A New Technology for Imparting Oak Character to Wine: Application to a New Zealand Chardonnay Wine

The University of Waikato
Te Whare Wānanga o Waikato

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Abstract

An analytical method using gas chromatography combined with mass spectrometry in selected ion mode (GC/MS-SIM) was developed to monitor the levels of aromatic woody compounds present in a New Zealand Chardonnay wine at the part per billion level. This technique was also used to determine the levels of compounds present in oak wood.

Sensory evaluations of experimental wines were carried out by experienced winemakers. Each assessment was complemented by the chemical analyses to provide an overall impression for the wines. The combination of winemaker's perception and chemical determinations were employed with all wine ageing and wooding trials carried out as part of the project.

A selection of ageing containers were chosen to monitor the ageing profiles of a Chardonnay wine under different conditions. New French oak barrels are the preferred ageing containers by the winemakers at Morton Estate Winery; especially those from Seguin Moreau and Dargaud & Jaegle. New French oak releases extractable compounds at at rate which is considered desirable. Wines which were aged in new French oak were described as 'elegant' and showed 'crisp, fresh oaky character.' Wines aged in used barrels and stainless steel scored lower in the sensory evaluations.

New French oak barrels contain elevated levels of aromatic compounds on the interior surface of each barrel due to thermal modifications which occur during the toasting process. The levels of these compounds are lower in used oak barrels.

It was found that heating used oak wood in an electric kiln increased the levels of aromatic compounds in the wood. Wrapping the oak in foil allowed longer toasting times and produced higher levels of thermal degradation products. These same products are considered desirable in higher quality wines.

The rates of release of extractable compounds from wood into ethanolic solutions and the effect of surface area was investigated. Toasted oak cubes of a particular dimension were found to release these compounds at a rate similar to that of new French oak. These cubes were suspended throughout the body of the wine using a new technology called the 'oak necklace.' The oak ageing trials over the 1993, 1994 and 1995 vintages provided encouraging evidence that Chardonnay wine exhibiting new oak character can be achieved using this new technology.

Acknowledgements

The completion of a research project such as this one requires the guidance and support of many people. I would like to express my grateful thanks to everyone who assisted me over recent years. Your on-going interest and enthusiasm ensured the completion of this thesis. I would especially like to thank the following people who, in your own way, had a positive influence on the outcome of this work.

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List of Abbreviations

GC	gas chromatography
GLC	gas/liquid chromatography
MS	mass spectrometry
GC/MS	gas chromatography/mass spectrometry
SIM	selected ion mode
HPLC	high performance liquid chromatography
FID	flame ionisation detector
UV	ultra violet
HETP	height equivalent to a theoretical plate
µg/L	microgram per litre
mg/L	milligram per litre
µg/g	microgram per gram
ppm	part per million
ppb	part per billion
nd	below 1 µg/L detection limit
nq	not quantified
id	internal diameter
sd	standard deviation
cv	coefficient of variability
D & J	Dargaud and Jaegle

Chapter One

Introduction and Literature Survey

1.0 Introduction

Grapes and wines are very important to the world.[1] Their magnitude of production, investment, tax revenue, employment and enjoyment reflect their importance and significance. Grapes are produced world-wide in greater tonnage than any other fruit. In countries such as Italy and France, grapes and wines represent a vital segment of the economy. These two countries alone produce one half of the world's wine. The top six wine-producing countries, including the United States, produce two thirds of the total.

The New Zealand wine industry is still maturing when compared to the the major wine-producing regions in Europe and America. In the last twenty years the industry has undergone some major changes. Before 1970, the bulk varietal wines were dominated by hybrid grape varieties - native American and French varietals which were thought to be suited to the New Zealand growing conditions. During the 1970s the industry was influenced by various German grape-growing practices and techniques. This influence saw a rapid increase in the amount of Muller Thurgau grapes being grown. For many years, Muller Thurgau was the most widely harvested grape in the country.

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[1] V. L. Singleton, 'Wine and Enology: Status and Outlook,' *Am. J. Enol. Vitic.* 43/4 (1992): 344-354.

The 1980s saw a move to plant more classical varieties such as Chardonnay and Sauvignon Blanc. It was realised that the success and security of the New Zealand wine industry would be dependent on its export market. The best opportunity to compete overseas lay with the promotion of premium table wines. The large-scale production output of the Australian and Californian wineries would always prove more cost effective at the lower end of the market; the New Zealand brands would lose out with higher cost per unit prices.

The 1990s has seen a shift towards producing up-market premium varietal wines which are being met with approval in overseas markets. In the past, the reputation of New Zealand wines has been confined to Sauvignon Blanc. More recently, New Zealand has gained recognition for its high quality Chardonnay wines. There has also been an increase in the proportion of red grapes being grown in New Zealand. Although the New Zealand climate does not suit the traditional red varieties, Merlot and Pinot Noir wines are beginning to gain popularity.

The success of Chardonnay wines in overseas markets has been due to a number of factors. Traditionally, the Chardonnay produced in New Zealand has not had a unique style. Winemaking practices in recent years, however, have adapted to allow a more subtle, more delicate style of wine to be produced. This elegant style of Chardonnay has emerged as being unique to New Zealand and is winning favour at international competitions.

The rise of Chardonnay as our most popular varietal has resulted from improved processing methods. Hand-harvested fruit and the whole-bunch pressing of fruit have replaced mechanised procedures resulting in less pick-up of undesirable material. Oak barrel fermentation of Chardonnay is now a common practice in New Zealand and lees stirring of the fermented juice and maturing wine removes particles from solution - the resulting wines show more distinctive fruity aromas.

Imparting oak character to wine is the winemaker's last chance to improve the quality of the final product. The practice of using oak chips in order to impart oak characteristics to wine at a rapid rate has been used extensively in Australia. The procedure has tended to be a relatively crude one and is normally used in the pre-fermentation or actual fermentation stages. Wines which are processed in this fashion are bulk-produced and tend to be stored only in stainless steel. This method gives the wine some oak character without the reliance on oak barrels. In New Zealand, the larger wineries have used chips purely for the economic gain of 'improving' the character of low quality wine. Higher prices can be demanded for 'wooded' styles as traditionally the degree of woody character has been a measure of wine quality.

Wood character is not the only indication of quality wine. It is the integration of fruit flavour and aromas complimented by the wood which determine the quality. The New Zealand style of Chardonnay has been complemented by a more effective use of the oak barrel for ageing purposes. Tighter grains of oak are now being used which have resulted in more subtle oak character - rather than oak dominant.

Both American and French oak barrels have been used to age New Zealand Chardonnay. French oak was seen as producing a more desirable style of wine but American oak was half the price. The major difference in price is due to the manual splitting of French oak versus the quarter sawing of American oak. Traditionally, American barrels were produced using the same technologies as those used for the production of Bourbon barrels. These techniques resulted in poor seasoning of the wood and heavy charring. Wines aged in these American casks have been described as 'coarse', 'sappy', and 'high in coconut and vanilla'.[2] In recent times, however, the French have had more input into the production of American barrels. There has been a considerable improvement in barrel-making procedures which has resulted in an improvement in quality. American oak has been grown in cooler climates resulting in tighter grains and the wood is now air-dried rather than kiln-dried.

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[2] J. Hancock, Personal communication, 5 November 1995.

Wineries throughout New Zealand use both French and American barrels to mature red and white wine. The slightly overpowering oak aromas characteristic of American oak can be offset by blending if required. The origin, seasoning and heat treatment of the oak vary depending on the cooperage. These factors are important in determining the best ageing container for the desired style of wine.

Under normal conditions, the lifetime of an oak barrel is 4-5 years. After this time, its capacity to impart woody compounds into the wine is significantly reduced. The average cost of a new French barrel is ca. $1000; the oak maturation process therefore demands a significant capital outlay. The total cost of 'wooding' a bottle of wine represents 25% of the total cost of production which is the same proportion as the cost of the grapes.

The reliance on new oak as a maturing vessel for Chardonnay is now well established in New Zealand. The French have used the traditional methods of maturing Chardonnay for over 200 years. Their methods for the production of high quality wine are completely reliant on new oak. It would appear that the production of New Zealand Chardonnay will also require new oak if its success in overseas markets is to continue. In the domestic market, however, the mystique which surrounds the oak ageing process could be overshadowed by a more cost-effective approach to wine ageing - an alternative procedure which still produces wine with the desirable oak aged character, but one which confronts the traditional methods of ageing wine.

1.1 Preliminary Wine Ageing Research

Analysis of a New Zealand Chardonnay wine took place at the University of Waikato.[3] The experimental wine was aged in both new French oak barrels and in

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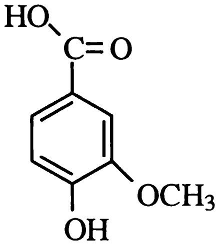
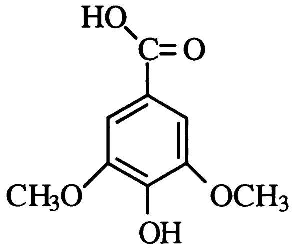
[3] C. P. Gribble, 'Some Studies of the Effects of Oak on the Ageing of Wine,' Masters Thesis, University of Waikato (1991).

stainless steel. The principal extractable organic compounds of this wine included malic acid, succinic acid and a range of fatty acids. The levels of these extracted compounds did not vary greatly in the different ageing containers throughout the ageing period.

Two aromatic acids, vanillic acid (**1**) and syringic acid (**2**) thought to be associated with the oak ageing process were detected in the wine at low levels. There was no attempt to quantify the levels of vanillic and syringic acids as the lower threshold limit for quantitation was reached (1 mg/L). Detection of these compounds was possible at lower levels but was not the focus of the work. One volatile woody compound, 2-furoic acid, was detected in wine aged in oak but not in wine aged in stainless steel so appeared to be introduced into the wine solely as a result of the oak ageing process.



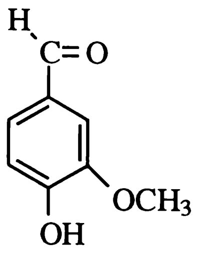
(**1**)



(**2**)

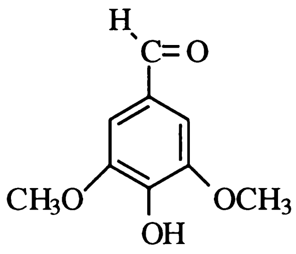
Extracts of new and used toasted and untoasted oak were also analysed. In the untoasted samples, fatty acids were the dominant compounds. Syringic acid was detected at a level of 20 μg/g in new untoasted oak. No other wood-derived aromatic compounds were found in the new or old untoasted samples.

New toasted oak showed the presence of wood-derived compounds, low molecular weight acids, fatty acids and aromatic compounds. Old toasted oak was depleted of the low molecular weight wood-derived compounds such as 2-furoic acid and 2-acetylfuran. The most notable effect of oak ageing on oak extractables was the depletion of an aromatic aldehyde, vanillin (**3**), which was present in high concentrations in new toasted oak but was found to be depleted in used toasted oak.



(3)

The presence of aromatic compounds such as vanillin were not detected in wine. The analytical technique employed for these experiments did not allow for accurate quantitation of compounds below the low part per million level. Moutounet et al.[4] identified a range of extractable compounds in a Chardonnay wine, including the aromatic aldehydes vanillin and syringaldehyde **(4)**. After 12 months ageing in new French barrels, the concentrations of vanillin and syringaldehyde were 0.2 mg/L and 0.4 mg/L respectively - in the part per billion range. A more sensitive method of detection would therefore be necessary to monitor the levels of these oak-derived extractables which were being introduced into the wine during the oak ageing process.



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1.2 Literature Survey

Flavour and aroma compounds in wine originate in the various production stages of the wine. The variety of grape, the fermentation process and degree of maturation each play their own special part in the formation of flavour and aroma. The nature and

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[4] M. Moutounet, Ph. Rabier, J. L. Puech, E. Verette and J. M. Barillere,'Analysis by HPLC of Extractable Substances in Oak Wood: Application to a Chardonnay Wine,' *Sciences des Aliments* 9 (1989): 35-51.

quantity of compounds formed during fermentation are affected by the fermentation conditions, which include temperature and level of yeast nutrients. Maturation establishes the sensory and organoleptic properties of the wine.

1.2.1 Analyses of Volatile Compounds in Wine

A headspace analysis of a kiwifruit wine and a Muller Thurgau wine was carried out by Craig.[5] Gas chromatography/mass spectrometry (GC/MS) revealed the presence of 47 compounds including alcohols and esters. Although the aroma profiles of the two wines looked similar at first, closer examination revealed differences in the concentrations of a range of volatile compounds.

The volatile compounds of grape must and the products of fermentation were reported by Bertrand.[6] These volatile compounds determine the aroma of wine and include alcohols, esters and volatile acids. A number of fermentation conditions were studied including the effect of oxygen and pH levels. Dubois[7] reported the significance of volatile phenols in red wine. A number of phenols reported were present in small amounts and possessed weak odours.

Edwards and Beelman[8] reported a modified method for the analysis of volatile compounds in wine. The method showed high recoveries for alcohols, esters and medium-chain fatty acids with good precision. Research and quality control were suggested as the potential applications of the method.

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[5] J. T. Craig, 'A Comparison of the Headspace Volatiles of Kiwifruit Wine with those of Vitis vinifera Variety Muller Thurgau,' *Am. J. Enol. Vitic.* 39/4 (1988): 321-324.

[6] A. Bertrand, 'Volatiles from Grape Must Fermentation,' *Flavour of Distilled Beverages:Originn and Development,* ed. J. R. Pigott, Chichester: Ellis Horwood, (1983): 93-109.

[7] P. Dubois, 'Volatile Phenols in Wine,' *Flavour of Distilled Beverages:Originn and Development,* ed. J. R. Pigott, Chichester: Ellis Horwood, (1983): 110-119.

[8] C. G. Edwards and R. B. Beelman, 'Extraction and Analysis of Volatile Compounds in White Wines Using Amberlite XAD-2 Resin and Capillary Gas Chromatography,' *J. Agric. Food Chem.* 38 (1990): 216-220.

A range of volatile and non-volatile compounds were identified by Gelsomini et al. using sorbent extraction techniques.[9] The extraction method was compared to liquid/liquid extraction and was reported as quicker, easier and less expensive. The extraction method combined with gas chromatography revealed 45 volatile compounds including acids and alcohols.

Continuous extraction techniques and gas chromatography/mass spectrometry revealed the presence of a large range of aroma components in a Spanish 'Verdejo' wine.[10] Alcohols, esters, terpenes and acids contributed to the 132 volatile components identified. The identification of these volatiles may be of interest for the characterisation of the wine.

1.2.2 Analyses of Acids in Wine

Acids contribute to the taste and stability of foods and beverages. The major acids found in grapes include malic and tartaric acids.

Both high performance liquid chromatography (HPLC) and gas chromatography (GC) have been used to identify carboxylic and fatty acids in wine. Calull et al.[11] described a reversed-phase HPLC method which identified three fatty acids present in wine. Before fermentation, no fatty acids were detected but caprylic and capric acids appeared during the fermentation process. Lauric acid was not detected at any stage of the fermentation process.

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[9] N. Gelsomini, F. Capozzi and C. Faggi, 'Separation and Identification of Volatile and Non-Volatile Compounds of Wine by Sorbent Extraction and Capillary Gas Chromatography,' *Journal of High Resolution Chromatography* 13/5 (1990): 352-355.

[10] T. Herraiz, G. Reglero, P. J. Martin-Alvarez, M. Herraiz and M. D. Cabezudo, 'Identification of Aroma Components of Spanish "Verdejo" Wine,' *J. Sci. Food Agric.* 55 (1991): 103-116.

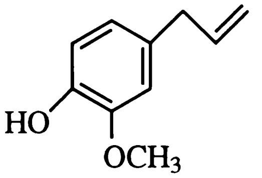
[11] M. Calull, F. Borrull, R. M. Marce and F. Zamora, 'HPLC Analysis of Fatty Acids in Wine,' *Am. J. Enol. Vitic.* 42/3 (1991): 268-273.

An optimised separation of carboxylic acids in wine was reported by Marce et al.[12] Good resolution of peaks was shown for a range of eluting solvents using an isocratic HPLC system. The determination of carboxylic acids in wine was said to be important because of their use in controlling the vinification process, and because of the influence of these acids in biological stability and organoleptic properties.

A range of carboxylic acids were identified for a number of different grape juices.[13] The HPLC method showed the presence of malic, tartaric, citric, succinic and other acids in grape juice. The purpose of the study was to use the composition of acids in grapes as a measure of authenticity of fruit juices.

1.2.3 Sensory Evaluation

Aiken and Noble[14] compared the sensory profiles of Cabernet Sauvignon wine aged in both glass and oak barrels. A panel of trained judges found two major differences between the wines. In the oak-aged wine, there was an increase in the intensity of spicy and vanilla aromas probably due to the extraction of phenolic compounds such as vanillin and eugenol (**5**) from the oak. The oak aged wine also showed a dramatic decrease in green bean/chillies aromas which may have resulted from degradation in the barrel.



(**5**)

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[12] R. M. Marce, M. Calull, J. C. Olucha, F. Borrull and F. X. Rius, 'Optomized Isocratic Separation of Major Carboxylic Acids in Wine,' *Journal of Chromatography*, 542 (1991): 277-293.

[13] T. Fuleki, E. Pelayo and R. B. Palabay, 'Carboxylic Acid Composition of Authentic Varietal and Commercial Grape Juices,' *Journal of AOAC International* 76/3 (1993): 591-599.

[14] J. W. Aiken and A. C. Noble, 'Comparison of the Aromas of Oak and Glass-Aged Wines,' *Am. J. Enol. Vitic.* 35/4 (1984): 196-199.

The aroma properties of some American and French oaks were analysed by Francis et al.[15] Both unseasoned and seasoned oaks were assessed either in Australia or in their country of origin. It was found that each of the treatments had influenced the aroma properties of the samples. Seasoning changed the green oak from having no significant character to having intense aroma. A hotter, drier and more humid seasoning environment appeared to promote the development of desirable wood aromas such as vanilla, caramel and cedar. French oak contained more intense aroma characteristics than the American oak. Overall, it was heat treatment which had the greatest effect on all the samples. Heating had a major effect on the sensory properties irrespective of the other variables.

1.2.4 The Oak Barrel: Its Contribution to the Ageing Process of Chardonnay Wine

Chardonnay wine styles originated in the Burgundy district of France. Classical styles such as the white Burgundies were matured in French oak barrels. Countries such as Australia and New Zealand also use French oak for the production of high quality Chardonnays. Many styles, in fact, are influenced by the contribution of oak barrel maturation. The wood gives the wine improved 'harmony' and a perfume of vanilla typical to quality wine.

The oak barrel has a significant role in the maturation of wine. Besides functioning as a physical storage vessel, the barrel provides extractable material to the wine and allows for chemical modifications to take place; both processes are associated with high quality wines. Oak wood imparts to the wine a typical woody character. The intensity and nature of the bouquet formed in the wood is dependent on oak origin, seasoning, barrel treatment and winemaking procedures.

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[15] I. L. Francis, M. A. Sefton and P. J. Williams, 'A Study by Sensory Descriptive Analysis of the Effects of Oak Origin, Seasoning, and Heating on the Aromas of Oak Model Wine Extracts,' *Am. J. Enol. Vitic.* 43/1 (1992): 23-30.

The barrel enriches the flavour, fragrance and character of wine by imparting a blend of volatile phenols, aromatic aldehydes, aromatic acids and tannins. These compounds marry with the wine and add complexity. By allowing a gradual penetration of oxygen, the barrel facilitates colour stabilisation, the decrease in tannin astringency, and the oxidation of species in the wine. The barrel also allows for the precipitation of tartrates and colloidal particles.

Wines aged in wood for extended periods may become unpleasant as the fruity character of the wine is masked by the dominant woody overtones. Wines suitable for oak ageing therefore require intensive fruit flavours to harmoniously combine the wood influence and subtly integrate the contributions of both to enhance the quality of the wine.

1.2.5 The Oak Barrel: Geographic Origin, Seasoning, 'Bousinage' and Cooperage

Among the 250 different species of oak, only three main species are used for the fabrication of barrels. A study by Miller et al.[16] examined the levels of aromatic aldehydes and acids found in different oak species from different sites. It is of interest to winemakers and coopers to know the variations in content of the wood and the influence this wood has on the final product. The results showed that there were considerable differences in the levels of aromatic content from the various samples. It was suggested that species is the most important factor in determining flavour components in oak wood.

Marco et al.[17] investigated the relationship between geographical origin and chemical

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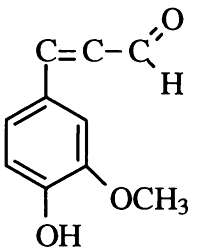
[16] D. P. Miller, G. S. Howell, C. S. Michaelis and D. I. Dickmann, 'The Content of Phenolic Acid and Aldehyde Flavor Components of White Oak as Affected by Site and Species,' *Am. J. Enol. Vitic.* 43/4 (1992): 333-338.

[17] J. Marco, J. Artajona, M. S. Larrechi and F. X. Rius, 'Relationship Between Geographical Origin and Chemical Composition of Wood for Oak Barrels,' *Am. J. Enol. Vitic.* 45/2 (1994): 192-200.

composition of oak wood. A number of woody compounds were targeted with the use of gas chromatography as indicators of geographic origin. The American samples had higher levels of vanillin and coniferaldehyde **(6)** while the French samples were higher in syringaldehyde and sinapaldehyde **(7)**.



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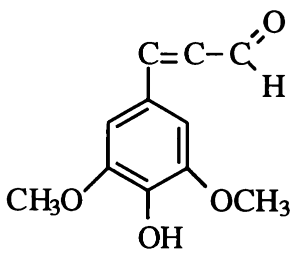

(7)

In France, the cooper's wood originates from four main regions: Limousin, Central, Burgandy and Vosges. Since the composition of the wood from different areas is variable (mainly in terms of density and porosity), the impact of the barrel on flavour and aroma is equally variable. The selection of the origin of the wood, the seasoning and the degree of toasting allows the winemaker to regulate the effects of wood on the wine.

Before oak is suitable for coopering it must have the moisture removed. This seasoning process is carried out over a period of 2-3 years. The wood is stacked in open yards exposed to all the climatic conditions. The stacks are designed to maximise the circulation of air in the centre of the stack.

Traditionally, the cooper would heat the barrel in order to decrease the resistance of the wood ('soften') and to reduce the risk of cracking the staves. Merely heating the wood is insufficient to alter the physical properties of compounds such as cellulose and hemicellulose which represent a large proportion of the wood's mass. These compounds are more sensitive to humidity. Staves are therefore bent into shape using both heat and steam.

Heating the internal surface of the barrel to alter its composition (oak wood toasting or 'bousinage') is a more recent operation, inspired by the experience of ageing brandies in oak cooperage. The toasting process goes beyond the main aim of the cooper whose intention is to produce a stable, watertight container. A deliberate technique is employed to alter the structure and composition of the wood. These chemical modifications generate a range of 'by-products' which contribute to wine flavour and aroma. Certain compounds are derived from the thermal degradation of lignin, which represents up to 20% of the wood mass. The phenolic aldehydes, notably vanillin, present an agreeable vanilla aroma. The concentrations of these lignin-derived compounds increase in concentration depending on the degree of toasting intensity. Untoasted wood already contains some phenolic compounds, but overall, the levels of phenolics are increased to a maximum at around medium toast (10-15 min toasting time).

The heating process was traditionally carried out manually using the heat of an open fire supplied with off-cuts of wood. Consistency of technique is a problem as the operation is performed differently from one cooper to another. During the toasting process the interior surface of the staves are exposed to relatively high temperatures (200°C). Some volatile odour compounds disappear rapidly as the wood is heated. Other substances, which are not present in the untoasted wood, develop during toasting.

1.2.6 Origin of Phenolic Compounds in Wine

Phenolic compounds are important constituents in wine contributing mainly to flavour and astringency. They play an important role in the treatment and ageing of wine.[18] The presence of 33 phenolic compounds in the must, skins and seeds of a white grape variety were reported by Fernandez de Simon et al.[19] The concentrations of benzoic

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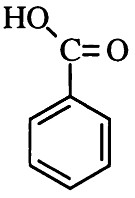
[18] M. Salagoity-Auguste and A. Bertrand, 'Wine Phenolics - Analysis of Low Molecular Components by High Performance Liquid Chromatography,' *J. Sci. Food Agric.* 35 (1984): 1241-1247.

[19] B. Fernandez de Simon, T. Hernandez and I. Estrella, 'Phenolic Composition of White Grapes. Changes During Ripening,' *Food Chemistry* 47 (1993): 47-52.

(8) and cinnamic acids **(9)**, and benzoic aldehydes were not present in appreciable quantities in the seeds. There were appreciable levels of all the phenolic components in the skin and in the must. These compounds such as vanillin, vanillic acid, syringaldehyde and syringic acid are associated with the oak ageing process. The levels of these compounds increased during the berry ripening process.



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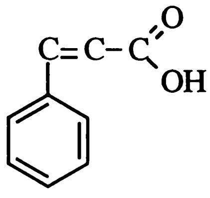
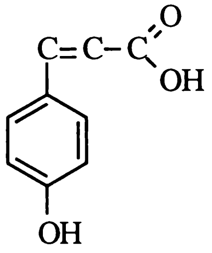


(9)

Ramos et al.[20] reported on the transformations undergone by phenolic compounds in anaerobic conditions - in the presence of CO_2. Rapid transformations take place during crushing and fermentation. The study was focused upon the berry skins which contain the highest concentrations of phenolic compounds. Important modifications take place in the presence of CO_2 which result in variations of the ratios of various compounds. There is a strong potential for the release of *p*-coumaric acid **(10)** which may be re-used in the metabolic pathways leading to the production of aromatic compounds.



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An extensive review of phenolic compounds and their relationship to wine flavour was carried out by Singleton and Noble.[21] In most wines, the polyphenolic compounds

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[20] T. Ramos, A. Fleuriet, M. Rascalou and J. J. Macheix, 'The Effect of Anaerobic Metabolism of Grape Berry Skins on Phenolic Compounds,' *Am. J. Enol. Vitic.* 44/1 (1993): 13-16.

[21] V. L. Singleton and A. C. Noble, 'Wine Flavor and Phenolic Substances,' *ACS Symposium*

are the main group of compounds that undergo oxidation during the ageing process. Thus, the processing and ageing of the wine produces direct changes in taste and odour by the chemical modification of the phenolic compounds.

1.2.7 Extraction of Phenolic Compounds from Oak Wood into Aqueous Media

Early work carried out by Singleton and Draper[22] analysed the rates of extraction of oak wood meal and oak chips into aqueous ethanol solutions. The extraction of solid material from meal into ethanolic solutions reached a maxima in 1 day. A further 16 days of extraction showed no increase in the levels of extracted material. The extraction rate of solid material from wood chips into solution was a little slower.

Phenolic extraction curves were determined for white wines aged in French and American oak barrels.[23] The experiments were carried out using three fill and three empty cycles. The time in barrel was 12 and 16 weeks for the first and second fills respectively; for the third fill, the ageing time was six months. The extraction rates for the first fills were rapid at the start, then decreased towards the end of the ageing period. This extraction profile suggests that the extraction rate is based on diffusion kinetics. By the third fill, all the diffusable phenols have been extracted from the wood surface, suggesting that remaining phenols were released into the wine by hydrolysis of bound and complexed wood phenols. The extraction rate now followed a linear relationship indicating hydrolysis kinetics.

Wine aged in new American oak was compared to the same wine aged in stainless steel

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Series, 26 (1976): 47-70.

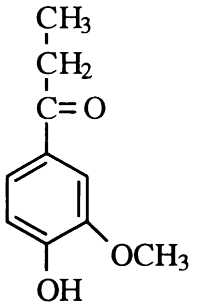
[22] V. L. Singleton and D. E. Draper, 'Wood Chips and Wine Treatment: the Nature of Aqueous Alcohol Extracts,' *Am. J. Enol. Vitic.*12 (1961): 152-158.

[23] C. Rous and B. Alderson, 'Phenolic Extraction Curves for White Wine Aged in French and American Oak Barrels,' *Am. J. Enol. Vitic.* 34/4 (1983): 211-215.

tanks and used barrels, both containing oak chips.[24] The concentrations of phenolic acids after different wood treatments appeared to remain constant, indicating minimal extraction of these compounds from barrel wood or oak chips.

Moutounet et al. (1989) analysed a solution of 12% alcohol-water at pH 3.2 after being stored in oak for 12 months. The solution showed the presence of aromatic acids and aldehydes at levels reaching 0.5 mg/L.

A comparison of the aromatic content of charred and uncharred oak wood chips extracted into 60% ethanol solutions was carried out by Nishimura et al.[25] The concentrations of vanillin and syringaldehyde in solution was 0.14 and 0.27 mg/L respectively for uncharred chips and 6.25 and 12.4 mg/L for charred chips. Other aromatic compounds showed similar results. The effect of toasting or charring of oak chips was also investigated. The results indicated that the levels of aromatic aldehydes and acids are increased with toasting, while untoasted chips contain few aromatics. The levels of propiovanillone **(11)** and acetosyringone **(12)** are also increased at higher temperatures.



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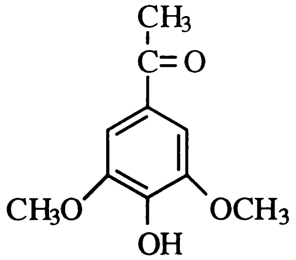

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[24] K. L. Wilker and J. F. Gallander, 'Comparison of Seyval Blanc Wine Aged in Barrels and Stainless Steel Tanks with Oak Chips,' *Am. J. Enol. Vitic.* 39/1 (1988): 38-43.

[25] K. Nishimura, M. Ohnishi, M. Masuda, K. Koga and R. Matsuyama, 'Reactions of Wood Components During Maturation,' *Flavour of Distilled Beverages:Originn and Development,* ed. J. R. Pigott, Chichester: Ellis Horwood, (1983): 241-255.

1.2.8 Analyses of Oak Wood and Alcoholic Beverages

The changes in wood extractives and the maturation of Scotch Malt Whisky was monitored by Conner et al.[26] During maturation, oxidation and ethanolysis result in a modification and breakdown of wood polymers that diffuse into the spirit producing colour, flavour and more complex sensory characters. To enhance the extent and rate of wood polymer breakdown, the casks are toasted or charred to produce both aerobic and anaerobic pyrolysis reactions that will generate solubles extracted by the aqueous ethanol.[27]

Conner et al. (1993) sampled staves from various casks which had different histories and originated from different oak species. Wood was sampled from staves at depths of 5 mm-25 mm from the interior of the cask. Shavings from each depth were extracted using Soxhlet extraction with chloroform and analysed by HPLC. It was found that the aromatic compounds vanillin, syringaldehyde, vanillic acid and syringic acid showed maxima at a depth of 5 mm from the charred surface. In new wood, syringaldehyde was present in levels 2-3 times higher than vanillin. There were low levels of aromatic compounds in used wood.

Puech[28] identified a range of aromatic aldehydes and acids in the oak ageing of Armagnac. Syringaldehyde and vanillin represented 50% and 30% respectively of the total aromatic aldehydes. In a later publication, Puech[29] compared the phenolic compounds found in oak wood extracts used to age brandy. Vanillic and syringic

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[26] J. M. Conner, A. Paterson and J. R. Piggott, 'Changes in Wood Extractives from Oak Cask Staves through Maturation of Scotch Malt Whiskey,' *J Sci Food Agric* 62 (1993): 169-174.

[27] V.L. Singleton, 'Some Aspects of the Wooden Container as a Factor in Wine Maturation,' *Chemistry of Winemaking*, ed. A. D. Webb (New York: ACS, 1974) 310.

[28] J. L. Puech, 'Extraction and Evolution of Lignin Products in Armagnac Matured in Oak,' *Am. J. Enol. Vitic.* 32/2 (1981): 111-114.

[29] J. L. Puech, 'Phenolic Compounds in Oak Wood Extracts Used in the Ageing of Brandies,' *J. Sci. Food Agric* 42/2 (1988): 165-172.

acids dominated the lignin-derived acids found in brandy. There were contrasting high and low levels of syringaldehyde and coniferaldehyde found in different oak wood extracts.

Vanillin, syringaldehyde, sinapaldehyde and coniferaldehyde were analysed from European oak and brandy matured in casks of this same wood.[30] In the brandy, the four aromatic aldehydes were present at 0.6 mg/L, 0.35 mg/L, 1.4 mg/L and 0.8 mg/L respectively. In oak, both vanillin and syringaldehyde were present in trace amounts, but coniferaldehyde and sinapaldehyde were not detected.

A range of phenolic compounds were detected in an ethanol-water extract and in a brandy by Puech and Moutounet.[31] HPLC revealed the presence of ellagitannins in the ethanol-water extract but not in the brandy. The brandy did contain lignin degradation products (aromatic aldehydes and acids).

Lehtonen and Jounela-Eriksson[32] used GC/MS and GLC to identify some non-volatile compounds in whisky, rum, cognac and an ethanol extract of oak chips. Gallic acid and syringaldehyde are dominating compounds in the flavour of Scotch Whisky and brandy. Vanillin plays an important role in whiskies other than Scotch. Syringic acid and syringaldehyde are the main phenolic compounds in rums.

1.2.9 Analyses of French and American Oak Wood

Hoey and Codrington[33] compared the relative contributions of new, shaved and old

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[30] K. Nabeta, J. Yonekubo and M. Miyake, 'Phenolic Compounds from the Heartwood of European Oak and Brandy,' *Mokuzai Gakkaishi* 33/5 (1987): 405-415.

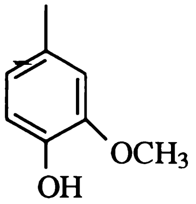
[31] J. L. Puech and M. Moutounet, 'Phenolic Compounds in an Ethanol-Water Extract of Oak Wood and in a Brandy,' *Lebensm.-Wiss. u.-Technol* 25 (1992): 350-352.

[32] M. Lehtonen and P. Jounela-Eriksson, 'Volatile and Non-Volatile Compounds in the Flavour of Alcoholic Beverages,' *Flavour of Distilled Beverages:Origin and Development*, ed. J. R. Pigott, Chichester: Ellis Horwood, (1983): 63-78.

[33] A. W. Hoey and I. D. Codrington, 'Contribution of Oak Barrel Maturation to Chardonnay Wine Styles,' *Proceedings of the Second International Cool Climate Viticulture and Oenology*

unshaven barrels. The effects of these different ageing conditions were monitored using sensory analysis and gas chromatography. The results of this study showed that new oak barrels allow for rapid diffusion of flavour and aroma compounds into the wine. The practice of shaving barrels allowed for quicker diffusion of the remaining oak compounds into wine.

A range of phenols, carbohydrate degradation products, lactones, terpenes and hydrolysable tannins were identified by Sefton.[34] The group of phenols based on the guaiacyl (**13**) and syringyl (**14**) structure are generally regarded as products of lignin degradation which makes up 25% to 35% of the dry weight of the wood.



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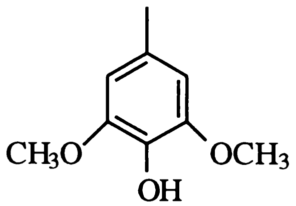

(14)

Laszlo[35] reported lignin-derived aromatic aldehydes such as vanillin and syringaldehyde as the the most important phenolic components originating from wood. Their extraction from the wood to the wine and conversion into the corresponding acids is an important feature of wood ageing. The amounts of vanillin and syringaldehyde in a range of French oaks was ca. 2 - 4 mg/g. The concentrations of some aromatic acids were also reported in wine aged in oak for one year. Vanillic and syringic acids were present at levels of 7 mg/L and 15 mg/L respectively.

Lebrun[36] compared the effect of a new French oak barrel, an old French oak barrel

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Symposium, Auckland, New Zealand (1988): 335-340.

[34] M. A. Sefton, 'How does Oak Barrel Maturation Contribute to Wine Flavor,' *Australian & New Zealand Wine Industry Journal*, Nov./Dec. (1991): 17-20.

[35] J. Laszlo, 'Wine Ageing in Oak Barrels,' *Wynboer*, Januarie (1984): 53-56.

[36] L. Lebrun, 'Used Barrels and Resulting Aromas,' *The Australian Grapegrower & Winemaker*, July (1991): 21-22.

and stainless steel on the character of wine. It was concluded that the aromatic transfer from oak to wine is far more efficient with new oak barrels. The concentration of vanillin could be multiplied up to 10 times by the use of a new oak barrel.

The concentrations of the total phenol content of seasoned and unseasoned French and American oaks were reported by Pocock et al.[37] The levels of phenolics were measured in model wine solutions and in wine stored in a range of oak containers. Sensory evaluations were also carried out on the various solutions. From the sensory findings it was found that the oak-derived volatiles had a much lower sensory threshold which suggests that these compounds may be the primary cue that wine has received oak treatment.

1.2.10 Analyses of Oak Chips

Puech[38] used HPLC to identify lignin degradation products in oak chips and in model solutions. Vanillin, syringaldehyde and coniferaldehyde were detected in oak chips at levels of 0.4 μg/g, 0.5 μg/g and 0.3 μg/g respectively. Vanillic and syringic acids were not detected in oak chips. In the model solutions, a more intense extraction of aromatic acids and aldehydes was observed in solutions buffered with acid. The amounts of vanillic and syringic acids in this medium were two and three times greater than those of the non-acidified medium.

Moutounet et al. (1989) analysed the effects of oak chips on a water-alcohol medium. The oak chips were agitated in the aqueous medium for eight hours. At this point, the levels of extractable compounds in the solution were similar to those found in a similar solution which was stored in oak for 12 months. HPLC revealed the presence of a

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[37] K. F. Pocock, M. A. Sefton and P. J. Williams, 'Taste Thresholds of Phenolic Extracts of French and American Oakwood: The Influence of Oak Phenols on Wine Flavour,' *Am. J. Enol. Vitic.* 45/4 (1994): 429-434.

[38] J. L. Puech, 'Extraction of Phenolic Compounds from Oak Wood in Model Solutions and Evolution of Aromatic Aldehydes in Wines Aged in Oak Barrels,' *Am. J. Enol. and Vitic.* 38/3 (1987): 236-238.

range of wood-derived compounds including vanillin, syringaldehyde, coniferaldehyde and sinapaldehyde.

1.2.11 Recent Publications

Reid et al.[39] used pyrolysis-mass spectrometry to assess the quality of scotch whisky and its correlation to the oak wood cask. A direct relationship was found between the quality of the whisky and the levels of phenolic extractives. Good quality whiskies showed elevated levels of phenolic compounds such as vanillic acid, syringic acid, syringaldehyde, vanillin and coniferaldehyde as compared with poor quality whiskies. It was suggested that further work could allow pyrolysis-mass spectrometry to provide a measure of whisky quality.

Clyne et al.[40] compared the chemical composition and aroma attributes of whisky distillates in charred and uncharred American oak casks over a three year maturation period. Sensory profiles were significantly different for the charred and uncharred samples; charred samples were described as ‘smooth’, ‘vanilla’ and ‘sweet’. All samples had different levels of phenolic compounds. Cask charring enhanced the levels of syringaldehyde but showed lower levels of coniferaldehyde, sinapaldehyde and vanillic acid.

1.3 Summary of Literature Survey

A complex mixture of volatile and non-volatile compounds are responsible for the aroma and flavour of wine. The oak ageing process enriches the flavour, aroma and

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[39] K. J. G. Reid, J. S. Swan and C. S. Gutteridge, ‘Assessment of Scotch Whisky Quality by Pyrolysis-mass spectrometry and the Subsequent Correlation of Quality with the Oak Wood Cask,’ *Journal of Analytical and Applied Pyrolysis* 25 (1993): 49-62.

[40] J. Clyne, J. M. Conner, A. Patterson and J. R. Piggott, ‘The Effect of Cask Charring on Scotch Whisky Maturation,’ *International Journal of Food Science and Technology* 28 (1993): 69-81.

character of the wine by providing extractable compounds which add to the complexity. The oak barrel also facilitates chemical transformations by allowing the gradual penetration of oxygen into the wine. The oak wood gives the wine a 'harmony' and a sweet perfume which characterises higher quality wines.

A number of extraction methods and analytical techniques have been employed to analyse the chemical profiles of wine. The same techniques, combined with various extraction methods, have been used to study the extractable compounds in various oak types, to monitor the effects of different coopering methods, and to determine the effects of heating oak wood during the oak toasting process. These techniques include HPLC, GC and GC/MS.

The volatile compounds found in wine including alcohols, esters and a large range of acids have been well described in the literature. The chemical changes which occur during wine ageing and the role of these volatile compounds was not the focus of this work. Preliminary work showed no observable correlation between the levels of acids and their derivatives throughout the oak ageing process of a New Zealand Chardonnay.

Other alcoholic beverages, typically Scotch Whisky, are stored in oak barrels for prolonged periods. The extractable compounds originating from the oak are part of the chemical changes which occur during the ageing process. The resulting spirit is mellow and pleasing to the palate. Aromatic compounds which are introduced into the spirit include vanillin, syringaldehyde, coniferaldehyde, vanillic acid and syringic acid. These compounds are used to describe the quality of the oak aged product.

The same compounds which enhance the flavour and character of whisky are important in the development of high quality wine. The toasting of the interior of the oak barrel provides elevated levels of aromatic lignin-derived compounds which are leached into the wine during exposure to oak. Preliminary work showed high levels of these compounds in new oak and low levels in used oak. Sensory profiles associate the 'vanilla-like' perfume of new oak with high quality wine.

The use of oak chips in order to impart the oak extractable compounds into the wine in a similar way to that of the traditional oak barrel has been tried. The oak chips appear to release their extractables at a rapid rate, however, not allowing time for the subtle integration of fruit and wood character to occur. As a result, the fruity flavours of the wine tend to to be overpowered by the coarseness of the wood and the wine may be described as 'disjointed'.

Traditional methods of ageing wine involve the use of toasted oak barrels which contain enriched levels of woody material at the wine/wood interface. The release of this material into the wine is a gradual process; consequently the ageing process takes a considerable length of time. In New Zealand, the ageing time for a Chardonnay wine depending on the desired style is 4-12 months. The amount of woody material leached into the wine during this time is also dependent on the type of barrel, the degree of toasting and the age of the barrel. The performance of an oak barrel decreases over time. Each subsequent filling results in further depletion of woody compounds from the interior of the barrel.

1.4 Morton Estate Winery

A project of this nature required a partnership with the industry to provide winemaking expertise and samples. John Hancock and Steven Bird from Morton Estate Winery were keen supporters of the project and played a vital role in the sensory evaluation of the experimental wines.

Morton Estate Winery is a medium-sized winery situated on the main highway between Tauranga and Katikati in the North Island of New Zealand. Since opening in 1983, John Hancock has built up a reputation centred around high quality barrel-fermented Chardonnay wines. Success too has come in the form of other varieties including Sauvignon Blanc, Cabernet Sauvignon and a leading Methode Champenoise.

1.5 Objectives of this Work

1. Develop an analytical technique to allow the extraction and analysis of target wood extractable compounds present in oak wood and in wine at the part per billion level.

2. Study the changes in the levels of these target extractable compounds over the ageing period using a range of ageing containers including: new French oak, new American oak, used oak and stainless steel. Carry out sensory evaluations to complement the chemical analyses.

3. Investigate the effects of the oak toasting process on the nature of organic extractable compounds in the oak wood. Develop a convenient method for toasting the rejuvenated oak which will provide the levels of aromatic compounds similar to that of new oak.

5. Explore the potential for imparting oak extractable compounds to the wine and enhancing character and complexity without reliance upon traditional ageing methods.

6. Develop an ageing technology based on rejuvenated oak which mimics the behaviour of the traditional new oak barrel.

Chapter Two

Experimental Methods and Materials

2.0 Introduction

Consideration of the various analytical methods available led to the decision to develop and use gas chromatography combined with mass spectrometry in selected ion mode (GC/MS-SIM) as a principal analytical technique for the work.

