OAK WOOD CONTRIBUTION TO WINE AROMA

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ABSTRACT

The aroma variability arising from the maturation of a Chardonnay wine (10 descriptors) and a Cabernet Sauvignon wine (12 descriptors), each in 24 new oak barrels for 55 and 93 weeks, respectively, was described using sensory ranking, and explained in terms of the natural and cultural variability to which the oak and the wines had been subjected. These wines, along with a model wine which was matured in 16 similar barrels and sampled at five different times over a 93 week period, were analysed for 20 oak wood–derived or associated volatile compounds using gas chromatography – mass spectrometry. Four compounds, cyclotene, maltol, 5–methylfurfuryl ethyl ether and vanillyl ethyl ether were quantified in a barrel aged wine for the first time.

Principal components analysis indicated the three main 'directions' of variation in the composition data. The oak lactones and eugenol were strongly associated with one another and were not associated with either coopering heat or microbial activity products. The seven coopering heat products targeted were strongly associated with one another except when affected by microbial activity. This activity yielded degradation products which constituted a third composition 'direction.'

Relationships among the composition and sensory data, along with an understanding of the genesis of the compounds, have suggested which of the natural and cultural variables are likely to have been involved in each of the aroma variations. Incorporated into these explanations are the imposed oak origin, seasoning location and cooper treatment effects, and the inferred coopering heat variability and wine microbial activity effects.

A novel data analysis method, involving compound concentration differences in relation to specific aroma differentiation, was developed. Each result is summarised graphically, as a specific aroma 'impact–pattern conformity' plot. The analysis tests for a naturally occurring association between compound concentration differences and specific aroma differentiation which is consistent with the existence of a causal relationship, and it estimates either the aroma potency of the compound or the concentration difference coinciding with the aroma impact of one or more unknown compounds.

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

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PUBLICATIONS ARISING

(to August 1998)

Sefton M.A., Spillman P.J., Pocock K.F., Francis I.L., Williams P.J. 1993 The influence of oak origin, seasoning, and other industry practices on the sensory characteristics and composition of oak extracts and barrel–aged white wines. *Australian Grapegrower & Winemaker*, **355**: 17–25.

Spillman P.J. 1994 Making sense of the oak flavour description of a white wine. *Australian Grapegrower & Winemaker*, **367**: 19–21.

Spillman P.J. 1995 Non–adhesion of oak flavour compounds to microbial cells. *Australian Grapegrower & Winemaker*, **379**: 19–22; and *errata* in *ibid,* **380**: 11.

Spillman P.J., Pocock K.F., Gawel R., Sefton M.A. 1996 The influences of oak, coopering heat and microbial activity on oak–derived wine aroma. In: *Proceedings 9th Australian Wine Industry Technical Conference*, Adelaide, Australia. Winetitles, Adelaide. pp. 66–71; and *errata* sheet.

Spillman P.J., Pollnitz A.P., Liacopoulos D., Skouroumounis G.K., Sefton M.A. 1997 Accumulation of vanillin during barrel–aging of white, red and model wines. *J. Agric. Food Chem.,* **45**: 2584–2589.

Spillman P.J., Pollnitz A.P., Liacopoulos D., Pardon K.H., Sefton M.A. 1998 Formation and degradation of furfuryl alcohol, 5–methylfurfuryl alcohol, vanillyl alcohol, and their ethyl ethers in barrel–aged wines. *J. Agric. Food Chem.,* **46**: 657–663.

Spillman P.J., Iland P.G., Sefton M.A. 1998 Accumulation of volatile oak compounds in a model wine stored in American and Limousin oak barrels. *Aust. J. Grape & Wine Research,* **4**: 67–73.

Chapter 1

Introduction, literature review and experimental design

Chapter outline

1.1 Introduction

The seasoned heartwood of several species of oak is used in the production of some wines, either as a material for container construction or as added pieces, to modify wine aroma, flavour and taste. Oak wood barrels have been used, historically, as containers for a variety of products, including wine, beer, spirits and oil. Their use as containers has declined, however, due to the introduction of lighter, cheaper or more inert alternatives. In the alcoholic beverage industries, stainless steel containers are now preferred for storage, unless the flavour and other contributions of oak wood are desired.

