*1*		2	2	2	**2**	*2*	2851tr					
121, 152, 190	4-hydroxybenzoic acid methyl ester	3301									3		**3**	*1*										6	**6**	*1*
95, 123, 179	3,4-dihydro-3-oxoactinidol#2	3317	4	5	3	4	**4**	*4*		3	7	4	**5**	*3*	1	5	7	4	**4**	*4*	12					*3*
95, 123, 179	3,4-dihydro-3-oxoactinidol#3	3362		16	7	7		*8*	4	5				*2*	3				**3**	*1*						
151, 123, 95, 208	Unknown				2799tr	2844tr					1	2		*1*	1	2	2929tr	2912tr	**1**	*1*	2934tr					
151, 123, 108, 194	Butyrovanillone	3400	6	15	7	9	**9**	*9*	6	7	5	4	**6**	*6*	5	8	10	2	**6**	*6*	2957tr	16	17	12	**15**	*11*
69, 121, 149, 175, 193, 208	3-hydroxy- -damascone	3420	14	38	21	24	**24**	*24*	18	22	22	15	**19**	*19*	9	17		8	**12**	*9*	32	75	39	52	**49**	*49*
136, 168, 108, 208	2,5-Dihydroxybenzoic acid methylester	3428	2	5	4	4	**4**	*4*	2	6	6	2	**4**	*4*		3	3	1	**2**	*2*	5	30	11		**15**	*11*
135, 107, 121, 151, 175, 190,	9-hydroxymegastigma-5,7-dien-3-one	3447																				2869tr	2894tr			
193, 175, 208	megastigm-5-en-7-yne-3,9-diol	3470	6	16	7	11	**10**	*10*	7	7	7	6	**7**	*7*	5	8	8	3	**6**	*6*	10	27	23	17	**19**	*19*
85, 57, 97, 125, 163, 181	Hydroxyactinidol #1	3511				4	**4**	*1*	2	1			**2**	*1*				3	**3**	*1*						
155, 170, 127, 112, 222	Unknown Norisoprenoid	3567								2989tr																
137, 194	Zingerone	3585															3133tr	6	**6**	*2*			25			*6*
168, 153	Vanillic acid	3610	10	41	30	35	**29**	*29*	15	7	8	17	**12**	*12*	4	10	22	2	**10**	*10*		153	5	75	**78**	*58*
125, 163, 181, 95, 57	Hydroxyactinidol #1																									
137, 182	Dihydroconiferyl alcohol	3634	16	229	165	41	**113**	*113*	18	22	21	9	**17**	*17*	21	28	53	34	**34**	*34*	114	32	43	3159tr	**63**	*47*
198, 183	isomer of syringic acid	3647	9	22	13	18	**16**	*16*	10	10	12	11	**11**	*11*	8	13	11	8	**10**	*10*	22	32	69	22	**36**	*36*
108, 69, 152, 123, 208	3-oxo-a -ionol	3687	2	20	5	12	**10**	*10*	5	7	8	6	**7**	*7*	3140tr	8	8	7	**8**	*6*	16	18		10	**15**	*11*
182, 181	Syringaldehyde	3705	7				**7**	*2*																		
137, 180	cis Coniferyl alcohol	3715														3151tr										
151, 110, 123, 194, 209	Dehydrololiolode	3740		3170tr		1	**1**	*0*	1	1	1	1	**1**	*1*	1	1	1	1	**1**	*1*	3245tr	4	3	3199tr	**3**	*2*
151, 123, 196	ethan-2-ol vanillone p #2																							8	**8**	*2*
151, 123, 182	2-(4-hydroxy-3-methoxyphenyl)2-oxoethanol	3794	2		3	3	**3**	*2*	1			1	**1**	*1*		3				*1*	5	10	8		**8**	*6*
109, 121, 137, 165, 193, 208	9-hydroxymegastigm-5-en-4-one	3815							1		2	1	**1**	*1*	2		2		**2**	*1*						
135, 123, 108, 150, 177, 195,	Blumenol C	3852	2	9	7	3204tr	**6**	*5*	3	4	5	3	**4**	*4*	3	4	5	3	**4**	*4*	4	3199tr				*1*
181, 212	Methyl syringate	3893	4	16	3222tr	5	**8**	*6*	6	6	7	7	**7**	*7*	3	7	8	4	**5**	*5*	11	17	24	16	**17**	*17*
170, 155	trimethyl naphthalene	3925	3	9	5	3	**5**	*5*	3	3	2	3	**3**	*3*	2	3	6	4	**4**	*4*	4	2	19	20	**11**	*11*
181, 196, 168	Acetosyringone	3934															5	1	**3**	*2*			3274tr			
137, 180	trans-coniferyl alcohol	3963	30	173	99	61	**91**	*91*	35	41	35	19	**32**	*32*	28	53	58	34	**43**	*43*	103	70	54	68	**74**	*74*
151, 123, 168, 180	Unknown					3363tr					5	15	**10**	*5*	4		3484tr			*1*				8	**8**	*2*
107, 166	1-phenyl-(4-propionic acid)	4007		3421tr		3382tr				3383tr			3337tr					1	**1**	*0*		16				*4*
178, 135, 147, 163, 194, 209	Coniferaldehyde	4025	2	8	8	3372tr	**6**	*5*	3367tr	1			**1**	*0*		2		2	**2**	*1*	5					*1*
181, 198, 226	ethylsyringate	4060	2				**2**	*0*															5		**5**	*1*
215, 159, 172, 230	MAS sesquiterpene	4074	1					*0*																		
181, 210	propiosyringone	4127		3502tr	3412tr					3464tr		3542tr	3414tr			3521tr	3491tr	3575tr	3559tr			3587tr				
181, 194, 224	butyrosyringone	4147	2	7	3		**4**	*3*	1	1	1	1	**1**	*1*	1	1	1		**1**	*1*		12	11	12	**12**	*9*
194, 179	cis -ferulic acid	4147									3		**3**	*1*									4	5		*2*
130, 161	Tryptophanol	4151	2	12	4	6	**6**	*6*	3	3	2	5	**3**	*3*	2	4	5	3	**4**	*4*	4			10	**7**	*4*
85, 71, 109, 124, 208	Unknown 17	4181				2	**2**	*0*	3507tr	1	1	1	**1**	*1*			3622tr	2	**2**	*1*		14			**14**	*3*
43, 123, 163, 209	Grasshopper Ketone	4234	60	209	92	90	**113**	*113*	22	75	39	36	**43**	*43*	44	93	99	41	**69**	*69*	129	117	105	100	**113**	*113*
151, 196, 123, 108	carboxyvanillate	4182	5	15	7	8	**9**	*9*	4	4	6	4	**5**	*5*	4	7	6	4	**5**	*5*	8		15		**11**	*6*
124, 168, 206	Vomifoliol	4326	10	58	23	21	**28**	*28*	11	16	21	12	**15**	*15*	9	18	119	11	**39**	*39*	22	82	53	43	**50**	*50*
198, 183	Syringic acid	4389	29	74	32	38	**43**	*43*	24	21	27	28	**25**	*25*	10	23	34	5	**18**	*18*		121	3676tr	98	**109**	*55*
168, 212	unknown shikimate	4414	7	52	30	13	**25**	*25*	7	9	10	7	**8**	*8*	6	11	16	9	**10**	*10*	25		80		**52**	*26*
110, 152, 170	Dihydrovomifoliol	4498	3	14	5	7	**7**	*7*	3	4	4	3	**3**	*3*	2	4	5	3	**4**	*4*	8		8		**8**	*4*
194, 179	trans ferulic Acid	4535	3804tr	3848tr	3752tr	3813tr			1	2	2	1	**1**	*1*		3841tr							3		**3**	*1*
181, 154, 212	2-(4-hydroxy-3,5-methylphenyl)-2-oxoethanol	4565	3818tr	3860tr	3768tr	3828tr			2	3827tr	1	1	**1**	*1*	1	2	3	3927tr	**2**	*2*			4	4		*2*
164, 163, 147, 212	trans-4-hydroxycinnamic acid	4593							3857tr	3859tr	1	3803tr	**1**	*0*												
151, 124, 164, 182	unkown shikimate	4627		16	10	3	**9**	*7*	6	4	7	5	**5**	*5*	4	5	12	3	**6**	*6*	8	28	33	19	**22**	*22*
73, 60	octadecadienoic acid																					17				*4*
181, 226	Unknown			11	4	4712tr	**7**	*4*	2	2		2	**2**	*2*	2	3	3	2	**3**	*3*	4		3	4	**4**	*3*
	TOTAL CONCENTRATION		677	1975	1132	1004	**1305**	*1197*	836	859	683	719	**908**	*774*	520	787	1182	2076	**1086**	*1141*	1644	2147	1776	1513	**2240**	*1770*