The techniques for extraction and the methods of analysis of ethanol/water samples, wine and oak wood samples employed for this work are discussed in this chapter. An overview of the various analysis methods investigated will also be discussed. The parameters affecting reproducibility and quality control such as detector linearity and efficiencies of recovery are included in the discussion.

Methods of sensory evaluation by controlled tasting, an important part of a project involving wine research are also described in this chapter.

2.1 Extraction of Low Volatile Compounds

Extraction of compounds from a sample matrix using a liquid to solubilise the target species is a common technique. The main advantages are: the ability to choose a range of extracting solvents, simple equipment, sample concentration, and samples which are dissolved in a phase compatible with the requirements of a chromatographic instrument.

The efficiency of the extracting solvent depends mainly on the affinity of the solute for the extracting solvent (distribution constant, K_D), the phase ratio (V) and the number of extractions (n). When continuous extraction techniques are employed then the value of n can be made large to maximise extraction efficiencies. When K_D is small, a large value for n allows for good recoveries of solute from the sample matrix. The fraction of solute extracted from the aqueous phase in n successive extractions is given by:

$$\varnothing_A = 1 - [\,1 / (1 + K_D.V]^{\,n}$$

where:

$\varnothing_A$ = fraction of solute A extracted

K_D = distribution constant ([A] in organic phase / [A] in aqueous phase)

V = phase ratio (volume of organic phase / volume of aqueous phase)

Continuous extractors are useful when the sample volume is large, the distribution constant is small, or the rate of extraction is slow. Extractions may be performed over many hours without the need for supervision. During this time, concentration factors of several orders of magnitude can be obtained.

Fernandez de Simon et al.[1] carried out a comparative study on the extraction efficiencies of diethyl ether and ethyl acetate using data from HPLC analysis. The recovery percentages for vanillic acid and syringaldehyde were 50% and 58% respectively for diethyl ether and 56% and 46% respectively for ethyl acetate. Other phenolic compounds showed similar extraction efficiencies. The standard deviation was calculated for each extracted compound and ranged from 4% to 10%.

Peters compared a range of extraction techniques for extracting trace organics from water.[2] Continuous liquid/liquid extractors achieved a 72% recovery for phenol. Other phenolics had recoveries between 72% and 98%. The extracting solvent was

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[1] B. Fernandez de Simon, J. Perez-Ilzarbe, T. Hernandez, C. Gomez-Cordoves and I. Estrella, 'HPLC Study of the Efficiency of Extraction of Phenolic Compounds,' *Chromatographia* 30, 1/2 July (1990): 35-37.

[2] T. L. Peters, 'Comparison of Continuous Extractors for the Extraction and Concentration of Trace Organics from Water,' *Anal. Chem.* 54 (1982): 1913.

dichloromethane.

A continuous extraction apparatus was described by Goldberg and DeLong.[3] High recoveries were achieved for the two chosen compounds; 2-pentanol and toluene. Recoveries for a range of extracting solvents including chloroform were reported.

Different liquid/liquid extraction techniques and designs have been described.[4,5,6] The use of continuous liquid/liquid extraction procedures for the analysis of wine was shown to be suitable for the recovery of neutral and acidic organic compounds with molecular weights in the range 100-400 atomic mass units (amu).[7] The continual replacement of solvent minimises partitioning effects and allows for good recovery of acids, aromatics and phenolic compounds.

2.1.1 Extraction of Wine Components by Liquid/Liquid Extraction

150 mL liquid/liquid extraction vessels and doubly distilled chloroform (ca. 250 mL) were used for wine and water/ethanol extractions throughout this work. Other extracting solvents were investigated (diethyl ether and dichloromethane); these solvents showed similar recoveries of the compounds of interest. Chloroform was shown to be pure after the double distillation process and was the most accessible solvent in the initial stages of the project so was used throughout the course of this

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[3] M. C. Goldberg and L. Delong, 'Extraction and Concentration of Organic Solutes from Water,' *Anal. Chem.* 45/1 (1973): 89-92.

[4] G. H. Morrison and H. F. Frieser, 'Solvent Extraction in Analytical Chemistry,' John Wiley and Sons (New York: 1966): 86-105.

[5] M. Ahnoff and B. Josefsson, 'Simple Apparatus for On-site Continuous Liquid-Liquid Extraction of Organic Compounds from Natural Waters,' *Anal. Chem* 46 (1974): 658.

[6] W. A. Hoffman, 'Compact, Variable Volume, Liquid/Liquid Extractor,' *Anal. Chem*, 50 (1978): 2158.

[7] C. P. Gribble, 'Some Studies of the Effects of Oak on the Ageing of Wine,' Masters Thesis, University of Waikato (1991).

work. Preliminary studies showed a 12 - 14 hr extraction period to be suitable for the recovery of organic compounds in wine.[8] For all extractions, however, an exhaustive extractive time was employed (ca. 24 hr). Longer extraction times showed similar recoveries of compounds from the sample matrix.

Chloroform is more dense than water and wine, so a liquid/liquid extraction system with appropriate siphon design was chosen (see Figure 2.1).

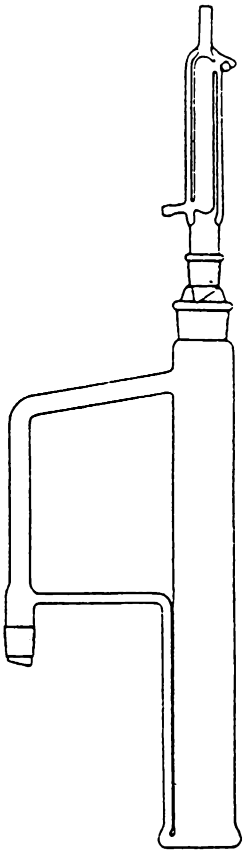

Figure 2.1 Liquid/liquid extraction system for liquid samples

The return of solvent to the round bottom flask is continuous. Extracted material is concentrated in the flask while clean solvent is constantly being recirculated.

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[8] I. Graves, 71.373 Report, University of Waikato (1988).

2.1.2 Extraction of Oak Wood Components by Soxhlet Extraction

Continuous liquid extraction of solid samples is carried out using a Soxhlet apparatus. The solvent is vapourised, condensed, then allowed to percolate through a solid sample contained in an extraction thimble. The return of solvent to the boiling flask is discontinuous; the solvent is returned using a syphon design, only when a certain volume of solvent is reached in the extraction chamber. All extractions involving oak chips, oak bodies and macerated oak samples used Soxhlet extractors with cellulose thimbles (see Figure 2.2).

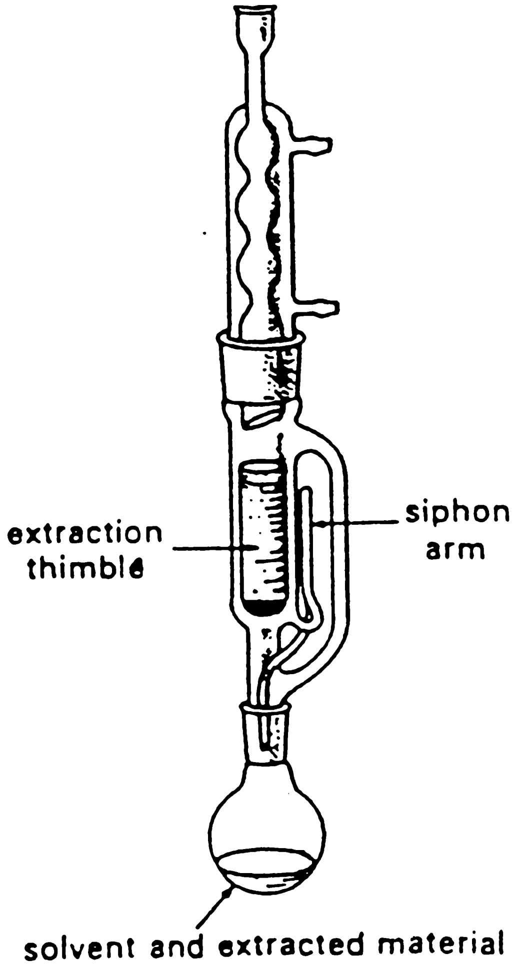

Figure 2.2 Soxhlet extraction system for solid samples

Like liquid/liquid extraction, the extracted material is concentrated in the flask while allowing constant recirculation of clean solvent.

2.2 *Preparation of Extracts for Analysis*

2.2.1 Concentration by Rotory Evaporation

At the completion of the 24 hour extraction period, the extract was dried with anhydrous MgSO4 and filtered through sintered glass. The 250 mL extract was concentrated before analysis took place.

Karasek et al.[9] compared rotary evaporation and the Kuderna-Danish evaporation method for the concentration of organic compounds. A range of organic compounds, including *n*-hexadecane and phenol were recovered at 85-90% using rotary evaporation. The same compounds were recovered at 90-92% using the Kuderna-Danish method. It was reported that The Kuderna-Danish method achieved better recoveries for compounds with higher volatilities. Practically, however, the rotary evaporation method was faster and achieved good recoveries of organics without the use of delicate and more specialised equipment.

Table 2.1 shows the recoveries of aromatic compounds using rotary evaporation followed with analysis by gas chromatography.

Table 2.1 Recoveries (%) of aromatic compounds after concentration by rotary evaporation

Sample	1	2	3	4	mean	sd
Vanillin	96	86	80	70	83	11
Acetovanillone	104	101	90	94	97	6.4
Vanillic acid	95	92	91	92	93	1.7
Syringaldehyde	100	72	73	68	78	15
Acetosyringone	99	100	92	88	95	5.7
Syringic acid	101	95	98	90	96	4.7

These recoveries were consistent with the findings of Karasek et al. They were

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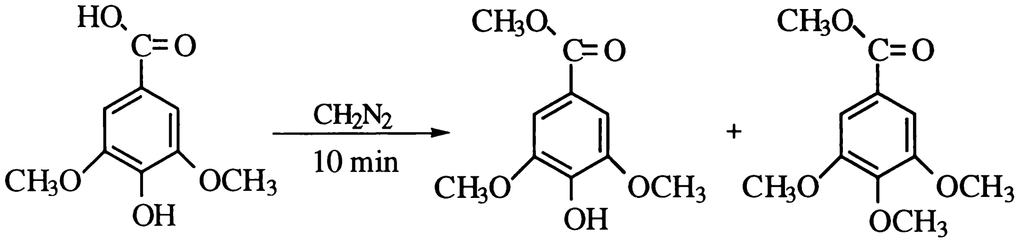
[9] F. W. Karasek, R. E. Clement and J. A. Sweetman, 'Preconcentration for Trace Analysis of Organic Compounds,' *Anal. Chem.* 53A (1981): 1050A.

considered suitable for the concentration of target species used in the wine and oak analyses. All extracts were therefore concentrated to approximately 1-2 mL using a glass rotary evaporator.

2.2.2 Sample Derivatisation

All samples were methylated with an ethereal solution of diazomethane. This derivatisation step was carried out to improve the stabilities and volatilities of compounds containing protonic functional groups. The derivatised sample exhibited improved peak shape due to the minimisation of column interactions associated with the polar acid functionality. While the carboxyl group in aliphatic compounds such as fatty acids methylated relatively quickly (ca. 10 min), it was noticed that if left for only 10 min, the methylation of phenolic hydroxyl group(s) in di- and tri-substituted aromatic compounds was variable (see Scheme 2.1). The Ar-OH group is less acidic than COOH groups and hence takes a longer time to methylate.

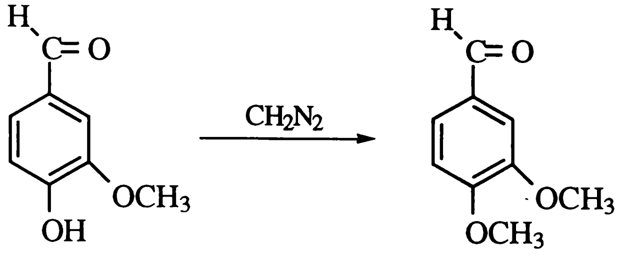


Scheme 2.1 Incomplete methylation of syringic acid

Exhaustive methylation (ca. 12 hr) was therefore carried out to ensure only the completely methylated product was present. Before any analyses took place, a test mix containing a range of compounds including aliphatic acids and substituted aromatic compounds was analysed by gas chromatography/mass spectrometry (GC/MS) to confirm the presence of the fully methylated components.

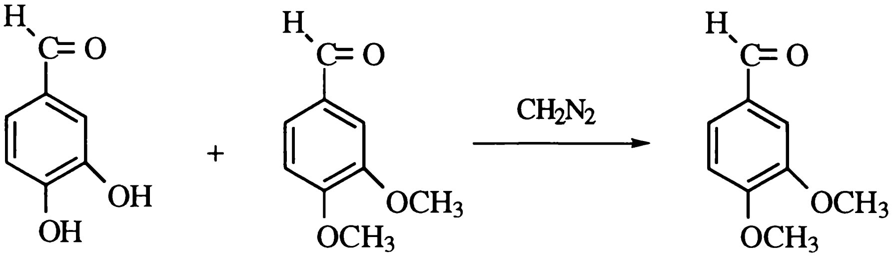
Excess diazomethane was removed using nitrogen gas. Samples were then sealed, labelled and refrigerated (ca. 4°C) until needed for analysis.

It was realised that the derivatisation step had the potential to lose the individual identity of some di- and tri-substituted aromatic compounds. The action of diazomethane converted components from the guaiacyl and syringyl series into their corresponding methylated analogues. Vanillin, for example, is converted into 3,4-dimethoxybenzaldehyde after methylation.



Scheme 2.2 Conversion of vanillin to 3,4-dimethoxybenzaldehyde with CH_2N_2

Two other aromatic compounds, however, 3,4-dimethoxybenzaldehyde and 3,4-dihydroxybenzaldehyde are represented as the same product after methylation.



Scheme 2.3 Conversion of 3,4-dihydroxybenzaldehyde and 3,4-dimethoxybenzaldehyde with CH_2N_2

Therefore, as a result of methylation, the detected methylated product is potentially a contribution of all three precursors. Other compounds in the guaiacyl and syringyl series undergo similar transformations in the presence of diazomethane.

Target compounds in wine and oak wood samples were analysed by detection of the methylated product. According to the literature,[10] the 3-methoxy, 4-hydroxy di-

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[10] M. A. Sefton, 'How does Oak Barrel Maturation Contribute to Wine Flavor,' *Australian & New Zealand Wine Industry Journal*, Nov./Dec. (1991): 17-20.

substituted product for the guaiacyl compounds and the 3,5-dimethoxy, 4-hydroxy tri-substituted product for the syringyl compounds were important extractable components from oak wood and were introduced into wine during the oak ageing process. It was decided to focus on these compounds throughout this work. Moutounet et al.[11] detected the presence of vanillin and syringaldehyde in Chardonnay wine stored in oak barrels at levels of 200 μg/L and 400 μg/L respectively. No other di- or tri-substituted aromatic aldehydes were detected.

Syringic acid and gallic acid are converted into the same product after derivatisation. Both of these acids are present in oak wood and are detected in wine during the ageing process. No attempt was made to determine the actual levels of these compounds individually - they were analysed as their common methylated analogue.

An ethereal solution of diazoethane was prepared and was used as the derivatising agent for some wine and oak wood samples. The action of diazoethane retained the individual identity of the di- and tri-substituted aromatic compounds. The use of diazoethane would allow for the actual concentration of each unique component to be determined.

Sample derivatisation was an essential step for analysis of wine and oak wood extracts using gas chromatography. The conversion of compounds to their methylated products improved the quality of GC traces. Reducing the total number of detected compounds allowed for well defined selected ion windows using GC/MS. Reducing the number of ions in the selected ion method allowed for greater sensitivity which was necessary to detect compounds in wine and oak wood extracts at low levels.

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[11] M. Moutounet, Ph. Rabier, J. L. Puech, E. Verette and J. M. Barillere, 'Analysis by HPLC of Extractable Substances in Oak Wood: Application to a Chardonnay Wine,' *Sciences des Aliments* 9 (1989): 35-51.

2.2.3 Preparation of Diazomethane

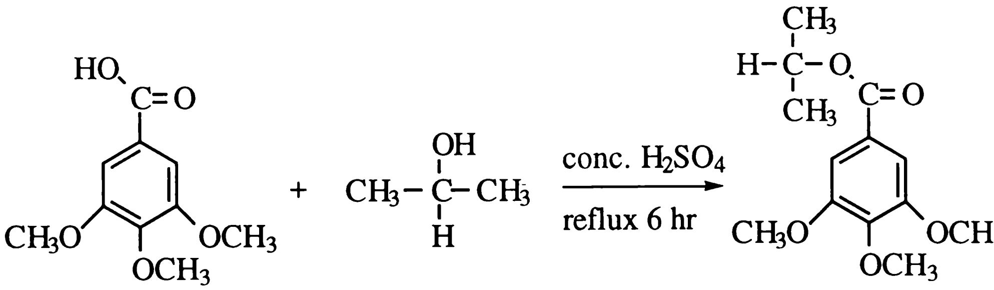
Diazomethane was prepared in the fume cupboard using a specially designed one-piece flask and condensor. Ca. 40 mL of aqueous KOH (25% w/v), ca. 10 mL of distilled diethyl ether and 2-3 g of nitrosomethylurea were added to the flask. The mixture was heated using hot water as the heat source. The yellow solution of ethereal diazomethane was collected in a glass vial and was stored in the refrigerator to reduce the rate of evaporation (Caution: CH_2N_2 is potentially explosive and is a known carcinogen).

Diazoethane was also prepared at different stages throughout the work. The preparation was carried out under the same conditions using nitrosoethylurea as the starting material.

2.2.4 Preparation of Internal Standards

An internal standard mixture of analytical grade octadecane in doubly distilled chloroform was prepared. The concentration of this mixture was 10,000 μg/mL. All samples were spiked with 100μL of the standard mixture. Each sample therefore contained 1000 μg of octadecane.

As the work progressed, more emphasis was placed on aromatic wood-derived compounds. At this point an internal standard was prepared which was chemically similar to these woody compounds. Trimethoxybenzoic acid was refluxed with 2-propanol in the presence of conc. sulphuric acid for ca. 6 hr resulting in the formation of the corresponding trimethoxybenzoic isopropyl ester (see Scheme 2.4).



Scheme 2.4 Preparation of isopropyl 3,4,5-trimethoxybenzoate

Although the new internal standard was used for quantitation, the octadecane standard was still added to all samples as a further check for correct addition of internal standard. No problems were encountered with the stability of the aromatic standard; however the chemically inert hydrocarbon, octadecane, provided an additional safeguard.

The concentration of the aromatic isopropyl ester internal standard was 100 μg/mL. To each sample, 100 μL was added. The amount of internal standard in each sample was therefore 10 μg.

Quantitation of individual peaks in the sample were carried out as follows:

$$\text{Concentration (A)} = \frac{\text{Peak area (A)} \times \text{C (I.S.)}}{\text{Peak area (I.S.)} \times \text{Rf (A)}}$$

where C (I.S.) = Concentration of internal standard
Rf (A) = Response factor of individual compound A

The response factor for each individual compound was obtained in using a separate response factor mixture.

2.2.5 Preparation of Response Factor Mixture

A response factor mixture was prepared by individually weighing 0.200 g of each of the following compounds: vanillin, acetovanillone, vanillic acid, syringaldehyde, acetosyringone, syringic acid, lauric acid, malic acid, octadecane and isopropyl ester. These compounds were transferred into a 1 L volumetric flask and made up to the mark with doubly distilled chloroform. The concentration of each component in the mixture was 0.2 g/L.

2.3 Choice of Analytical Techniques

Three methods for the analysis of wine and oak wood extracts were considered; HPLC, GC/FID and GC/MS. The literature has shown these methods to have useful applications in identifying and quantifying wine-related compounds.[12,13]

2.3.1 High Performance Liquid Chromatography

The essential components of a modern high performance liquid chromatography system are shown in Figure 2.3.

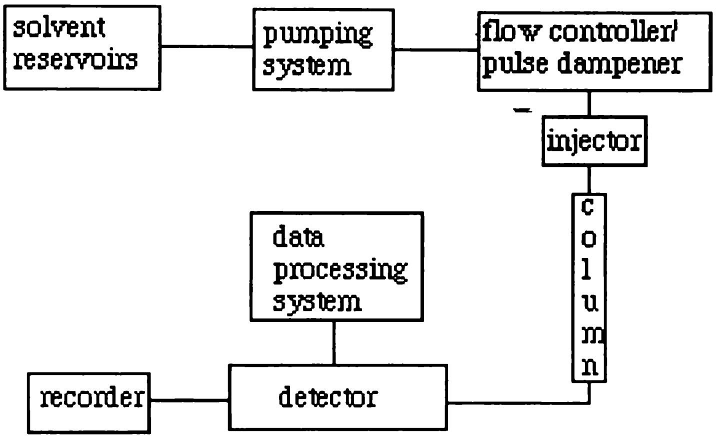


Figure 2.3 Components of an HPLC system

Ideally, the separating power of an HPLC system should depend on the efficiency of the column and be independent of the instrument. In practical terms, however, band broadening will arise due to the presence of void volumes in the injector, connecting ferrules and detector cell.

Solvents for HPLC should be pure, filtered to remove suspended particles, and degassed to remove any dissolved gases. Air bubbles in the solvent stream lead to

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[12] J. M. Conner, A. Paterson and J. R. Piggott, 'Changes in Wood Extractives from Oak Cask Staves through Maturation of Scotch Malt Whiskey,' *J Sci Food Agric* 62 (1993): 169-174.

[12] M. Lehtonen and P. Jounela-Eriksson, 'Volatile and Non-Volatile Compounds in the Flavour of Alcoholic Beverages,' *Sciences Des Aliments Series*, ed. J. R. Pigott, 63-78.

degradation of pump components and poor detector performance. Dissolved oxygen may also cause oxidative degradation of samples and reduction in sensitivity of ultraviolet (UV) detectors. Oxygen absorbs in the low uv and may cause baseline drift and random noise.

The pump is an important component in an HPLC system as its performance directly affects retention times and reproducibility. A typical pump needs to provide constant flow rates of 0.5 to 10 mL/min at pressures up to 5000 p.s.i. (350 kg/cm^2).

Ideally, the sample injection device should introduce the sample into the solvent stream as a sharp slug. Presently, microvolume sampling valves are the most widely used devices. The sample is introduced into an external sampling loop of fixed volume which, when in the 'load' position, is by-passed by the solvent stream. When rotated to the 'inject' position, the solvent path is channeled via the sample loop and, in turn, sweeps the sample slug onto the front of the column. The nature of a microvalve injector means that injection reproducibility is essentially independent of the operator; errors associated with injection may be less than 1%.

A universal detector has yet to be found for HPLC. UV-visible detectors are a popular choice, however, as most organic compounds have a useful absorption in the uv region. Sensitivity is dependant on the strength of absorption and a transparent mobile phase at the absorption maximum is preferred. The operation of the detector is based on the absorbance of monochromatic light by an eluting compound in accordance with the Beer-Lambert law.

The column provides the means of separation for the HPLC system. The stationary phase is a liquid bonded to a solid support and separations take place depending on the partition of sample between the stationary and mobile phases. In conventional chromatography, the stationary phase was polar and the mobile phase non-polar. In HPLC, it has become more common to use a non-polar chemically-bonded stationary phase (‘liquid-liquid’ or ‘bonded-phase’) with an aqueous (polar) mobile phase containing a proportion of miscible organic modifier. This system is referred to as 'reversed-phase' chromatography to distinguish the procedure from conventional or 'normal' systems. Reversed-phase chromatography is a widely used technique for the separation of polar and non-polar organic molecules.

Reversed-phase separations for the analysis of target compounds in wine were carried out using a Shimadzu LC-5A liquid chromatography system combined with a Linear uvis 203 detector. Compounds were eluted isocratically using 30% methanol/70% water at a flow rate of 1 mL/min. The system was fitted with a 100 x 4.6 mm Econosphere C8 column.

2.3.2 Gas Chromatography with Flame Ionisation Detection (GC/FID)

The essential components of a gas chromatograph (GC) include a supply of carrier gas, a sample vapourising chamber (injector), a column contained in an oven and devices for the detection and recording of eluting compounds.

The mixture is first introduced into the GC via the sample injector whereby the carrier gas sweeps the injected sample onto the column - the fundamental component of any chromatographic procedure. The separation of compounds in the mixture takes place inside the column by the partitioning between the inert mobile gas phase and the stationary phase. In the case of non-polar columns, separation is determined essentially on boiling point. More volatile molecules spend less time interacting with the stationary phase and thus elute from the column more quickly. With polar columns, separations are more selective depending on the functionality of individual components in the mixture.

The flame ionisation detector (FID) is a useful method of detection as it responds to the presence of nearly all organic compounds. The FID is close to a universal detector - only compounds such as the inert gases, nitrogen oxides, CO_2, CS_2, NH_3, water and formic acid provide an insufficient detector response. The FID has high sensitivity and a large linear response.

In the FID, the formation of positive ions are produced as a function of combustion of organic compounds in a hydrogen/air flame. A collector electrode is located a few millimeters from the flame and the ion current is measured by establishing a potential between the jet tip and electrode. Typically, a background current of 10^{-14} amperes would increase to 10^{-12} to 10^{-9} in the presence of an eluting vapour. The small signal currents are amplified and sent to a recorder. The increase in current is proportional to the amount of positive charge produced in the flame which is, in turn, dependent on the amount of eluting compound. The change in current and resulting peak height/area are a direct measure of the original concentration of compounds in the injected sample. The sensitivity of the detector can be optimised by adjusting the ratio of air to hydrogen.

Temperature programming is a convenient procedure whereby the volatilities of larger molecules can be increased in order to obtain suitable elution times.

Analyses of wine and oak wood samples were carried out using a Hewlett Packard 5890B gas chromatograph equipped with FID detection. The GC was fitted with a 20 metre 0.25 i.d. HP-1 (methyl silicon gum) column. Analyses were carried out under the following GC conditions:

initial temperature	70°C
temperature programme	70°C to 270°C at 6°C/min
grob load time	0.3 min
split flow rate	40 - 50 mL/min
injection volume	1 µL
carrier gas	hydrogen
injector temperature	250°C
detector temperature	250°C

2.3.3 Gas Chromatography/Mass Spectrometry (GC/MS)

After separation on the GC column, the components of the sample move through the interface into the mass selective detector. This type of detector gives considerably more information on the structure of individual compounds contained in the sample as compared with conventional GC with a flame ionisation detector.

In the mass selective detector, molecules in the gaseous state are bombarded with a beam of electrons with energies in the range of 5 to 100 eV. A value of 70 eV is typical. Since most organic compounds have ionisation potentials of 7 to 20 eV, the energy transferred on collision between the electron and a neutral molecule is sufficient to cause both ionisation and extensive fragmentation. Conditions on modern electron impact (EI) mass spectrometers are set up to optimise the production of singly-charged parent ions or molecular fragments. These ions are repelled from the ion source, focused and accelerated to provide an ion beam capable of traversing the mass analyser section of the mass spectrometer.

The bombardment of a neutral molecule with a beam of high energy electrons often results in the loss of electrons and the formation of a molecular ion. This ion provides valuable information on the structure of a compound as it defines its molecular weight. Other useful structural data can be obtained from the resulting fragmentation patterns which are characteristic of individual compounds, groups of compounds and homologous series. Figure 2.4 shows the components of a GC/MS system.

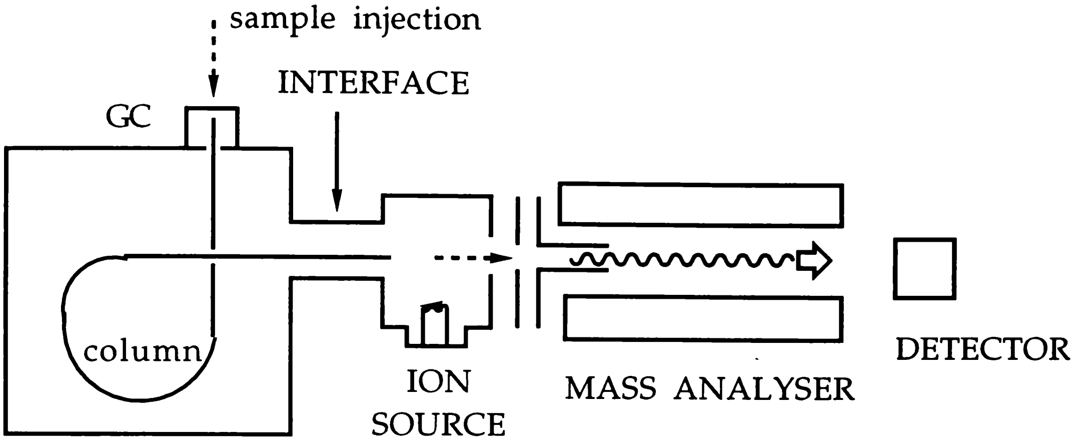


Figure 2.4 Components of a GC/MS system

The Hewlett Packard 5970 mass analyser, a quadrapole mass spectrometer consisting of four rods aligned parallel to the electron beam, provides mass separation of the ion beam leaving the ion source. Mass analysis is achieved by changing the Rf fields and DC voltage applied to the quadrapole rods. The detector is an electron multiplier which measures the ion current resulting from ions of the selected mass (or masses) hitting the photomultiplier. This current is very small and must be typically amplified by several orders of magnitude. This signal is then processed and sent to the data station. In normal operating mode or 'total ion mode', the relative abundances of the ions within a defined mass range are tabulated in a mass spectrum for each individual peak eluting from the GC column. A typical GC/MS trace (total ion) is shown in Figure 2.5.

```
File        : A:\CPGTA02A.D
Operator    : CPG
Acquired    : 18 Dec 95   1:21 am using AcqMethod DATA:CRAIG.M
Instrument  :   MS_5970
Sample Name:
Misc Info   :
Vial Number: 2
```

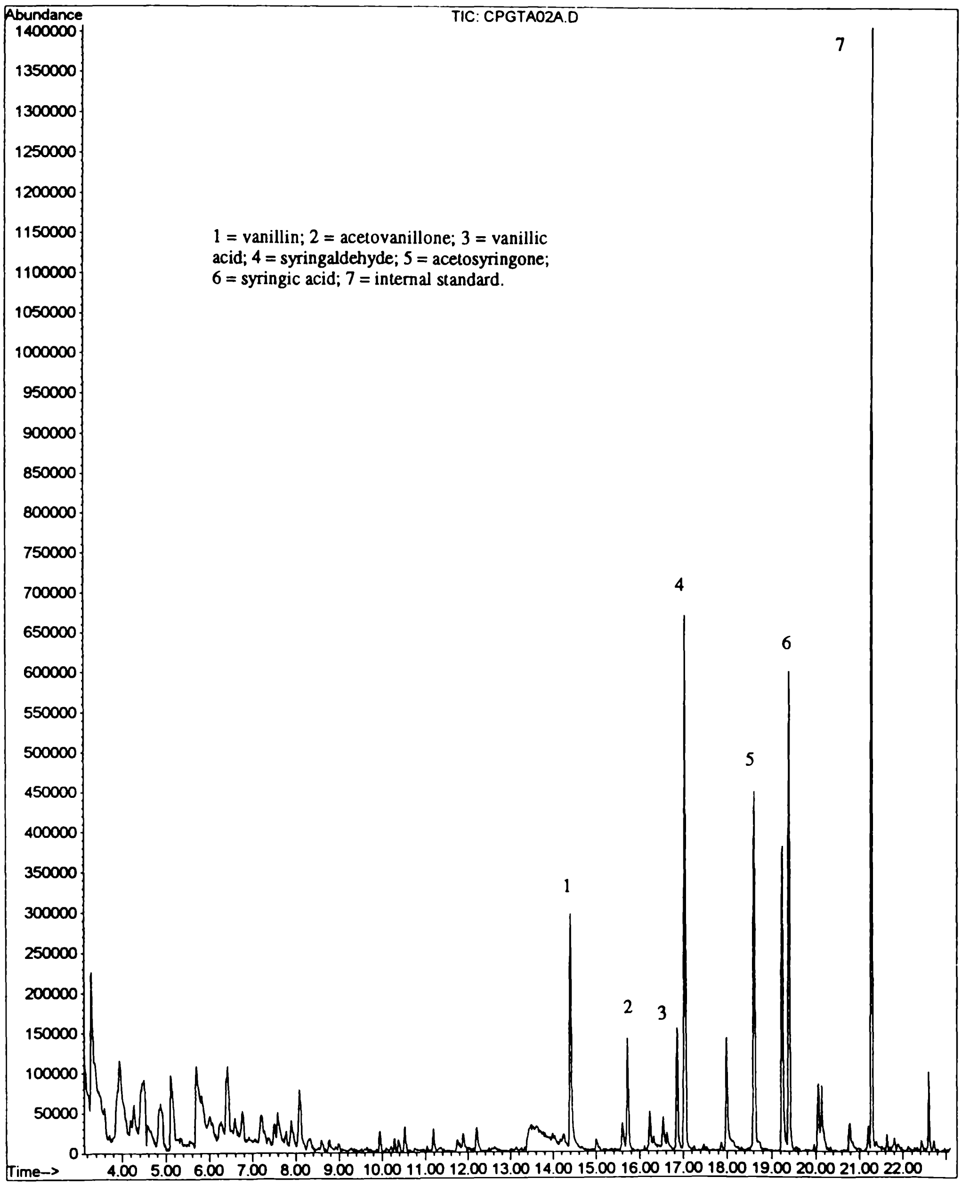


Figure 2.5 Typical GC/MS (total ion mode) chromatogram of an oak wood extract

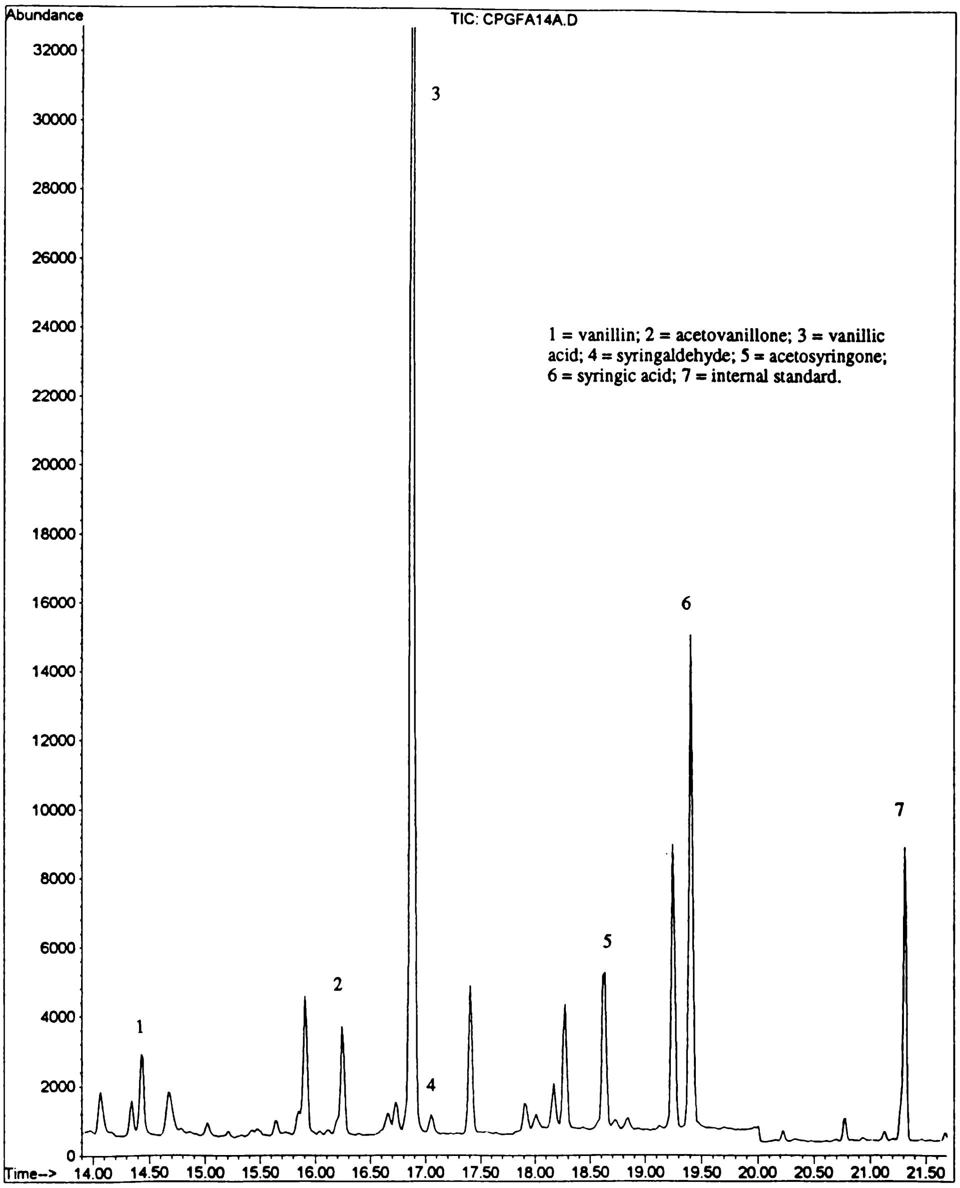
2.3.4 Analysis by Gas Chromatography/Mass Spectrometry in Selected Ion Mode (GC/MS-SIM)

GC/MS is a powerful analytical tool for the separation, identification and quantitation of complex mixtures of organic compounds in small sample volumes. Structural elucidation of unknown compounds from mass spectral data is made easy with the use of pure individual compounds. Combining this technique with the separating power of the gas chromatograph is therefore a logical choice.

Previously, GC/MS had allowed for the detection and quantitation of woody compounds down to low ppm levels. Preliminary work showed the presence of aromatic woody compounds present in wine at levels below 1 mg/L (Gribble 1991). These compounds are associated with desirable oak character in high quality wine. A more sensitive method of detection was required to accurately quantify these aromatics compounds at the low ppb level - GC/MS-SIM.

The mass spectrometer can be tuned to monitor a single ion or to switch at high frequency between several preset *m/z* values throughout a chromatographic separation. When several ions are recorded sequentially the specificity for a particular compound is increased as the probability of two compounds having the same retention times and the same mass ion ratios is small. The result is a considerable gain in sensitivity as the dwell time for each ion current is increased compared to the normal scan mode. The sensitivity of selected ion monitoring can be 100 times greater than that of scan mode. Figure 2.6 shows a typical selected ion chromatogram of target compounds in wine.

```
File        : A:\CPGFA14A.D
Operator    : CRAIG
Acquired    : 16 Dec 95   2:16 am using AcqMethod DATA:CPGSIM.M
Instrument  :   MS_5970
Sample Name:
Misc Info   :
Vial Number: 14
```



1 = vanillin; 2 = acetovanillone; 3 = vanillic acid; 4 = syringaldehyde; 5 = acetosyringone; 6 = syringic acid; 7 = internal standard.

Figure 2.6 Typical selected ion chromatogram of target compounds in wine

2.3.5 Summary of Analytical Procedures

Three analytical techniques for the qualitative and quantitative determinations of organic compounds in wine and oak wood were considered. These techniques were HPLC, GC/FID and GC/MS. All three techniques have been shown to have useful applications for the analysis of compounds present in wine and oak wood. The universal detection of compounds using the different techniques is restricted only by the requirement for compounds to have a suitable absorption in the uv region in the case of HPLC and by the need for careful choice of selected ions in GC/MS using SIM mode.

HPLC has the advantage of not requiring a sample derivatisation step. Samples may be injected directly into the HPLC system provided that a compatable or preferably identical extracting solvent has been used. The nature of the modern HPLC injection system allows for good reproducibility of samples as the injection technique is almost independent of the operator. The resolution of eluting compounds in HPLC is inferior to the resolution obtained using gas chromatography as the ability to resolve compounds (column efficiency) is dependent on the internal diameter of the column.

GC/FID provides almost universal detection of organic compounds. There is no scope with FID, however, to selectively detect a particular compound or group of compounds, or to eliminate peaks from the GC trace which are not of interest. Narrow bore capillary columns on a modern GC/FID give superior resolving power resulting from high plates per meter counts when compared to packed GC columns and HPLC columns.

GC/MS provides a powerful means of resolving complex mixtures with the added advantage of structural information for each individual eluting compound. Individual compounds or classes of compounds may be selectively targeted using SIM mode. In complex mixtures, this technique identifies only the compounds of interest and consequently simplifies the GC profile. The loss of structural information using SIM is compensated for by a 100 fold increase in sensitivity.

GC/MS was therefore the preferred technique for the analysis of wine and oak wood samples. The GC/MS system was fitted with an autosampler which provided good injection reproducibility and the scope for carrying out overnight analyses.

2.3.6 Development of Selected Ion Method

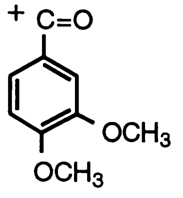
The use of GC/MS in SIM mode allowed for the detection of aromatic woody compounds in wine. SIM provided the necessary sensitivity to detect aromatic compounds such as vanillin in wine at the low ppb level. Previously, vanillin and other aromatic compounds were not quantified as GC/MS in the normal scanning mode allowed for accurate quantitation only to the low ppm level.

Vanillin has the basic guaiacyl structural skeleton and also contains an aldehyde group. After derivatisation, the AR-OH is converted to the corresponding methylated product (see Scheme 2.2). The derivatised product of vanillin has a molecular weight of 166 amu. Inside the ionisation chamber of the mass spectrometer, the proton of the aldehyde group is easily removed (characteristic of aldehydes). This product therefore shows a strong *m/z* 165 fragment ion.

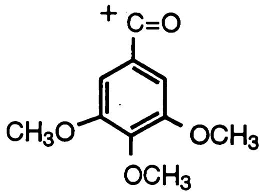
The parent ion, *m/z* 166 and the *m/z* 165 ion are the dominant ions in the ion chromatogram of vanillin. This result is typical of the fragmentation of aromatic compounds. The aromatic nature and associated resonance stability mean that the parent ion is often strongly observed. Aliphatic compounds, which do not exhibit resonance effects, fragment more readily. In these cases, the parent ion may not be observed. A knowledge of the nature of fragmentation of individual compounds is essential to developing a selected ion method. The detected ion current for a group of selected ions for a particular compound and their relative ratios to one another provides a means of qualitative analysis additional to that of retention times.

The *m/z* 165 ion was a useful target ion for the SIM mode operation as other aromatic compounds (from the guaiacyl series) associated with wood also have the characteristic *m/z* 165 fragment. This target ion was also used to detect the presence of acetovanillone and vanillic acid.

Compounds with a syringyl type base structure are also associated with oak wood extractives (Sefton 1991). After derivatisation, compounds of this type show a strong *m/z* 195 fragment ion. This ion was used in the selected ion method to identify target compounds with the syringyl structure.



m/z 165 ion



m/z 195 ion

Initially, the *m/z* 165 ion (for the methylated form of vanillin) and the *m/z* 254 ion for the internal standard (octadecane) in SIM provided a suitable response for the detection and quantitation of some aromatic compounds. A response factor determination relating the GC response of the *m/z* 165 ion (aromatic compounds) and the *m/z* 254 (octadecane) was carried out in SIM. This value was used to calculate the actual concentrations of target compounds in wine.