The combination of oak and wine, in aroma and flavour terms, has become so well accepted as to be indispensable for some wine styles. The red and white dry table wine styles of Bordeaux and Burgundy, which have been emulated by many of the so–called new world wine producing countries (principally in North and South America, South Africa and Australasia), are the best examples.

The contribution that oak wood can make to wine composition and aroma is variable because the oak wood, the wine, the nature of their contact and the perception among consumers varies in more than a few ways.

1.2 Volatile composition overview

The primary constituents of oak wood are cellulose, hemicellulose, lignin and hydrolysable tannins (Maga 1989b). However, the substances extracted by wines are of more direct interest. To consider the compositional effects of oak on wines, the headspace or solvent extract of the wine must be analysed, not the wood. Analysis of the wood itself may also be useful, however, since it might help to elucidate the mechanisms of wine–soluble compound formation.

Over two hundred volatile oak wood compounds have been identified (Maga 1989b) but a much smaller number have also had their flavour or aroma impacts elucidated. Presently, substantial composition data and a limited understanding of the flavour impact of these

compounds have led to some understanding of the relationship between oak wood–derived wine composition and flavour.

The volatile compounds may be categorised according to their biogenetic or chemical origin (*e.g.* Sefton 1991) but, to be consistent with the categorisation used throughout this thesis, the review considers these compounds with reference to their natural or cultural origins.

Compounds present in oak wood prior to coopering

The *cis*– and *trans*– isomers of the so–called oak lactone (β–methyl–γ–octalactone) are currently thought to be some of the most substantial contributors to oak wood aroma in wine. Masuda and Nishimura (1971) have established that, in alcoholic beverages, these two isomers "are derived only from oak wood." Their formation is thought to be through lipid oxidation (Maga 1989b).

There is some confusion in the early literature regarding the isomeric assignment when authors have discussed various aspects of the *cis*– and *trans*–oak lactones. This has resulted in apparently contradictory reports. Masuda and Nishimura (1981) have reported the correct isomeric configurations and absolute stereochemistry. They can be distinguished by gas chromatography in that the *trans* isomer is eluted before the *cis* isomer. It is the *cis* isomer which is more important in terms of its effect on aroma as it is found in higher concentrations and has a reported aroma threshold in white wine around five times lower than that of the *trans*: 92 compared with 460 μg/L (Chatonnet *et al.* 1992c).

Nishimura *et al.* (1983) have isolated a different lactone from oak — γ–nonalactone — which they suggested might contribute to the matured flavour of spirits.

Eugenol, a compound of the volatile phenol group discussed in the following section, is present prior to coopering (Sefton *et al.* 1993a, Chatonnet *et al.* 1994b). This compound may arise from the degradation of lignin but, unlike compounds of similar origin which arise most substantially as a result of the heat applied to the wood during coopering, its concentration is determined largely prior to coopering. Thus, an alternative to the genesis by heat seems to be involved.

Terpenes represent another group of oak wood compounds present prior to coopering. Monoterpenes, sesquiterpenes and 9– , 11– and 13–carbon norisoprenoids have been identified as oak wood constituents (Nishimura *et al.* 1983, Nabeta *et al.* 1986, Sefton *et al.* 1990b) but their role in oak wood–derived flavour has not been established.

Sefton *et al.* (1990b) identified 31 norisoprenoids in oak wood extracts, one of which, β–ionone, had previously been reported as an oak wood component (Nishimura *et al.* 1983). The norisoprenoids may prove to have some organoleptic importance since they have been reported as important to the flavour of tobacco, tea and some fruits (Schreier 1984). Perhaps a reason for the scarcity of reports regarding this group of compounds in oak wood might lie in the fact that they are also known to occur in both black and white grape varieties as glycosidic conjugates (Abbott *et al.* 1989, Sefton *et al.* 1989, Winterhalter *et al.* 1990) and as such may have had their source assigned to grapes.

Hydrolysable tannins, though not directly contributing to aroma, are present in oak wood, and may impact on aroma through catalysis of oxidation (Chatonnet *et al.* 1991). They also contribute to red wine colour and astringency changes (Vivas and Glories 1996). Hydrolysable tannins are composed of esters of gallic acid (gallotannins) and/or ellagic acid (ellagitannins) with a sugar core, predominantly glucose (Deschamps 1989). Around 5 to 10 % of oak wood dry weight is made up of the hydrolysable tannins which are unstable at wine and spirit pH, breaking down to form gallic acid and, more predominantly, ellagic acid. This latter compound is sparingly soluble in aqueous alcohol and precipitates from solution (Pocock *et al.* 1984).