Appendix D. 5 Mean values of the tasting scores: two tasting dates combined for high and low density plantings.

	HIGH DENSITY			LOW DENSITY		
	Nil Thinned	Ripped Th. + Rip.		Nil Thinned	Ripped Th. + Rip.	
Overall score /20	15.05	14.97		15.02	14.95	
	14.15	14.47	***-	15.66	15.20	*+
Aroma /7	5.15	5.09		5.16	5.12	
	4.76	4.89	***-	5.31	5.16	
Taste /10	6.94	6.91		6.87	6.84	
	6.39	6.58	***-	7.36	7.05	**+

Mean values of tasting score and their significance: tasting date 1, replicates I and II for high and low density planting

	HIGH DENSITY			LOW DENSITY		
	Nil Thinned	Ripped Th. + Rip.		Nil Thinned	Ripped Th. + Rip.	
Overall score /20	15.05	14.73		15.11	15.11	
	14.27	14.27	**-	15.31	15.45	
Aroma /7	5.19	5.00		5.14	5.22	
	4.83	4.80	*-	5.13	5.22	
Taste /10	6.92	6.81		6.98	6.91	
	6.44	6.47	**-	7.20	7.25	

Mean values of tasting score and their significance: tasting date 2, replicates III and IV for high nd low density plantings

	HIGH DENSITY			LOW DENSITY		
	Nil Thinned	Ripped Th. + Rip.		Nil Thinned	Ripped Th. + Rip.	
Overall score /20	15.06	15.20		14.92	14.80	
	14.03	14.67	**+	16.0^ X	14.49	
					**-	
Aroma /7	5.11	5.19		5.17	5.02	
	4.69	4.98	**-	5.48	5.09	*+
Taste /10	6.95	7.02		6.75	6.78	
	6.34	6.69	*-	7.52 X	6.84	

1. Asterisks at lower right of the four values denote significance of the difference between the thinned and unthinned treatments. Similarly, asterisks above the top right value shows the significance of the ripping effect. 2. The plus or minus signs show the direction of the effect compared with unthinned or unripped 3. no asterisks, *, ** and *** denotes not significant, significant at $p<0.05$, $p<0.01$ and $p<0.001$ respectively. 4. X denotes a significant ($p<0.05$) interaction between thinning and ripping