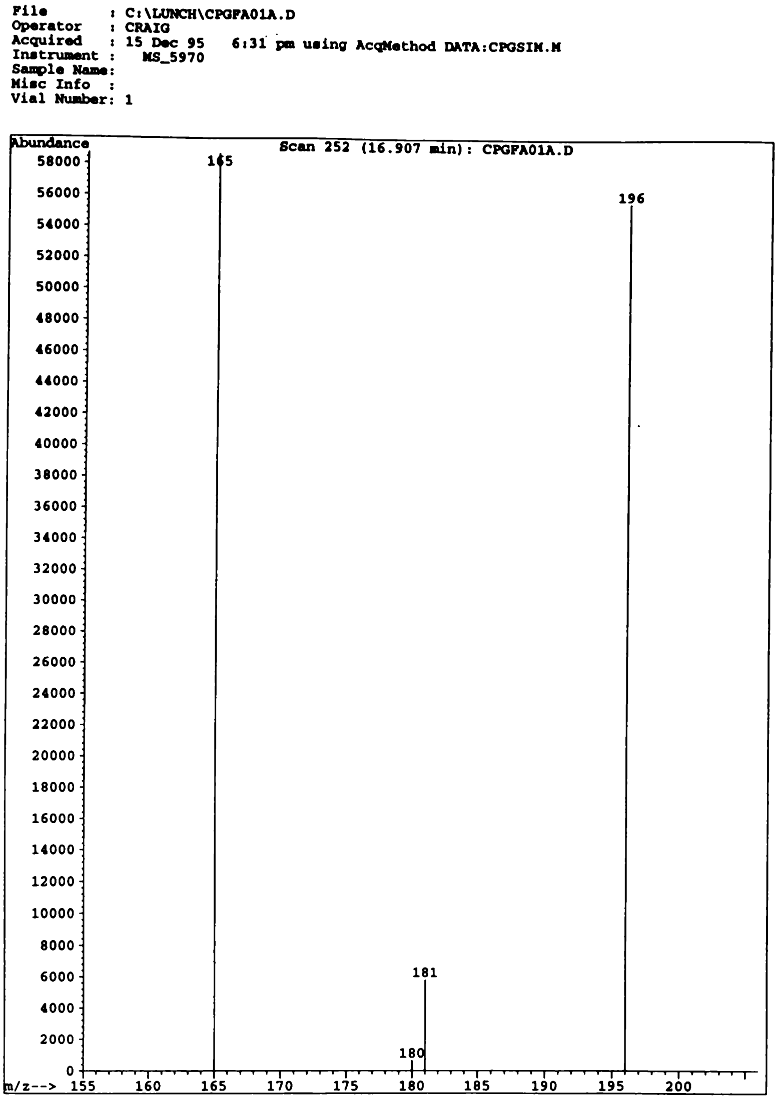
As the work progressed, a new internal standard (isopropyl 3,4,5-trimethoxybenzoate) was prepared which was chemically similar to the compounds of interest. The new internal standard was an aromatic compound also of molecular weight 254. This result meant that the same SIM conditions could be maintained for the detection of both the new aromatic internal standard and the inert hydrocarbon used for previous work. The two standards eluted at similar retention times in the GC profile.

For analyses carried out in total ion mode, a 'class' response mix was adequate as different compounds from the same class gave a similar GC response. In SIM mode, however, the response of a particular compound may be quite different from another compound from the same class, depending on the ion or group of ions selected. A response factor for each individual compound in the selected ion trace was determined. Therefore, a response factor mix including the two internal standards, a fatty acid and a selection of aromatic compounds used for targeting key wood extractives was prepared. This response factor mix was analysed before any GC/MS analyses were attempted. The results from these test traces were also used to check the performance of the GC/MS.

Although sensitivities of several orders of magnitude greater than total ion monitoring can be obtained using SIM, a disadvantage is the loss of structural information due to a reduced number of ions being detected. The identification of compounds can still be varified using both the retention times of compounds and their corresponding ion ratio abundances. A three ion ratio match was considered adequate for the positive identification of a compound.

Firstly, the relative ion ratios were determined for different target compounds in total ion mode. Three abundant ions from the total ion trace were then chosen for the SIM analyses. The relative abundances of each ion remain constant in the two modes. Conveniently, the most abundant ions for many of the target woody compounds are similar for a range of compounds. This result allows for a selection of the same target ions to be used for the detection and identification of target species in SIM.

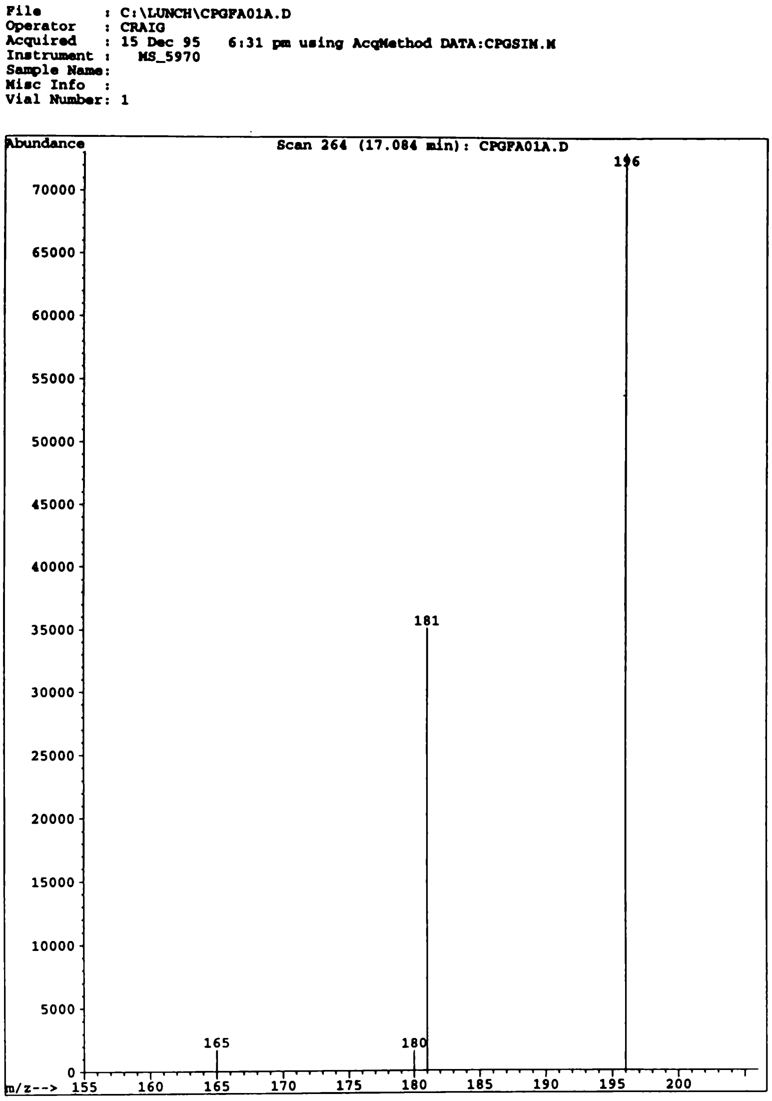
A comparison of two compounds, vanillic acid and syringaldehyde shows the usefulness of the ion ratio method. The methylated form of these two compounds both have the same molecular weight and show a parent ion of *m/z* 196. The *m/z* selected ions of 165, 181 and 196 were chosen to distinguish between these two compounds. Figure 2.7 shows the relative ion ratios of the three target ions of vanillic acid. This mass spectrum was obtained from a standard solution of vanillic acid.



File : C:\LUNCH\CPGFA01A.D
Operator : CRAIG
Acquired : 15 Dec 95 6:31 pm using AcqMethod DATA:CPGSIM.M
Instrument : MS_5970
Sample Name:
Misc Info :
Vial Number: 1

Figure 2.7 Selected ion mass spectrum for vanillic acid (methyl ester)

The ratio of the ion count for the 165/196 ions was typically equal to 1. For the *m/z* 181/196 ratio, a typical value was 0.1. Vanillic acid (methyl ester) was recognised by its similar proportions of *m/z* 165 and *m/z* 196 ions and a low *m/z* 181 ion count compared to the counts associated with the *m/z* 196 ion. Figure 2.8 shows the relative ion ratios of the three target ions of syringaldehyde (methylated) obtained from a standard solution of syringaldehyde.



```
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Operator   : CRAIG
Acquired   : 15 Dec 95   6:31 pm using AcqMethod DATA:CPGSIM.M
Instrument :   MS_5970
Sample Name:
Misc Info  :
Vial Number: 1
```

Figure 2.8 Selected ion mass spectrum for syringaldehyde (methylated)

Syringaldehyde showed a low ratio of *m/z* 165 ion to the *m/z* 196 ion - typically 0.05. The ratio of the *m/z* 181 ion to the *m/z* 196 ion for syringaldehyde was significantly greater than for vanillic acid - typically 0.5. These three selected ions and their relative abundances were used along with retention times to confirm the identity of eluting peaks.

The relative ion ratios of target compounds were not identical for each sampling time as the values for each target ion were dependent on the tuning profile of the mass spectrometer. The values for individual ions could also vary for different samples as overlapping peaks could potentially contribute to the ion count of a particular selected ion if the overlapping *m/z* value was the same. These effects were minimised by optimising the performance of the GC/MS system and ensuring adequate resolution of eluting peaks.

All wine, ethanol/water, and oak wood analyses were carried out using a Hewlett Packard 5890A gas chromatograph coupled with a Hewlett Packard 5970 mass selective detector. The GC was fitted with a 20 metre 0.25 i.d. HP-1 (methyl silicon gum) column. The following GC conditions provided good separation of compounds from the sample mixture:

initial temperature	70°C
temperature programme	70°C to 270°C at 6°C/min
Grob load time	0.2 min
split flow rate	40 - 50 mL/min
MS solvent delay	3 min
injection volume	1 μL
carrier gas	helium
carrier gas flow rate	30 cm/s
injector temperature	250°C
MS interface temperature	280°C

The following SIM method was typical for the detection of target compounds in all samples:

Time (min)	Selected ions	Target compounds
14-16	95, 165, 166	vanillin
16-18	165, 180, 181, 196	acetosyringone vanillic acid syringaldehyde
18-20	195, 210, 211, 226	syringic acid acetosyringone
20-22	254	internal standards

Any alteration to the conditions inside the GC oven required the SIM method to be adapted as the detection of compounds using the selected SIM time window is dependent on the elution time of compounds from the GC.

All samples were introduced into the gas chromatograph using an autosampler. This injection mechanism combined with Grob injection conditions provided good reproducibility and reduced the potential for discrimination in the injector of the GC.

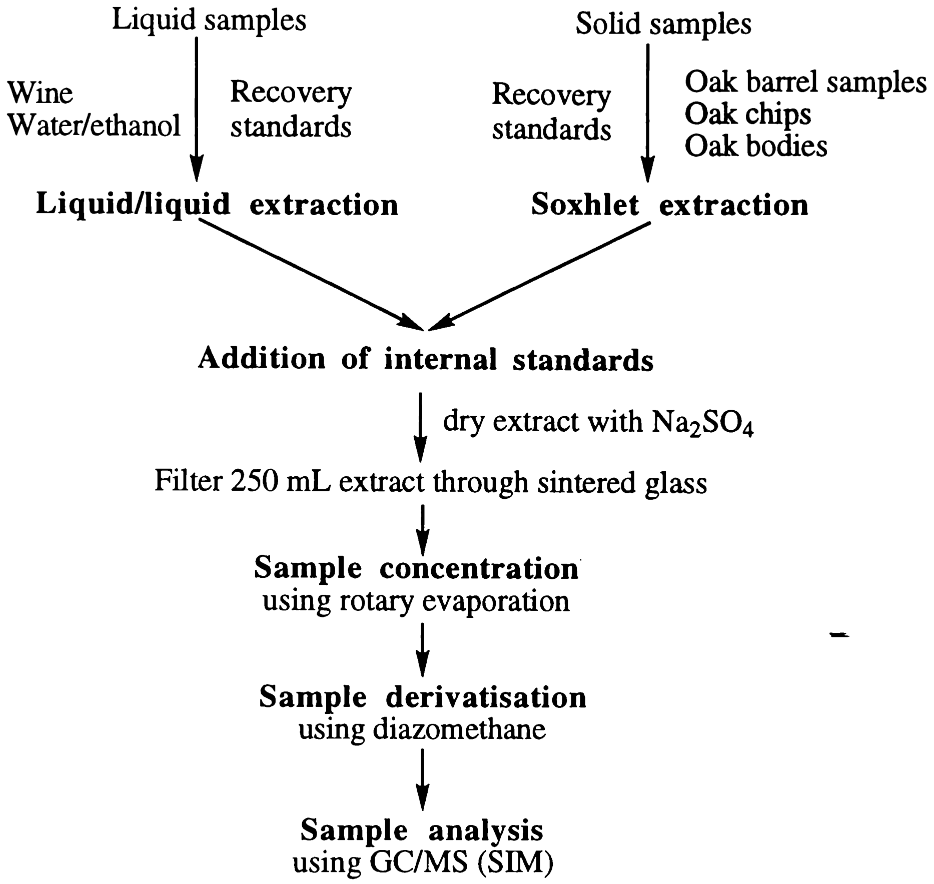
2.3.7 Summary of Sample Preparation and Analyses Procedures

1. Liquid/liquid extraction for liquid samples and Soxhlet extraction for solid samples were suitable for the recovery of target non-volatile compounds. The potential losses of highly volatile material using these techniques were not the focus of this work.

2. Rotary evaporation was the preferred method for the concentration of target compounds.

3. GC/MS provided the sensitivity, resolution and unequivical identification of individual components in the sample matrix. These three advantages meant that the GC/MS system was the preferred technique over HPLC and GC/FID.

The analytical protocol established for this work is summarised in Scheme 2.5.



Scheme 2.5 Analytical protocol for the analysis of wine and oak wood extracts

2.4 Reproducibility and Quality Control

The reliability of the analytical procedure was tested by replicate determinations and an estimate of method standard deviation.

A Chardonnay wine was sampled six times from an oak barrel. All six samples were prepared and analysed using the same analytical procedure. This experiment was carried out in order to determine the standard deviation of the sampling, extraction, concentration, derivatisation and analyses steps. The results of this experiment are shown in Table 2.2.

Table 2.2 Concentration of target compounds (μg/L) in wine from six samples

Sample	1	2	3	4	5	6	mean	sd
Vanillin	28	31	31	24	37	42	32	6.4
Acetovanillone	30	35	36	36	43	44	37	5.3
Acetosyringone	26	21	25	33	35	33	29	5.6
Vanillic acid	780	1100	1100	800	1200	1100	1000	180

The purpose of this experiment was to investigate the error associated with all steps in the analytical procedure. For the target compounds vanillin, acetovanillone, acetosyringone and vanillic acid, the coefficients of variability were 20%, 14%, 19% and 18% respectively. It was thus concluded that the analytical methods were reliable to about 20%

2.4.1 Detector Linearity

In order to carry out analyses over a large concentration range, the linearity of the mass spectrometer detector needed to be evaluated. A set of standards containing key compounds at various concentrations was prepared. The compounds of interest present in wine and oak wood ranged from the low ppb level through to the mid ppm level. This experiment determined the linear response range of the selected compounds and confirmed the suitability of the detector and the GC/MS system for analyses over an extended concentration range. The amount of sample injected into the GC was plotted against actual concentration obtained by calculation using the internal standard method employed for all analyses. Figure 2.9 shows the results obtained for one of the target compounds, vanillin. The response curves for other target compounds are shown in Appendix 1.

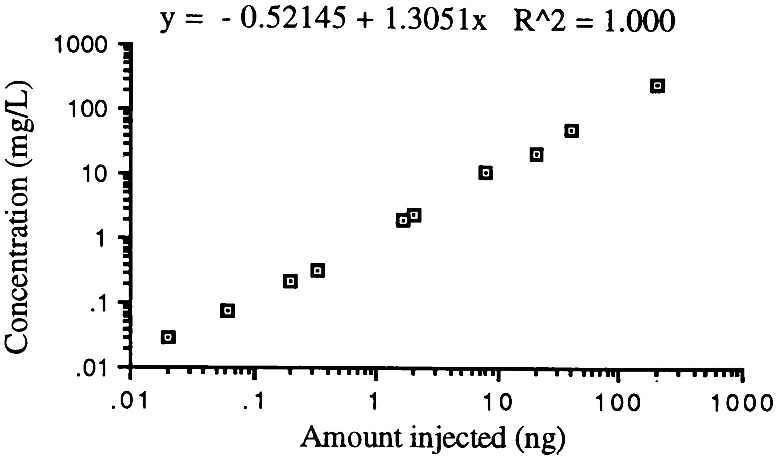


Figure 2.9 Response curve for vanillin

Analyses of all the target compounds were carried out over the same range. A least squares regression of the response graphs gave R^2 values between 0.997 and 1.000, which showed the linear range of the MS detector was accurate over a 10^4 or better linear response range. Injection amounts of 20 pg through to 200 ng would be suitable for the detection of compounds in wine and oak wood.

2.4.2 Efficiency of Recovery

Some loss of target compounds will occur during the extraction, concentration, derivatisation and analyses steps. Vanillin, syringaldehyde, acetosyringone and syringic acid were chosen to determine the recoveries of these compounds after all steps in the analytical process had taken place. A 12% alcoholic solution buffered to pH 3.3 was prepared and the solution was spiked with the target compounds present at 5 mg/L. After the extraction time, the samples were analysed by the same methods as those employed for the analysis of wine. Table 2.3 shows the results from this experiment.

Table 2.3 Recovery (%) of compounds at the 5 mg/L level

Sample	1	2	3	4	5	mean	sd
Vanillin	78	72	87	82	76	79	5.7
Acetovanillone	62	84	83	75	78	76	8.8
Vanillic acid	61	77	72	80	85	75	9.1
Syringaldehyde	55	84	70	86	56	70	15
Acetosyringone	63	82	80	82	70	75	8.5
Syringic acid	61	79	84	85	79	78	9.7

These results show that the liquid-liquid extraction techniques employed provided good recoveries of compounds from solution. The experiment was repeated using target compounds present at 100 µg/L. The results from this experiment are shown in Table 2.4.

Table 2.4 Recovery (%) of compounds at the 100 µg/L level

Sample	1	2	3	4	5	mean	sd
Vanillin	77	69	73	65	72	71	4.5
Acetovanillone	77	79	73	82	74	77	3.7
Vanillic acid	82	63	80	78	70	75	7.9
Syringaldehyde	60	53	46	52	48	52	5.4
Acetosyringone	79	79	76	80	74	78	2.5
Syringic acid	73	70	72	75	69	72	2.4

These results show that at different concentrations, the recoveries of compounds were also good using liquid-liquid extractors.

The efficiency of recovery from solid samples was also investigated. A sample of raw untoasted oak wood was extracted for 18 hours with chloroform, allowed to dry then ground into a coarse powder. The same raw oak wood was then spiked with a solution of target compounds at known concentration; the solution was surface-absorbed onto the oak wood. The sample was then analysed by GC/MS. This experiment was carried out with four raw oak wood samples. The results for this experiment are shown in Table 2.5.

Table 2.5 Recovery (%) of compounds extracted from solid samples

Sample	1	2	3	4	mean	sd
Vanillin	68	83	78	85	79	7.6
Acetovanillone	60	75	55	63	63	8.5
Vanillic acid	83	72	70	78	76	5.9
Syringaldehyde	70	65	79	68	71	6.0
Acetosyringone	73	60	78	70	70	7.6
Syringic acid	88	85	78	55	77	15

Soxhlet extraction techniques for solid samples were shown to achieve good recoveries for the target compounds absorbed onto oak wood.

2.4.3 Effect of Ethanol/Water Composition

While commercial Chardonnay wines typically have an alcohol content of about 12% ethanol, some variation in alcohol composition can be expected. An experiment was carried out to evaluate the effect on extraction of compounds from an ethanol/water medium of different composition. Three ethanol/water compositions were prepared: 0% ethanol/water (samples 1 and 2), 12% ethanol/water (samples 3 and 4) and 20% ethanol/water (samples 5 and 6). All prepared solutions were buffered to approx. pH 3.3 with citric acid. Each composition was analysed in duplicate. Table 2.6 shows the results and a statistical analysis for this experiment.

Table 2.6 Recovery (%) of compounds from different ethanol/water media

Sample	1	2	3	4	5	6	mean	sd	cv
Vanillin	64	71	72	66	72	73	70	3.7	5.3%
Acetovanillone	85	51	62	82	63	68	69	13	19%
Vanillic acid	76	53	54	62	58	58	60	8.4	14%
Syringaldehyde	62	79	80	81	79	59	73	10	14%
Acetosyringone	74	80	80	81	82	77	79	3.0	3.8%
Syringic acid	71	76	80	78	76	74	76	3.1	4.1%

It would appear from these results that a small change in the amounts of ethanol present in various wine compositions would not effect the extraction of aromatic compounds.

2.4.4 Determination of Optimum Carrier Gas Flow Velocity

The efficiency of a chromatographic system is dependent on the carrier gas flow rate. It was essential to optimise the carrier gas flow rate to allow for the resolution of compounds with similar boiling points. If the carrier gas flow rate is too fast, there is insufficient time for partitioning between sample compounds and the stationary phase. Alternatively, if the flow is too slow, diffusion of the sample matrix occurs resulting in peak broadening and a subsequent reduction in system efficiency.

A *n*-decane standard was prepared and analysed in the GC/MS using a range of flow rates. The number of effective theoretical plates (Neff) were calculated for different carrier gas flows using the following formula:

$$\text{Neff} = 5.54(\text{tx}'/\text{w})^2$$

where:

tx' = corrected retention time for *n*-decane
w = peak width at half height of the *n*-decane peak

The height equivalent to a theoretical plate (HETP) is a measure of column efficiency. HETP was calculated using the following formula:

$$\text{HETP} = \text{L}/\text{Neff}$$

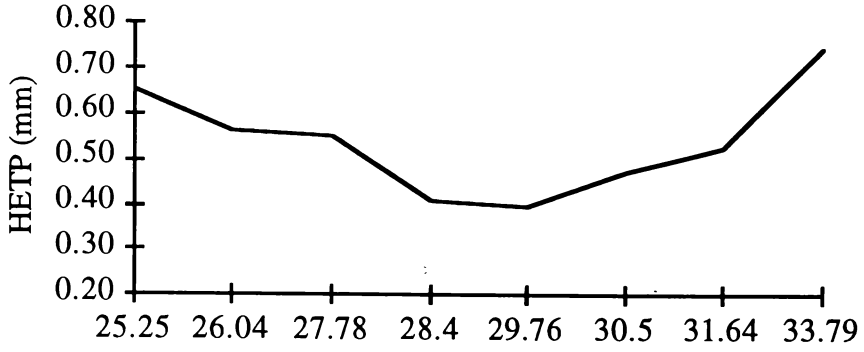
where L = length of the column

Table 2.7 lists the results of Neff and HETP calculations at various flow rates.

Table 2.7 Column efficiency parameters

Pressure (psi)	Linear flow (cm/sec)	tx (min)	to (min)	tx' (min)	k	peak width (min)	Neff	HETP
10	25.3	5.393	1.650	3.743	2.3	0.045	38328	0.652
11	26.0	5.274	1.600	3.674	2.3	0.041	44485	0.562
12	27.8	5.125	1.500	3.625	2.4	0.040	45499	0.549
13	28.4	5.027	1.467	3.560	2.4	0.034	60736	0.412
14	29.8	4.906	1.400	3.506	2.5	0.033	62531	0.400
15	30.5	4.808	1.366	3.442	2.5	0.035	52958	0.472
16	31.6	4.730	1.317	3.413	2.6	0.037	47139	0.530
18	33.8	4.566	1.233	3.333	2.7	0.043	33285	0.751

These results allowed a Van Dempter plot to be constructed (see Figure 2.10). This plot shows the relationship between column efficiency (HETP) and carrier gas flow rate. The column is most efficient when the value for HETP is minimised.



Carrier gas flow rate (cm/sec)

Figure 2.10 Carrier gas flow rate versus HETP

The Van Dempter curve for this chromatographic system shows that maximum efficiency occurs when the carrier gas flow rate is ca. 30 cm/sec. All analyses were carried out at this optimum carrier gas flow rate.

2.4.5 Reference Standard

A certified reference standard was not available for this work. An 'in-house' reference standard was prepared from a wine sample under the same conditions as all other samples analysed during this work. This reference standard was routinely analysed in the GC/MS as part of any major batch analyses. The reference standard was used as a quality control measure to ensure that the storage conditions of samples were adequate and that the GC/MS system was providing on-going reproducibility.

Every attempt was made to analyse batches of prepared samples at times when the GC/MS system was operating most efficiently. Analyses were normally carried out immediately after a regular maintainance shutdown had taken place. At this stage a new column was normally installed in the GC and the MS detector had undergone a routine clean.

At the beginning of the work, the reference standard was analysed 6 times to obtain a mean and standard deviation for each of the target compounds in the reference standard. These results are shown under sample batch 1 in Table 2.8 and were calculated as 180 (27), 110 (21), 2200 (220), 170 (30), 140 (15) and 850 (140) µg/L for vanillin, acetovanillone, vanillic acid, syringaldehyde, acetosyringone and syringic acid respectively. Table 2.8 shows the concentrations of these compounds over 8 major batch analyses.

Table 2.8 Concentration of target compounds (µg/L) in the test mixture over time

Sample batch number	1	2	3	4	5	6	7	8
Vanillin	180	170	140	140	190	220	170	200
Acetovanillinone	110	160	160	150	100	120	93	110
Vanillic Acid	2200	2600	2800	2300	1800	2300	2100	2300
Syringealdehyde	170	160	160	220	220	220	210	250
Acetosyringeone	140	170	200	240	150	150	100	120
Syringic Acid	850	1200	1300	1000	890	770	740	760

Table 2.9 shows the statistical results for the level each target compound in the reference standard calculated over time. These results compare favourably with the replicates determined at the beginning of the work.

Table 2.9 Statistical results for the levels of compounds in the reference standard

	mean	sd	cv
Vanillin	180	26	14%
Acetovanillone	120	25	21%
Vanillic acid	2300	280	12%
Syringaldehyde	200	33	17%
Acetosyringone	160	44	28%
Syringic acid	940	210	22%

A control chart was constructed to show the concentration of vanillin over time in the reference standard. The control charts for other components are shown in Appendix 2.

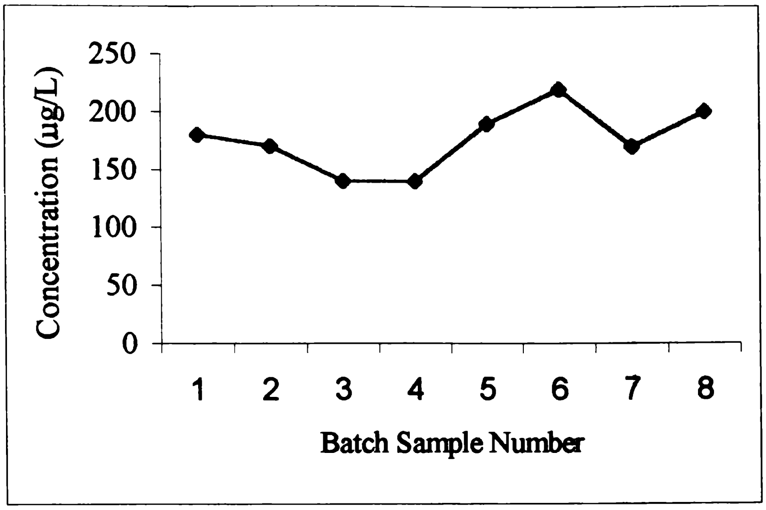


Figure 2.11 Control chart for vanillin

The upper and lower limits shown in Figure 2.11 were set at two standard deviations from the mean. The results shown in Figure 2.11 are those obtained after the appropriate attention had been given to the GC/MS system. At different times during the course of the work there were difficulties in detecting target compounds, especially aromatic compounds. Analysis of the reference standard at these times showed significantly lower levels of test compounds, especially vanillin. It was noticed that aromatic compounds were succeptible to absorption problems in the injection system of the GC/MS. It was necessary to use a cleaned silated glass injector liner for all batch analyses to ensure that adsorption of target compounds onto the liner did not occur. Normally, after the routine checks and injector clean-up, the quantitation of individual compounds in the reference standard fell within two standard deviations of the mean.

A response factor mixture was also analysed in the GC/MS in total ion mode and then inspected before any analyses were carried out. The relative responses of compounds in the mixture provided valuable information on the operating conditions of the GC/MS system. A typical GC profile of the mixture is shown in Figure 2.12.

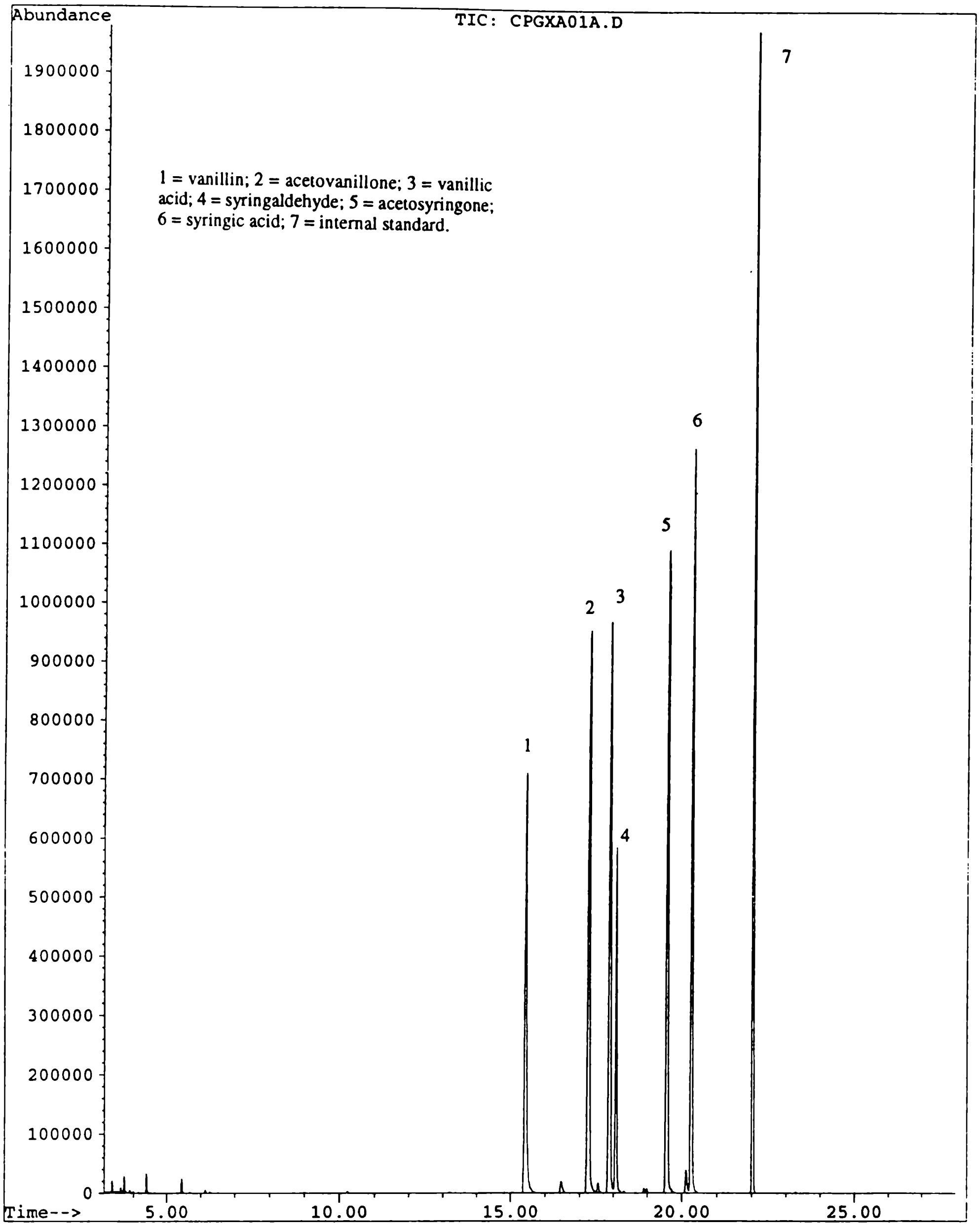


Figure 2.12 Relative responses (total ion) of compounds in response factor mixture.

The relative response of each individual component in the mixture was compared to the response of the hydrocarbon, octadecane. Typically, the relative response ratio of aromatic compounds to octadecane was ca. 0.4-0.5. Inefficiencies in the GC/MS system resulted in low response ratios. A further GC analysis was carried out using SIM in order to establish response factors in SIM mode for the purpose of quantitation.

2.5 Sensory Evaluation

Sensory determinations of wine are carried out routinely at Morton Estate Winery. Many decisions which determine the style of wine produced are based solely around the sensory judgement of the winemakers. Often there are no chemical determinations which assist the winemakers in their decisions. The required time for oak ageing a typical Chardonnay wine is normally between 6 and 9 months. The winemakers carry out frequent sensory evaluations to determine the exact barrel ageing time. Wines are sampled from their ageing containers into clean glassware. Each individual wine is evaluated and discussed. At the completion of the tasting, a decision is made to determine what action will be taken to achieve the desired style of wine

It was realised that the procedures undertaken in wine competitions to evaluate wines would include a panel of judges followed by rigorous statistical tests. Although this type of process would have produced results with greater statistical significance the routine operations of the winery do not allow for these considerations. The winemakers base their judgements on wine style, potential and a knowledge of consumer appeal. Their skills in sensory evaluation allow for major decisions affecting each wine to be based on single samples.

A programme of sensory evaluation was set up. At each sampling time, a tasting of the experimental wines took place. These evaluations were carried out by John Hancock, Steve Bird and Warren Gibson - the winemakers at Morton Estate Winery. All three winemakers are Roseworthy Agricultural College (Australia) graduates and experienced wine judges.

At formal tastings, the wines were evaluated 'blindly' and evaluations were carried out in duplicate to check reproducibility. The wines were scored using a points system which allocated 3 points for colour, 7 for aroma and 10 for taste and general impression. The marks were tabulated and an average mark for each wine was determined. All wines were tasted immediately after being sampled from the ageing container. Clean glasses were used for each individual wine.

At informal tastings, the individual wines were also evaluated 'blindly'. The wines were not allocated a mark but the overall impression and development of the wines were recored in tasting notes. A full set of tasting notes is shown in Appendix 3.

2.6 Conclusions

The extraction techniques, sample preparation and methods of analysis for identifying and quantifying aromatic compounds in water/ethanol, wine and oak wood samples have been discussed. It was not the intention of this work to use these analytical procedures to replace the traditional sensory testing by winemakers. The purpose of these analytical techniques were to establish a chemical profile of each wine which would complement the winemaker's assessments. The principal conclusions of this chapter are as follows:

1. Continuous liquid/liquid extractors and Soxhlet extractors allowed for good recovery of aromatic compounds from water/ethanol, wine and oak wood samples.

2. Addition of internal standards, sample derivatisation and concentration of samples using rotary evaporation were important steps in sample preparation. All samples were prepared and analysed under the same conditions. Stored samples were labelled, sealed and kept refrigerated.

3. GC/MS in selected ion mode provided the sensitivity and resolution necessary for the detection of aromatic compounds in samples down to low µg/L (ppb) levels. Selected ion profiles of target compounds and the relative ion ratios for individual components allowed for the accurate assignment of GC peaks in addition to the information gained from retention times.

4. The experimental error including all extraction, sample preparation and analyses steps gave rise to a standard deviation of about 20%.

5. Steps were taken throughout the course of the work to ensure that the operating conditions of the GC/MS were optimum and that the results obtained from analyses were reproducible over time. Before analyses were performed, the GC/MS system was tuned and routine checks of the injection system and column were carried out. A reference standard and reponse factor mixture were routinely analysed using identical GC/MS conditions before any further analyses of samples took place.

6. A programme of controlled sensory evaluations were conducted to ensure that each experimental wine was described and scored according to the sensory skills of the winemaker. Each assessment was complemented by chemical analyses using the advanced analytical techniques outlined in this chapter. The combination of winemaker's perception and chemical determinations were employed with all wine ageing and wooding trials carried out as part of the project.

Chapter Three

Development of Chemical and Sensory Characteristics in Chardonnay Wine

3.0 Introduction

The work described in the this chapter was designed to obtain data for chemical extractives to complement sensory evaluation of the effect of wood treatment during ageing of wine. Winemakers generally have a well developed concept of the wine style and wood character they are trying to achieve in a wine and are able to judge the development of desired characteristics through periodic tasting. Modern analytical techniques such as GC/MS-SIM may be able to contribute to the assessment of the maturing of a wine during ageing through the detection of component changes before they impact significantly on the sensory characteristics of the wine. In the work described in this chapter, GC/MS-SIM is used to monitor the changes in target compounds as ageing of wine in oak barrels progresses. The results of the chemical studies are compared with sensory evaluations of the wine using conventional tasting.

The three aromatic woody compounds vanillin, acetovanillone and vanillic acid were chosen as target species to monitor the wine ageing performance of a range of ageing containers. To confirm their significance to the oak ageing process, their concentrations were determined in a wine aged in new French oak and in a low priced casked wine which had not received oak treatment. The levels of vanillin, acetovanillone and vanillic acid in the oak aged wine were 150, 190 and 3300 μg/L respectively. In the casked wine there was no detectable vanillin. Acetovanillone and vanillic acid were present at 64 and 260 μg/L respectively. Even though the two wines were made from different grape varieties by different winemakers, the absence of key extractives provided good evidence that the casked wine had not been oak aged. These target compounds are also present in new oak wood and are considered important components of quality wine.[1]

In previous work,[2] the threshold for quantitation of these wood-derived compounds by GC/MS using total ion was found to be in the low part per million range using realistic sample sizes. Typically, the woody compounds introduced into the wine as the oak ageing process progresses are present in concentrations below ppm levels. Thus they could not be determined quantitatively by GC/MS in total ion mode. More sensitive detection achieved through GC/MS-SIM allowed the target species to be identified and individually quantified. The methodology described in Chapter Two was developed to allow routine analysis of wood-derived compounds down to ppb or μg/L levels.

A Chardonnay wine from the 1992 vintage was stored for ageing in 14 different barrels from various origins and cooperages and monitored by a programme of sensory evaluation and chemical analysis.

The purpose of this study was to obtain data on the performances of a range of ageing containers by targeting key extractable compounds as chemical determinants of the degree of oak ageing. The sensory findings of the winemakers would also be used to determine the most preferred ageing container for the desired style of wine. Ideally the chemical profile and the sensory evaluation would be correlated and could then be used separately or in combination to investigate alternative methods of ageing wine that did not rely upon the traditional new oak cask. In particular, it was envisaged that it might

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[1] J. Laszlo, 'Wine Ageing in Oak Barrels,' *Wynboer*, Januarie (1984): 53-56.

[2] C. P. Gribble, 'Some Studies of the Effects of Oak on the Ageing of Wine,' Masters Thesis, University of Waikato (1991).

be possible to impart oak character to the wine aged in old or stainless steel barrels by adding rejuvenated used oak bodies to enhance the oak character of the wine.

3.1 Barrel Ageing of 1992 Chardonnay Wine

An experimental wine (from Hawkes Bay grown Chardonnay grapes vinted by Morton Estate Winery) from the 1992 vintage was stored for 7 months in 10 new French barrels, a new American barrel, 2 old French barrels (including one which had undergone a re-shaving process) and a stainless steel cask to provide a control. Sensory evaluation and chemical analysis of this wine was carried out from June through to December. The wine was subsequently racked, transferred to a tank, and then bottled.

This survey was designed to investigate the oak ageing performance of a range of containers using target compounds as chemical indicators to complement conventional tasting. Dargaud & Jaegle (D&J) and Seguin Moreau new French oak barrels were the principal ageing containers of Chardonnay wine at Morton Estate Winery. Eight of these barrels were used in this survey. Francois Freres and American oak barrels were also included.

The ageing containers used are listed below:

1.	Re-shaven D&J:	re-shaven old French oak (3 previous vintages)
2.	Stainless steel control cask:	220 L like a typical oak barrique
3.	D&J Allier:	old French oak (3 previous vintages)
4.	D&J Tonnelleriere BM HT:	new French oak
5.	D&J Tonnelleriere VM HT:	new French oak
6.	D&J Tonnelleriere Voges:	new French oak
7.	D&J Tonn. B:	new French oak
8.	Seguin Moreau Voges:	new French oak
9.	Seguin Moreau Allier HT:	new French oak
10.	Seguin Moreau Troncais:	new French oak

11. Seguin Moreau Troncais : new French oak
12. Francois Freres Tonn. Voges MT: new French oak
13. Francois Freres Allier M: new French oak
14. American oak: new American oak

Fermentation of the experimental Chardonnay was complete in May 1992 and the first sampling took place in June.

3.2 Sensory Evaluation During Barrel Ageing

An informal wine tasting took place at the time of the first sampling in June 1992. Differences were evident in each of the wines after one month in the various ageing containers. Formal evaluation at the one month sampling was not performed as some wines were still cloudy and fermentation odours were still present The wines stored in stainless steel and old oak were easily identified at the informal tastings (see Table A3.1, Appendix 3). They were described as 'lacking fresh new oak character'. The wine stored in stainless steel showed some unpleasant aromas on the nose. The term 'sulphidic' was a typical descriptive used to characterise this wine. The wines stored in new oak were typically described as 'fruity' and 'pleasant'. The wines stored in the old oak casks were easily identified and were described as 'lacking crispness'.

In early August (1992), the second sampling took place along with a formal wine tasting using the three winemakers at Morton Estate (see Table A3.2, Appendix 3). At this stage, the wines had undergone three months ageing. The wine tastings were set up so that the winemakers did not know the ageing container from which the samples originated. Each wine was tasted in duplicate.

The third and fourth wine tastings and samplings took place respectively in October and December of 1992 (see Tables A3.3 and A3.4, Appendix 3).

After three months, all the wines, with the exception of the wine aged in stainless steel, had developed a deeper colour. The stainless steel aged wine was clearly lacking in complexity when compared with the wines aged in oak. It appeared dull and watery, and tasted 'insipid' (see Table A3.2, Appendix 3). The wine was easily recognised by its 'reductive' and 'sulphidic' character.

The formal tasting after 3 months revealed differences in the overall appeal of the 14 wines (see Table A3.2, Appendix 3). The wines aged in stainless steel and old oak, including the wine aged in the re-shaven barrel, were not considered to have developed desirable character at this stage of the ageing process. Essentially, the winemakers were looking for a balance between the fruit flavours of the grapes and the fresh oak character which the oak wood imparts. The obvious lack of fresh toasty oak meant wines aged in stainless steel and in old oak scored poorly in the tastings.

The wines aged in the D&J and Seguin Moreau new French barrels were favoured by the winemakers. These wines were typically described as having 'good flavour' and showing 'nice balance'.

The wine aged in the American barrel was said to show 'strong vanillin'. Surprisingly, the level of vanillin in the wine was found to be 15 µg/L (see Section 3.3) which was less than the levels in the wines aged in new French oak barrels. The apparent 'high vanillin' as described by the sensory profiles did not appear to correlate with the chemical analysis in this instance.

A summary of the sensory findings at the final wine tasting after 7 months ageing is given in Table 3.1. A complete list of tasting notes is shown in Table A3.4 of Appendix 3.

Table 3.1 Summary of tasting notes for wine aged for 7 months

Ageing container	Tasting notes
Stainless steel control cask	Sulphidic, mercaptan, grapefruit pungency. Not pleasant to taste. Bitterness on palate. Dirty. Unpleasant. H_2S. Mercaptan/H_2S. Feijoa-like. Shows some mealy, biscuity, yeasty character underneath H_2S.
Re-shaven D&J barrel	Slight dusty nose. Good mouth feel. Quite high alcohol finish. Fruit is a little dull. Oak is quite obvious at start. Fruit showing on finish, moderate length and good mouth feel. Good fruit. Slight green characters.
Old used D&J barrel	A little mercaptan. Wood and fruit not integrated. Fruit slightly honeyed. Pinacolada. Ripe fruit followed by toffee apple aroma. Oak not obvious. Lingering fruit. Plain old oak character - lacks freshness. Quite nice fruit flavours.
D&J 4 (new French oak)	Good body. Good fruit and oak. Good finish. Big nose. Well integrated fruit and oak. Good fruit on palate and nice wood flavours. Excellent mouth feel and good length. Lacks oak on nose but nice oak on finish.
Seguin Moreau 1 (new French oak)	Nose subdued. Subtle oak. A little dull. Savoury oak showing through. Good extract. Clean and fresh. Nice fresh balanced oak on nose. Good fruit. Soft palate.
Francois Freres 1 (new French oak)	Nice fresh nose - fruit and oak well integrated. Subtle oak on palate and fruit dominant. Not complex but good. Francois Freres? Slightly burned character. Oak and fruit disjointed. Overpowering toasty oak. A little fruit showing through. A little hazelnut/toasted nut character.
American oak (new American oak)	Oak character too overpowering. Too much tannin. Oak very dominant. Lots of vanillin. Too much wood flavour. American oak? Coconut/vanilla - quite attractive but strong. Coarse oak tannins on palate.