Chatonnet *et al.* (1991) have reported that the maturation of a white wine in oak wood resulted in an increase in polyphenol content (D280), and it is widely believed that the reported increases in polyphenol content due to oak wood maturation are accompanied by increases in astringency (Moutounet *et al.* 1989). However, Quinn and Singleton (1985) have suggested that this may not be so and that the sensory importance of the ellagitannins require further investigation. Pocock *et al.* (1994) reported a sensory study that suggested that the taste impact of non–volatile oak wood compounds in wine was likely to be subtle,

at most, at the concentrations commonly found. Sefton (1991) has suggested the possibility that non–tannin components of oak wood may affect the perception of astringency in wines.

Compounds arising during the coopering of oak wood

Some oak wood–derived volatile phenols found in wine arise most substantially, if not entirely, as a result of coopering heat, some as a result of the action of microorganisms during wine maturation (discussed below), and one, eugenol (mentioned above), is present in oak wood in significant concentration before these processes. Of those arising as a result of coopering heat, vanillin, guaiacol and 4–methylguaiacol are perhaps the most important. Boidron *et al.* (1988) have suggested that, while these latter two compounds are unlikely to impact, individually, on wine aroma, they are likely to contribute in concert with other volatile phenols.

Considered the most studied group of volatile oak extractives (Sefton *et al.* 1990a), the volatile phenols are mainly derived from the degradation of lignin, a complex and heterogenous structural polymer of dihydroconiferyl and dihydrosinapyl alcohols (Leisola and Fiechter 1985). The volatile phenols are based on the guaiacyl or syringyl nucleus. Nishimura *et al.* (1983) suggested that the lignin degradation products are generally developed by two alternate pathways. Oak wood lignin can be degraded by charring, the products extracted, then oxidised and/or esterified. Alternatively, oak wood lignin can be extracted through ethanolysis, with the lignin portion of the ethanol–lignin complex evolving into the aromatic aldehydes (Reazin *et al.* 1976). The former pathway is dominant in spirits such as bourbon whisky which is stored in *charred* barrels whereas the latter pathway is dominant in scotch whisky and cognac (Nishimura *et al.* 1983) which are stored in non–charred (only toasted) barrels. Much higher amounts of the aromatic aldehydes are found in oak aged spirits than in oak aged wines (Dubois 1983), the most abundant being vanillin, syringaldehyde, coniferaldehyde and sinapaldehyde (Puech and Moutounet 1992).

Carbohydrate degradation products also arise during coopering of oak wood. Cellulose and hemicellulose undergo thermal degradation to form carbohydrate degradation products, mainly furan and pyran derivatives (Sefton 1991). Relatively large quantities of the furan aldehydes, furfural and 5–methylfurfural, are formed in oak wood during heating but these

are unlikely to be important to the aroma of most wines because they are readily reduced to their corresponding alcohols in the presence of microbial activity (Chatonnet *et al.* 1989).

Maltol and cyclotene are thought to contribute to the sweet and toasty aroma of toasted oak wood (Nishimura *et al.* 1983). These compounds are pyrolysis products of sucrose (Johnson *et al.* 1969). Cyclotene can also arise from the acid hydrolysis of glucose.

Protein in wood can serve as the nitrogen source necessary for the formation of thermally induced nitrogen–containing heterocyclic flavour compounds such as pyrazines (Maga 1989b) which are frequently associated with roasted, nutty, coffee, chocolate flavours and which may also be produced by bacterial action (Kempler 1983).

Compounds arising during the wood–wine contact period

Many of the compounds arising from coopering are susceptible to modification by microorganisms. The aldehydes — furfural, 5–methylfurfural and vanillin — are all susceptible to biochemical reduction (by yeast and/or bacteria in wine) to the corresponding alcohols. The high sensory thresholds of these compounds — furfuryl alcohol, 5–methylfurfuryl alcohol and vanillyl alcohol — relative to the concentrations at which they are present in wine, indicate that they are unlikely to impact upon wine aroma (Boidron *et al.* 1988, Chatonnet *et al.* 1992c).