After each wine was tasted, the wine was marked using a scale which allocated a possible 3 marks for colour, 7 for aroma and 10 for taste and general impression; a total of 20 possible marks. Most wines scored the maximum 3 marks for colour except for the wine aged in the stainless steel cask. The lack of extracted material in this wine clearly distinguished it from all the other wines. Table 3.2 shows a summary of the results for the various ageing containers. Each wine was tasted blind and in duplicate. The mean score was calculated for each wine.

Table 3.2 Judges scores for each wine at the final wine tasting

	Judge 1	Judge 2	Judge 3	Average score
Re-shaven D&J	16.0	15.5	15.5	15.7
Stainless steel	10.0	9.5	8.5	9.3
Old used D&J	14.0	14.5	14.0	14.2
D&J 1	17.0	17.5	17.5	17.3
D&J 2	17.0	17.0	17.0	17.0
D&J 3	17.0	16.0	16.5	16.5
D&J 4	18.0	17.0	17.0	17.3
Seguin Moreau 1	16.0	16.0	16.0	16.0
Seguin Moreau 2	17.0	18.0	17.5	17.5
Seguin Moreau 3	16.5	16.5	16.3	16.4
Seguin Moreau 4	16.5	15.5	16.5	16.2
Francois Frere 1	14.5	15.0	14.5	14.7
Francois Frere 2	14.3	14.5	14.5	14.4
American oak	14.5	14.0	14.5	14.3

Wine aged in the stainless steel control cask was characterised by its sulphidic aroma and unpleasant taste and consequently scored poorly with all judges. The wine aged in the old used D&J oak barrel showed good fruit but lacked the crispness and freshness associated with new oak character.

The wine aged in the re-shaven D&J barrel scored higher than the wine aged in the other old barrel, the stainless steel cask, and the wines aged in both Francois Freres and American oak barrels. The wine was described as 'slightly stale', however, and on average scored lower than the wines aged in new French oak.

The wines aged in D&J containers showed fresh, pleasant character with a good balance of toasty oak. The judges scored these wines highly. The judges found the wines aged in Seguin Moraeu barrels also to be attractive and pleasant. The oak characteristics were described as 'sweet', 'fresh', and 'well integrated' (see Table A3.4, Appendix 3).

The wines aged in the Francois Freres barrels showed some new oak character but were judged to be slightly 'disjointed' and 'clumsy'. Consequently, these two wines scored lower than the the wines aged in other new French oak containers. The wine aged in the American oak barrel showed obvious oak character but was judged to be too strong and dominant. The oak aroma and flavour in the wine was typically described as 'coarse' and 'overpowering'.

3.3 Concentrations of Target Extractives During Barrel Ageing

The three aromatic woody compounds: vanillin, acetovanillone and vanillic acid were chosen as target species to monitor the wine ageing performance of a range of ageing containers.

Aromatic compounds, particularly vanillin, are considered important extractable compounds in the oak ageing process and are often used to describe aromas and flavours associated with high quality wines.[3] Samples from the barrel ageing experiment were taken at one month, three months, five months and seven months. Data for the concentrations of vanillin, acetovanillone and vanillic acid in the 14 ageing containers for each sampling time - June (1 month ageing), August (3 month ageing), October (5 month ageing) and December (7 month ageing) are summarised in the Tables 3.3 to 3.6.

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[3] J. Hancock and S. Bird, Personal communication, 1992.

Table 3.3 Concentration of target compounds (µg/L) in wine aged for one month

Ageing container	Vanillin	Acetovanillone	Vanillic acid
Re-shaven D&J	nd	140	930
Stainless steel	nd	82	890
Old Used D&J	13	96	810
D&J 1	39	160	1100
D&J 2	34	130	730
D&J 3	41	160	910
D&J 4	25	190	1300
Seguin Moreau 1	22	150	880
Seguin Moreau 2	22	130	840
Seguin Moreau 3	24	140	750
Seguin Moreau 4	23	150	1300
Francois Frere 1	3.5	16	1000
Francois Frere 2	16	175	930
American oak	4.0	130	1100

nd = below 1 µg/L detection limit

The wine stored in the stainless steel control cask (with no exposure to oak) showed no detectable vanillin. The levels of acetovanillone and vanillic acid in this wine were 82 and 890 µg/L respectively. These compounds were present at about this concentration in wine after fermentation and were expected to be found at these levels in all the wines at the start of the ageing process.

The wine stored in the re-shaven D&J barrel showed no detectable vanillin despite the barrel having undergone a re-shaving process. This attempt to rejuvenate old oak barrels was part of an experiment conducted by the winemakers at Morton Estate Winery. Normal practice requires that barrels used for 3-4 vintages are discarded as the performance of a barrel decreases significantly over time (Hancock and Bird 1992). The old used D&J barrel showed detectable levels of vanillin and acetovanillone. All three ageing containers showed similar levels of vanillic acid.

All the wines aged in D&J barrels showed similar enhanced levels of vanillin and acetovanillone at this first sampling time. The concentration of vanillic acid appeared to be somewhat variable over the four ageing containers but the variability was not much more than the 20% expected for the reproducibility of the analysis.

As in the D&J containers, the wines aged in Seguin Moreau barrels showed similar and enhanced levels of vanillin and acetovanillone, though the level of vanillin was somewhat less than that for the D&J barrels. The Seguin Moreau 4 barrel showed what appeared to be a significantly higher level of vanillic acid.

Wines aged in Francois Freres barrels and in American oak gave low levels of vanillin at this early stage of the ageing process. The wine aged in the Francois Freres 1 barrel was unusual amongst the new French oak barrels in that the level of acetovanillone was much lower.

Table 3.4 shows the levels of target compounds after ageing for three months.

Table 3.4 Concentration of target compounds (µg/L) in wines aged for three months

Ageing container	Vanillin	Acetovanillone	Vanillic acid
Re-shaven D&J	nd	120	1100
Stainless steel	nd	100	990
Old used D&J	18	210	1300
D&J 1	19	280	1400
D&J 2	27	290	1600
D&J 3	38	240	1400
D&J 4	31	280	1300
Seguin Moreau 1	10	260	1300
Seguin Moreau 2	14	320	1300
Seguin Moreau 3	14	190	1400
Seguin Moreau 4	13	270	1500
Francois Frere 1	23	270	1500
Francois Frere 2	22	300	1800
American oak	15	320	1300

nd = below 1 µg/L detection limit

After three months ageing, the re-shaven cask and the stainless steel control cask still showed no detectable vanillin. The wine aged in stainless steel was easily recognised by its comparatively pale colour. All the other wines had begun to develop a deeper ‘straw’ colour. The wine aged in the old used D&J barrel displayed a further increase of vanillin and acetovanillone.

All the wines stored in D&J containers showed similar levels of the target compounds similar to those reported in the first sampling. After three months ageing the levels of vanillin appear to remain constant over the four wines but there was a significant increase in the levels of acetovanillone - on average, ca. 100 μg/L for each of the wines. There were also notable increases in vanillic acid for the wines in three of the four D&J containers. The level of vanillic acid in the D&J 4 wine was unchanged from the first month of ageing.

There were no changes in the vanillin concentrations in the Seguin Moreau barrels. Their extractive profiles for vanillin were similar to those of the wines aged in D&J barrels. There were notable increases in the levels of both acetovanillone and vanillic acid in all four wines.

After three months, the levels of target compounds in the wines aged in Francois Freres and American oak barrels approached those attained in other new oak barrels. The rates of extraction for these barrels appeared to be slower over the initial ageing period.

Table 3.5 shows the levels of target compounds in wines after ageing for 5 months.

Table 3.5 Concentration of target compounds (µg/L) in wines aged for five months

Ageing container	Vanillin	Acetovanillone	Vanillic acid
Re-shaven D&J	220	170	1300
Stainless steel	nd	93	1000
Old used D&J	40	220	1600
D&J 1	100	230	1200
D&J 2	120	240	1400
D&J 3	160	510	2600
D&J 4	80	230	1300
Seguin Moreau 1	110	260	1300
Seguin Moreau 2	90	260	1500
Seguin Moreau 3	nq	nq	nq
Seguin Moreau 4	40	270	1600
Francois Frere 1	30	260	1500
Francois Frere 2	50	290	1500
American oak	40	280	1800

nd = below 1 µg/L detection limit
nq = not quantified

Significant increases in the levels of vanillin and acetovanillione were observed in the wine aged in the re-shaven cask. The extractives in the wine aged in the old used cask showed little change. The wine aged in stainless steel showed no detectable vanillin.

The wines stored in D&J casks showed considerable increases in the levels of vanillin over the 2 month additional ageing period. The D&J 3 wine also had increased vanillic acid. In the other wines, the levels of vanillic acid were similar to those levels detected after three months ageing. The wine stored in the D&J 3 cask showed high levels of all three target compounds.

The 5 month samples showed the development of vanillin in all the wines stored in Seguin Moreau containers. The levels of both acetovanillone and vanillic acid were similar to those detected after three months ageing. A complication at the extraction step of analysis prevented the quantitation of target compounds in the wine aged in the Seguin Moreau 3 cask.

The results from previous sampling times showed the levels of extracted compounds in the wines aged in Francois Freres and American oak barrels to be lower than wines in other new casks. Although the levels of vanillin were higher than at the beginning of the ageing process, the release of vanillin appeared to be slower. The concentrations of both acetovanillone and vanillic acid were similar to those detected after three months ageing.

At the five month ageing stage, significant differences in the extractive profile from each ageing container were observed. The concentration of vanillin followed a trend consistent with the sensory findings of the winemakers (see Table A3.3, Appendix 3). The final sampling and tasting was carried out in December - representing seven months ageing time. The results from the chemical analysis is shown in Table 3.6.

Table 3.6 Concentration of target compounds (μg/L) in wines aged for seven months

Ageing container	Vanillin	Acetovanillone	Vanillic acid
Re-shaven D&J	120	260	2100
Stainless steel	nd	140	2100
Old used D&J	90	150	1900
D&J 1	110	280	1500
D&J 2	120	320	2300
D&J 3	120	300	2400
D&J 4	150	200	2100
Seguin Moreau 1	130	300	2600
Seguin Moreau 2	250	340	2500
Seguin Moreau 3	110	210	2000
Seguin Moreau 4	100	270	2300
Francois Frere 1	90	300	2100
Francois Frere 2	110	230	2200
American oak	210	350	2500

nd = below 1 μg/L detection limit

The wine aged in the re-shaven D&J barrel showed a similar level of vanillin when compared to the five month sampling time. The wine aged in the old used D&J barrel continued to show a slow release of vanillin. All three wines showed elevated levels of vanillic acid of about 2 mg/L.

The four wines aged in D&J containers showed similar extractive profiles at the end of the ageing process. The levels of vanillin in the wines were between 110 and 150 µg/L. The level of vanillin in the D&J 4 container increased from 80 to 150 µg/L. The concentration of vanillin and acetovanillone appeared to plateau after about three months ageing. The wines stored in the D&J 2 and D&J 4 containers both showed an increase in the level of vanillic acid. The levels of vanillic acid over the entire ageing period doubled to greater than 2 mg/L in three of the four containers.

Three of the four wines aged in Seguin Moreau containers showed similar levels of extracted compounds. The Seguin Moreau 2 wine, however, showed a significant increase in the amount of vanillin in the wine. The levels of acetovanillone for all wines were similar to those detected at the last sampling. Vanillic acid doubled to levels exceeding 2 mg/L over the course of the experiment.

The extractive profiles of the wines aged in Francois Freres and American oak barrels after 7 months ageing had profiles which resembled those of wines aged in the other new oak containers. The wine aged in the American oak barrel showed a higher level of vanillin and acetovanillone. The concentration of acetovanillone, detected at 350 µg/L was higher than any of the wines aged in new French containers.

Wines aged in new French and American barrels showed higher levels of vanillin compared with those wines aged in old barrels and in the stainless steel control cask. After three sampling times, the level of vanillin was over 100 µg/L in wines aged in new French oak barrels in contrast with those wines aged in old barrels and in the stainless steel control cask with levels of 40 µg/L and 'not detected' respectively. At the end of the ageing period (4th sampling) all wines stored in new French oak barrels showed levels of vanillin to be greater than 100 µg/L - the wine aged in American oak had 210 µg/L of vanillin. The level of vanillin in the wine aged in old oak had increased to 90 µg/L.

At the completion of the ageing process, the wine aged in the re-shaven D&J cask showed a similar chemical profile to wines aged in new French casks.

The new French oak barrels, the new American oak barrel and the re-shaven D&J barrel appeared to impart elevated levels of aromatic woody compounds to the wines which were identified in the chemical fingerprints. These findings were also reflected in the sensory descriptions of the winemakers (see Table A3.4, Appendix 3). The wines stored in the stainless steel control cask and the old used oak barrel showed lower levels of extracted material at the completion of the ageing period. The old barrel showed the slowest rate of release of target compounds into the wine over the ageing period. Wines aged in the D&J barrels showed the most rapid release of compounds into the wine compared to the other new French oak containers.

While levels of target compounds gave an indication of the degree of oak ageing, the differences were often not much greater than 20% - the experimental error given to each of the determinations. It appeared that the levels of target compounds in the wines aged in D&J and Seguin Moreau new French oak barrels had similar chemical profiles over the entire ageing process and it was these wines which had scored most favourably in the wine tastings. The data obtained for these 8 wines were combined to provide an average chemical ageing profile for a typical new French oak barrel. The data is summarised in Table 3.7 and is shown graphically in Figures 3.1, 3.2 and 3.3. The bracketed numbers in Table 3.7 show the standard deviation for each value calculated from the 8 wines.

Table 3.7 Average concentrations of target compounds (μg/L) in wines aged in new French oak over ageing time (weeks)

Ageing time (weeks)	0	4	12	20	28
Vanillin	nd	28 (8.0)	21 (10)	100 (37)	140 (48)
Acetovanillone	90	150 (20)	270 (39)	290 (100)	280 (50)
Vanillic acid	890	980 (230)	1400 (110)	1600 (480)	2200 (350)

nd = below 1 μg/L detection limit

This average profile showed the gradual increase in vanillin and vanillic acid over the ageing period. The level of acetovanillone appeared to increase up to 12 weeks and plateau for the remaining period.

3.4 Kinetics of Extraction

A better impression of the rate of release of the target compounds over the ageing period is given by concentration versus time plots. These were constructed for wines aged in the re-shaven oak barrel, old used D&J barrel and new American oak barrel. Also included on the plots were the average values for the wines aged in D&J and Seguin Moreau new French oak barrels. Error bars were determined by calculating 20% of the coordinate as the error associated with each point.

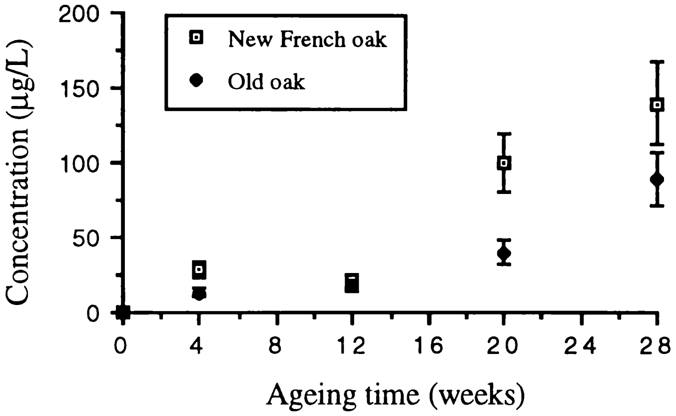

Figure 3.1 Extraction rate of vanillin in wine aged in new French oak barrels and in an old used D&J barrel

The release of vanillin into the wine from the old oak was gradual and remained at a level which was lower than that observed in the wines aged in new French oak.

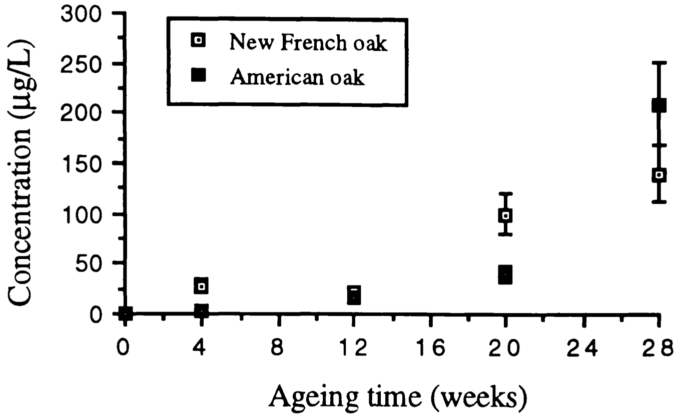

Figure 3.2 Extraction rate of vanillin in wine aged in new French oak barrels and in a new American oak barrel

The development of vanillin in the wine aged in American oak was slow during the initial stages of ageing, but there was a significant increase in the extraction rate after ca. 20 weeks ageing.

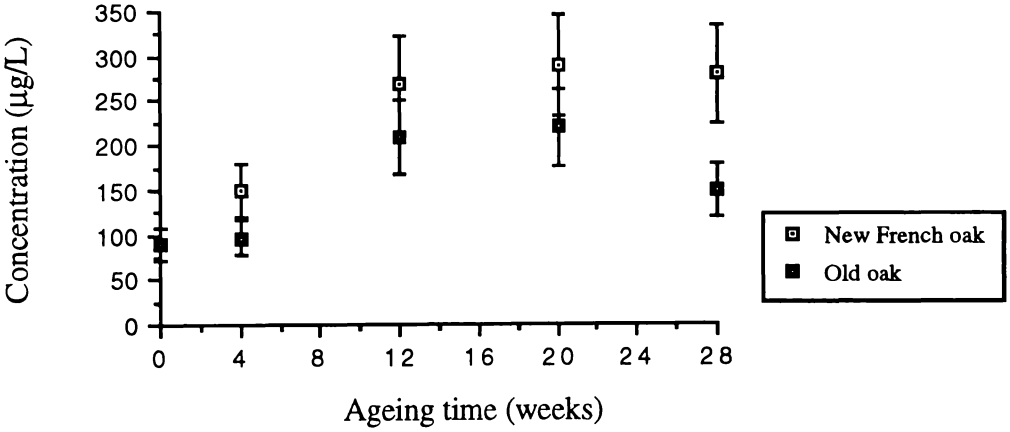

Figure 3.3 Extraction rate of acetovanillone in wine aged in new French oak barrels and in an old D&J oak barrel

The wines aged in both new French oak barrels and the old D&J barrel showed a gradual increase in the concentration of acetovanillone over the ageing time. The maximum level of acetovanillone in the wine aged in the old D&J oak barrel, however, appeared to be less. The rate of extraction appeared to plateau after 12 weeks ageing time.

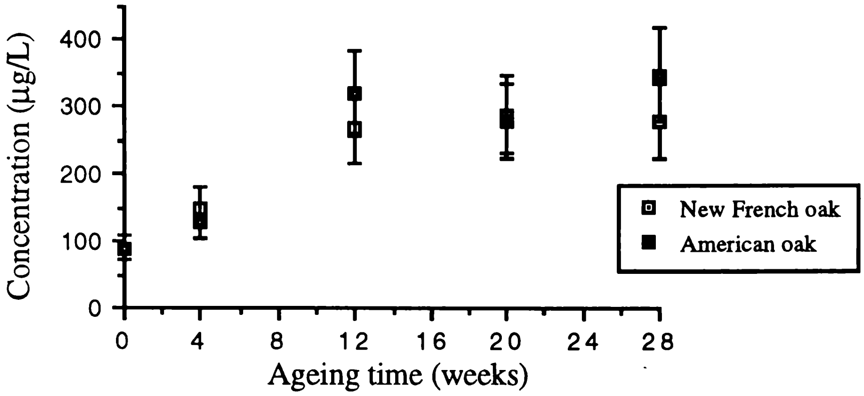

Figure 3.4 Extraction rate of acetovanillone in wine aged in new French oak barrels and in a new American oak barrel

The extraction profiles for the release of acetovanillone into wine aged in New French oak barrels and new American oak barrels were similar.

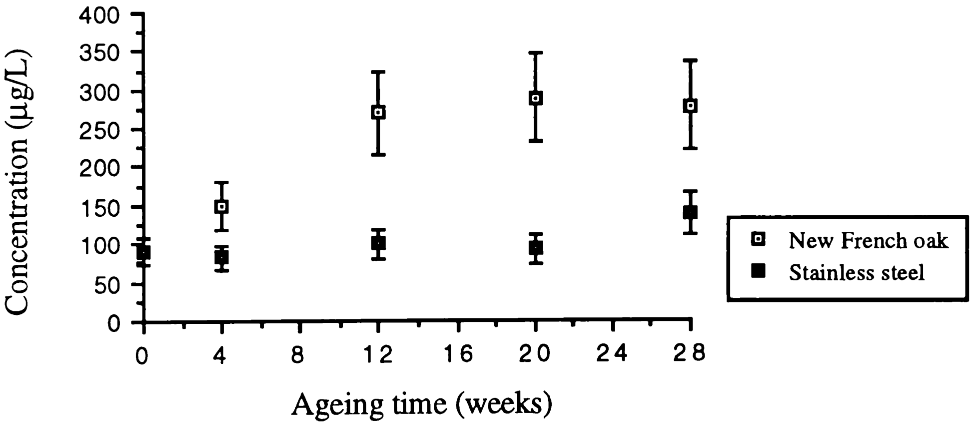

Figure 3.5 Extraction rate of acetovanillone in wine aged in new French oak barrels and in a stainless steel control cask

The extraction rate of acetovanillone in the wine aged in the stainless steel control cask did not follow the trend typical of the wines aged in new French oak barrels. Acetovanillone was present at ca. 90 µg/L at the beginning of the ageing time. Over the rest of the ageing time, the level remained within the 20% error associated with analysis.

3.5 Comparison of Sensory Evaluation and Chemical Analysis

The levels of extractable compounds provided a useful analytical basis for distinguishing the wines aged in new oak, old oak and stainless steel barrels. Maga[4] reported the taste thresholds for some phenolic compounds in alcoholic media. A panel of 20 sensory judges found that the taste threshold for vanillin was at the low part per billion level. It was concluded that the low taste threshold values obtained for compounds such as vanillin confirmed their significant contribution to sensory properties in alcoholic beverages.

It was not within the scope of this work to determine the taste thresholds of target compounds or to examine the actual contribution of these compounds to aroma and flavour. The purpose of the sensory profiling was to evaluate whether the chemical analyses could be used to complement sensory findings. Ideally the chemical parameters could then be employed to establish the nature and performance of the ageing container.

The experimental Chardonnay showed a significant increase in the target wood extractives in all the wines aged in oak barrels. Those wines stored in new French oak barrels scored highly in the tastings and their sensory profiles were described as showing 'integrated oak' and 'subtle oak aromas'. The levels of extracted compounds thus provided useful target levels to be achieved by alternative oak ageing technologies. The concentrations of extracted compounds in wine aged in the stainless steel control barrel and the used oak barrels were lower than those determined in wine aged in new oak barrels. The sensory profiles of the former wines were described

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[4] J. A. Maga, 'Flavor Contribution of Wood in Alcoholic Beverages,' *Proceedings of the 4th Weurman Flavor Research Symposium*, (1984): 409-416.

respectively as 'lacking freshness or crispness' and 'showing stale oak character'. The new American oak barrel appeared to release higher levels of the target compounds towards the end of the ageing process. In the sensory findings, this wine was labelled 'coarse', 'aggressive' and 'overpowering'.

The preferred ageing containers were the D&J new French oak barrels, closely followed by the new Seguin Moreau French oak barrels. The wines aged in the Francois Freres and American oak barrels tended to score lower in the sensory evaluations. The wine aged in the re-shaven D&J barrel showed a similar extractive profile to those wines aged in new French oak barrels and its performance was judged to be better than the old used unshaved D&J barrel.

These results would suggest that both D&J new French oak barrels and Seguin Moreau new French oak barrels are best suited to producing the desired style of Chardonnay at Morton Estate Winery. Combined with good fruit, these ageing containers appear to integrate fruit flavour and aroma with crisp toasty oak character. The resulting wine shows a good balance of fruit and oak.

The wines stored in new French oak barrels from D&J and Seguin Moreau scored highly in all cases. The sensory evaluation of wood character correlated with the preference of the judges. At the end of the ageing process, the levels of vanillin, acetovanillone and vanillic acid averaged over the 8 wines stored in new French oak barrels were 140, 280, and 2200 μg/L respectively. The wine aged in the old oak barrel had levels of vanillin, acetovanillone, and vanillic acid of 90, 150, and 1900 μg/L respectively. With these levels the wine was described as 'dull' and 'stale. The wine stored in the new American oak barrel showed levels of 210, 350, and 2500 μg/L respectively. This wine was described as 'coarse' and 'overpowering'. The preferred wines (those stored in the D&J and Seguin Moreau new French oak barrels), had intermediate levels of target compounds. Of the compounds monitored, vanillin and acetovanillone appeared to be relatively more important in determining oak character. Typically, the levels of these compounds in new French oak barrels were

120-150 μg/L for vanillin and 250-300 μg/L for acetovanillone. The level of vanillic acid in all the wines was similar. It is clear from the results of this study that levels of the target compounds chosen, by themselves, do not provide sufficient criteria on which to evaluate desired wood character.

Generally, the sensory evaluation and chemical characterisation data displayed similar trends. However, in the case of the wines aged in the new French Francois Freres and new American oak barrels there was no similarities. Even at the early stages of the ageing process, these wines were described as having 'high vanillin' and 'powerful oak' character (see Table A3.2, Appendix 3). Yet, the levels of target compounds were lower than any of the other wines stored in new French oak containers.

3.6 Conclusions

The work covered in this chapter set out to investigate the changes in the levels of a limited number of target extractable compounds in wine stored in a range of ageing containers. Chemical analyses and sensory evaluations were carried out on a Hawkes Bay Chardonnay wine in order to achieve this objective. The principal conclusions of this chapter are outlined below:

1. The three aromatic compounds vanillin, acetovanillone and vanillic acid increase in concentration as the barrel-ageing process proceeds. Their development in wine is greater when the wine is stored in new oak barrels as opposed to old oak barrels. Vanillin was not detected in wine aged in stainless steel. Wines aged in stainless steel and old oak barrels appear to lack the freshness and crispness associated with wines aged in new French oak.

2. New French oak barrels are the preferred ageing containers by the winemakers at Morton Estate Winery; especially those from Seguin Moreau and D&J. Wines aged in new French Francois Freres barrels and new American oak barrels scored lower in the

sensory analyses. Wines aged in new French Seguin Moreau and D&J barrels show a gradual release of woody compounds into the wine over the ageing time.

3. It is clear from the results of this work, that while chemical analyses give an indication of the progress of oak ageing, the process is too subtle to be described adequately in terms of the limited chemical parameters chosen. Sensory evaluation will still be required in studies of alternative oak ageing technologies. It is possible that more indicative results could be obtained from chemical analyses by targeting a greater range of compounds. Such an approach will be investigated later in the work.

Chapter Four

Investigation of the Regeneration of Used Oak by Controlled Heat Treatment

4.0 Introduction

Old oak barrels are less effective at imparting oak character to wine than new oak barrels. After 3-4 seasons of use, oak barrels do not facilitate the desired transformations at a cost effective rate. Old barrels are discarded and replaced with new barrels. The purpose of this chapter is to investigate the possibility of regenerating used oak wood by heat treatment, using oak wood obtained from these discarded oak barrels.

Target compounds were used to monitor the effects of heat treatment on the used oak wood. These compounds were also used to compare the chemical profiles of oak wood which had undergone traditional heat treatments. The series of target compounds was expanded to include the aldehyde, ketone and acid from the syringyl series of compounds. Pure standards of these compounds were obtained and a response factor mixture was prepared. The new set of target compounds included: vanillin, acetovanillone, vanillic acid, syringaldehyde, acetosyringone and syringic acid.

As part of the work, the chemical profile of a new French oak barrel was determined. This profile was used to provide target levels of key compounds in toasting experiments.

4.1 Analysis of Toasted Oak from a New French Oak Barrel

In order to establish the levels of target extractable compounds in oak from a new French barrel, the chemical profile of the toasted wood from the interior of a new French oak barrel was studied. The profile of new French toasted oak wood would provide an indication of the desired levels of target compounds that would need to be achieved in regenerated wood.

4.1.1 Distribution of Target Compounds on Interior Surface of Barrel

A new Seguin Moreau French oak barrel was dismantled. When the head of the barrel was removed a distinctively strong toasted wood aroma was noticed. Samples were collected from the central point of six different staves around the toasted interior of the barrel. Care was taken to ensure that each sample contained only the surface toasted oak; the part of the barrel that forms the wine/wood interface. The six samples were prepared using Soxhlet extraction techniques and analysed by GC/MS to determine the levels of target compounds present in new oak. A mean, standard deviation (sd) and coefficient of variability (cv) were calculated for each target compound. The results from this experiment are shown in Table 4.1.

Table 4.1 Concentration of target compounds (µg/g) in new toasted French oak

Sample	1	2	3	4	5	6	mean	sd	cv
Vanillin	320	180	150	240	290	120	210	79	38%
Acetovanillone	52	55	28	67	45	32	47	15	32%
Vanillic acid	96	94	58	110	110	71	90	21	23%
Syringaldehyde	890	500	340	570	1100	700	680	270	40%
Acetosyringone	350	340	160	330	320	230	290	74	26%
Syringic acid	330	290	160	380	380	250	300	85	28%

While it was realised that the traditional toasting process would not toast all interior parts of the barrel to the same degree, the determined levels of compounds were relatively constant over the central sampling points of six individual staves.

Coefficients of variability were typically 20% - 40% which indicated relatively uniform concentrations of target compounds given the 20% experimental error associated with analyses. These values provided target values to be aimed for in toasting procedures carried out in further experiments.

The ratios of aldehyde to acid for each target series were determined. For vanillin/vanillic acid, the ratio was between 1.7 and 3.3 (average 2.3). For syringaldehyde/syringic acid, the ratio was between 1.5 and 2.9 and also averaged 2.3. This result shows that about double amounts of aldehyde are present relative to acid in new French toasted oak.

Figure 4.1 shows a typical chromatographic trace for the analysis of new French oak wood.

4.2 Preliminary Toasting Experiment

A preliminary experiment was carried out to investigate the effect of toasting used oak wood from a used barrel using a method similar to the traditional firing process.

4.2.1 Preparation of Used Oak

A selection of oak blocks were prepared by dismantling a used French oak barrel (D&J barrel purchased in 1989 and used for 4 years), removing each individual stave, sanding the staves to expose visually unmodified wood, and then cutting the staves into blocks of dimensions 4 x 2 x 2 cm. All oak wood used throughout this section of the work was prepared in this manner.

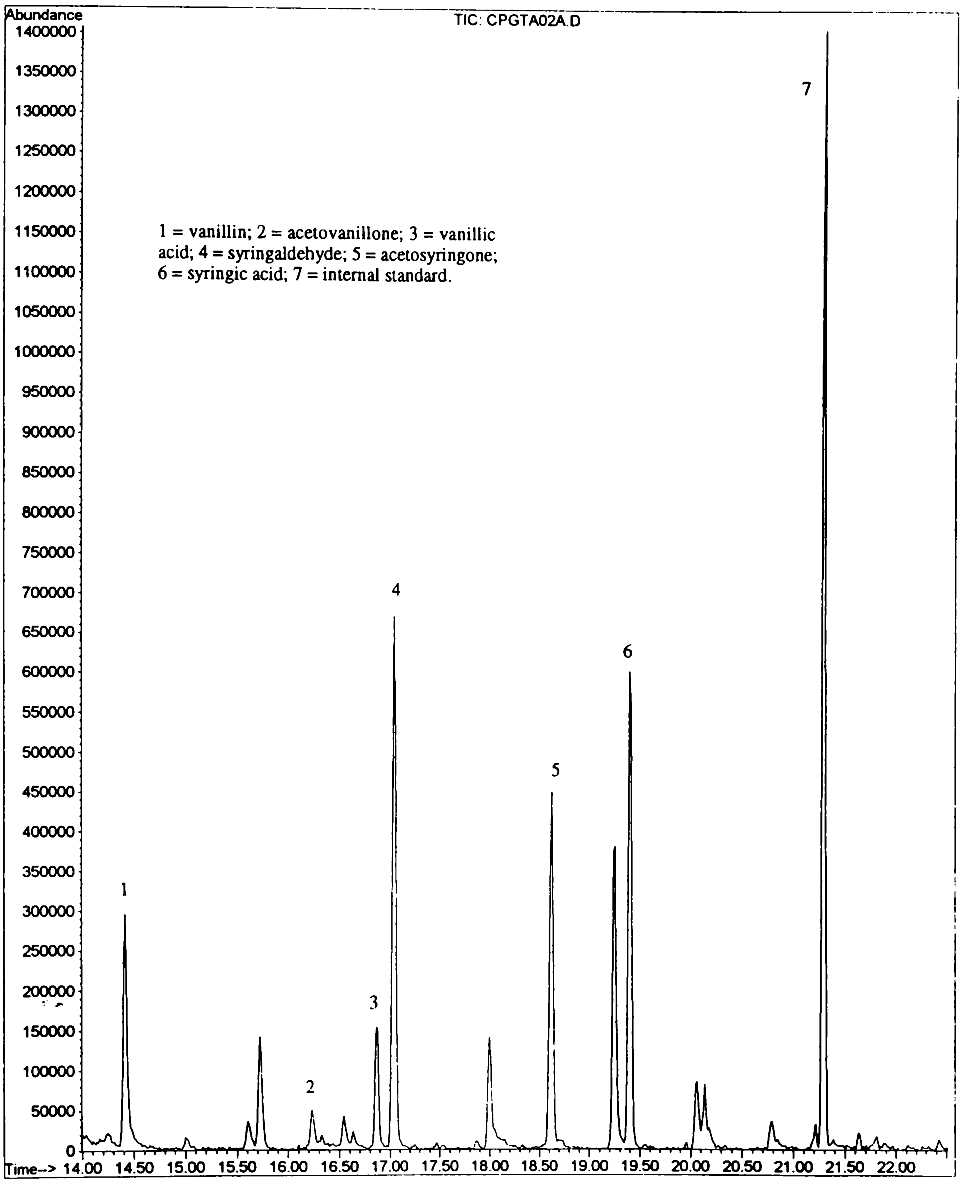

Figure 4.1 Typical GC/MS chromatogram of new French toasted oak

4.2.2 Preliminary Toasting Procedure

The blocks were toasted over a flame fuelled by oak shavings which had been scraped from another stave of the same barrel. The blocks were turned frequently in order to receive maximum heat without burning. After 5-10 min, the blocks were removed from the heat before the onset of charring. Controlling the heat of the flame and monitoring the degree of toasting was difficult so reproducibility was poor.

After toasting, the blocks were chopped finely to make them suitable for extraction purposes. A random selection of used oak blocks which had not received heat treatment were also extracted to provide untoasted control samples. Table 4.2 shows the levels of target compounds in the samples of untoasted oak.

Table 4.2 Concentration of target compounds (μg/g) before toasting used French oak wood

Sample	A	B	C	D	mean	sd
Vanillin	0.9	4.6	3.6	3.1	3.1	1.6
Acetovanillone	nd	nd	nd	nd		
Vanillic acid	3.8	4.5	3.6	7.5	4.9	1.8
Syringaldehyde	5.5	8.5	5.9	10	7.5	2.2
Acetosyringone	1.1	1.1	0.8	0.7	0.9	0.2
Syringic acid	4.0	6.2	4.1	15	7.3	5.2

nd = not above 1 μg/g threshold for quantitation

Table 4.3 shows the levels of target compounds after the toasting process.

Table 4.3 Concentration of target compounds (μg/g) after toasting used French oak wood

Sample	A	B	C	D	mean	sd
Vanillin	27	12	9.8	11	15	8.1
Acetovanillone	7.6	4.7	3.1	2.9	4.6	2.2
Vanillic acid	15	16	11	4.7	11	5.1
Syringaldehyde	42	12	12	19	21	14
Acetosyringone	25	7.8	4.8	5.5	11	9.6
Syringic acid	36	28	22	13	25	9.7

This experiment showed that the levels of aromatic compounds in the used oak wood could be enhanced through exposing the wood to elevated temperatures. All compounds in the toasted samples showed levels of target compounds higher than in the untoasted samples.

4.3 Characterisation of Extractives in Used French Oak

The levels of target compounds throughout various parts of the used oak were determined to establish a baseline against which toasting procedures could be measured. These levels would be used to determine the suitability of used oak as a precurser material for oak toasting experiments.

4.3.1 Variation Along a Single Stave

A single stave was prepared by sanding to unmodified wood as described in Section 4.3.2. The variation in levels of target compounds at six points along the interior length of the stave was determined. This experiment was carried out to determine the reproducibility of sampling at random points along a stave. The six samples contained wood from all depths at each sampling point. The results are shown in Table 4.4. A-F represent the six sampling points along the stave.

Table 4.4 Concentration of target compounds (µg/g) along a single stave of the used D&J barrel

Sample	A	B	C	D	E	F	mean	sd
Vanillin	15	16	14	13	18	15	15	1.7
Acetovanillone	1.7	1.8	1.5	1.7	2.0	1.1	1.6	0.3
Vanillic acid	13	13	12	14	18	9.2	13	2.9
Syringaldehyde	28	24	28	22	34	30	28	4.3
Acetosyringone	3.5	3.4	3.1	3.1	3.6	2.7	3.2	0.3
Syringic acid	15	12	14	16	21	10	15	3.8

This result showed that the distribution of compounds over a single used oak stave was essentially the same throughout the stave. All parts of the used staves should be usable in toasting experiments.

4.3.2 Variation between Staves

It was necessary to determine if the levels of compounds were constant throughout different staves of a used oak barrel. A used D&J oak barrel (purchased in 1989 and used for 3 years ageing) was dismantled and collapsed to provide a selection of individual staves. Each stave was sanded to expose visually unmodified wood. Six different staves were sampled at the central point of each of the staves. The central sampling point on each stave included wood from all depths through the stave from around the sampling point. It appeared from the colour of the wood that these samples of used oak had not been affected by heat treatment during the original firing process. The results are shown in Table 4.5.

Table 4.5 Concentration of target compounds (μg/g) from six oak staves from the used D&J oak barrel

Sample	A	B	C	D	E	F	mean	sd
Vanillin	7.6	12	9.3	12	12	6.5	10	2.3
Acetovanillone	1.2	1.3	1.1	1.7	1.7	0.9	1.3	0.3
Vanillic acid	7.2	8.5	8.0	12	11	5.2	8.7	2.5
Syringaldehyde	13	20	14	21	22	11	17	4.7
Acetosyringone	2.8	3.1	2.3	3.4	4.1	1.2	2.8	1.0
Syringic acid	8.5	11	12	16	15	6.4	12	3.7

Similar levels of compounds were present in staves from different parts of the barrel. Individual staves were prepared in the same way for use as experimental oak wood which would be used in further experiments.

4.3.3 Depth Profile of Used French Toasted Oak

It was of interest to investigate the effect of the original toasting process on used French toasted oak and the extent to which the wood is modified through the staves of the barrel. The variation with depth of target compound concentration was investigated using samples of used oak taken from two used oak barrels. This experiment would provide an indication of the extent to which subsurface used oak had been modified by winemaking practises.

Two used oak barrels were dismantled and four staves from each barrel were used to carry out two separate depth profile experiments. The four staves from each barrel were cut into 5 mm slices from the interior through to the exterior wood. The samples for each depth were combined with samples from the other staves of the same barrel in order to provide an average sample for each depth for each barrel. The same process was carried out for the second used barrel. In addition, a 2 mm 'surface film' was scratched from the interior surface of each barrel to obtain a sample of wood which formed the wine/wood interface. The two used French barrels were:

Barrel #1	D&J French oak barrel purchased in 1988 and used for 3 vintages.
Barrel #2	Radoux French oak barrel purchased in 1988 and used for 3 vintages. At the end of the 1991 season, the interior surface of the barrel was re-shaven and the barrel had not been used for ageing since the treatment.

Tables 4.6 and 4.7 show the concentrations of target compounds at various depths through the staves. The 0-2 mm sample represents the thin film sample scratched from the interior surface of the barrels.

Table 4.6 Concentration of target compounds (μg/g) from the interior surface film to exterior of used D&J barrel

Depth	0-2 mm	0-5 mm	5-10 mm	10-15 mm	15-20 mm	20-exterior
Vanillin	nd	140	22	nd	nd	nd
Acetovanillone	nd	6	nd	nd	nd	nd
Vanillic acid	nd	150	180	140	96	78
Syringaldehyde	nd	290	3	nd	nd	nd
Acetosyringone	nd	49	54	nd	nd	3
Syringic acid	nd	55	98	13	40	45

nd = < 1 μg/g

No target compounds were detected in the oak wood which had been in contact with wine (0-2 mm). At depths greater than 5 mm, some target compounds were present. Samples taken from oak closer to the exterior of the barrel showed low levels of target compounds. Evidence of original toasting during barrel manufacture was apparent in the levels of target compounds at the 0-5 and 5-10 mm depths.

Table 4.7 Concentration of target compounds (μg/g) from the interior surface film to exterior of used Radoux barrel

Depth	0-2 mm	0-5 mm	5-10 mm	10-15 mm	15-20 mm	20 - exterior
Vanillin	nd	nd	nd	nd	nd	nd
Acetovanillione	nd	nd	nd	nd	nd	nd
Vanillic acid	nd	nd	34	170	190	180
Syringaldehyde	4.0	nd	130	78	68	41
Acetosyringone	nd	nd	37	24	25	26
Syringic acid	nd	nd	51	68	73	57

nd = < 1 μg/g

Apart from a low level of syringaldehyde (4 μg/g) present at the 0-2 mm depth, no target aromatic compounds were detected in the oak wood which formed the wine/wood interface. Unlike Barrel #1, the target compounds appeared to be totally depleted in the region between the interior surface of the barrel through to a depth of 5-10 mm. There was no evidence of the effects of the original toasting process.

It was clear that target compounds which were shown to be present in new toasted

French oak were depleted in samples of used French oak at the wine/wood interface.

4.3.4 Discussion

The chemical profiles of used French oak were investigated. It was anticipated that the re-shaven Radoux barrel (see Section 4.3.1) may show elevated levels of aromatic compounds on the interior surface having undergone the re-shaving process. Experimental data did not show this result. The shaving process did not expose increased surface concentrations of target compounds as these compounds were depleted to depths of about 10 mm.

The depth to which wine extracts compounds from the barrel appeared to be 5-10 mm. The oak wood sampled towards the exterior of the barrel contained less aromatic compounds. This wood was likely to have received little heat treatment during the original barrel firing process.

Conner et al.[1] reported the concentration and location of phenolic extractives in cask wood. The amounts of vanillic and syringic acids and the respective aldehydes were determined. Extracts of used wood showed a depletion of acid and aldehyde (< 20 μg/g) at the interior of the stave. Greater depths showed a gradual increase in the levels of extractives. The present work also showed low levels of aromatic compounds at the interior surface of the barrel. Higher levels of these compounds were detected at greater depths through the wood.

Oak wood prepared by sanding staves from a used barrel gave similar levels of target compounds throughout the barrel. This used wood was of sufficiently uniform composition to offer a suitable material to determine the effects of heat treatment on used oak.

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[1] J. M. Conner, A. Paterson and J. R. Piggott, 'Changes in Wood Extractives from Oak Cask Staves through Maturation of Scotch Malt Whisky,' *J. Sci. Food Agric.* 62 (1993):169 - 174.

4.4 Kiln Toasting of Used French Oak

A more controllable method of toasting was developed using an electric kiln as a heat source. The kiln was a McGregor 200A muffle furnace and allowed for control of toasting temperatures to within 10°C.