A second group of microbially derived compounds — comprising the volatile phenols, 4–vinylguaiacol, 4–vinylphenol, 4–ethylguaiacol and 4–ethylphenol — arises from the precursors ferulic and *p*–coumaric acid. Dubois (1983) has suggested that these are the most important of the volatile phenols in terms of their effect on alcoholic beverage flavour. The precursors are contributed most substantially by the grape component of wines but Miller *et al.* (1992) have shown that oak wood is an additional source. Chatonnet *et al.* (1992b, 1993, 1995) have explained the sensory importance of these compounds and the chemical and microbiological factors responsible for their evolution in wines.

1.3 Compounds selected for study

A review of the literature, dealing with the occurrence of, or the sensory impact of oak wood–derived or associated volatile compounds in alcoholic beverages (*e.g.* Boidron *et al.* 1988), led to a selection of 17 compounds for study. A further three (the ethyl ethers which were found to form when furfuryl alcohol, 5–methylfurfuryl alcohol and vanillyl alcohol were present with ethanol in wine) were selected in the early stages of the study. The 20 compounds, along with a selection of their reported aroma detection thresholds and likenesses, are shown in Table 1.1, and their molecular structures are shown in Figure 1.1.

Cyclotene and maltol, compounds extracted from oak wood, are thought to be aroma–active in whisky (Nishimura *et al.* 1983). In this thesis, quantities of these two compounds in wine are reported, apparently for the first time. No wine–related aroma threshold data have been reported.

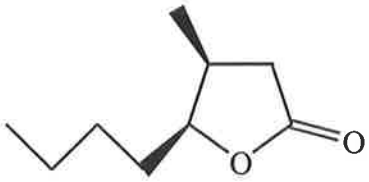
Quantities of 5–methylfurfuryl ethyl ether and vanillyl ethyl ether in wine are reported, also apparently for the first time in this thesis. Furfuryl ethyl ether was reported earlier (Bertuccioli and Viani 1976) and is thought to be associated with staling in beer (Harayama *et al.* 1995). No wine–related aroma threshold or likeness data have been reported for these ethyl ethers. These three compounds were identified by comparison with synthetic samples by others in this laboratory (Spillman *et al.* 1998).

Some compounds reported to be commonly present in oak wood–aged wines at concentrations below their estimated aroma threshold were quantified (*i.e.* particularly, furfural, 5–methylfurfural, furfuryl alcohol and vanillyl alcohol). Nevertheless, these aroma thresholds, determined in wines different from those of this study, do not necessarily mean that the compounds could not influence the aroma of other wines. Nor do they mean that they can not have contributed to aroma effects in combination with other compounds.

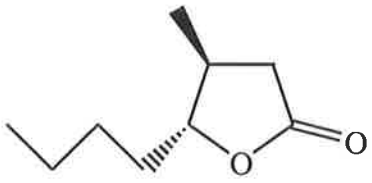
Table 1.1. A selection of the published aroma characteristics for the 20 target–compounds.

Compound	Detection threshold (µg/L) in various media, and description (Boidron *et al.* 1988, unless indicated at right)					Other references
	Water	Model wine	White wine	Red wine	Aroma likeness	
cis–oak lactone†	28	25	92	74	coconut, oak	Chatonnet *et al.* 1992c
trans–oak lactone†	64	110	460	320	coconut, oak	Chatonnet *et al.* 1992c
eugenol	7	15	100	500	clove	
guaiacol	5.5	20	95	75	smoke	
4–methylguaiacol	10	30	65	65	burning wood, ash	
vanillin	105	65	400	320	vanilla	
cyclotene					caramel, maple	Shigematsu *et al.* 1975
maltol	7100				fragrant, caramel	Keith and Powers 1968, Hodge 1967
furfural	8000	15000	65000	20000	almond	
5–methylfurfural	6000	16000	52000	45000	grilled almond	
furfuryl alcohol	1000	15000	35000	45000	mouldy hay	
5–methylfurf.alc.*						
vanillyl alcohol	>50000	>50000	>50000	>50000		Chatonnet *et al.* 1992c
furfuryl ethyl ether						
5–methylfurf.e.e.*						
vanillyl ethyl ether						
4–vinylguaiacol	32	130	440	380	pink pepper, clove	
4–ethylguaiacol	25	47	70	150	smoke, spice	
4–vinylphenol	85	180	770	1500	datura	
4–ethylphenol	130	440	1100	1200	horse, horse stable	