The used oak was prepared in the same way as described in Section 4.2.1. A selection of staves from a used Seguin Moreau oak barrel (purchased in 1988, and used for 4 vintages) were sanded then cut into small blocks (4 x 2 x 2 cm). All blocks used in these experiments were from this same used Seguin Moreau barrel.

The kiln was pre-heated to the toasting temperature. A selection of blocks were laid out on the bottom of the kiln with a small spacing between each cube. It was noticed that the sides and top of the blocks toasted at a faster rate than the bottom. Every 2-3 min, the blocks were turned in an attempt to provide consistent heating to all sides of the blocks.

These experiments were designed to determine the optimum combination of time and temperature to produce toasted oak wood with levels of aromatic compounds similar to the levels previously observed in samples of new French oak (see Section 4.1.1).

4.4.1 Used Oak Toasted at 200°C

Vanillin and vanillic acid were used in a preliminary experiment to investigate the effect of gentle heating on the levels of compounds in the wood. The blocks were placed in the kiln for 15 minutes at a temperature of 200°C. The results are shown in Table 4.8. Six blocks were used to check reproducibility. An average value for the levels of vanillin and vanillic acid in untoasted oak is shown for comparison.

Table 4.8 Concentration of vanillin and vanillic acid (µg/g) in used French oak before and after toasting at 200°C for 15 min

Sample	untoasted oak	1	2	3	4	5	6
Vanillin	3.1	11	6.3	14	13	20	11
Vanillic acid	4.9	6.8	8.3	13	8.2	23	14

This experiment showed that these conditions did increase the levels of vanillin and vanillic acid in used oak wood. However the increased levels obtained were significantly lower than those obtained in the analysis of new French oak. The colour of the toasted samples was not significantly different to the colour of untoasted samples. Further experiments were carried out which varied the time and temperature variables.

4.4.2 Used Oak Toasted at 300°C

The experiment outlined in Section 4.4.1 was repeated except the toasting temperature was increased to 300°C and the selection of target compounds was expanded to include other aromatic compounds. The toasting time was varied from 0 through to 18 min. After 18 min, charring of the wood began to take place and the risk of combustion increased. The oak blocks were removed from the heat at this point. The results for this experiment are shown in Table 4.9.

Table 4.9 Concentration of target compounds (µg/g) after toasting used oak at 300°C for 0-18 min

Toasting time (min)	0	2	5	10	12	15	18
Vanillin	3.1	13	5.2	34	39	31	28
Acetovanillone	nd	1.5	0.9	5.1	6.2	5.8	9.2
Vanillic acid	4.9	9.3	5.7	20	25	21	27
Syringaldehyde	7.5	18	9.0	74	86	65	80
Acetosyringone	0.9	2.0	1.3	16	24	19	42
Syringic acid	7.3	9.2	4.8	40	58	45	81

Increases in concentration of target compounds are shown in Figures 4.2 and 4.3.

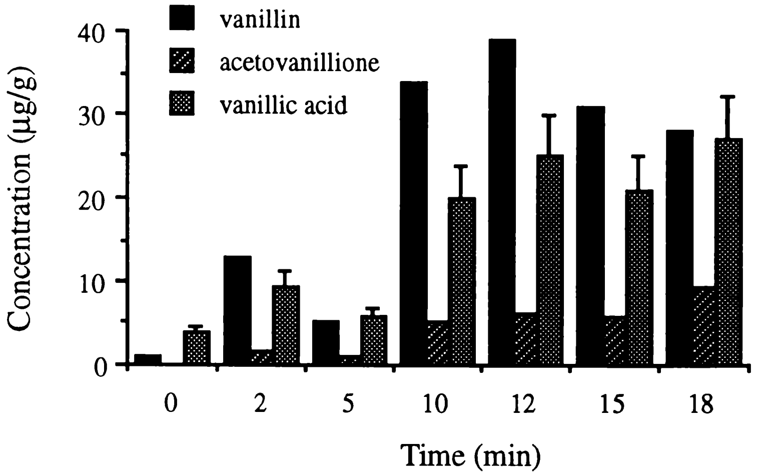

Figure 4.2 Concentration versus toasting time for the vanillin, acetovanillone and vanillic acid at 300°C

It appears that changes in concentration become significant only after an induction period of 5-10 min. This is possibly the time taken for the oak cube to heat to furnace temperature. For vanillin, increases in concentration occur over the next 5 min then appear to level off. This leveling off may be due to the depletion of precurser material or losses due to evaporation. At the same time, levels of acetovanillone and vanillic acid may still be increasing.

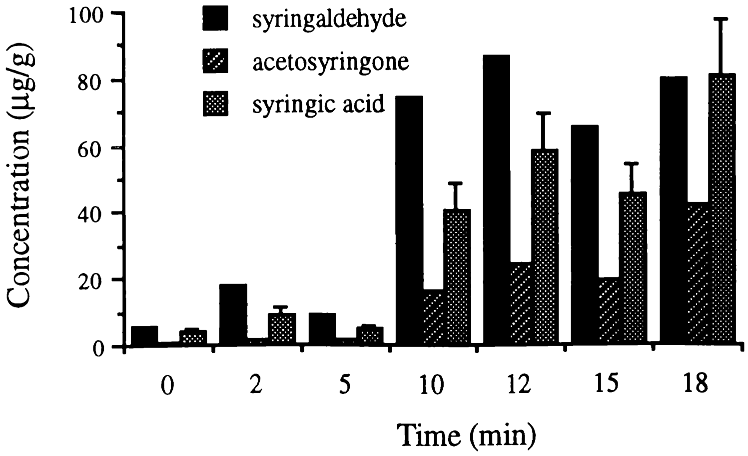

Figure 4.3 Concentration versus toasting time for syringaldehyde, acetosyringone and syringic acid at 300°C

In the case of acetosyringone and syringic acid, it appeared that concentrations may still be increasing after 18 min. After 18 min the experiment was terminated because of visible charring of the oak.

4.4.3 Used Oak Toasted at 400°C

The experiment was repeated at 400°C. The results are shown in Table 4.10. The oak blocks were toasted for as long as possible without extensive charring. At this higher temperature, the wood began to char after 10-12 min and so the experiment was terminated at this time.

Table 4.10 Concentration of target compounds (μg/g) after toasting used oak at 400°C

Toasting time (min)	0	2	4	6	8	10
Vanillin	3.1	1.0	3.7	4.0	3.8	2.2
Acetovanillone	nd	nd	1.5	3.6	4.6	6.8
Vanillic acid	4.9	5.0	4.9	9.6	7.2	9.2
Syringaldehyde	7.5	7.0	10	40	22	19
Acetosyringone	0.9	3.0	3.4	15	18	23
Syringic acid	7.3	5.0	6.8	22	15	20

In general, the final levels of aromatic compounds after heating at 400°C were less than those acheived at 300°C. Changes in the concentrations of target compounds are shown in Figures 4.4 and 4.5.

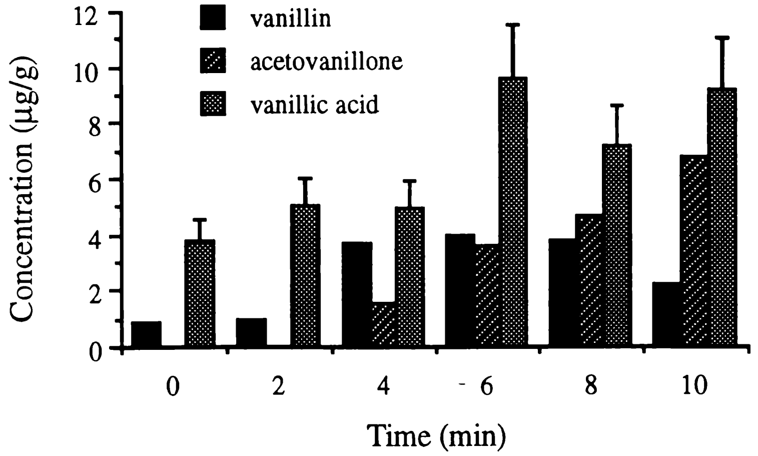

Figure 4.4 Concentration versus toasting time for vanillin, acetovanillone and vanillic acid at 400°C

In the case of acetovanillone and vanillic acid, the concentrations still appeared to be increasing after 10 min heating. The level of vanillin reached a maximum after 6 min.

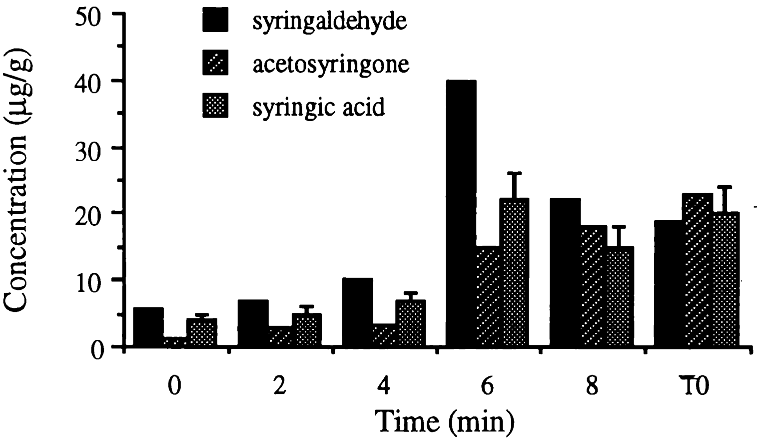

Figure 4.5 Concentration versus toasting time for syringaldehyde, acetosyringone and syringic acid at 400°C

The levels of acetosyringone and syringic acid appear to be still increasing after the onset of charring. Syringaldehyde reached a maximum after 6 min heating.

The aromatic aldehydes, vanillin and syringaldehyde appear to be most affected by overheating. After 6 min heating the levels of these compounds decrease. The other target compounds still appear to be increasing after the onset of charring.

4.4.4 Used Oak Toasted at 500°C

At 500°C, the oak blocks charred extensively after 5 min, and combusted after a total of 8 minutes in the kiln. To determine the effect of extensive charring on the levels of target compounds, the blocks continued to be heated after the onset of charring. At 0.5 minute intervals, a selection of blocks were removed for sampling. Results are shown in Table 4.11.

Table 4.11 Concentration of target compounds (μg/g) after toasting used oak at 500°C

Toasting time (min)	0	5	5.5	6.0	6.5	7.0	7.5	8.0
Vanillin	3.1	14	18	17	8.5	6.0	2.9	1.5
Acetovanillone	nd	1.4	11	14	10	4.9	3.3	1.2
Vanillic acid	4.9	21	15	18	11	6.5	4.2	1.6
Syringaldehyde	7.5	16	64	61	42	36	18	11
Acetosyringone	0.9	2.6	40	50	38	22	15	8.0
Syringic acid	7.3	24	46	53	33	17	14	6.7

Toasting at 500°C produced lower levels of target compounds than those observed at lower toasting temperatures. The levels peaked after 5-6 min, then decreased dramatically at longer toasting times particularly after 7-8 min when the blocks began smouldering. The blocks combusted after the 8 minute sampling.

4.4.5 Used Oak Toasted at 600°C

The experiment was repeated at 600°C. The oak blocks combusted after 10 seconds in the kiln. No sampling was carried out on used oak wood toasted at this temperature.

4.4.6 Discussion

Nishimura et al.[2] reported the effects of heat treatment on oak chips. Untoasted chips contained few aromatic compounds but increased temperatures caused a significant increase in aromatic content. After charring, the oak chips showed lower levels of aromatic compounds. The effect of toasting on American and Limousin oak was carried out by Hoey.[3] Studies showed the increase in other aroma compounds such as furfural. The present work also shows significant increases in the levels of aromatic compounds at elevated toasting temperatures. These compounds are regarded as

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[2] K. Nishimura, M. Ohnishi, M. Masuda, K. Koga and R. Matsuyama, 'Reactions of Wood Components During Maturation,' *Flavour of Distilled Beverages:Originn and Development*, ed. J. R. Pigott, Chichester: Ellis Horwood, (1983): 241-255.

[3] A. Hoey, 'Why be Concerned about Oak Barrel Maturation,' The Australian Grapegrower & Winemaker, Nov. (1986):14-20.

products of lignin degradation resulting from the toasting of oak wood.[4,5,6] Figure 4.6 is a model of lignin according to Adler[7] which shows the complex network of aromatic monomers that make up the lignin molecule.

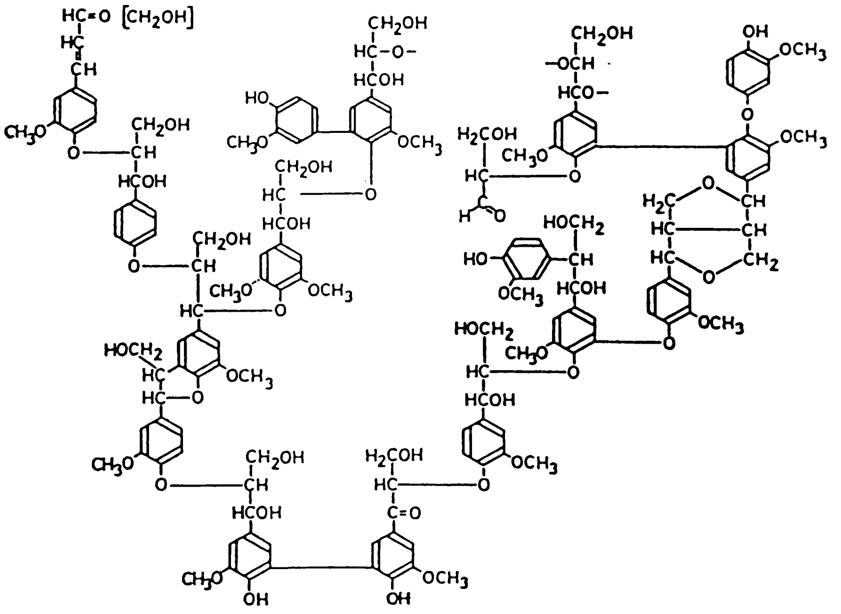

Figure 4.6 The constitution of lignin (Adler, 1977)

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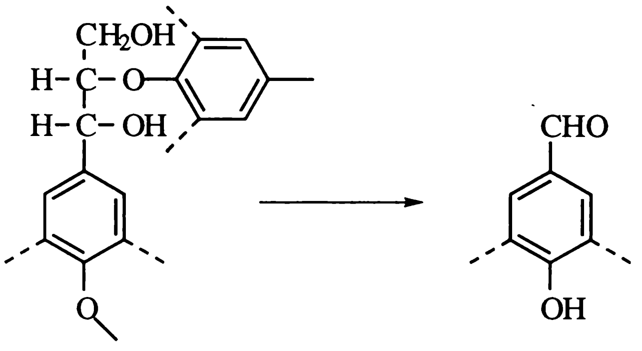
[4] M. A. Sefton, 'How does Oak Barrel Maturation Contribute to Wine Flavor?' *Australian & New Zealand Wine Industry Journal,* Nov./Dec. (1991): 17-20.

[5] J. Laszlo, 'Wine Ageing in Oak Barrels.' *Wynboer,* Jan. (1984):53-56.

[6] J.L. Puech, 'Extraction of Phenolic Compounds from Oak Wood in Model Solutions and Evolution of Aromatic Aldehydes in Wines Aged in Oak Barrels.' *Am. J. Enol. Vitic.* 38/3 (1987):236-238.

[7] E. Adler, *Wood Sci. Technol.* 11 (1977):169-218.

The process of lignin acidolysis was described by Lundquist.[8] The heating of lignin in the presence of acid causes the selective cleavage of ß-aryl ether linkages. The majority of derived acidolysis monomers originate from arylglycerol ß-aryl ether components (see Scheme 4.1). A range of mono, di and tri-substituted aromatic compounds are liberated.



Scheme 4.1 Formation of aromatic aldehydes from ß-aryl cleavage of arylglycerol ß-aryl ether

It is likely that these types of transformations occured in oak wood during thermal treatment. The presence of acid in the wood, moisture content, and the elevated temperatures are the conditions required for acidolysis. Details for the mechanistic pathways for these types of tranformations have been previously reported.[9] General schemes for the presence of phenolic acids and aldehydes are also outlined.[10] It is likely that the occurence of these compounds result from transformations via numerous pathways as many monomeric aromatic compounds are present in the complex lignin molecule. The cleavage of these compounds provide a range of aromatic pre-curser compounds which may then be oxidised to simple monomeric components such as

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[8] K. Lundquist, 'Acidolysis', *Springer Series in Wood Science,* ed. Lin and Dence (1992):290-300.

[9] K. Lundquist, 'Low Molecular Weight Lignin Hydrolysis Products,' *Appl. Polymer Symp.* 28 (1976):1393-1407.

[10] K. J. G. Reid, J. S. Swan and C. S. Gutteridge, 'Assessment of Scotch Whisky Quality by Pyrolysis-mass spectrometry and the Subsequent Correlation of Quality with the Oak Wood Cask,' *Journal of Analytical and Applied Pyrolysis* 25 (1993): 49-62.

vanillin. In the present work, however, it was found that the ratio of aldehyde to acid was ca. 1 indicating the presence of similar amounts of aldehyde and acid in lignin. This ratio was constant throughout the heating process suggesting that potential transformations such as oxidation of aldehydes to acids were not occurring under the conditions outlined.

During the toasting process, the levels of acetovanillone, vanillic acid, acetosyringone and syringic acid were still increasing after the onset of charring. Enrichment of these compounds may have been occurring at greater depths inside the blocks. The levels of vanillin and syringaldehyde appeared to level off after 10 min toasting time at 300°C.

Uniform heating throughout the whole oak block was difficult to achieve. The surface of the block heated more quickly than the interior. From the colour of the blocks after toasting, it was clear that while the edges of the blocks may have charred, wood below the surface was less affected. Subsurface changes could account for continued increases in levels of target compounds after the onset of charring.

The toasting experiment carried out at 500°C showed that it was not until extensive charring or smouldering of the wood took place that a decrease in the levels of target compounds would occur. It appeared that ca. 15 min of 300°C heat provided optimum toasting conditions allowing for the heating of the whole block without extensive charring and the potential loss of desired pyrolysis products.

4.5 Preparation of Toasted Oak Cubes for Wine Ageing Experiments

A large batch of toasted oak cubes were prepared as described in Section 4.4 from used oak obtained from the used Seguin Moreau barrel. The used oak staves were sanded back to expose unmodified oak wood. This wood was cut into 1.5 cm side length cubes. These dimensions were chosen because the depth profile experiment described in Section 4.3.3 showed the penetration of wine into the wood to be ca. 5 mm. If these blocks were added to wine, the degree of penetration should expose most of the wood.

4.5.1 Effect of Wrapping Cubes in Foil

In an attempt to make the degree of heating more uniform, the cubes were wrapped in foil. About 15 cubes were wrapped together and placed on the bottom of the kiln. The cubes were then toasted in the kiln for 25 minutes at 300°C. At 5 minute intervals throughout the toasting time, the packaged cubes were turned. A selection of the toasted cubes were taken for sampling then ground into a coarse powder for extraction purposes. Three samples of the powder were extracted and the results of analysis are shown Table 4.11 as A, B, and C. Table 4.12 also shows the levels of target compounds in untoasted used oak as a comparison.

Table 4.12 Concentration of target compounds (µg/g) in untoasted oak and in foil wrapped toasted oak cubes toasted at 300°C for 25 min

Sample	untoasted	A	B	C	mean
Vanillin	3.1	170	150	220	180
Acetovanillone	nd	120	37	50	69
Vanillic acid	4.9	330	130	200	220
Syringaldehyde	7.5	170	320	470	320
Acetosyringone	0.9	360	130	190	230
Syringic acid	7.3	590	230	390	400

The effect of toasting used oak wrapped in foil showed significantly high levels of target compounds in the cubes. The foil allowed for the cubes to be exposed to the heat treatment for a longer period without extensive charring taking place. Levels of target compounds achieved using these toasting conditions approached those determined in new French oak.

Vanillin/vanillic acid and syringaldehyde/syringic acid ratios both averaged ca. 0.8 indicating similar amounts of aldehyde and acid were produced during the toasting process.

Figures 4.7 and 4.8 show typical chromatographic traces for the analyses of untoasted oak wood and wood taken from the toasted oak cubes.

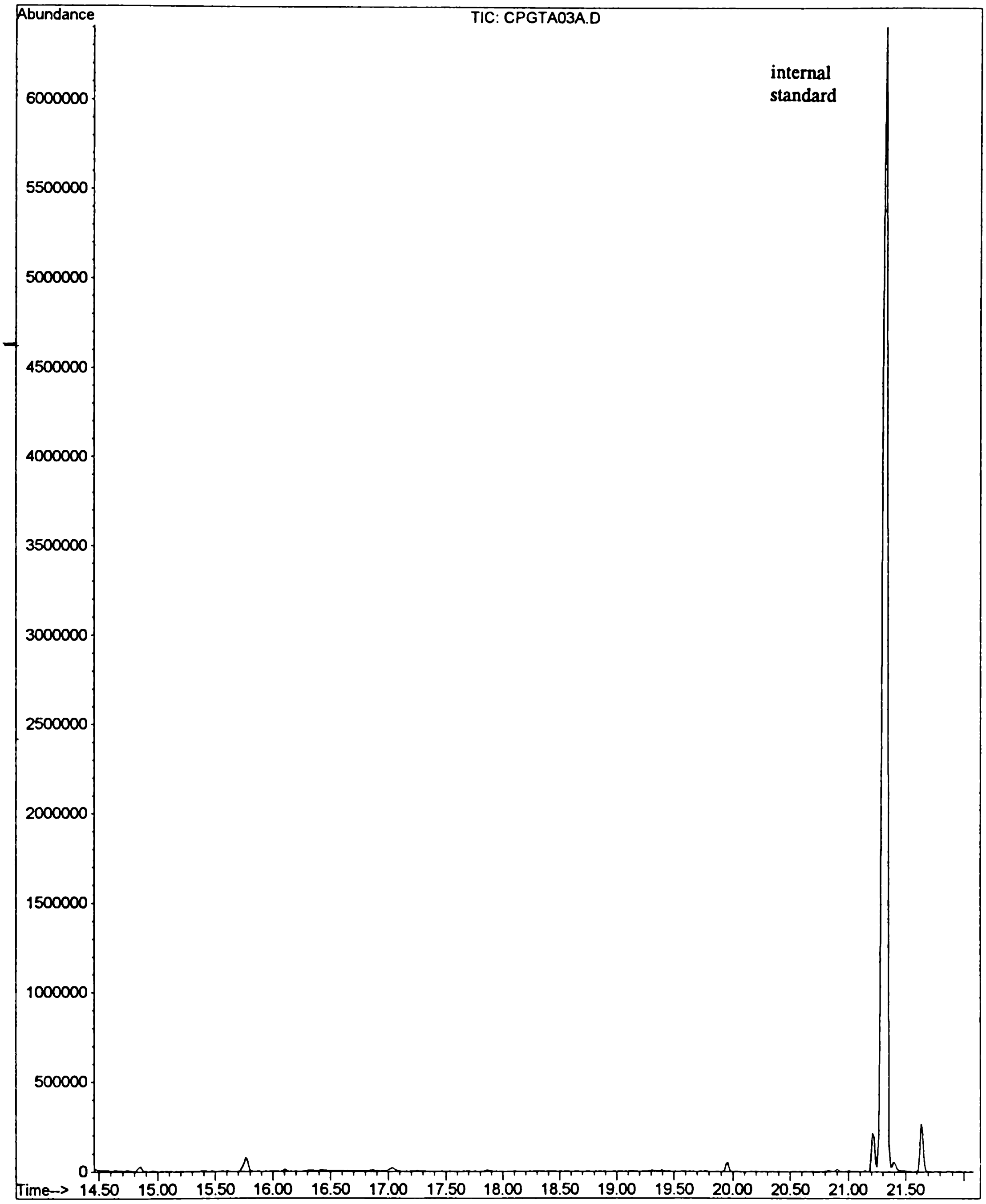

Figure 4.7 Typical GC/MS chromatogram for untoasted oak wood

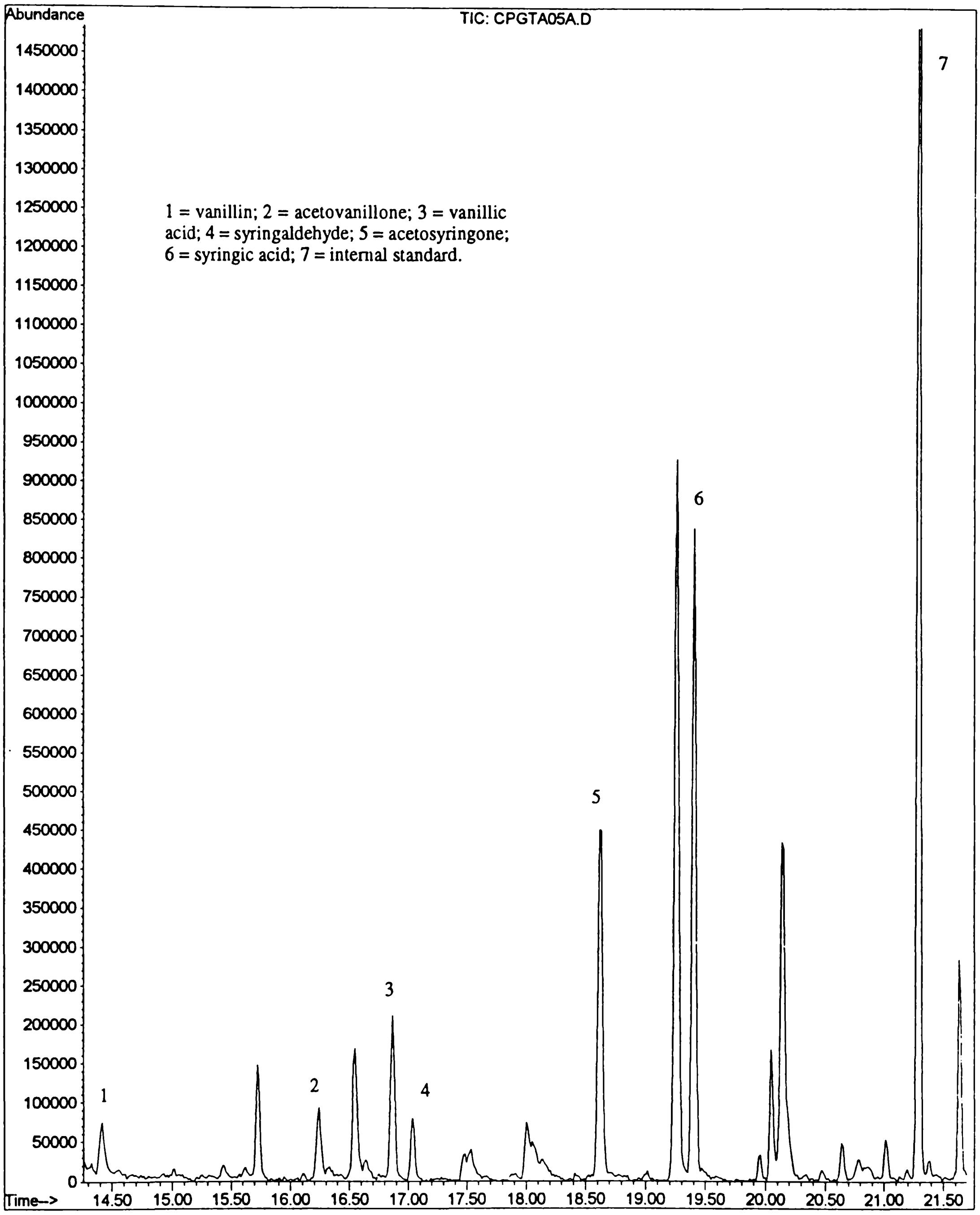

Figure 4.8 Typical GC/MS chromatogram for wood from toasted oak cubes

4.5.2 Effect of Atmosphere

In order to check the effect of atmospheric O_2 on the extractive profile of the toasted oak cubes, the kiln was flushed with nitrogen gas. Other conditions were as described in Section 4.5.1. Table 4.13 shows the results from this experiment.

Table 4.13 Concentration of target compounds (µg/g) in toasted cubes after flushing kiln with nitrogen gas

Sample	untoasted	1	2	3	mean
Vanillin	3.1	120	80	74	91
Acetovanillone	nd	49	32	41	41
Vanillic acid	4.9	110	66	88	88
Syringaldehyde	7.5	190	140	120	150
Acetosyringone	0.9	170	110	130	140
Syringic acid	7.3	210	140	180	180

All target compounds in these samples showed elevated levels. The levels were, however, lower than those achieved by using air as the atmosphere as was the case in previous experiments. It appeared that oxygen facilitated the transformation of precurser compounds into target compounds.

4.6. Effect of Oak Body Size on Extraction Kinetics

The main purpose of this study was to investigate the effect of oak material size and surface area on the rate of release of compounds into an ethanolic solution.

4.6.1 Experimental Method

The rate of release of wood related compounds from the toasted oak wood of varying size into a 12% ethanol/water medium was investigated. A 50 L plastic carboy was filled with a 12% ethanolic solution and was buffered to ca. pH 3.5 using citric acid. The toasted oak material was added to the solution at 2 g/L. A sample volume of 150 mL was taken at each sampling time. Three types of toasted oak material were investigated: commercial oak chips, toasted oak pieces and 1.5 cm toasted oak cubes.

The ethanolic solution was gently stirred each day to simulate convection in an oak barrel. No attempt was made to control the amount of oxygen present inside the plastic carboy. The carboy was sealed after each stirring and sampling time.

4.6.2 Kinetics of Extraction for Commercial Toasted Oak Chips

Toasted oak chips were obtained from a commercial supplier. Typically the oak chips were small shavings of toasted oak and weighed on average 0.1 g/chip. Chip dimensions were typically 15 x 2 x 1 mm. The chip samples also contained large amounts of dusty material which would contribute to the total surface area of the sample. Table 4.14 shows the levels of target compounds in the ethanolic solution after the addition of 'Oak Plus' chips.

Table 4.14 Concentration of target compounds (μg/L) into ethanolic solution with 'Oak Plus' chips versus time (weeks)

Time (weeks)	0	0.5	1.0	2	3	6	8	10	14	16	22
Vanillin	0	360	490	360	560	440	490	440	400	460	680
Acetovanillone	0	40	53	42	68	75	66	83	51	90	140
Vanillic acid	0	120	160	110	330	330	300	350	250	440	660
Syringaldehyde	0	780	980	660	990	740	830	660	740	600	800
Acetosyringone	0	100	150	130	130	110	120	130	90	190	280
Syringic acid	0	300	400	270	350	350	340	370	260	600	820

It is clear that after a few days of exposure to the oak chips, the ethanolic solution showed elevated levels of target compounds. Extended exposure did not produce significant further increases.

4.6.3 Kinetics of Extraction for Toasted Oak Pieces

The purpose of this experiment was to establish if the toasting conditions described in Section 4.5.1 produced toasted oak wood which allowed for similar levels of target compounds to be released when compared to the release from the 'Oak Plus' chips.

The experiment outlined in Section 4.6.2 was repeated using toasted oak pieces. The 20 x 5 x 5 mm pieces were cut from toasted oak cubes prepared as described in Section 4.5.1. The pieces typically weighed 0.5 g/piece. The surface area of these pieces was thus less than the 'Oak Plus' chips. The results are shown in Table 4.15.

Table 4.15 Concentration of target compounds (μg/L) into ethanolic solution with toasted oak pieces versus time (weeks)

Time (weeks)	0	0.5	1	3	5	7	10	14
Vanillin	0	50	110	110	100	210	380	350
Acetovanillone	0	nd	nd	23	60	90	140	140
Vanillic acid	0	nd	nd	70	160	240	420	430
Syringaldehyde	0	80	170	340	230	550	820	860
Acetosyringone	0	nd	40	70	200	320	500	410
Syringic acid	0	60	150	160	310	530	900	780

nd = < 1 μg/L

The rate of release of compounds from the pieces was not as rapid as observed for the commercial 'Oak Plus' chips. This result is consistent with the larger dimensions and lower surface area. Vanillin was chosen to compare the rates of release of the 'Oak Plus' chips and the toasted oak pieces. The result is shown in Figure 4.9.

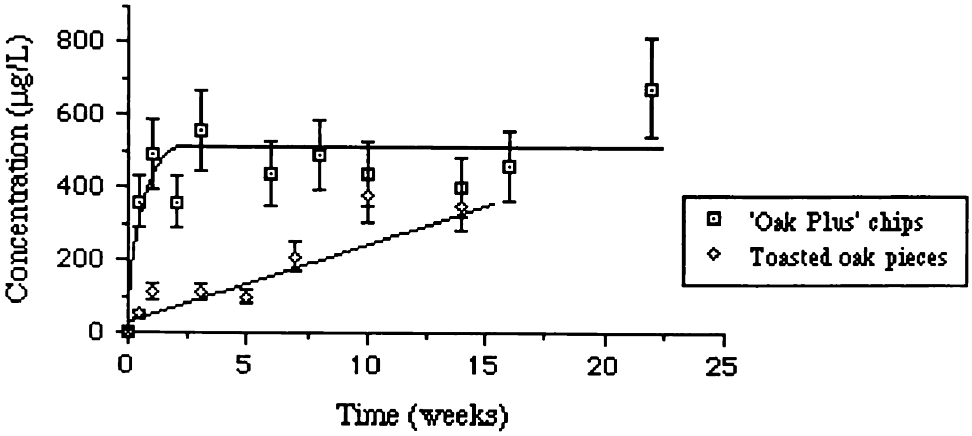

Figure 4.9 Concentration versus time for vanillin with 'Oak Plus' chips and toasted oak pieces in ethanolic solution

The toasted oak pieces showed a slower release of target compounds into solution.

4.6.4 Kinetics of Extraction for Toasted Oak Cubes

The experiment outlined in Section 4.6.2 was repeated using 1.5 cm toasted oak cubes prepared by the method described in Section 4.5.1. Table 4.16 shows the extraction of target compounds from the cubes into the 12% ethanolic solution. The toasted oak cubes remained in the solution for 32 weeks which represents a typical barrel ageing time for wine.

Table 4.16 Concentration of target compounds (µg/L) into ethanolic solution with toasted cubes versus time (weeks)

Time (weeks)	0	1	3	6	9	13	16	20	22	25	28	32
Vanillin	0	nd	nd	40	43	24	250	300	360	570	510	580
Acetovanillone	0	17	50	71	200	120	54	51	51	100	100	120
Vanillic acid	0	34	89	140	340	210	91	110	110	310	380	420
Syringaldehyde	0	nd	nd	40	130	180	810	1300	1700	1500	1700	1600
Acetosyringone	0	37	120	210	570	320	160	230	260	270	330	460
Syringic acid	0	60	160	300	710	400	250	370	390	460	570	800

nd = < 1 µg/L

These results showed the steady release of target compounds from the toasted oak cubes into the ethanolic solution over time. After 6 weeks the toasted cubes had begun to impart higher levels of all target compounds into the solution. Vanillin was again chosen to compare the rates of release into the ethanolic solutions using both the commercial oak chips and the toasted oak cubes. The results are shown in Figure 4.10.

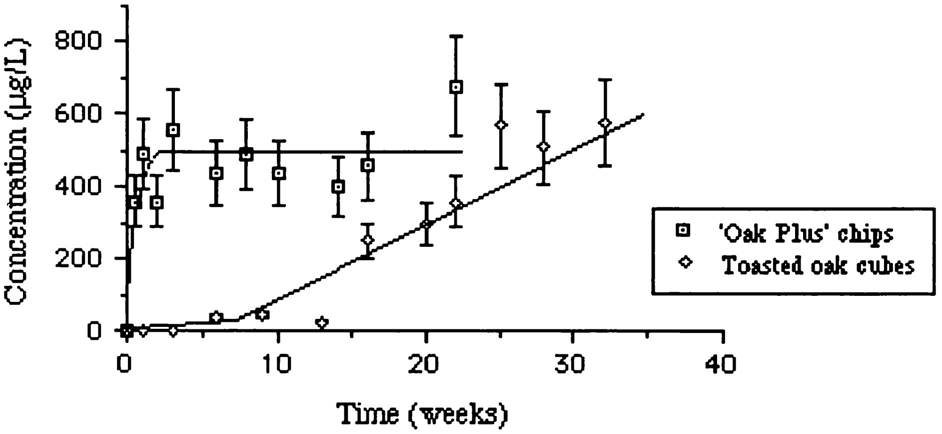

Figure 4.10 Concentration versus time for vanillin with 'Oak Plus' chips and toasted oak cubes in ethanolic solution

The effect of oak body size and surface area was investigated. The commercial 'Oak Plus' chips released target compounds into the ethanolic solution at a rapid rate. After one week of exposure to the chips, this solution showed similar levels of extracted compounds to the solution containing the toasted oak cubes after 32 weeks exposure. The rates of release are consistent with diffusion kinetics. The larger cubes with longer diffusion paths give slower rates of release.

4.6.5 Exposure of End-grain Wood

A feature of the toasted oak cubes was the presence of end-grain wood which is not exposed in the interior of barrels. The way in which barrels are coopered means that only side-grain wood is in contact with the wine. The side-grain wood (from the interior surface of a barrel) and end-grain wood (from a toasted oak cube) were examined under a scanning electron microscope. Figures 4.11 and 4.12 show the images from the 2 wood samples.

Figure 4.11 Side-grain wood from the interior surface of a barrel

Side-grain wood shows evidence of the cavities extending lengthwise across the wood. The rest of the wood shows little porosity.

Figure 4.12 End-grain wood from a toasted oak cube

End-grain wood shows the cavities extending into the wood making it more penetrable. The more porous nature of the end wood may allow for greater accessibility of compounds at greater depths in the wood.

4.6.6 Discussion

Moutounet et al.[11] reported diffusion kinetics of oak chip phenolic compound release into 12% alcohol-water solutions at pH 3.2. Macerated oak wood chips were agitated in 250 mL flasks and extracted material reached a maximum after only eight hours. In the present work, the rates of release were in order: 'Oak Plus' chips, toasted oak pieces, 1.5 cm toasted oak cubes. The diffusion controlled kinetics from the toasted oak cubes allowed for controlled release of compounds into solution. These compounds were extracted from throughout the entire oak material rather than from a limited zone like the wine/wood interface in an oak barrel.

The amount of toasted oak cubes added to the ethanolic solution was 100 g in 50 L (2 g/L). The concentrations of target compounds in the toasted cubes were averaged from the results shown in **Table 4.12**. A mass transfer calculation showed that for 4 of the 6 compounds, the amount of material extracted into the ethanolic solution was consistent with the original amount present in the cubes. Vanillin and syringaldehyde, however, showed a statistically higher level in solution than expected. It appeared that the original 100 g of toasted oak cubes contained higher levels of vanillin and syringaldehyde.

The vanillin/vanillic acid and syringaldehyde/syringic acid ratios averaged 1.4 and 2.0 respectively. In ethanolic solution, these compounds were extracted from the cubes at a similar rate.

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[11] M. Moutounet, Ph. Rabier, J. L. Puech, E. Verette and J. M. Barillere,'Analysis by HPLC of Extractable Substances in Oak Wood: Application to a Chardonnay Wine,' *Sciences des Aliments* 9 (1989): 35-51.

The use of oak chips to impart oak character to wine has been tried with varying degrees of success[12]. The rate at which these chips release extractable compounds into the wine is considered to be too rapid. This rapid release has been confirmed. As a result, the fruit flavours of the wine become 'disjointed' relative to aroma and flavour contributions of the wood.

Wines aged in new French oak barrels show a gradual increase in the levels of extracted material over time (see Table 3.7). The toasted oak cubes provided a gradual release of target compounds into solution similar to that of new French oak .

4.7 Conclusions

This chapter set out to investigate the possibility of regenerating used oak wood by controlled heat treatment. The principal conclusions are as follows:

1. New French toasted oak contains elevated levels of aromatic compounds on the interior surface of the barrel which forms the wine/wood interface.

2. Target compounds were depleted in used oak to depths of 5-10 mm from the interior surface. Re-shaving old oak did not appear to replenish aromatic compounds in the wood. Used wood obtained from used barrels showed similar levels of target compounds at different sampling points around the staves of the barrel.

3. Toasting used oak wood with a flame enhances the levels of target compounds in the used oak wood. Controlling the heat of the flame and the degree of toasting was difficult.

4. Heat treatment in an electric kiln allowed for better control of toasting conditions. The used oak was heated to moderate temperatures (300°C) and removed before the

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[12] J. Hancock and S. Bird, Personal communication, 1992.

onset of severe charring. Wrapping the oak in foil allowed longer toasting and produced higher levels of desired thermal degradation products.

5. The kinetics of extraction into ethanolic solutions were consistent with diffusion controlled extraction.

Chapter Five

A New Technology for Imparting Oak Character to Wine

5.0 Introduction

New French toasted oak wood imparts to Chardonnay wine unique woody character which enhances the wine's aroma and flavour. The toasting of the interior oak wood is an important step in the physical preparation of the barrel and also in the development of aromatic compounds which are produced in the wood as a result of the toasting process. Traditionally the wine is stored in oak barrels and over time these woody compounds are extracted from the wood and into the wine. The winemakers expertise in sensory assessment determines the amount of time required for the wine to be stored in oak barrels in order that the desired amounts of woody material may be imparted.

Although the traditional barrel toasting operation was carried out using an open fire fuelled by oak shavings, it was found that thermally heating raw oak under controlled conditions also significantly increased the levels of woody compounds in used oak wood. It was also found that the rate of release of these compounds into wine needed to be gradual so that the aroma and flavour contributions of the wood were not 'disjointed' relative to the the fruit flavours of the wine.

While chemical monitoring of key wood extractives in wine may provide an indication of the overall ageing profile of the wine, the processes involved are more subtle. Chemical data alone was not sufficient to accurately assess the wine's potential.

Sensory evaluation by the winemaker remains the most sensitive and diagnostic detection of wine character necessary for accessing the development of a wine.

Changes in the levels of target compounds in wine during the ageing process followed trends which were consistent depending on the ageing container. Although the sensory contribution of these compounds is subtle, certain target compounds do provide some indication of the more complex transformations occurring during the ageing process.

The principal objective of this chapter is to describe the development of a new technology for imparting new oak character into wine without the use of the traditional new oak barrel. The technology is based on the use of toasted oak cubes prepared from used oak wood which was developed over a three year period and is reported in Section 4.5.1.

Hawkes Bay Chardonnay wines vinted in the years 1992, 1993, 1994 and 1995 were used in the trial. The principal result from the 1992 vintage was an indication of levels of oak extractives that can be expected after six months' ageing in new oak barrels, old oak barrels and in stainless steel.

The first attempt to impart oak character through the use of oak cubes was with the 1993 vintage. Encouraging results were obtained but data was limited to the first sixteen weeks of ageing. The experiment was repeated in 1994. Sensory evaluation of the wines indicated a favourable oak contribution from the cubes, but the levels of indicator compounds determined analytically showed evidence of poor extraction of compounds from the cubes. In this experiment, the cubes were allowed to sink into the lees at the bottom of the barrel and the kinetics of release may have been affected. When the experiment was repeated in 1995, the cubes were suspended throughout the body of the wine on a stainless steel wire attached to the top of the barrel. This approach has successfully allowed for almost complete extraction and also gave favourable sensory profiles.

5.1 Materials and Methods

The basic strategy of the work described in this chapter was to use sensory evaluation to determine the effectiveness of using oak cubes in the ageing of Chardonnay wine. Chemical analysis was also used to monitor the changes in levels of target compounds associated with oak ageing. It was clear from early results that the levels of these compounds did not always correlate with sensory findings. The analytical data was considered valuable to complement, but not replace the sensory evaluation. Details of methodology for tasting panels are outlined in section 2.5. Details of methodology for extracting and analysing target compounds are outlined in Sections 2.1, 2.2 and 2.3.

5.2 Vintage 1992

The results for the levels of target compounds extracted after six months' ageing from various ageing containers are outlined in Section 3.3. These results provide a quantitative measure of the effects of the different ageing conditions on the chemical profile of the wine.

5.3 Vintage 1993

Five barrels were chosen for this trial. A description of each ageing container is outlined below.

#1. Seguin Moreau Allier: new French oak
#2. D&J: new French oak
#3. D&J: used French oak (used for 3 vintages)
#4. D&J: used French oak (used for 3 vintages) + 2 g/L toasted oak cubes
#5. D&J: used French oak (used for 3 vintages) + 2 g/L toasted oak cubes

This selection of barrels was made for the following reasons. Two new oak barrels from different cooperages were included to supplement the trial form the 1992 vintage. Three identical used barrels from the same cooperage (D&J) were used to investigate the effect of adding toasted oak cubes. Two of these used barrels had oak cubes added and the third was used as a control. The control barrel was used to monitor the changes in levels of target compounds from used oak excluding the contribution of either new oak or the addition of cubes.

A selection of toasted oak cubes were prepared based on preliminary findings outlined in Section 4.4.2. At the time the work was carried out, the rate of release of compounds from the cubes into solution was not understood. The wine was fermented in a stainless steel tank then transferred to barrels after fermentation was complete. One sample was taken from the tank before the transfer to provide a time = 0 value. The toasted oak cubes were added to wine #4 and wine #5 initially at 2 g/L which was based on industry experience with the use of oak chips. After 4 weeks ageing time, the Chardonnay wines were sampled and analysed for target compounds. Also, at this time, an additional 4 g/L of toasted oak cubes was added to wine #4. This addition was made to investigate the effect of increasing the amount of toasted oak material. Two further samplings took place at six weekly intervals subsequent to the four week sampling.

5.3.1 Sensory Evaluation

A formal tasting of the five experimental wines took place after the last sampling time (16 weeks ageing). The wines aged in the new French oak barrels (wine #1 and #2) scored highest in the sensory analysis (see Table A3.5, Appendix 3). These wines were said to show 'subtle' and 'well integrated' oak character. The wine aged in the used D&J oak barrel (wine #3) scored lowest in the sensory analysis. This wine was said to show 'good fruit' but was 'a little stale'.

Wines #4 and #5 were also aged in used D&J oak barrels, but had received the additional oak treatment from the toasted oak cubes. The winemakers preferred these two wines to the wine aged in the used D&J oak barrel (without oak cubes) and scored them higher in the sensory analysis. Wine #4 which had received an additional 4g/L of toasted oak after 4 weeks ageing was described as 'slightly clumsy' and 'disjointed'.

After 4 months ageing, the wines stored in the five different ageing containers showed significantly different sensory profiles. The wines aged in the new French oak barrels from Seguin Moreau and D&J, like wines from the 1992 vintage, were preferred by the winemakers. The fresh fruit complimented by the sweet, crisp toasty character of new oak was pleasing to the palate.

The wine aged in the used barrel (wine #3) showed only a slow development of extractable compounds. After four months ageing the levels of these compounds were significantly below those levels detected in the wines aged in new oak. The sensory analysis of this wine still revealed the 'good fruit characteristics' (arising from good quality fruit) but lacked the integrated oak character which clearly distinguished the wines aged in new French oak.

5.3.2 Chemical Analysis

Table 5.1 shows the concentrations of target compounds for the wines over the 16 week ageing period.

Table 5.1 Concentration of target compounds (µg/L) in wines aged in different ageing containers (1993 Vintage)

Ageing time (weeks)	0	4	10	16
Wine #1 (new oak)				
Vanillin	nd	50	60	60
Acetovanillone	66	90	80	90
Vanillic acid	2200	2200	2500	1700
Syringaldehyde	nd	nd	nd	nd
Acetosyringone	37	50	60	60
Syringic acid	360	460	470	550
Wine #2 (new oak)				
Vanillin	nd	40	65	72
Acetovanillone	66	120	130	150
Vanillic acid	2200	3400	2900	2100
Syringaldehyde	nd	nd	nd	nd
Acetosyringone	37	55	150	90
Syringic acid	360	650	700	650
Wine #3 (used oak)				
Vanillin	nd	nd	44	43
Acetovanillone	66	65	30	83
Vanillic acid	2200	2000	3400	2500
Syringaldehyde	nd	nd	nd	nd
Acetosyringone	37	160	200	130
Syringic acid	360	310	620	(150)
Wine #4 (used oak)*				
Vanillin	nd	nd	37	140
Acetovanillone	66	83	110	150
Vanillic acid	2200	3500	3300	2300
Syringaldehyde	nd	nd	nd	120
Acetosyringone	37	120	89	240
Syringic acid	360	390	550	1000
Wine #5 (used oak)*				
Vanillin	nd	nd	49	110
Acetovanillone	66	76	120	110
Vanillic acid	2200	3000	2800	2100
Syringaldehyde	nd	nd	nd	79
Acetosyringone	37	66	210	140
Syringic acid	360	350	620	760

* with toasted oak cubes

( ) anomalously low result

The five wines show the changes in the levels of target compounds in wines aged in new oak, and in used oak, with and without the addition of toasted oak cubes. The levels of vanillin and acetovanillone over time are shown graphically in Figures 5.1, and 5.2. After the 4 week sampling time, the wines containing the toasted oak cubes had similar extractive profiles. It was at this point that the additional 4 g/L of toasted oak cubes were added to wine #4. The effect of this addition is also shown in Figures 5.1 and 5.2.

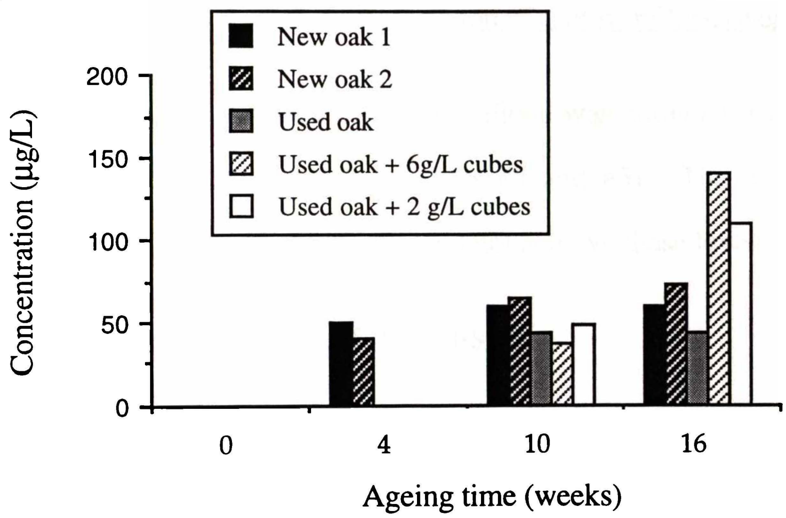

Figure 5.1 Concentration of vanillin over time (1993 Vintage)

Vanillin was not detected at the first sampling time in the wines aged in used oak (with or without the additional toasted oak cubes). After 16 weeks ageing, wine #4 (containing 6 g/L toasted oak cubes) showed a significant increase in the level of vanillin. This increase may have been due to the additional 4 g/L of toasted oak cubes added after week 4.

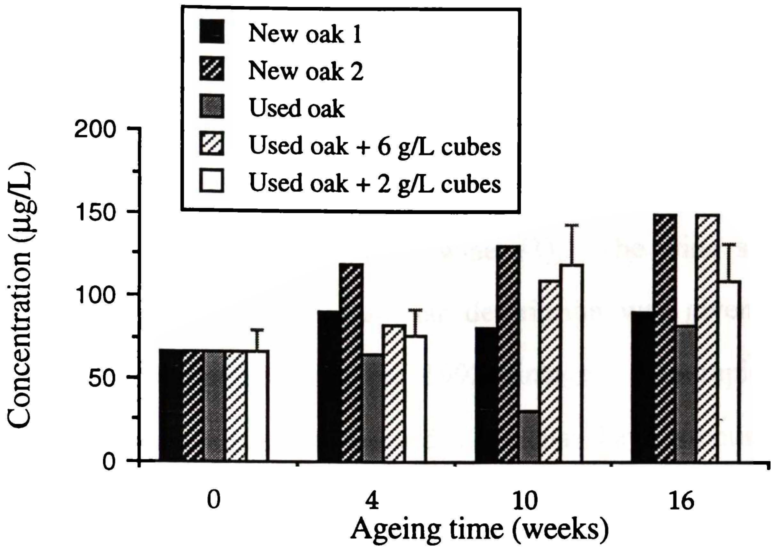

Figure 5.2 Concentration of acetovanillone over time (1993 Vintage)

A gradual increase in the level of acetovanillone was shown for the wines aged in the used barrels containing oak cubes (wines #4 and #5). The wine aged in used oak without the additional oak cubes (#3) did not achieve these levels.

5.3.3 Discussion and Conclusion

Wine #2 showed extractive profiles similar to wines stored in new French oak as previously observed (see Section 3.3). There was only a slow development of vanillin in the wine aged in used oak (wine #3). After the first sampling time there was no detectable vanillin. This result may have been due to the prior depletion of woody compounds at the wine/wood interface. After an extended ageing time, the level of vanillin increased, possibly indicating the presence of vanillin at greater depths in the wood.

The effect of adding an additional 4 g/L of toasted oak cubes to wine #4 was apparent at the 16 week sampling time. For example, the level of vanillin in this wine had increased from ‘not detected’ to 140 µg/L, which was greater than the level of vanillin in the wines aged in the two new oak barrels (60 and 72 µg/L respectively).

The feasibility of adding the toasted cubes to wine in order to impart oak character to the wine has been established.

The wines aged in barrels #4 and #5 (both used D&J oak) showed elevated levels of extracted compounds at the end of the 16 week trial. It appeared that the toasted oak cubes provided additional extractable material to the wines which distinguished them from the wine aged in used oak (wine #3). The wine stored in barrel #4 was described as 'disjointed' - a similar description was given to the wines aged in Francois Freres barrels from the 1992 vintage. The rapid increase in extracted material from the additional toasted cubes may have accounted for the 'disjointed' description given to the wine. With additional time, a more favourable sensory profile may have been established as the various extractable species established an equilibrium with the fruit flavours of the wine.

This trial showed that wines aged in new French oak progressively developed new oak character. The winemakers preferred the freshness of these wines and described them as 'elegant'. The wine aged in used oak, in comparison, did not develop these characteristics at nearly the same rate. These wines lacked freshness and were described as slightly 'stale' or 'dull'.

It appeared to be possible to impart into a wine some new oak characteristics normally associated with new oak barrel ageing by re-toasting used oak and adding this wood to wine. The use of the experimental toasted oak cubes in conjunction with used barrels elevated the levels of target compounds in the wine. The sensory perception of these experimentally 'oak cube treated' wines was favourable. They were preferred to the wine aged the in used oak barrel without the additional oak material.

5.4 *Vintage 1994*

Results from the 1993 vintage experiments indicated that toasted oak cubes produced from used oak provided favourable oak character to Chardonnay wine. A longer trial over 24 weeks was designed for the 1994 vintage.

This experiment set out to determine the effects of adding toasted oak cubes to wine for a typical ageing period. A Chardonnay wine was aged in a new French oak barrel, a used D&J barrel and another used D&J barrel containing toasted oak cubes. Also, for the first time, toasted oak cubes were added to a wine stored in a 220 L stainless steel cask. The wines were fermented in their ageing containers without racking. A description of the ageing containers is shown below.

1. Seguin Moreau new French oak barrel
2. Used D&J oak barrel (purchased in 1989 and used for 4 years)
3. Used D&J oak barrel (as above) + 2 g/L toasted oak cubes
4. 220 L stainless steel cask + 2 g/L toasted oak cubes

The 2 g/L addition of toasted oak cubes was based on industry experience with oak chips. The cubes were added after fermentation was complete.

5.4.1 Sensory Evaluation

Formal sensory evaluations were carried out after 12 and 24 weeks ageing (see Tables A3.6 and A3.7). After 12 weeks ageing, the wine in new French oak was showing 'good balance' of fruit and oak character. The other three wines, however, did not show this typical new oak character and scored lower in the sensory evaluation. The wine aged in stainless steel was said to show 'no obvious oak'. The wines aged in the used barrels lacked the freshness and crispness which new oak provides.

After 24 weeks, the wine aged in new French oak was easily recognised for its well integrated blend of fresh fruit and toasted oak flavours. The wines in used oak were described as 'a little stale' and showed 'some old oak character'. The wine aged in stainless steel was also easily recognised as showing little oak character. The scores for each of the wines reflected these sensory findings.

5.4.2 Chemical Analysis

The chemical analysis of the wines is shown in Table 5.2.

Table 5.2 Concentrations of target compounds (µg/L) in wine aged in different ageing containers (1994 Vintage)

Ageing time (weeks)	0	6	12	16	24
Aged in new oak					
Vanillin	40	90	130	130	190
Acetovanillone	29	15	29	120	190
Vanillic acid	790	1200	1900	1400	2700
Syringaldehyde	nd	66	66	67	80
Acetosyringone	140	160	170	180	270
Syringic acid	720	920	900	900	1300
Aged in used oak					
Vanillin	43	72	59	44	110
Acetovanillone	25	13	85	66	100
Vanillic acid	1000	780	1600	1700	1900
Syringaldehyde	nd	nd	nd	nd	nd
Acetosyringone	21	19	23	20	36
Syringic acid	500	380	370	320	490
Aged in used oak *					
Vanillin	31	70	74	57	91
Acetovanillone	20	16	87	93	160
Vanillic acid	1100	970	1700	1500	2200
Syringaldehyde	nd	nd	nd	nd	49
Acetosyringone	20	33	38	48	120
Syringic acid	430	430	430	440	690
Aged in stainless steel *					
Vanillin	nd	48	49	51	120
Acetovanillone	22	10	90	80	120
Vanillic acid	1100	800	1600	1100	2500
Syringaldehyde	nd	nd	nd	nd	nd
Acetosyringone	20	15	30	33	65
Syringic acid	470	200	390	380	620

* with toasted oak cubes

Figure 5.3 shows the increase in the levels of vanillin in the 4 ageing containers.

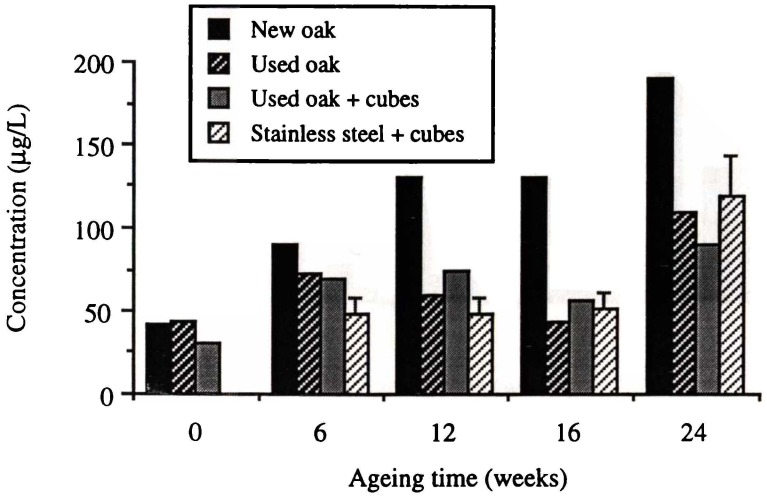

Figure 5.3 Concentration of vanillin over time (1994 Vintage)

The wine aged in the new oak barrel showed a steady increase in the levels of vanillin over time. The wines aged in the used barrels, including the barrel with the toasted oak cubes did not show this increase. It was evident that the levels of acetovanillone and acetosyringone increased gradually as the ageing time progressed in all of the ageing containers. These changes are shown graphically in Figures 5.4 and 5.5.

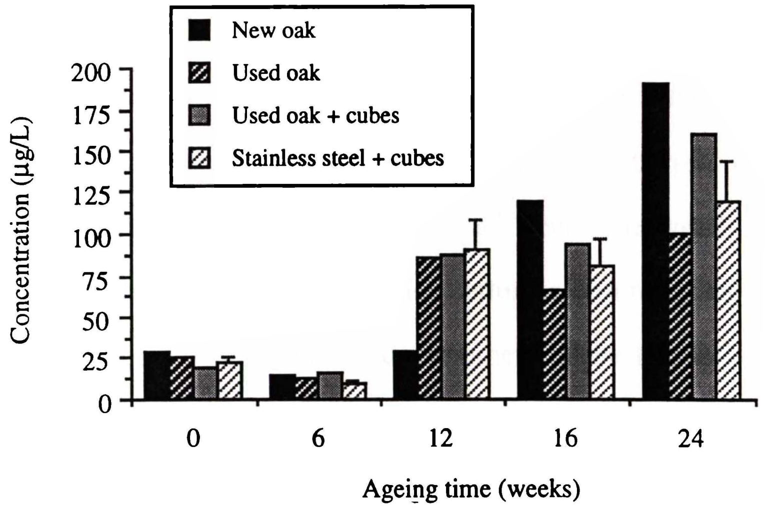

Figure 5.4 Concentration of acetovanillone over time (1994 Vintage)

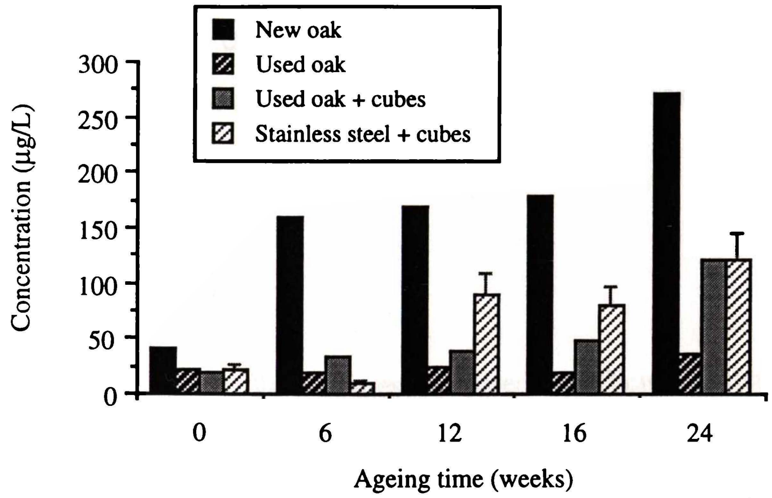

Figure 5.5 Concentration of acetosyringone over time (1994 Vintage)

5.4.3 Discussion and Conclusion

The effect of the toasted oak cubes on wine for the 1994 vintage was disappointing. The sensory findings showed that the wine aged in new French oak was superior to all the other wines. The oak cubes did not provide the typical oak character associated with barrel-aged wine. The chemical analysis showed that after 24 weeks ageing, the amount of material extracted from the oak cubes into wine did not reach the levels attained by the wine aged in new French oak.

A consequence of barrel fermentation was realised for the 1994 vintage. It was apparent that the toasted oak cubes added to the wine in barrels sunk soon after exposure to the wine. Because the wines had been fermented in their ageing containers, they had not been racked. At the bottom of each ageing container there was a thick layer of lees. The anaerobic environment of the lees is not typical of the wine/wood interface inside an oak barrel.[1] The chemical processes of extraction from the toasted cubes into the wine may have been affected.

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[1] S Bird, Personal communication, 1994.

An additional problem arose when pumping the wine from barrels back to stainless steel holding tanks. The cubes were small enough to be sucked into the pump housing. A new method for controlling the position of the cubes was required.

5.5 Vintage 1995

Data from the 1993 and 1994 vintages indicated insufficient extraction of target compounds from toasted oak cubes over the ageing period. Poor circulation of wine about or around the sunken cubes which were presumably covered with lees may have contributed to poor extraction in both vintages. As explained in Section 5.4, the presence of large amounts of lees in the ageing containers may account for slower release of compounds from the cubes into the wine. A means of suspending the cubes throughout the bulk of the wine was needed.

5.5.1 Cube Suspension Method

A new method of handling and positioning the cubes was developed. A hole was drilled through the centre of each toasted cube and the cubes were fed onto a piece of stainless steel wire. In order to improve circulation past all surfaces, each cube was separated on the wire with a 4 mm x 2 mm ceramic bead (see Figure 5.6). The 'necklace' could then be added or removed from the barrel or stainless steel container through the bung hole. This method allowed for easy handling of the cubes and prevented them from sinking into the chemically different and non-circulating environment of the lees.

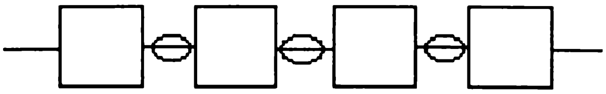

Figure 5.6 Toasted oak cubes and ceramic beads forming a toasted oak 'necklace'

The lengths of stainless steel wire which supported the cubes were cut to approximately 1 metre. One end of the wire was fastened to the outside of the ageing container. This length of wire allowed the cubes to be suspended throughout the main body of the wine in a similar environment to the wine/wood interface of a barrel.

5.5.2 Weight of Cubes Required

The weight of oak cubes used in the 1993 and 1994 vintages was based upon the weight of oak chips used in the wine industry. By the 1995 vintage, the toasting of cubes had been optimised, the levels of target extractives determined and the kinetics of extraction by alcoholic solutions established. It was therefore possible to estimate the amount of oak that would be needed over the ageing period in order to yield the levels of target compounds similar to those obtained after ageing wine in new barrels. Although these target compounds may not be definitive indicators of wine character, the assumption implicit in the calculation below followed that unidentified flavour and aroma components sensed by the winemakers would be extracted similarly.

The weight of toasted oak cubes to be included in the ageing containers was calculated using the levels of target compounds extracted from the toasted oak cubes prepared in Section 4.5.1. An example of this calculation using vanillin as the target compound and 220 L as the sample volume is as follows:

Vanillin concentration in wine aged in new French oak: 190 μg/L (see Table 5.2)
Total weight of vanillin extracted: 190 μg x 220 L = 41800 μg
Average amount of vanillin extracted from toasted oak cube: 180 μg/g (see Table 4.12)
Weight of cubes required: 41800 μg / 180 μg/g = 232 g
Average weight of 1 cube: 2.2 g
Total amount of cubes required: 232 / 2.2 = 106 cubes

Data for the weight and number of cubes based upon other target compounds, calculated in the same manner is summarised in Table 5.3

Table 5.3 Oak cubes required to replicate new oak extractive levels

Target compound	Weight of cubes (g)	Number of cubes
Vanillin	232	106
Acetovanillone	255	116
Acetosyringone	258	117
Syringic acid	341	155
mean	272	124

The mean weight and number of cubes obtained from these calculations (272 g/220 L or 1.2 g/L) compares favourably with the 2 g/L used in the 1993 and 1994 vintages.

An alternative approach to the calculation could be based upon a consideration of surface area. The interior stave surface area of a 220 L barrel was estimated to be 14700 cm^2.

Average internal diameter of barrel:	60 cm
Average length of barrel:	78 cm
Surface area of barrel staves:	60 x 3.142 x 78 = 14700 cm^2

The surface area of a toasted oak cube (1.5 cm side length) is 13.5 cm^2. Thus from surface area considerations, 1100 cubes (14700/13.5) would be required to give the same area of wood contact as is achieved in a barrel. Allowing for a 20% reduction for the bottom side of the barrel (covered with lees) and for the top side of the barrel (not in contact with the wine), a surface area of 12000 cm^2 is approximated. The number of cubes to achieve this area is 890 (12000/13.5).

The effect of surface area of oak contact on wine character was not investigated. For this reason, it was decided to use the calculation based on chemical extractives. It was also realised that the cubes exposed end-grain wood (see Section 4.6.5) which could allow for greater accessibilty of extractives than side-grain wood in the interior of barrels. However in order to ensure that the number of cubes added would produce an unambiguous effect, it was decided to add more than the number determined by the weight-based calculation. The final number of cubes added was

300 (3 g/L) for the stainless steel cask and 200 (2 g/L) for the used oak barrel. The reduced number added to the used barrel allowed for a contribution from the barrel.

5.5.3 Materials and Methods

As was the case in the 1992, 1993 and 1994 vintages, a Hawkes Bay Chardonnay was selected for this trial together with the new toasted oak necklaces.

The experimental Chardonnay was aged in three different ageing containers:

1. New Seguin Moreau French oak cask
2. Stainless steel cask combined with toasted oak necklace at 3 g/L.
3. Used French oak cask (Seguin Moreau barrel purchased in 1990 then used for 3 years) combined with toasted oak necklace at 2 g/L.

The wine was fermented in a stainless steel tank then transferred to the three ageing containers for the next six months. Over this time, the three containers were sampled regularly and analysed by the method described in Sections 2.1, 2.2 and 2.3. In addition, sensory analyses were carried out as the ageing process progressed by the method described in 2.5. Chemical and sensory data was obtained one week after the completion of fermentation and after 4, 8, 14, 20 and 24 weeks ageing.

5.5.4 Sensory Evaluation

Formal sensory assessments were carried out after 4, 20 and 24 weeks ageing. Informal tastings took place after 8 and 14 weeks ageing. Full sets of tasting notes for these sensory evaluations are shown in Tables A3.8, A3.9, A3.10, A3.11 and A3.12 of Appendix 3.

A summary of the 4 week and 24 week formal evaluations is shown in Table 5.4.

Table 5.4 Sensory analysis of wines after 4 weeks and 24 weeks ageing

Wine (4 weeks ageing)	Description of barrel	Sensory description of wine	Average Score
1	Seguin Moreau Allier - new French oak	Subtle aromas. Some oak but not obvious. Attractive nose. Signs of extract.	16.3
2	Stainless steel cask with toasted oak necklace	Lactic (buttery). Simple. Fermentation esters dominant.	14.8
3	Used Seguin Moreau cask with toasted oak necklace	Nice fruit. Pleasant aromas. Good on palate.	15.7
Wine (24 weeks ageing)			
1	Seguin Moreau Allier - new French oak	Attractive fruit. Tropical fruit. Nice sweet oak. Fresh toasty aromas. Elegant wine. Nice finish. Soft on palate.	17.0
2	Stainless steel cask with toasted oak necklace	Nice fruit flavours and subtle oak. Oak more subtle. Stainless steel? Peachy fruit. Light oak on finish.	15.3
3	Used Seguin Moreau cask with toasted oak necklace	Lots of flavour. Fruit showing on finish. Sweet toasty oak aromas and flavours. Nice mouthfeel. Excellent potential. Elegant wine. Delicate oak flavours. New oak?	17.0

In the initial stages of the ageing process, the winemakers found no difficulty in distinguishing between each of the three wines as they immediately recognised the powerful oaky aromas which characterised the wine aged in new oak. At the first sampling, the levels of vanillin in the wines aged in new oak, stainless steel and used oak were 110 µg/L, 48 µg/L and 80 µg/L respectively. At the same sampling time, the levels of acetovanillone in the three wines were 130 μg/L, 77 μg/L and 120 μg/L respectively. At this early stage in the ageing process, the wine aged in new oak showed higher levels of all target compounds. The chemical findings were consistent with the sensory evaluations of the winemakers. At this point in the ageing process the winemakers recognised the lack of oak character in the wines aged in stainless steel and in used oak.

As the ageing time progressed, the wines aged in stainless steel and used oak received their dose of woody compounds provided by the toasted necklaces which was consistent with the rate of release experiments outlined in Section 4.6. At this stage it became more difficult for the winemakers to determine the exact ageing conditions of each of the wines. All three wines showed 'good fruit' and 'pleasant toasty oak'. The wines aged in stainless steel and used oak were not 'discarded' as in previous tastings and achieved higher scores in the formal tastings.

When evaluating the sensory properties of wines from previous vintages, the winemakers also found the wines aged in stainless steel and used oak could be easily distinguished from those aged in new oak. Although wines not aged in new oak often showed good fruit flavour and aromas, they lacked the freshness and complexity which new oak provides. As a result, these wines scored poorly in the formal tastings as the desired style of Chardonnay is a more complex combination of fruit aromas and flavours married together with woody undertones.

The most favourable aspect of the sensory evaluation summarised in Table 5.4 is the used Seguin Moreau barrel with the toasted oak necklace which produced a wine that scored as highly as the wine aged in new French oak. In previous vintages, wines aged in used oak were always distinguishable and were described as 'lacking fresh oak character'. The toasted oak necklace appeared to provide the 'freshness' to this wine which resulted in the wine being indistinguishable from the wine aged in new oak.

At the end of the ageing time, the three wines were found to have similar sensory properties. All three wines were described as having similar fruit and wood character and consequently scored highly. The wine stored in the old oak barrel was said to have the 'most potential' ahead of the wine stored in new oak.

In previous vintages the wines aged in stainless steel did not reach a level which was considered desirable by the winemakers. At the end of this trial, however, the wine aged in stainless steel with the inclusion of the toasted oak necklaces had matured to a stage where it became more difficult to distinguish it from other wine aged in the traditional way. The toasted oak necklaces appeared to provide fresh oak character to the wine which the winemakers associated with quality wine aged in new French oak barrels.

5.5.5 Chemical Analysis

Table 5.5 shows the levels of target compounds present in the wines throughout the 24 week ageing period. Unfortunately, a sample of wine was not taken from the stainless steel holding tank before the wine was transferred to the three ageing containers. Consequently, the initial concentrations of target compounds could not be determined exactly. A good approximation was used, however, which was the concentration of compounds in the stainless steel barrel at the one week sampling time before the addition of the toasted oak 'necklaces'. Based upon previous experiments, the ageing of wine in stainless steel would not have affected the levels of target compounds after only one week.

Table 5.5 Concentration of target compounds (µg/L) in wine aged in different ageing containers (1995 Vintage)

Ageing time (weeks)	0	1	4	8	14	20	24
Aged in new oak							
Vanillin	48	110	90	160	120	140	150
Acetovanillone	77	130	100	140	130	170	190
Vanillic acid	1100	2400	2100	2700	1600	3100	3300
Syringaldehyde	nd	nd	nd	nd	61	64	130
Acetosyringone	nd	92	120	170	180	200	250
Syringic acid	300	630	490	630	660	870	1000
Aged in stainless steel*							
Vanillin	48	48	60	130	130	140	150
Acetovanillone	77	77	100	120	170	170	130
Vanillic acid	1100	1100	1800	1700	1800	2400	1800
Syringaldehyde	nd	nd	nd	43	56	83	160
Acetosyringone	nd	nd	60	120	190	150	180
Syringic acid	300	300	380	570	850	920	800
Aged in used oak *							
Vanillin	48	80	120	210	190	170	180
Acetovanillone	77	120	100	160	180	170	170
Vanillic acid	1100	2100	1300	2000	1900	2600	2100
Syringaldehyde	nd	nd	nd	98	80	61	110
Acetosyringone	nd	42	110	190	250	210	290
Syringic acid	300	430	450	810	840	970	950

* with toasted oak necklaces

Figures 5.7, 5.8 and 5.9 show typical chromatographic traces for the three experimental wines at the 24 week ageing time.

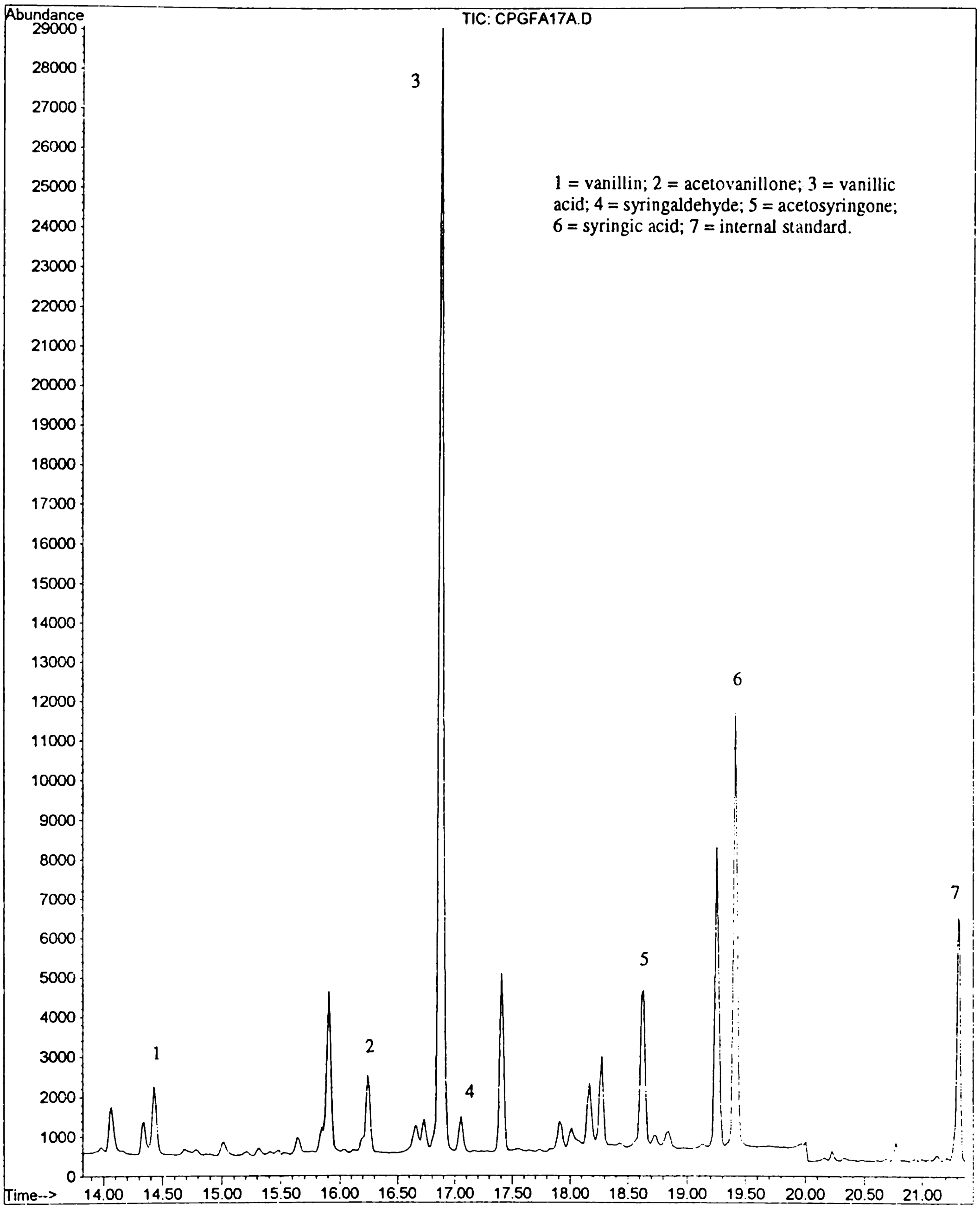

Figure 5.7 GC/MS-SIM chromatogram of the wine aged in new French oak (24 weeks ageing)

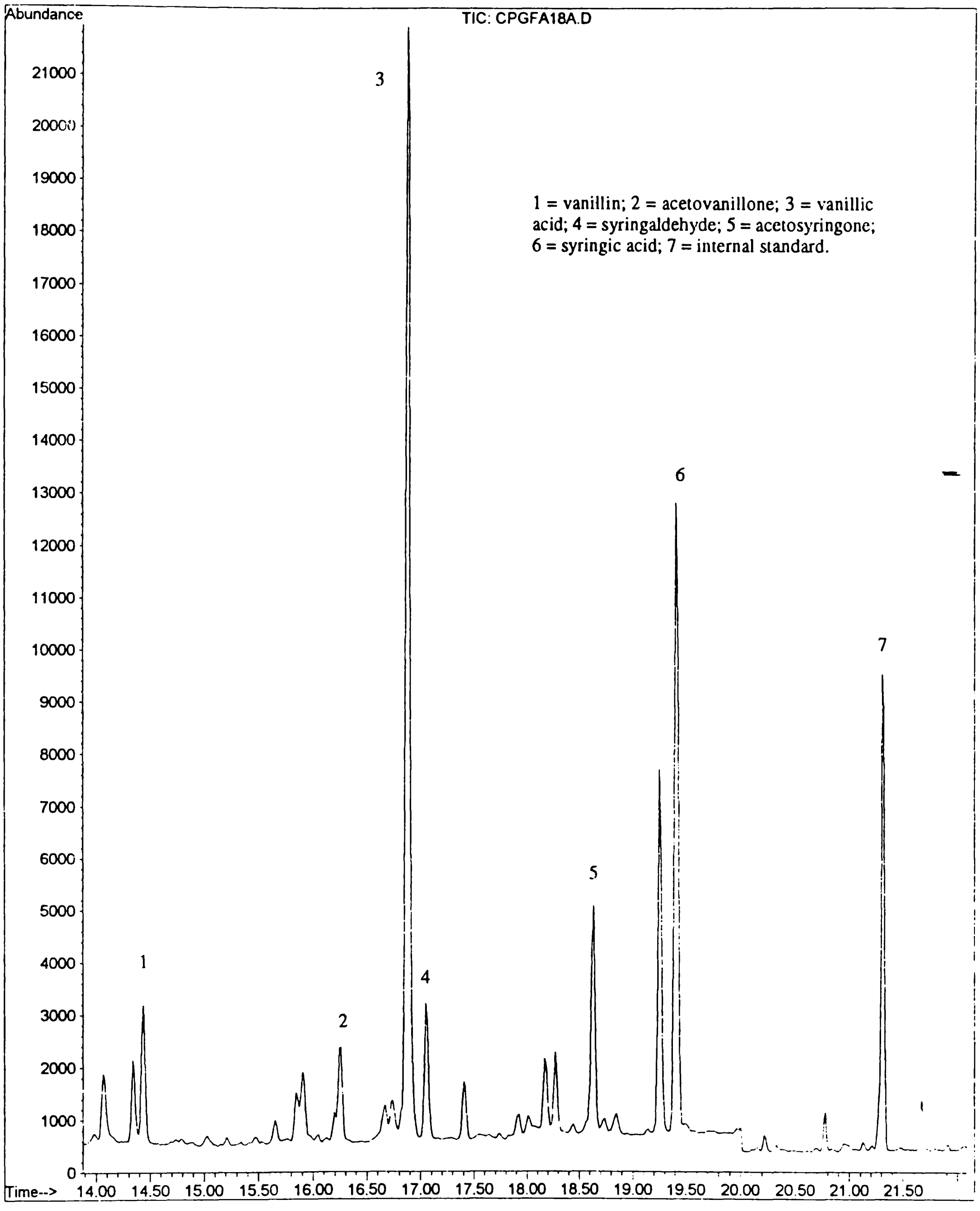

Figure 5.8 GC/MS-SIM chromatogram of the wine aged in stainless steel (24 weeks ageing)

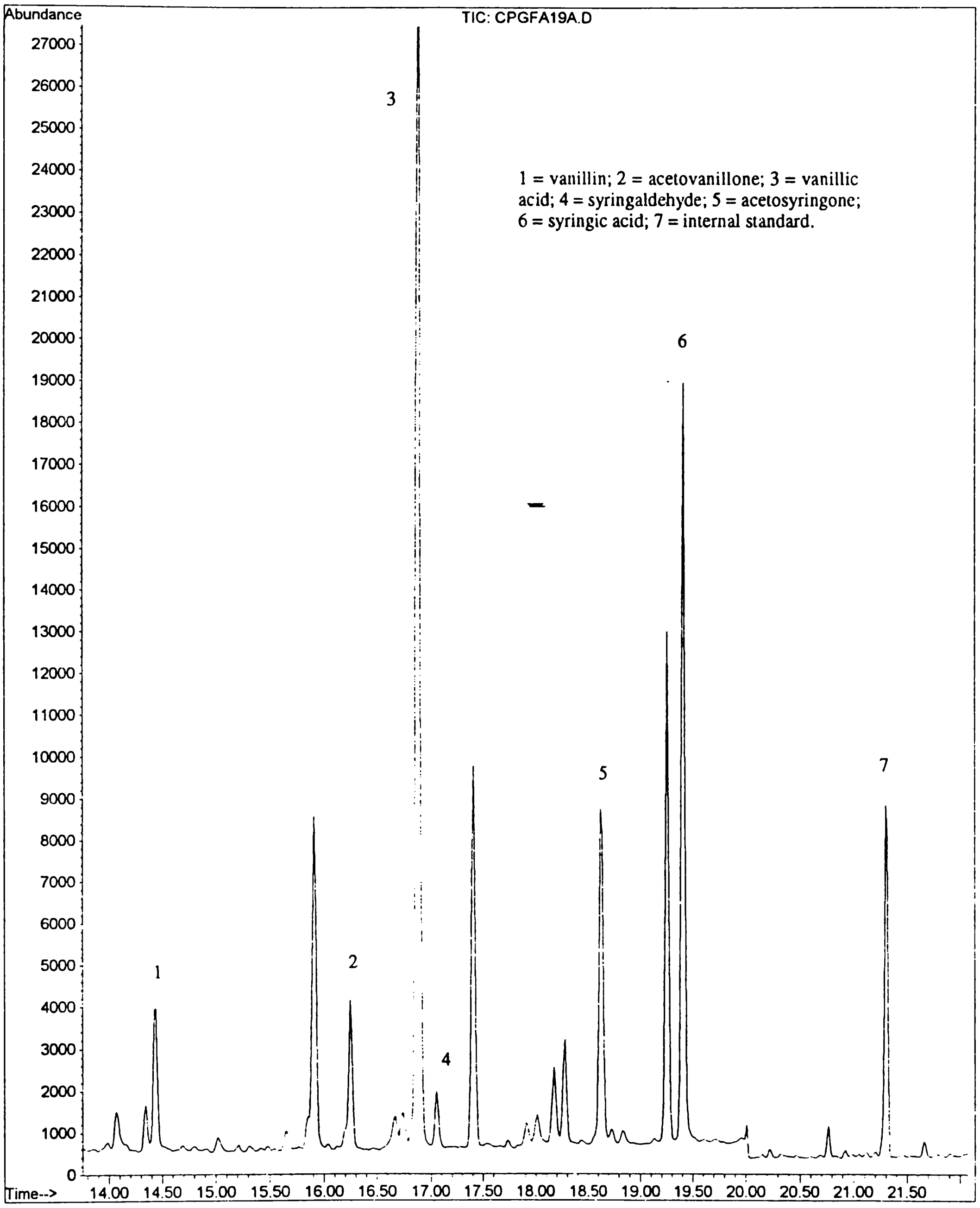

Figure 5.9 GC/MS-SIM chromatogram of the wine aged in used oak (24 weeks ageing)

The changes in the levels of target compounds over the 24 week ageing period are shown graphically in Figures 5.10, 5.11, 5.12, 5.13, 5.14 and 5.15.

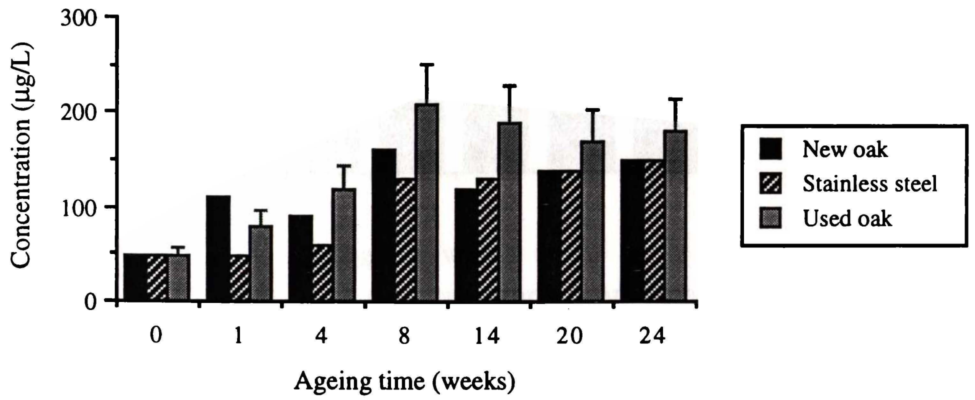

Figure 5.10 Concentration of vanillin over time(1995 Vintage)

A significant increase in vanillin was seen in all three wines at the 8 week sampling time. After this initial increase the levels remained similar for the rest of the ageing time.