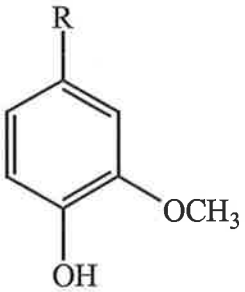
† Oak lactones = *cis*– and *trans*–β–methyl–γ–octalactone
* 5–methylfurf.alc. = 5–methylfurfuryl alcohol; and 5–methylfurf.e.e. = 5–methylfurfuryl ethyl ether.



cis-oak lactone

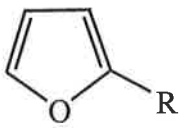


trans-oak lactone

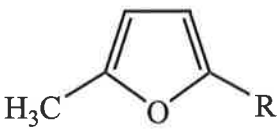


eugenol:	R = CH_2CHCH_2
guaiacol:	R = H
4-methylguaiacol:	R = CH_3
4-ethylguaiacol:	R = CH_2CH_3
4-vinylguaiacol:	R = $CHCH_2$
vanillin:	R = CHO
vanillyl alcohol:	R = CH_2OH
vanillyl ethyl ether:	R = $CH_2OCH_2CH_3$

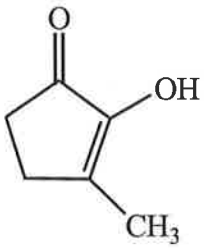
4-vinylphenol:	R = $CHCH_2$
4-ethylphenol:	R = CH_2CH_3



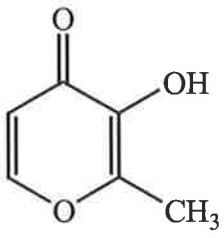
furfural:	R = CHO
furfuryl alcohol:	R = CH_2OH
furfuryl ethyl ether:	R = $CH_2OCH_2CH_3$



5-methylfurfural:	R = CHO
5-methylfurfuryl alcohol:	R = CH_2OH
5-methylfurfuryl ethyl ether:	R = $CH_2OCH_2CH_3$



cyclotene



maltol

Figure 1.1. Molecular structures of the 20 target-compounds.

1.4 Current oak wood usage choices, perceived consequences and apparent mechanisms

Oak wood extractive components have been found to vary considerably from one lot of wood to another, and this has been attributed to variation in tree species and age, growth conditions, seasoning, coopering heat, pre–use conditioning and previous use history for used barrels. However, much of the research which has led to these conclusions has not satisfactorily isolated each variable from confounding by other variables.

The considerable variability of wine composition, too, can play a part in the amounts of compounds extracted and the transformations which they can undergo. Such factors as alcohol content, pH, redox potential, temperature, and the influence of microorganisms have been cited as active in this regard and are discussed below. The function of contact duration, and the effect of barrel structure relative to the structural effects of non–barrel oak, *e.g.* oak chips, are also considered.

Oak wood source

For the purpose of cooperage, oak trees are felled and sawn into lengths of approximately 1.5 metres, corresponding to slightly more than barrel stave length. The cylinders of wood are then either split or sawn into rough stave shape and dried before the barrel making process. Non–barrel oak (*e.g.* chips) destined for use in winemaking comes from the same source but, since it does not need to possess the same structural characteristics, it may differ from barrel stave wood in some ways.

Oak wood variability may be defined in terms of structural and compositional differences but these things are hard to define and measure. More obvious differences, such as the forest from which the wood originated, have been targeted by coopers and winemakers when attempting to understand the variability of oak wood influence on wine composition and flavour. Many winemakers have particular preferences for barrels made of wood from certain locations, for their different wines, but there seems to be little consistent evidence regarding the relative merits of the various sources, and it seems likely that uncontrolled

variables such as seasoning and coopering conditions have contributed to the inconsistency of reports and variability of preferences.

Attempts at defining oak wood variability primarily by origin are numerous (*e.g.* Onishi *et al.* 1977, Rous and Alderson 1983, Marco *et al.* 1994). Indeed, this is not surprising given that barrels are constructed from different hybrids and species of the genus *Quercus* which grow under a variety of environmental conditions (Mosedale 1995, Schahinger and Rankine 1992 pp. 10–12).