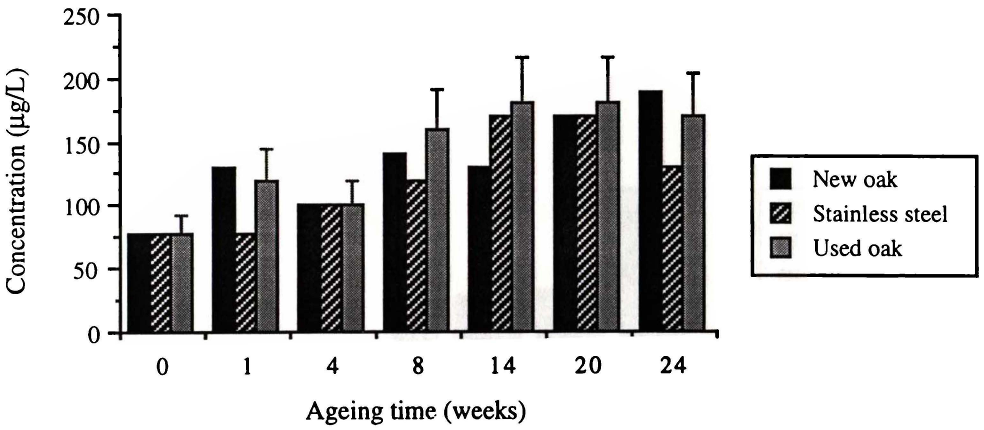

Figure 5.11 Concentration of acetovanillone over time(1995 Vintage)

The level of acetovanillone in the wines increased gradually as the ageing time progressed.

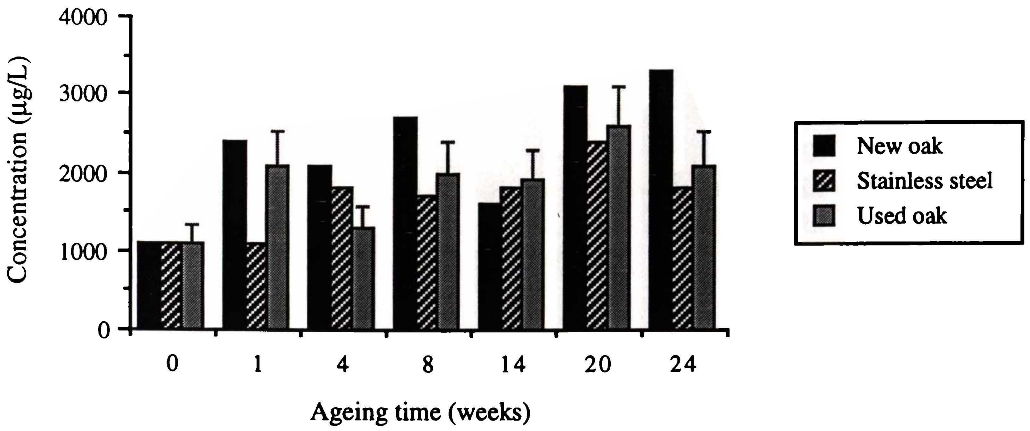

Figure 5.12 Concentration of vanillic acid over time (1995 Vintage)

There were significant levels of vanillic acid present in the wines after fermentation. These levels appeared to increase gradually as the ageing process continued. The wine aged in the new oak barrel showed a consistently higher level of vanillic acid compared to the wines in the other containers. This difference did not provide the winemakers with sufficient information to distinguish between the wines in the sensory evaluations.

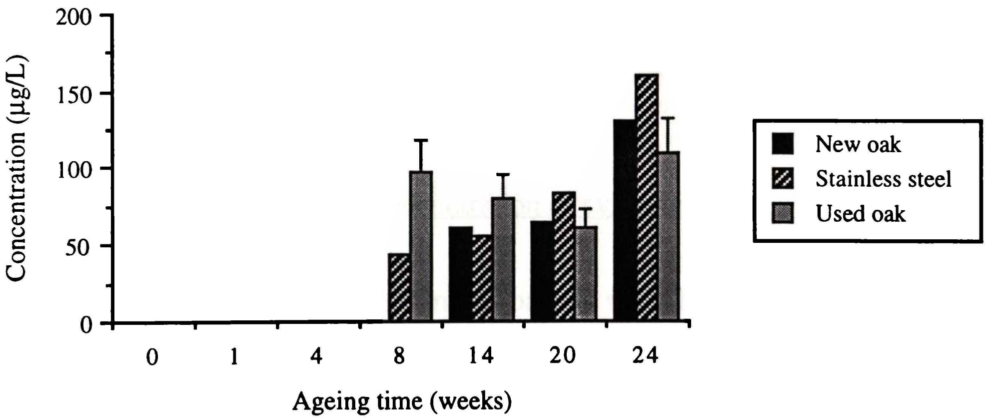

Figure 5.13 Concentration of syringaldehyde over time (1995 Vintage)

Syringaldehyde was not detected in the wine aged in new oak for 14 weeks. In the wine aged in used oak and in stainless steel, syringaldehyde was detected after 8 weeks ageing and appeared to level off. All wines showed an overall increase after 24 weeks ageing.

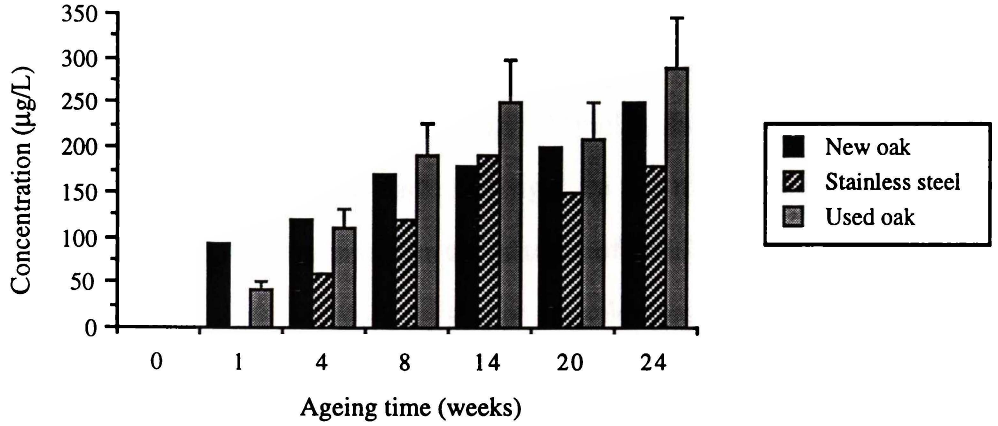

Figure 5.14 Concentration of acetosyringone over time (1995 Vintage)

Acetosyringone was not detected after fermentation in any of the three wines. During ageing, the levels of acetosyringone in each of the wines showed a steady increase.

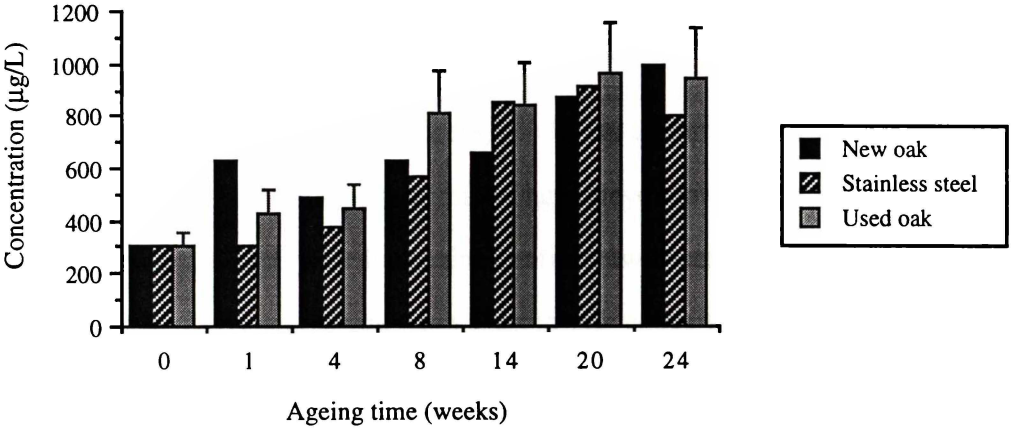

Figure 5.15 Concentration of syringic acid over time (1995 Vintage)

It was expected that the wines aged in oak barrels would have higher levels of vanillic and syringic acids because of the potential oxidation taking place through the pores of the wood. At the end of the ageing time, the levels of syringic acid in the wines aged in new oak, stainless steel and used oak were 1000 µg/L, 800 µg/L and 950 µg/L respectively. These values fall within the boundaries of experimental error. The levels of vanillic acid in the wines aged in the three containers were 3300 µg/L, 1800 µg/L and 2100 µg/L respectively. The levels of vanillic acid in the wine aged in new oak was consistently higher than the wine aged in stainless steel.

5.5.6 Discussion of Analytical Data

The experimental wines aged under different conditions showed similar levels of target compounds after 24 weeks ageing. Vanillin, for example, was detected at levels of 150 μg/L, 150 μg/L and 180 μg/L in the three containers at the final sampling. These values were within the bounds of the 20% experimental variability. Other target compounds showed similar developments as the ageing process continued. It appeared that the target compounds were showing similar trends in the three wines and this trend was reflected in the sensory findings of the winemakers.

The original calculations to determine the amount of oak cubes to add to the wines was carried out to achieve a final concentration of ca. 190 μg/L vanillin. Similar targets were obtained for the other key compounds in the wines apart from vanillic acid and syringic acids which were already present in high concentrations in grapes.

At the completion of the ageing process, the levels of syringaldehyde were detectable in all three wines. The development of syringaldehyde in wines aged in oak barrels is slow and was not detected in these wines until midway through the ageing time. The rate of release of syringaldehyde from toasted oak cubes to an ethanol/water medium was also slow and took 6 weeks before detectable levels were recorded.

While some variability of the data was apparent, particularly at short ageing times, all target compounds showed a similar steady increase in all the wines over the ageing period. After the first week, the wine in the new French oak barrel showed a rapid increase in levels of extracted material. Further increases were gradual over the ageing time. After the 24 week ageing time the levels of compounds in the three wines were generally within experimental error. It appeared that the oak necklaces provided levels of target compounds similar to those of new oak. This result is a significant improvement to results obtained in other vintages.

Previously, the levels of extractives from wines exposed to toasted cubes were less than those levels in wine aged in new oak barrels. The levels were also lower than the amounts of extractable material known to be contained in the cubes. The method of suspending the cubes using the necklace improved the efficiency of extraction.

It was not possible to descriminate between the wines solely on the basis of analytical data. From the sensory evaluation, however, the winemakers were able to identify the wine aged in stainless steel. Although scoring lower than the barrel-aged wines, this wine still showed desirable oak characteristics.

At the early stages of the ageing process, the two wines stored in new and used oak barrels showed higher levels of target compounds. These wines had received some exposure to oak as they were transferred into barrels immediately after fermentation was complete. The wine aged in stainless steel and the wine aged in the used barrel had a toasted oak necklace added to the wine after 1 week in the ageing containers. The kinetics of release experiments described in Section 4.6.4 showed that the release of all target compounds from the toasted oak cubes into solution was slow in the early stages of exposure. It would be several weeks into the ageing process before the toasted oak necklaces would contribute significantly to the levels of target compounds in the wine.

After 4 weeks of ageing, the wines began to show an extractive profile which was similar over all 3 containers. Although the actual concentrations of target compounds were not identical, the wine aged in stainless steel, in particular, had begun to show levels of extracted compounds approaching those of wine aged in new oak.

After two months ageing, the wine aged in all three containers showed a significant increase in all extracted target compounds. This result was typical of wines aged in new oak barrels as observed in other vintages and from the results of kinetics of release experiments oulined in Section 4.6.4.

At the completion of the ageing period the three wines showed similar chemical profiles.

5.6 Conclusions

The oak ageing trials over the 1993, 1994 and 1995 vintages have provided encouraging evidence that Chardonnay wine exhibiting new oak character can be achieved using used barrels and toasted oak cubes. Suspending the cubes on a necklace significantly improved the ease of handling and allowed for higher extraction efficiency of desired compounds. Results from the 1995 vintage, in which this technology was used, showed that:

1. Target compounds are released into the wine in a controlled manner over the course of the ageing period.

2. The levels of target compounds in wines aged in the used oak barrel and the stainless steel cask were similar to those aged in new French oak.

3. The two wines aged in oak barrels scored identically in the sensory evaluation carried out at the end of the ageing time. This result indicated that the oak cubes compensated for the pre-use of the barrel.

4. The cubes, when added to the stainless steel container provided subtle oak character to the wine. These sensory qualities meant the wine scored more highly than other wines aged in stainless steel without exposure to oak.

A major aim of this experiment was to determine whether the addition of toasted oak necklaces to wines aged in stainless steel and used oak barrels produce desirable qualities in the wines normally associated with those wines aged in new French oak barrels. The data from these trials indicate that this objective was acheived. The oak extractable compounds extracted into the wine from the toasted oak necklaces did enhance the character of the wines so that they resembled those which had undergone ageing in the traditional way.

Chapter Six

Conclusions and Recommendations

6.0 Introduction

The wine industry in New Zealand is gaining world-wide recognition for its ability to produce quality wines. The shift towards the production of premium varietal wines has seen the increasing success of varietals such as Sauvignon Blanc, Chardonnay and Pinot Noir. This success has been due to modified winemaking practices which have allowed for more delicate, subtle styles of wine to be produced.

In order to achieve the desired style of wine, the winemaker must consider all aspects of the winemaking process: from choice of fruit and harvesting methods through to pressing, filtering and maturation. Although all the steps in this process are important, the maturation step allows the winemaker a final opportunity to improve the quality of the wine by providing the wine with unique oak-aged character. The choice of ageing container is therefore a critical decision in the overall determination of wine style.

The new French oak barrel has been used by winemakers in New Zealand and throughout the world to produce quality wines with a typical oak maturation character. This wine ageing process has been the traditional method of ageing for many years. It was not the intention of this project to attempt to replace this traditional method of ageing wine. It was realised that some consumers, despite the expense, were prepared to pay for and would demand wines which had undergone ageing in the traditional manner. There is little scope for altering the ageing processes which produce these styles of wines. It was the underlying objective of the work, however, to produce a high quality wine which showed typical oak-aged character without reliance on the traditional methods. This chapter summarises some major conclusions and describes the potential for implementing the findings of this work on an industrial scale.

6.1 Development of Analytical Method

The methods for analysis of compounds found in alcoholic beverages and oak wood have been extensively reported.[1,2,3] Both HPLC and GC are now common analytical tools for the accurate quantitation of these compounds. Recent publications have reported GC/MS as the preferred technique for analysis.[4,5] The MS detector in combination with GC has the advantage of providing structural information in addition to that information gained from retention times.

The analytical protocol established in this work combined liquid/liquid extraction techniques with GC/MS in selected ion mode (SIM) to selectively target key compounds in wine and oak wood. SIM allowed for greater sensitivity towards compounds of interest while excluding other peaks from the chromatographic

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[1] M. Moutounet, Ph. Rabier, J. L. Puech, E. Verette and J. M. Barillere,'Analysis by HPLC of Extractable Substances in Oak Wood: Application to a Chardonnay Wine,' *Sciences des Aliments* 9 (1989): 35-51.

[2] J. L. Puech, 'Phenolic Compounds in Oak Wood Extracts Used in the Ageing of Brandies,' *J. Sci. Food Agric* 42/2 (1988): 165-172.

[3] J. M. Conner, A. Paterson and J. R. Piggott, 'Changes in Wood Extractives from Oak Cask Staves through Maturation of Scotch Malt Whiskey,' *J Sci Food Agric.* 62 (1993): 169-174.

[4] S. P. Arrhenius, L. P. McCloskey and M. Sylvan, 'Chemical Markers for Aroma of Vitis vinifera Var. Chardonnay Regional Wines,' *J. Agric. Food Chem.* 44 (1996): 1085-1090.

[5] Jonathon R. Mosedale and Andrew Ford, 'Variation of the Flavour and Extractives of European Oak Wood from Two French Forests,' *J. Sci. Food Agric.* 70 (1996): 273-287.

trace. Selected ion profiles of target compounds allowed for the accurate assignment of eluting peaks while quantitation was performed using an internal standard method.

Precision of the analytical procedure was monitored throughout the course of the work with the use of a reference standard and appropriate response factor mixtures. Before analyses were performed, the GC/MS system was tuned and routine checks of the injection system and column were carried out. The analytical protocols set up for sampling, extraction and analysis were strictly adhered to during the course of the work.

Chemical data obtained from analyses was used to complement the sensory evaluation of each experimental wine. In oak aged Chardonnay wines, the ageing potential and the conditions for ageing can be determined from the sensory skills of the winemaker. The winemaker's sensory assessment of each wine combined with the relevant chemical data were used to describe all wine ageing and toasted oak cube trials carried out as part of the project.

6.2 Target Compounds as Sensory and Chemical Indicators

A recent report by Arrhenius et al. (1996) provides correlations between more volatile aroma compounds in wine and sensory data. The focus of this report refers to regional uniqueness of target compounds found in wines from different regions. Phenolic compounds including aromatic acids and aldehydes have previously been reported in wine and oak wood.[6,7,8] However, they have not

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[6] V. L. Singleton and A. C. Noble, 'Wine Flavor and Phenolic Substances,' *ACS Symposium Series*, 26 (1976): 47-70.

been used together with sensory profiling, to produce a technology that mimics the complex transformations occurring during barrel-toasting and the wine ageing processes.

The potential for target compounds to be used in quality assessment of alcoholic beverages was suggested by Reid et al.[9] Analysis by pyrolysis mass spectrometry provided good correlation between Scotch whisky quality and target compounds. Vanillin and syringaldehyde were present at higher levels in good quality whiskies. Puech[10] reported that lignin is degraded to syringaldehyde and vanillin during the wine ageing process. It was concluded that vanillin provided the perfume of vanilla typical to oak-aged wines and that these aromatic compounds could be used as a means of assessing wines stored in oak barrels.

A range of ageing containers were selected in the present work to allow the levels of target compounds in experimental wines to be determined. A typical chemical profile for a wine aged in new French oak was established using a mean value obtained from these wines. This profile was compared with other profiles from wines aged in different ageing containers. Moutounet et al. (1989) reported the levels of vanillin and syringaldehyde in a Chardonnay wine stored in an oak barrel to be 200 μg/L and 400 μg/L respectively. Similar results were obtained for a

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[7] M. A. Sefton, 'How does Oak Barrel Maturation Contribute to Wine Flavor,' *Australian & New Zealand Wine Industry Journal,* Nov./Dec. (1991): 17-20.

[8] K. F. Pocock, M. A. Sefton and P. J. Williams, 'Taste Thresholds of Phenolic Extracts of French and American Oakwood: The Influence of Oak Phenols on Wine Flavour,' *Am. J. Enol. Vitic.* 45/4 (1994): 429-434.

[9] K. J. G. Reid, J. S. Swan and C. S. Gutteridge, 'Assessment of Scotch Whisky Quality by Pyrolysis-mass spectrometry and the Subsequent Correlation of Quality with the Oak Wood Cask,' *Journal of Analytical and Applied Pyrolysis* 25 (1993): 49-62.

[10] Puech, Jean-Louis. 'Extraction of Phenolic Compounds from Oak Wood in Model Solutions and Evolution of Aromatic Aldehydes in Wines Aged in Oak Barrels.' *Am. J. Enol. Vitic.* 38/3 (1987): 236-238.

water/ethanol solution stored in an oak barrel. These results are consistent with the present work.

Target extractable compounds present in both new toasted oak barrels and in quality Chardonnay wine were used as sensory and chemical indicators of the oak ageing process. During the sensory assessment of experimental wines, the winemakers spoke specifically of 'vanillin' with reference to the degree of wood-derived material extracted from oak wood. Specific detail was obtained concerning the type of barrel and the extent of toasting, from the sensory profile of particular wines. The chemical data from these wines indicated that the differences in the levels of target compounds were more subtle. Determining variables such as barrel type and toasting conditions was therefore not possible using chemical data alone. The levels of compounds in the wine from various ageing containers (such as stainless steel versus oak barrels) were sufficiently different however to allow this chemical data to determine the ageing conditions of a wine.

The three experimental wines aged under different conditions during the 1995 vintage (see Table 5.5) showed similar levels of target compounds throughout the 24 week ageing period. Vanillin, for example, was detected at levels of 150 µg/L, 150 µg/L and 180 µg/L in the three containers at the final sampling. The sensory findings for these three wines were also similar, especially for the two wines aged in oak barrels. Both wines were described as showing 'nice fruit', 'toasty oak aromas' and 'elegance'. The wine aged in stainless steel showed 'subtle oak' and achieved a lower score.

It did appear that although the levels of selected target compounds from chemical data could not distinguish between the subtle differences in ageing methods or the small changes in sensory quality, they were a reasonable indicator of the degree of oak ageing. The importance of compounds such as vanillin in barrel-aged beverages has been previously reported.[11,12,13,14] The literature is not clear, however, about the role of specific phenolic compounds in the sensory quality of wine. In the present work, wine which had not been stored in new oak barrels showed a different chemical profile and could be distinguished from wine aged in new oak using chemical data. The most powerful sensory tool, however, was still the evaluating skills of the winemaker.

6.3 Toasting of Used Oak Wood

-It became evident that the toasting of a new oak barrel thermally modifies only a small proportion of the total amount of oak wood. There still remains a large portion of un-modified wood in used barrels suitable for precursor material.

Nishimura et al.[15] reported the levels of aromatic aldehydes, ketones and acids in charred oak chips. At 200°C, the level of vanillin was 13.5 ppm. Methods for extraction and analysis and information concerning the length of exposure of the chips to ethanol solutions were not reported. It maybe that incomplete extraction of compounds into solution occured. A similar experiment was carried out in the

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[11] J. W. Aiken and A. C. Noble, 'Comparison of the Aromas of Oak and Glass-Aged Wines,' *Am. J. Enol. Vitic.* 35/4 (1984): 196-199.

[12] V. L. Singleton and A. C. Noble, 'Wine Flavor and Phenolic Substances,' *ACS Symposium Series*, 26 (1976): 47-70.

[13] J. L. Puech, 'Extraction and Evolution of Lignin Products in Armagnac Matured in Oak,' *Am. J. Enol. Vitic.* 32/2 (1981): 111-114.

[14] M. Lehtonen and P. Jounela-Eriksson, 'Volatile and Non-Volatile Compounds in the Flavour of Alcoholic Beverages,' *Flavour of Distilled Beverages:Origin and Development,* ed. J. R. Pigott, Chichester: Ellis Horwood, 63-78.

[15] K. Nishimura, M. Ohnishi, M. Masuda, K. Koga and R. Matsuyama, 'Reactions of Wood Components During Maturation,' *Flavour of Distilled Beverages:Originn and Development,* ed. J. R. Pigott, Chichester: Ellis Horwood, (1983): 241-255.

present work also at 200°C. Six samples of oak wood were analysed after toasting and the average result for the level of vanillin was 12.6 µg/g (ppm).

Mosedale and Ford (1996) reported the levels of phenolic and other wood-related compounds from 14 cm x 1 cm x 1 cm heat modified sticks extracted into ethanol solutions. It was not surprising that high levels of compounds were found considering the large amounts of oak wood added to the solution. The toasting temperature for the sticks was 200°C like that used by Nishimura (1983). An important result from the present work found that the toasting temperature could be increased to 300°C without the onset of extensive charring. The higher temperature allowed for increased levels of wood-related compounds.

Traditionally, the staves which make up an oak barrel are heated to allow the staves to be bent into shape. This heating process also allows for the thermal degradation of lignin compounds into aromatic compounds. The levels of wood-derived aromatic compounds present in both new French oak and in used oak were determined. The new oak wood showed elevated levels of these compounds compared to the used wood. Laszlo[16] reported the levels of aldehydes in a range of oak types. These levels were greater that those reported by Nishimura (1983) and those found in the present work. The methods for extraction and analysis were not discussed. In an earlier publication, a similar table reported the level of aromatic aldehydes in oak wood. The units of concentration changed between the two tables from mg/g to µg/g. The levels of compounds in oak wood established in the present work fall in between those reported in the two tables.

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[16] J. Laszlo, 'Wine Ageing in Oak Barrels,' *Wynboer*, Januarie (1984): 53-56.

The literature is clear that the presence of high levels of aromatic aldehydes and acids in toasted oak wood are derived from the thermal degradation of lignin.[17,18] Thermal treatment of oak wood results in cleavage of aromatic monomers from the lignin molecule. The conditions underwhich the toasting process occurs appear suitable for the hydrolysis of aromatic compounds which may then be solubilised into ethanolic solutions. It was not considered within the scope of this work to investigate the pathways by which these transformations occur. One objective of the work was to produce levels of compounds in the toasted oak wood similar to those found in new French oak barrels. It was found that the ratios of the levels of compounds produced during the toasting process remained constant indicating that further transformations must occur after these compounds are exposed to wine. Further work could employ the use of pyrolysis techniques to investigate the fate of the lignin molecule and the degree to which degradation has occurred.

Traditionally, oak wood was heated over a flame fuelled by oak shavings. Controlling the temperature and achieving consistent heating was difficult. In the present work, an electric kiln was used to carefully control the temperature which allowed for constant heating over all surfaces of the oak wood. Enhanced levels of target compounds in the wood were achieved at a temperature of 300°C. Wrapping the oak wood in foil allowed the toasting time to be increased and slowed down the onset of charring. Charred wood showed lower levels of target compounds indicating the potential loss of desirable compounds at higher temperatures.

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[17] K. Nishimura, M. Ohnishi, M. Masuda, K. Koga and R. Matsuyama, 'Reactions of Wood Components During Maturation,' *Flavour of Distilled Beverages:Originn and Development*, ed. J. R. Pigott, Chichester: Ellis Horwood, (1983): 241-255.

[18] K. Lundquist, 'Acidolysis', *Springer Series in Wood Science*, ed. Lin and Dence (1992):290-300.

6.4 Rate of Release of Target Compounds

Moutounet et al. (1989) reported low levels of phenolic compounds in oak chips. These chips, however, were taken from the external part of a barrel where little thermal modification would have occurred. Also, the aromatic aldehyde concentration was shown to be approximately ten times greater in wine stored in an oak barrel than in a water/ethanol solution containing these oak chips. This result is consistent with the present work, as oak barrels, following heat treatment, show elevated levels of aromatic compounds. Levels of these compounds in wood taken from external parts of a barrel are low.

In the present work, it was found that the rate of release of compounds from oak material into solution is dependent on the surface area. Moutounet et al. (1989) showed similar results for the release of compounds from oak chips into solution. After a few hours, most of the extractable material had been transferred into solution. Further investigations were carried out in this work on oak material with a lower surface area.

Imparting oak character to wine has been carried out in the past using oak chips. This practice allows for rapid transfer of woody material from the chips into the wine. The sensory assessment of these oak treated wines, however, has been unfavourable. Consequently, these wines have been described as 'coarse' and 'disjointed' - the flavour and aroma contribution from the chips have not been integrated with the fruity flavours of the wine.

The effect of oak material size on the rate of release of target compounds was carried out using oak wood material of different sizes submerged in buffered ethanolic solutions. It was found that 1.5 cm^3 oak cubes, toasted under the appropriate conditions, released target compounds into solution at a similar rate to new French oak. These cubes formed the basis for the new technology for imparting oak character to wine.

6.5 A New Technology for Oak Ageing Wine

Although the use of oak chips in the wine ageing process has been reported,[19,20] the literature does not report the use of heat modified oak material of a particular dimension for the purpose of providing wine with oak characteristics associated with barrel-aged wine.

An important aspect of this work was to select target compounds which would provide a quantitative measure of the degree of oak ageing undergone by a particular wine. The actual differences between the levels of target compounds in the experimental wines was not as great as originally hoped. Sensory testing by the winemakers showed differences in the various wines which could not be distinguished solely on the basis of chemical analyses. The monitoring of target chemical extractives, however, did provide valuable data on the levels of compounds present in Chardonnay wine and for the determination of extractable material found in oak wood. This data was used in the development of a technology for imparting oak character based on the addition of toasted cubes to wine.

The toasted oak cubes were suspended in a way which allowed for gradual release of wood-derived compounds from the cubes into the wine. An outcome of the work was a technology that produced a wine with traditional oak aged

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[19] V. L. Singleton and D. E. Draper, 'Wood Chips and Wine Treatment: the Nature of Aqueous Alcohol Extracts,' *Am. J. Enol. Vitic.* 12 (1961): 152-158.

[20] K. L. Wilker and J. F. Gallander, 'Comparison of Seyval Blanc Wine Aged in Barrels and Stainless Steel Tanks with Oak Chips,' *Am. J. Enol. Vitic.* 39/1 (1988): 38-43.

characteristics without the reliance on new oak barrels. The wines aged by this new method achieved favourable responses from the winemakers who formed the sensory judging panel. These wines, before the oak cube technology, were clearly seen as inferior to the wines aged in new oak. The oak cube 'necklaces' allowed for a release of compounds into the wine at an appropriate rate which provided the oak characteristics normally associated with wines aged in new French oak barrels.

6.6 Industrial Application

The toasted oak 'necklaces' used in the wine ageing trials were made on a small scale using simple equipment. If these cubes were to be used on an industrial scale there would need to be improvements on the way batches of cubes are produced in order to make the process more efficient.

The steps taken to prepare the used oak wood for toasting were carried out manually using power saws and an electric planer. These steps allowed an old oak barrel to be converted into a large quantity of oak cubes in a reasonable time.

The most time consuming part of the process was the toasting of the cubes. For this project, a small batch of cubes were wrapped in foil and placed inside the muffle furnace. The amount of toasted cubes required to age one 220 L barrel of wine requires up to three of these batches. To produce the cubes in this manner to age many barrels of wine would be a tedious process.

The toasting process could be made more efficient with the use of a larger oven combined with a conveyor belt system which would move the cubes through the oven at the required rate. Heating elements above and below the conveyor belt would allow for more constant heating through each surface of the cubes.

Ideally, if the belt vibrated, the continual shaking would help to reduce the tendency for the cubes to smoulder.

After the cubes had been toasted, they were individually strung onto a piece of stainless steel wire. Inserted between each cube was a ceramic bead. This method of making the 'necklace' also required many hours of labour. Automating this step along with the toasting process would result in a considerable labour saving.

The advantage of application of this technology would be improving the quality of wine from a product which shows little or no oak ageing character to one which shows oak aromas and flavours typical of higher quality wines. The presence of this oak character allows the price of a bottled wine to be increased accordingly to reflect the oak-aged quality. Some wines do not undergo ageing in new barrels due to the cost involved with the purchase of new oak. The use of the oak 'necklace' would allow these wines to develop some oak aged character, in used barrels which would have normally been discarded. The technology therefore allows for less reliance on new oak as some used barrels provide the storage containers while others provide the raw material for the toasted oak 'necklaces'.

It was found that one used barrel provided enough oak wood to produce oak 'necklaces' for about ten other barrels of wine. Presently, the industry sells used barrels for flower pots and recovers less than ten percent of the original cost of the barrel. This technology provides an opportunity for the winemaker to utilise previously discarded barrels and effectively extend their life-span in a way which is beneficial to the industry.

A new technology which deviates from the traditional methods could be expected to meet some resistance from discerning consumers who demand wines aged in new French oak barrels. It was not the intention of this work to produce a wine which competed in this market. A wine which shows some characteristics of higher quality at a lower price, however, may have a place in the market. By not attempting to replace the traditional French oak barrel, but marketing an alternative to it, may allow for wine styles with different ageing methods to be accepted by the consumer.

This research has shown that a used oak barrel in combination with toasted oak 'necklaces' allows for the transfer of desirable compounds into wine while still facilitating the subtle oxidation processes and other transformations which take place through the pores of the wood. Wines with oak aged character can also be produced using stainless steel containers in combination with oak 'necklaces', thus allowing even greater economy for oak wood use by the industry.

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Appendix 1: Response Curves for Target Compounds

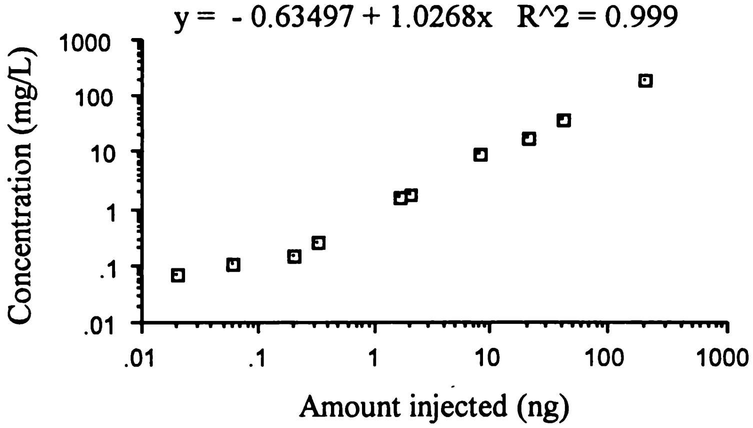


Figure A1.1 Response curve for acetovanillone

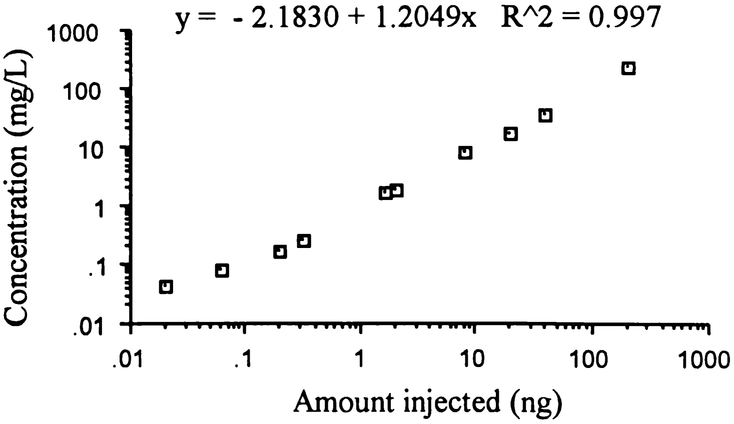


Figure A1.2 Response curve for vanillic acid

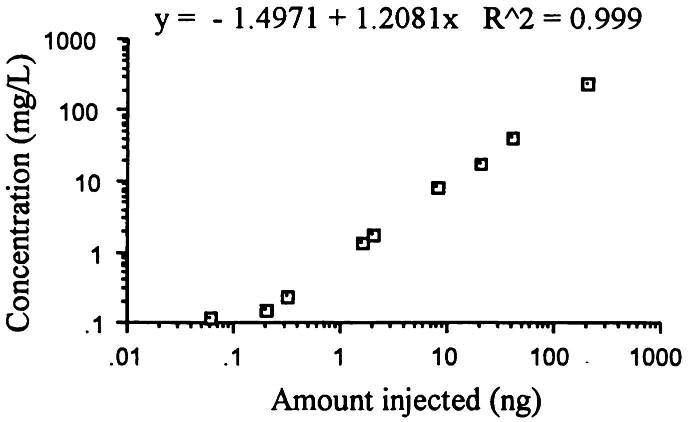


Figure A1.3 Response curve for syringaldehyde

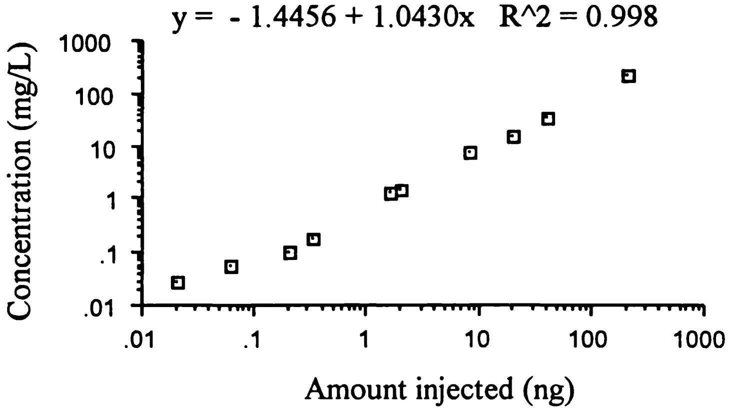


Figure A1.4 Response curve for acetosyringone

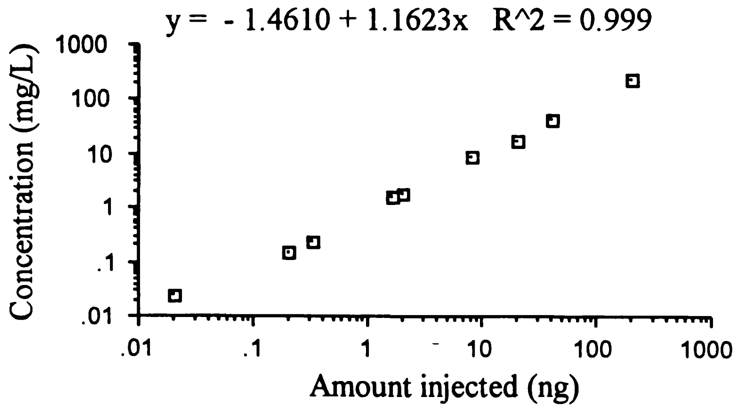


Figure A1.5 Response curve for syringic acid

Appendix 2: Control Charts

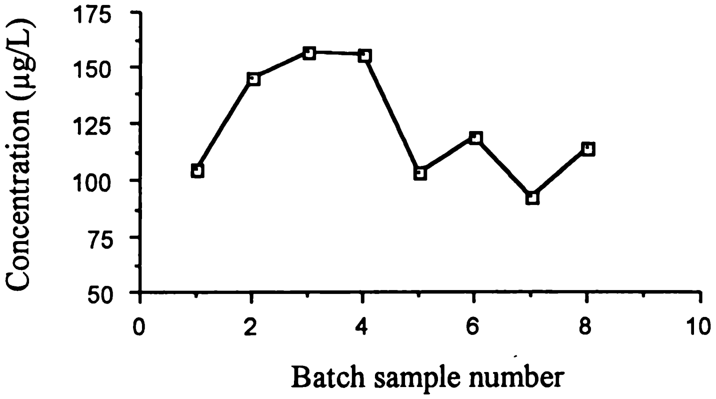


Figure A2.1 Control chart for acetovanillone

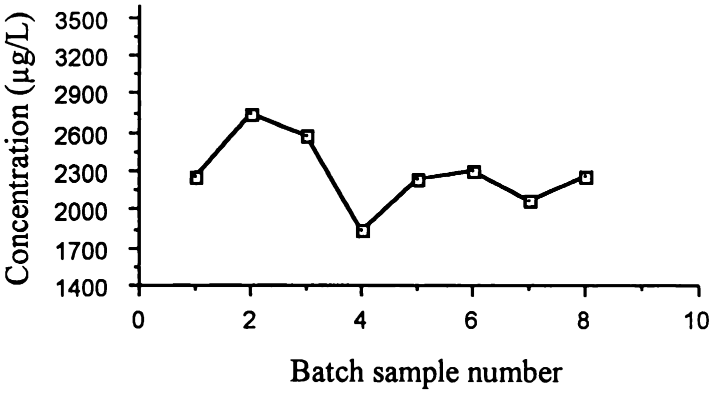
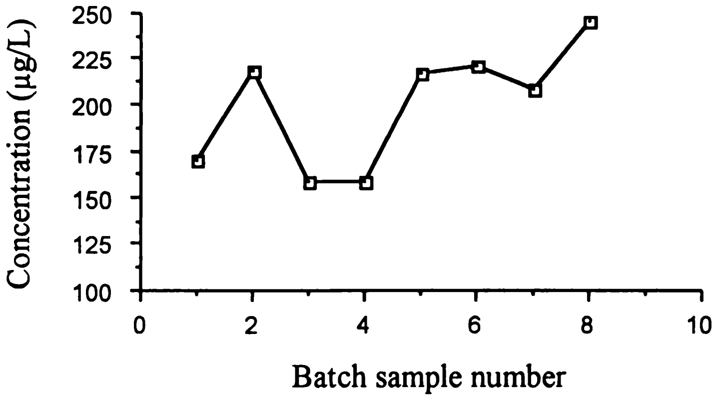


Figure A2.2 Control chart for vanillic acid



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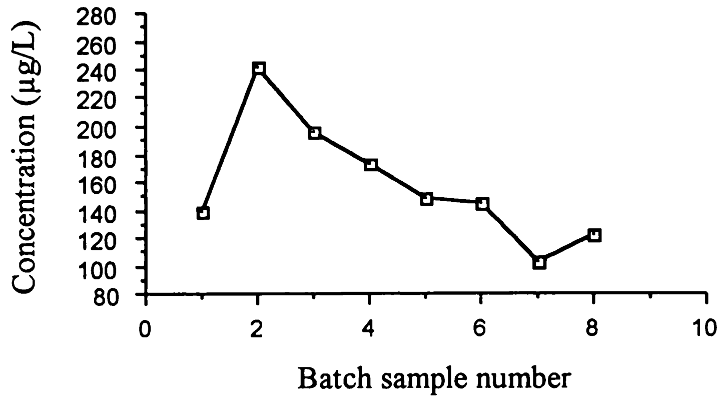

Figure A2.4 Control chart for acetosyringone

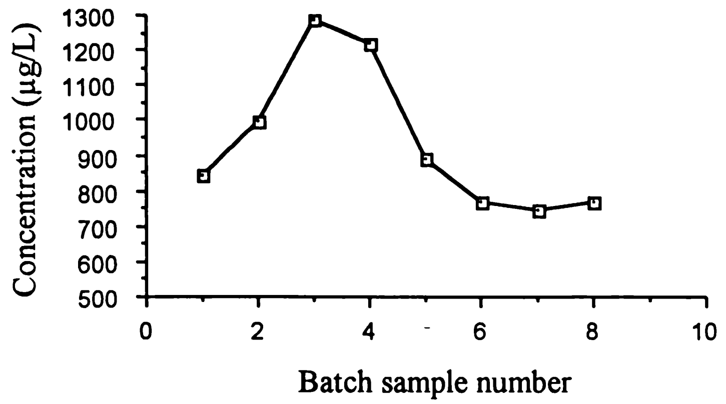

Figure A2.5 Control chart for syringic acid

Appendix 3: **Wine Tasting Notes**

Table A3.1 Tasting notes from first sampling 1992 (one month ageing)

Barrel	Description	Tasting notes	Average score
Re-shaven D&J barrel	D&J barrel used for 3 vintages then had interior surface re-shaven	Nice fruit. Pleasant.	
Stainless steel cask	220 L stainless steel control cask	Obvious fermentation odours. Sulphidic.	
Old used D&J barrel	D&J barrel used for 3 vintages	Nice fruit flavours. A little stale	
D&J 1	D&J Tonn. BM HT (new French oak)	Nice fruit and some toasted oak character	
D&J 2	D&J Tonn. VM HT (new French oak)	Nice fruit flavours	
D&J 3	D&J Tonn. Voges (new French oak)		
D&J 4	D&J Tonn. B. (new French oak)		
Seguin Moreau 1	Seguin Moreau Voges (new French oak)	Pleasant. Some toasty oak.	
Seguin Moreau 2	Seguin Moreau Allier (new French oak)	Toasty oak aromas and flavours	
Seguin Moreau 3	Seguin Moreau Troncais (new French oak)		
Seguin Moreau 4	Seguin Moreau Troncais (new French oak)		
Francois Freres 1	F. F. Tonn. Voges MT (new French oak)		
Francois Freres 2	F. F. Allier M (new French oak)		
American oak	new American oak barrel	Good fruit.	

Table A3.2 Tasting notes from second sampling 1992 (three month ageing)

Barrel	Description	Tasting notes	Average score
Re-shaven D&J barrel	D&J barrel used for 3 vintages then had interior surface re-shaven	Nose not uplifted. Sweet fruit - melons/figg. Fruit dominant. Light oak characteristics. Wood not obvious on nose. Pineapples/bananas well lifted. Slight extract/tannin finish.No obvious fruit flavours. Leesy old oak. Yeasty. Some clove/cinnamon. Lacks freshness.	15.0
Stainless steel cask	220 L stainless steel control cask	Insipid. Reductive. Unpleasant. Garlic. No oak. Sulphidic. Spoilled. Fruit hidden. No oak. Some buttery diacetyl flavours.	11.2
Old used D&J barrel	D&J barrel used for 3 vintages	Complex nose - bubblegum. Sweet fruit flavours with oak subdued. Fresh peachy/appley aromas with little oak.	15.4
D&J 1	D&J Tonn. BM HT (new French oak)	Estery fruit. Good flavours. Obvious charring. Oak finish. Fresh fruity nose with subtle oak. Slightly nutty/toasty. Smokey oak flavours dominate fruit.	16.5
D&J 2	D&J Tonn. VM HT (new French oak)	Moderate intensity of aromas. Sweet fruit with astringency. Finishes charry. Good mouthfeel. Subtle oak toasty flavours and aromas - perfumed nose. Toasty/woody finish. Light delicate fruit. Sweet oak slightly smokey.	16.0
D&J 3	D&J Tonn. Voges (new French oak)	Fruit dominant/estery bouquet. Nutty/biscuit flavour. Good complexity. Toasted oak finish. Lots of fruit. Citrus/stone fruit aromas with light oak. Light oak flavours on pallate.	15.6
D&J 4	D&J Tonn. B. (new French oak)	Sweet fruit flavours with light charry oaking. Biscuit/perfumed nose. Good fruit. Oak is subtle and subdued. Good delicate oak - light &toasty. Good fruit/melon on pallate. Good oak/fruit balance.	16.2

Seguin Moreau 1	Seguin Moreau Voges (new French oak)	Banana/estery fruit dominant nose. Nutty flavours. Some sawdust. Good extract. Woody finish. Fresh fruit aromas with some oak. Some oak on pallate - smokey and toasty.	15.8
Seguin Moreau 2	Seguin Moreau Allier (new French oak)	Nose not uplifted. Good flavours &nice balance. Light toasting. Fruit dominates nose. Oak on pallate as extract but not as flavour. Subtle light fruit. Lacks fresh oak.	16.3
Seguin Moreau 3	Seguin Moreau Troncais (new French oak)	Complex aromas. Melons/tropical. Nice balance. Peachy/pineapple nose. Oak not obvious on nose but distinct on pallate. Estery/ferment yeast. Melon & grapefruit aromas. Well-balanced. Attractive oak. Light toasty aromas.	16.8
Seguin Moreau 4	Seguin Moreau Troncais (new French oak)	Complex nose - bubblegum/estery. Some charry oak. Well balanced oak and fresh fruit. Good extract and fruit on pallate with toasty finish. Lacks depth. Some oak on pallate.	16.1
Francois Freres 1	F. F. Tonn. Voges MT (new French oak)	Oaky charred nose - attractive. Vanillin & coconut aromas. Attractive fruit balanced with oak. Slightly green and sappy on the nose with oak up front. Burned or smokey on pallate dominates fruit. Good fresh oak with slight vanillin. Smokey/toasty oak flavours. Cedery. Francois Freres?	15.4
Francois Freres 2	F. F. Allier M (new French oak)	Attractive oaky nose - nutty. Sweet oak. Obvious heavy toasted oak. Obvious smokey burned oak. Some coarseness. Lacks balance. Oak a little powerful. Slightly phenolic pallate. Clumsy oak. Francois Freres?	15.1
American oak	new American oak barrel	Heavy coconut bouquet. Obvious American oak. Long flavour - coconut/vanillin. Fruit masked by sweet oak. Toasty finish on nose. Fresh fruit (peaches and melons) and strong vanillin on pallate. Powerful coconut masking fruit.	14.4

Table A3.3 Tasting notes from third sampling 1992 (five month ageing)

Barrel	Description	Tasting notes	Average score
Re-shaven D&J barrel	D&J barrel used for 3 vintages then had interior surface re-shaven	Pleasant toasted nutty nose. Lacks fruit on palate. Good extract. Nice savoury palate. Fruit and oak linger. Slightly cheesy, leesy nose with some oak. Lacks fresh fruit. Some biscuitty MLF character with oak on palate.	15.2
Stainless steel cask	220 L stainless steel control cask	Melon fruit. A touch smelly. Citrus style fruit. No oak. Off odours. Some delicate fruit underneath the pong (mercaptan). Thin, empty feel on palate.	10.0
Old used D&J barrel	D&J barrel used for 3 vintages	Subtle melon nose. Fruit lacking on palate. Oak subdued. Lacks freshness. Nutty toasty character with fruit up front. Slightly leesy nose. Lacks fresh fruit and oak (H_2S?). Soft, sweet fruit palate with no oak.	14.8
D&J 1	D&J Tonn. BM HT (new French oak)	Melon fruit. Subtle pleasant oak. Buttery finish. Fresh fruit lifted nose. Estery. Good extract. Toasty lingering oak and fruit. Good palate length. Fruit subdued on nose initially then opens up. Some spicey aromas Strong toasty oak flavours with smokey finish.	16.3
D&J 2	D&J Tonn. VM HT (new French oak)	Tropical fruit. Obvious oak. Slightly dull. Levels of tannin or extract. Some obvious oak flavours. Nice fruit with strong coconut/vanillin on nose. Slightly thin and short. Quite strong oak flavours.	15.7
D&J 3	D&J Tonn. Voges (new French oak)	A little dull and musty. Subdued fruit on nose. Slightly coarse. Lacks freshness on palate. Slightly oaky finish.	15.0
D&J 4	D&J Tonn. B. (new French oak)	Subtle oak. Estery. Fruit dominant. Fresh clean lifted subtle oak on nose. Oak extract obvious from mouthfeel. Clean finish. Well balanced. Clean fresh fruit with light oak on nose. Slightly biscuitty/toasty palate - good oak.	15.3

Seguin Moreau 1	Seguin Moreau Voges (new French oak)	Closed nose at first. Estery. Obvious fruit. Wood on nose. Fresh tropical fruit shows after wood palate. Less intense. Lovely light fruit on nose(peaches). Soft palate with nice fruit and oak. Nice toasty/smokey flavours.	15.3
Seguin Moreau 2	Seguin Moreau Allier (new French oak)	A little dull. Good toasty oak on nose. Clean fresh fruit. Well-balanced aromas. More obvious wood with balanced fruit on palate. Vanillin/toasty oak nose dominant over nice melon fruit. Slightly coarse, but nicely integrated oak with good length. Biscuitty/meally flavours.	15.8
Seguin Moreau 3	Seguin Moreau Troncais (new French oak)	Uplifted nutty nose. Firm acid finish. Well integrated oak. Less oak extract (mouthfeel). Moderate fruit on palate with strong wood flavours. Fruit subdued. More oak dominated nose. Good balance. Nice oak flavours.	14.8
Seguin Moreau 4	Seguin Moreau Troncais (new French oak)	Closed fruit on nose. Subtle oak. Lovely fruit, almost perfumed. Some wood character. Well integrated. Some toasty buttery oak on finish. Good fruit with little oak on nose. Good, soft palate. Fresh fruit and bubblegum. Light oak flavours.	15.0
Francois Freres 1	F. F. Tonn. Voges MT (new French oak)	Pleasant biscuitty buttery palate. Powerful fruit and oak in good balance. Complex. Perhaps oak slightly more dominant. Fresh vanillin and good fruit on nose. Slightly hard palate. Strong Francois Freres oak. High levels of toasty/smokey flavours.	15.2
Francois Freres 2	F. F. Allier M (new French oak)	Oak a little overpowering on nose and palate. Very obvious toasty oak, almost dusty. Fruit masked. Palate overpowered by wood. Fresh lifted peachy melon fruit but strong. Heavy toast finish. I dont like this F/F oak!	14.2
American oak	new American oak barrel	Pleasant buttery nose. Harsh oak. Fruit masked by oak. Obvious vanillin. Lifted oak (eg American). Oak totally dominates nose. Oak very strong on palate. Not unattractive but very obvious.	14.8

Table A3.4 Tasting notes from final sampling 1992 (seven month ageing)

Barrel	Description	Tasting notes	Average score
Re-shaven D&J barrel	D&J barrel used for 3 vintages then had interior surface re-shaven	Slight dusty nose. Good mouth feel. Quite high alcohol finish. Fruit is a little dull. Oak is quite obvious at start. Fruit showing on finish, moderate length and good mouth feel. Good fruit. Slight green characters. Slight stale character.	15.7
Stainless steel cask	220 L stainless steel control cask	Sulphidic, mercaptan, grapefruit pungency. Bitterness on palate. Dirty. Feijoa like. Shows some mealy, biscuity, yeasty character underneath H_2S.	9.3
Old used D&J barrel	D&J barrel used for 3-vintages	A little mercaptan. Wood and fruit not integrated. Fruit slightly honeyed. Pinacolada. Ripe fruit followed by toffee apple aroma. Oak not obvious. Lingering fruit. Plain old oak character - lacks freshness. Quite nice fruit flavours.	14.2
D&J 1	D&J Tonn. BM HT (new French oak)	Uplifted buttery, toasty, marshmellow nose. Lacking a little tannin at finish but fruit /oak integration good. Powerful fruit over subtle oak. Lifted tropical fruit flavours. Good extract with wood flavours at finish. Soft and round. Moderate complexity. Good length. Slightly dominant strong sweet new oak character. Toasty. Some vegemite, yeast character. Attractive.	17.3
D&J 2	D&J Tonn. VM HT (new French oak)	Coconut character on nose. Pleasant oak. Fruit a little subdued - opens up with time. Firm finish. Good fruit and some toasty oak. Quite strong oaky flavours on palate - smokey/toasty.	17.0
D&J 3	D&J Tonn. Voges (new French oak)	Subtle fruit character with good oaky nose. Slightly disjointed but not overpowering. Fruit and oak coming together. Slightly unclean. Reasonable fruit. Lacks complexity. Slightly darker colour. Some tropical/peach fruit aromas and flavours. Nice fruit.	16.5

D&J 4	D&J Tonn. B. (new French oak)	Good body. Good fruit and oak. Good finish. Big nose. Well integrated fruit and oak. Good fruit on palate and nice wood flavours. Excellent mouth feel and good length. Lacks oak on nose but nice oak on finish.	17.3
Seguin Moreau 1	Seguin Moreau Voges (new French oak)	Nose subdued. Subtle oak. A little dull. Savoury oak showing through. Good extract. Clean and fresh. Nice fresh balanced oak on nose. Good fruit. Soft palate.	16.0
Seguin Moreau 2	Seguin Moreau Allier (new French oak)	Palate lacking fruit in middle. Pleasant oak. Oak a little more up front. Toasty/bacon aromas. Pencil shavings and a little disjointed, however, lots of flavour. Good sweet oak aromas and nice tropical fruit. Good light oak flavours and finish.	17.5
Seguin Moreau 3	Seguin Moreau Troncais (new French oak)	Pleasant but simple. Clean.Good fruit. Mealy. Some pencil shavings character. Oak a little aggressive. Flavours are good. Attractive peachy fruit. Soft palate and nice mouthfeel. Light oak flavours. Lacks complexity but a nice wine.	16.4
Seguin Moreau 4	Seguin Moreau Troncais (new French oak)	Subdued nose. A little dull. Fresh tropical fruit - delicate and elegant. Good extract and well integrated oak. Toasty finish. Good length. Not complex. Lacks complexity but is developing. Slightly flat. Nice wine but lacking oak.	16.2
Francois Freres 1	F. F. Tonn. Voges MT (new French oak)	Nice fresh nose - fruit and oak well integrated. Subtle oak on palate and fruit dominant. Not complex but good. Francois Freres? Slightly burned character. Oak and fruit disjointed. Overpowering toasty oak. Alittle fruit showing through. A little hazelnut/toasted nut character.	14.7
Francois Freres 2	F. F. Allier M (new French oak)	Aggressive oak on nose. Overpowering oak. Heavy toast overpowers fruit. Slightly burned. Clumsy oak. F/F?	14.4
American oak	new American oak barrel	Oak character too overpowering.Too much tannin. Oak very dominant. Lots of vanillin. Too much wood flavour. American oak? Coconut/vanilla - quite attractive but strong. Coarse oak tannins on palate.	14.3

Table A3.5 Tasting notes from 1993 Vintage Chardonnay

Wine	Description of barrel	Sensory description of wine	Average Score
#1	Seguin Moreau Allier - new French oak	Pleasant. Good aromas. Cedary. Subtle oak. Good mouthfeel.	16.5
#2	D&J - new French oak	Good fruit flavour. Crisp toasty oak, integrated fruit/oak. Elegant. Lovely fresh new oak.	16.8
#3	D&J - old French oak	Fruit dominant. Good fruit flavours. Dull. A little yeasty. Estery. Slightly stale oak. Lacks freshness.	13.5
#4	D&J - old French oak with toasted oak pieces	Good on palate. Nice fruit. Slightly clumsy oak. Slightly disjointed.	14.7
#5	D&J - old French oak with toasted oak pieces	Nice fruit. Pinacolada. Some oak. Old oak? Slightly plain oak character.	15.0

Table A3.6 Tasting notes from 1994 Vintage Chardonnay (12 weeks)

Wine	Description of barrel	Sensory description of wine	Average Score
#1	Seguin Moreau Allier - new French oak	Lovely fresh new oak. Pleasant wine. Good aromas. Nice fruit with subtle oak. Good balance.	16.8
#2	D&J - used French oak (used for 4 vintages)	Wood not obvious. Lacks freshness. A little yeasty. Some nice fruit.	14.5
#3	D&J - used French oak (used for 4 vintages) + 2 g/L toasted oak cubes	Nice fruit flavours. Some oak flavours. Quite nice wine.	14.5
#4	220 L stainless steel cask + 2 g/L toasted oak cubes	Good fruit. Clean. Nice flavours. No obvious oak. Stainless steel?	13.0

Table A3.7 Tasting notes from 1994 Vintage Chardonnay (24 weeks)

Wine	Description of barrel	Sensory description of wine	Average Score
#1	Seguin Moreau Allier - new French oak	Crisp toasty oak flavours. Lovely fruit with fresh oak. Well integrated. Balanced. Soft palate with toasty oak.	17.0
#2	D&J - used French oak (used for 4 vintages)	Some pleasant fruit flavours. Slightly stale oak. Nutty. Some old oak character. A little stale?	14.5
#3	D&J - used French oak (used for 4 vintages) + 2 g/L toasted oak cubes	Nice fruit with some oak. Some old oak character. Lacks a little freshness.	15.0
#4	220 L stainless steel cask + 2 g/L toasted oak cubes	Clean but simple. Subtle light fruit. Lacks fresh oak. Slightly thin on palate. Stainless steel?	13.8

Table A3.8 Tasting notes from 1995 oak necklace experiments (4 weeks ageing)

Wine	Description of barrel	Sensory description of wine	Average Score
1	Seguin Moreau Allier - new French oak	Subtle aromas. Some oak but not obvious. Attractive nose. Signs of extract.	16.3
2	Stainless steel cask with toasted oak necklace	Lactic (buttery). Simple. Fermentation esters dominant.	14.8
3	Used Seguin Moreau cask with toasted oak necklace	Nice fruit. Pleasant aromas. Good on palate.	15.7

Table A3.9 Tasting notes from 1995 oak necklace experiments (8 weeks ageing)

Wine	Description of barrel	Sensory description of wine	Average Score
1	Seguin Moreau Allier - new French oak	Good extract. Nice fruit. Toasty finish. Good wine.	
2	Stainless steel cask with toasted oak necklace	Good flavours. Fresh fruit with some subtle oak.	
3	Used Seguin Moreau cask with toasted oak necklace	Nice balance of fruit and subtle oak. Attractive oak. Light and toasty.	

Table A3.10 Tasting notes from 1995 oak necklace experiments (14 weeks ageing)

Wine	Description of barrel	Sensory description of wine	Average Score
1	Seguin Moreau Allier - new French oak	Nice balance of fruit and oak flavours.	
2	Stainless steel cask with toasted oak necklace	Nice fruit on nose. Signs of extract.	
3	Used Seguin Moreau cask with toasted oak necklace	Crisp and light. Clean. Nice toasted oak.	

Table A3.11 Tasting notes from 1995 oak necklace experiments (20 weeks ageing)

Wine	Description of barrel	Sensory description of wine	Average Score
1	Seguin Moreau Allier - new French oak	Uplifted nose. Fresh fruit with toasty oak. Integrated oak. Good length on finish. Clean and fresh.	16.7
2	Stainless steel cask with toasted oak necklace	Good fruit on nose. Toasty oak on finish. Some nice fruit flavours. Oak a little subdued. Stainless steel?	15.0
3	Used Seguin Moreau cask with toasted oak necklace	Nice mouthfeel. Pleasant toasted nutty nose. Light oa flavours. Soft on palate. Good balance.	16.3

Table A3.12 Tasting notes from 1995 oak necklace experiments (24 weeks ageing)

Wine	Description of barrel	Sensory description of wine	Average Score
1	Seguin Moreau Allier - new French oak	Attractive fruit. Tropical fruit. Nice sweet oak. Fresh toasty aromas. Elegant wine. Nice finish. Soft on palate.	17.0
2	Stainless steel cask with toasted oak necklace	Nice fruit flavours and subtle oak. Oak more subtle. Stainless steel? Peachy fruit. Light oak on finish.	15.3
3	Used Seguin Moreau cask with toasted oak necklace	Lots of flavour. Fruit showing on finish. Sweet toasty oak aromas and flavours. Nice mouthfeel. Excellent potential. Elegant wine. Delicate oak flavours. New oak?	17.0

Appendix 4: Mass Spectra for Target Compounds

All compounds are represented as their methylated analogues.

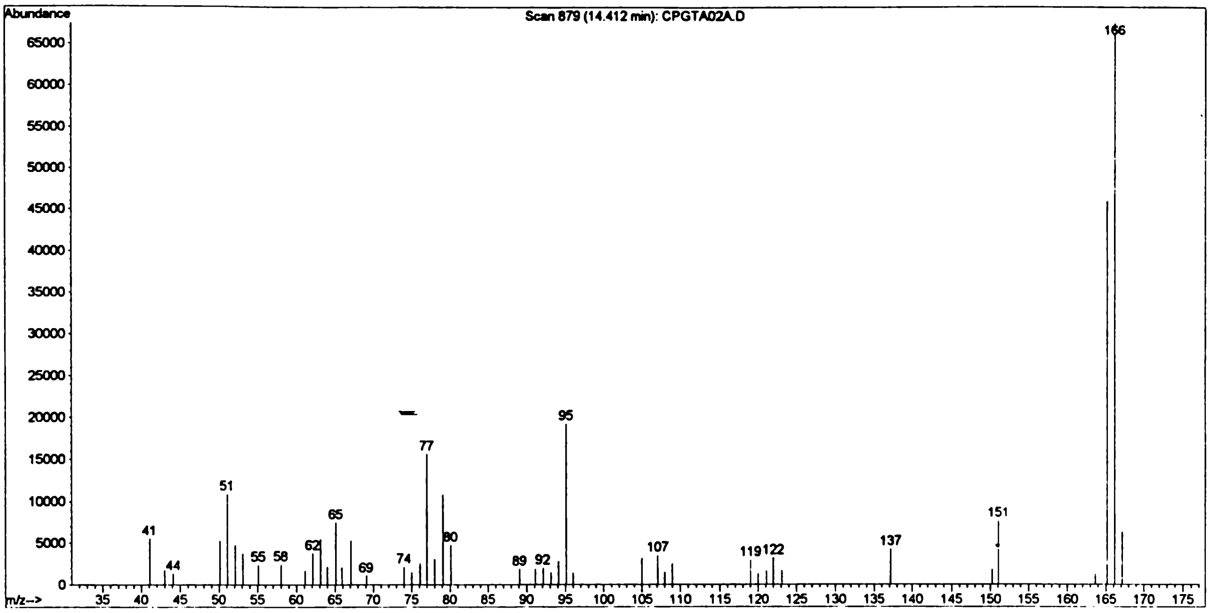

Figure A4.1 Total ion mass spectrum for vanillin

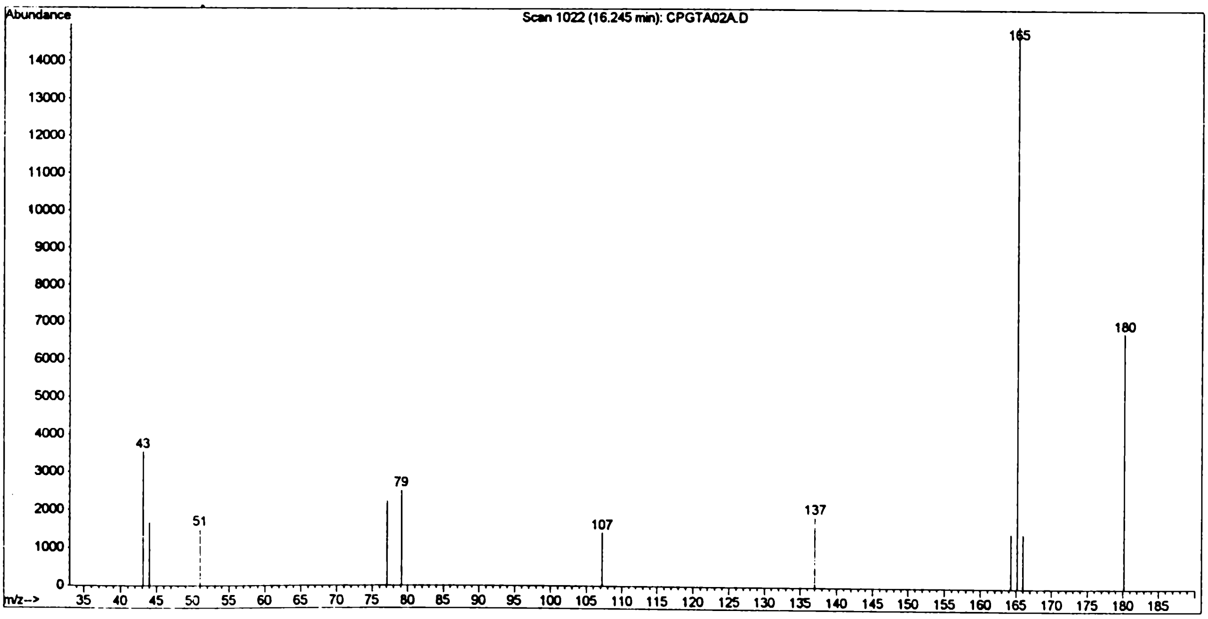

Figure A4.2 Total ion mass spectrum for acetovanillone

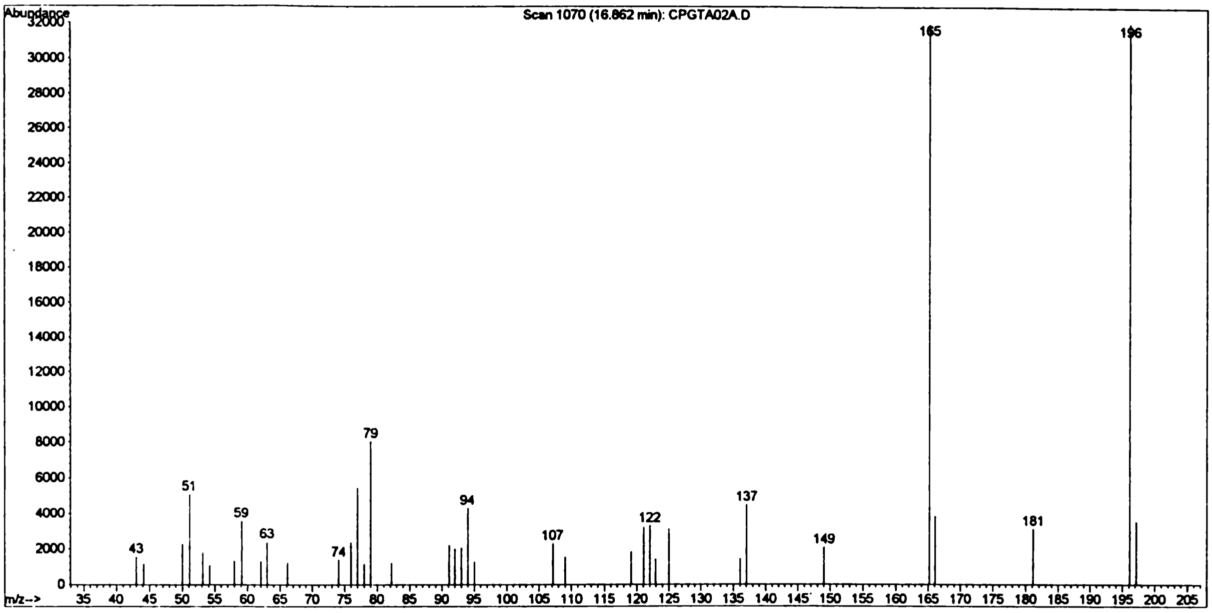

Figure A4.3 Total ion mass spectrum for vanillic acid

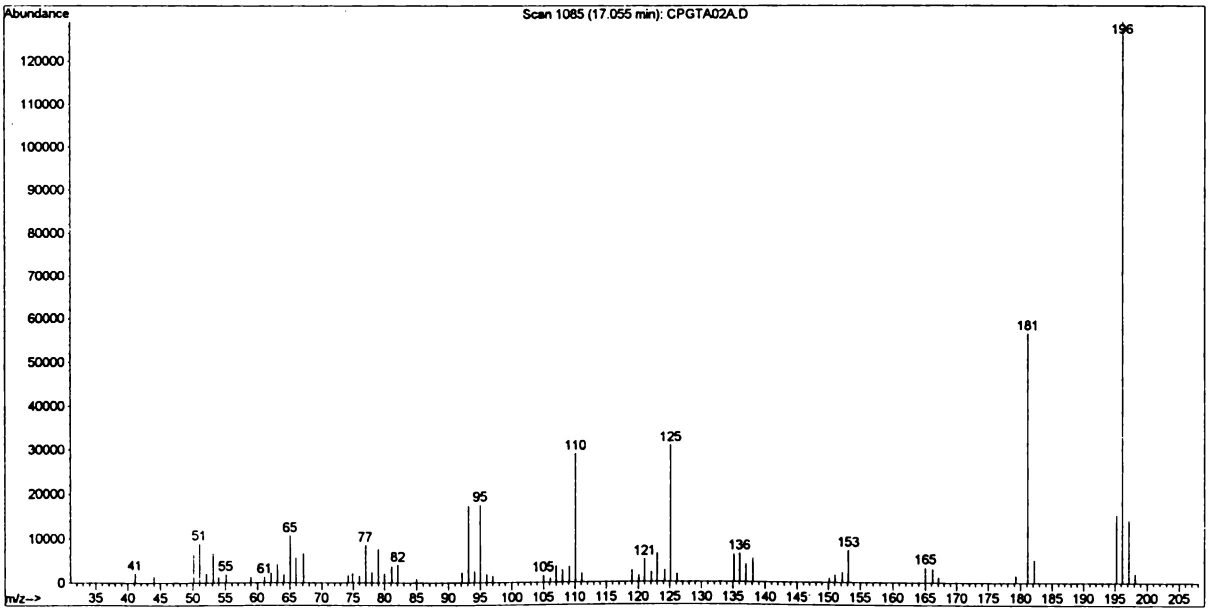

Figure A4.4 Total ion mass spectrum for syringaldehyde

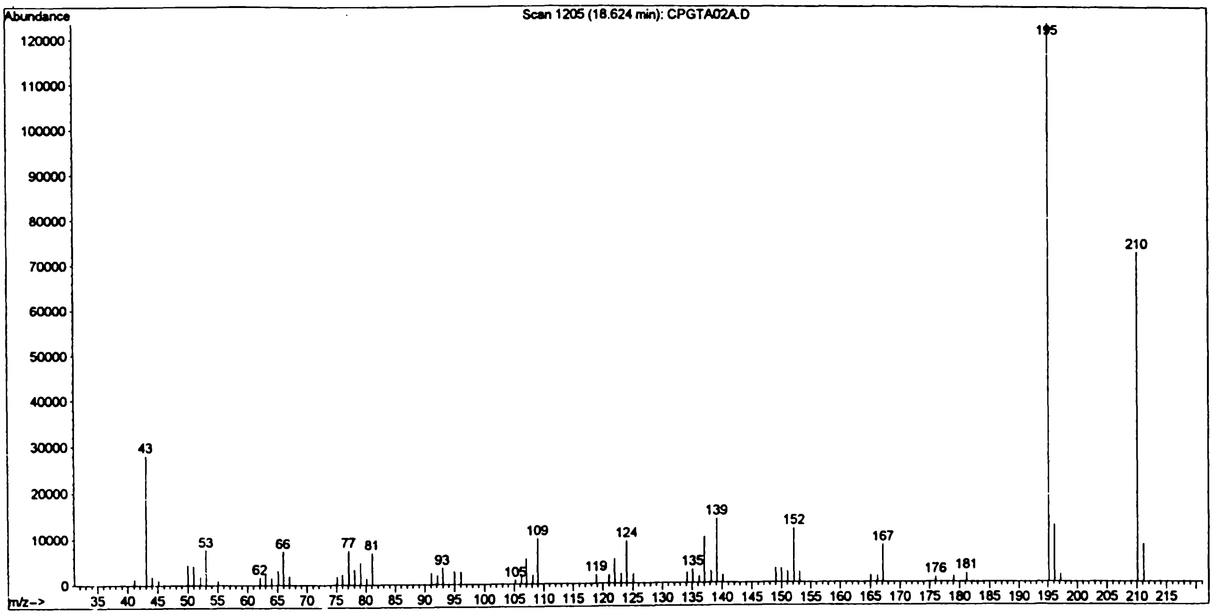

Figure A4.5 Total ion mass spectrum for acetosyringone

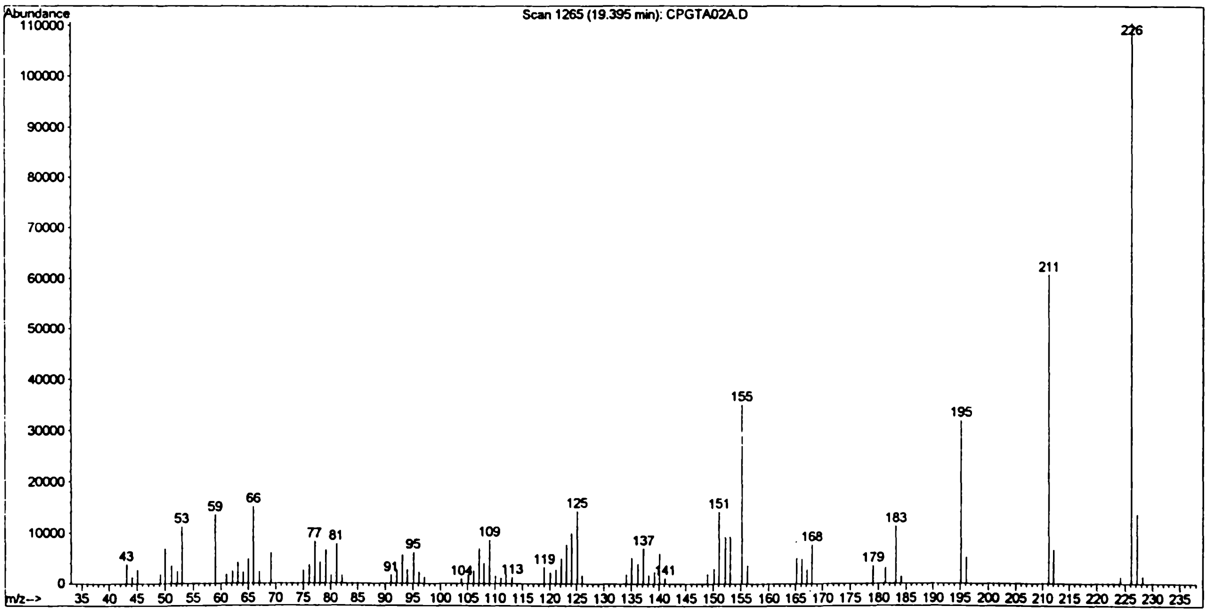

Figure A4.6 Total ion mass spectrum for syringic acid

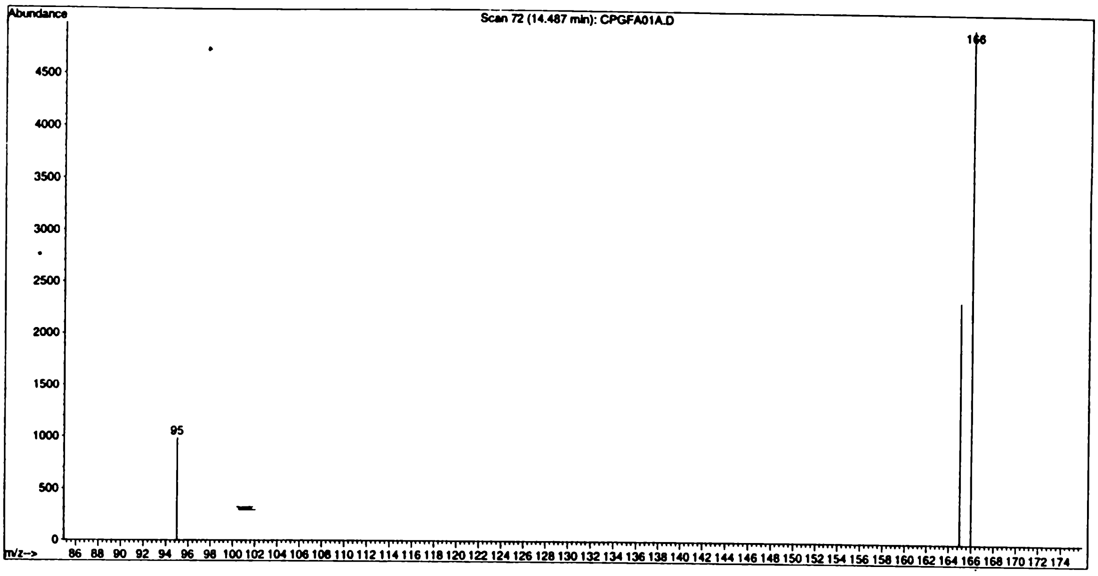

Figure A4.7 Selected ion mass spectrum for vanillin

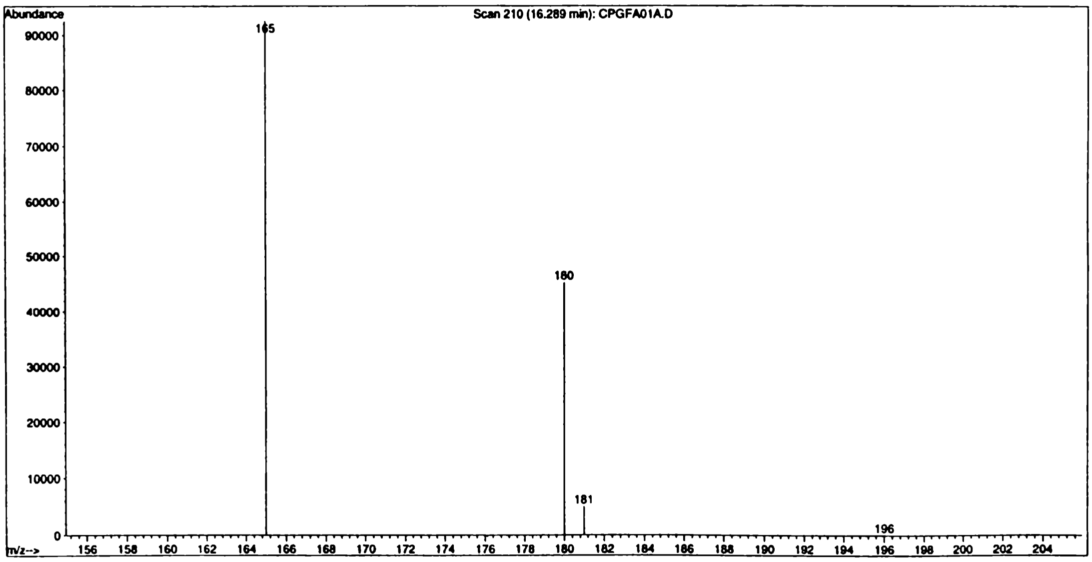

Figure A4.8 Selected ion mass spectrum for acetovanillone

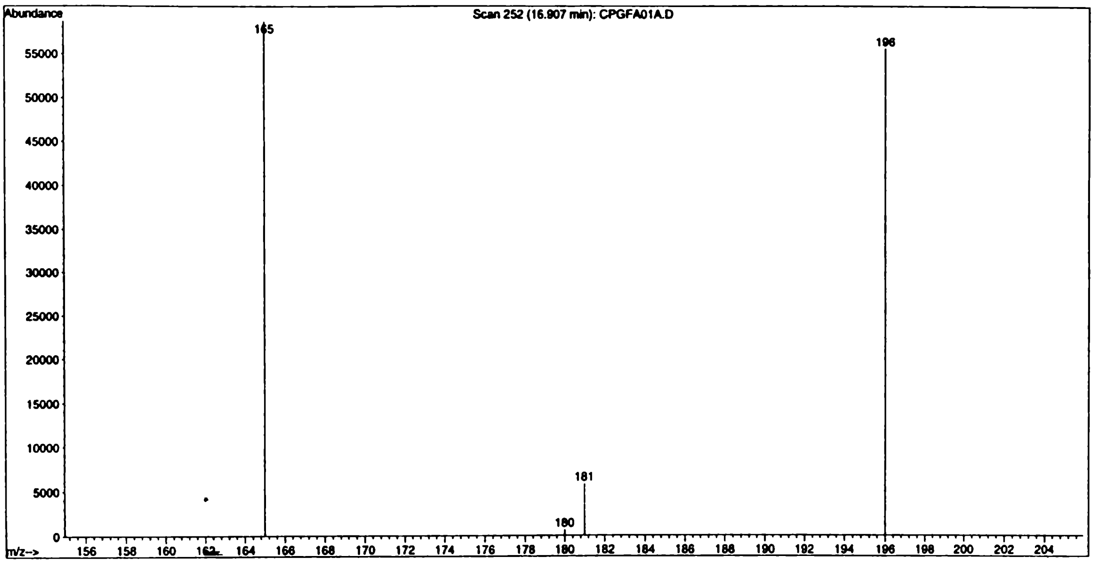

Figure A4.9 Selected ion mass spectrum for vanillic acid

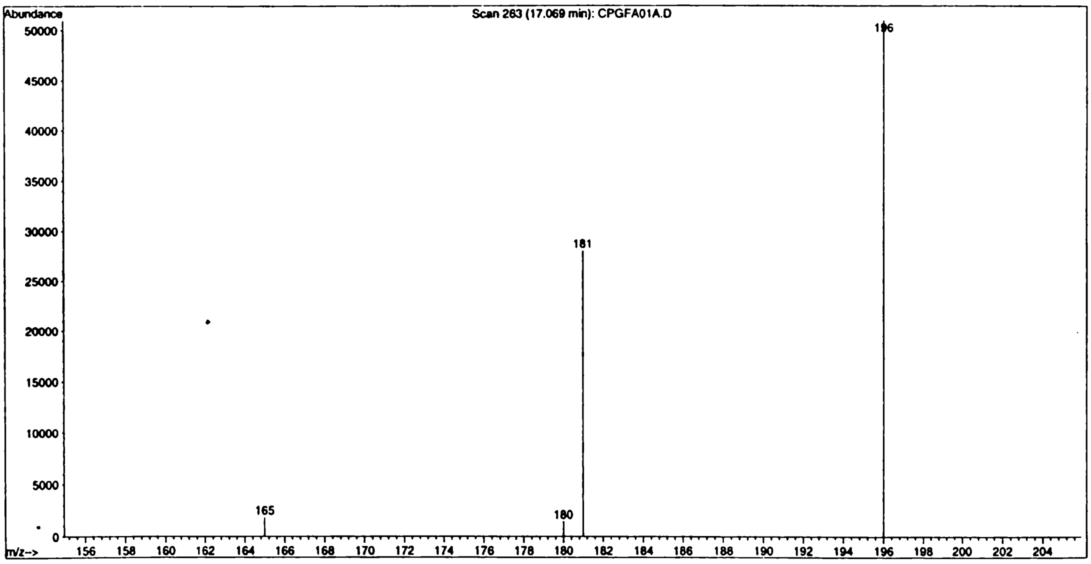

Figure A4.10 Selected ion mass spectrum for syringaldehyde

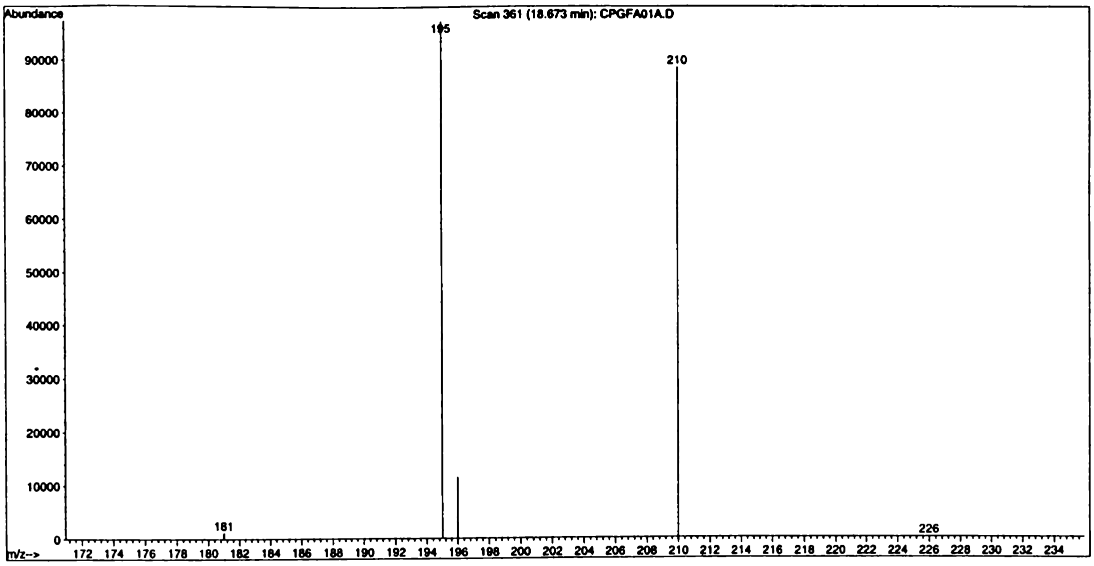

Figure A4.11 Selected ion mass spectrum for acetosyringone

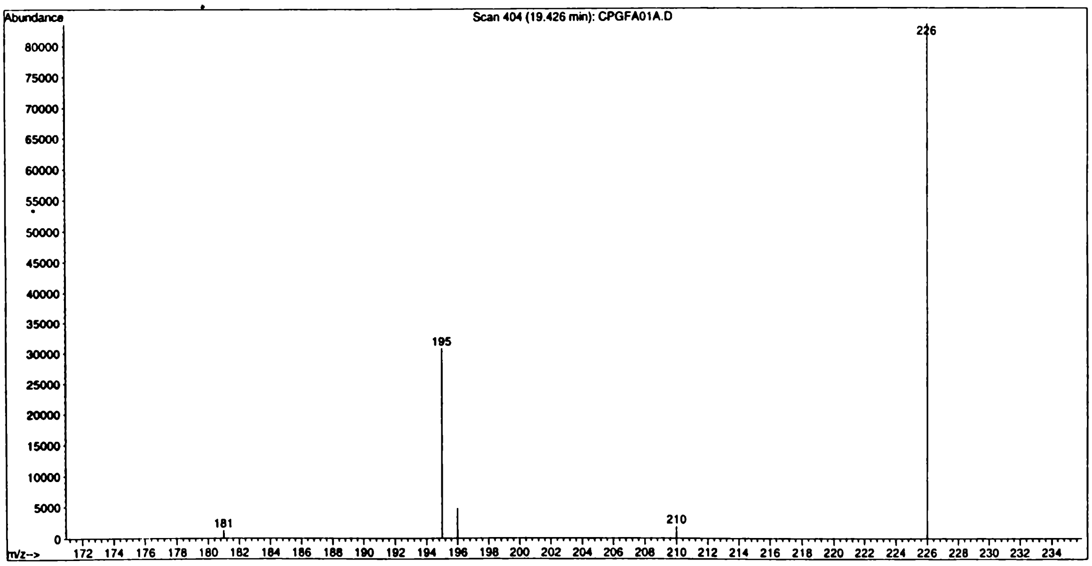

Figure A4.12 Selected ion mass spectrum for syringic acid