The main species used in American cooperage is *Q. alba*, while those in France are *Q. robur* (= *Q. pedunculata*) which predominates in the south–west (*e.g.* Limousin), and *Q. petraea* (= *Q. sessiliflora* = *Q. sessilis*) which predominates in the centre (*e.g.* Tronçais, Nevers, Allier) and north–east of France (*e.g.* Vosges) (Puech and Moutounet 1990). Little is known of species variability as an influence on wine composition or aroma effect.

Miller *et al.* (1992) isolated species effects (*Q. alba* and *Q. robur*) from site effects for phenolic acids and aldehydes extracted from oak wood which was grown in Michigan, USA. However, oak wood age, a variable now known to affect some of the compounds quantified (Viriot *et al.* 1993), was not constant between species treatments. Of the compounds quantified, only vanillin is likely to contribute directly to wine aroma, and this compound increases substantially with the application of heat during coopering (Chatonnet *et al.* 1989), a process which was omitted from the experiment.

Mosedale and Savill (1996) identified an oak wood species effect for the oak lactones in German forests. With the experimental design balanced for site, seed origin and tree age, the *Q. petraea* wood yielded more of both oak lactone isomers than the *Q. robur* wood.

Characterisation of oak wood composition and sensory effects according to geographical origin are more numerous than characterisations based on botanical origin. Onishi *et al.* (1977), in analysing brandies matured in either French or American barrels, found that compounds arising mostly or entirely from oak wood (furfural, 5–methylfurfural, diethylsuccinate and *cis*– and *trans*–oak lactone) were more abundant in brandies from the American barrels. Aiken and Noble (1984), however, found no significant flavour difference

between American and Nevers oak wood–aged Cabernet Sauvignon wine in an experiment that involved controlled coopering.

There has generally been no distinction made within the USA regarding the forest from which cooperage wood is sourced; it has been referred to simply as American oak. On the other hand, French oak sources are clearly defined (Schahinger and Rankine 1992 pp. 10–11).

Sefton *et al.* (1993a) measured concentrations of eugenol, vanillin and the oak lactones in oak wood from four origins — America, Limousin, Tronçais and Vosges — over a period of three years open–air seasoning either in the country of origin or in Australia. The Tronçais and Vosges samples contained around twice the concentration of eugenol of the Limousin and American samples and these decreased on average by about two–thirds in the French woods but only slightly in the American wood over three years. Vanillin was observed at similar concentrations in all samples; these origin and seasoning variables showed little effect. The oak lactones showed increases and decreases, identified by these authors as being dependent on oak origin and seasoning time and location. The authors concluded that, for the constituents measured, oak origin was a more important source of variation than length or location of seasoning.

Oak lactone concentrations have often been the subject of attempts at typifying oak wood according to geographical definitions. Marco *et al.* (1994) found that most of their American oak wood samples were richer in the oak lactones than a variety of French samples, with the exception of those from Nevers which were comparable to the American samples. Chatonnet (1989), however, found Nevers samples to be relatively poor in the oak lactones, and suggested that, like grape quality, both genetic and environmental factors are important to the development of oak wood quality.

Waterhouse and Towey (1994) arrived at a conclusion using the statistical significance of a difference in the *cis*– and *trans*–oak lactone isomer ratio between American and French oak wood without consideration of the variance which these authors had acknowledged was present within at least one of their statistical units. Further, the treatment effects were not isolated from oak wood seasoning and coopering differences, each thought to be associated

with oak lactone concentration variations (Chatonnet *et al.* 1989, Sefton *et al.* 1993a). However, their conclusion was supported by data reported by Guichard *et al.* (1995).

Most of the norisoprenoids identified by Sefton *et al.* (1990b) were noted, by the same authors, to vary considerably between woods from different origins. The American oak contained "substantial quantities" of 3–oxonorisoprenoids compared with no detection or traces only in the Vosges oak. Conversely, a 4–oxonorisoprenoid, and β–ionone, a compound known to have a pleasant fruity aroma and very low sensory threshold, were found exclusively in the Vosges oak.

Some coopers are of the opinion that the origin of oak wood is a highly overrated factor, and that French coopers pay more attention to the general nature of the wood, *i.e.* whether it is course– or fine–grained (Deves 1988).

A large French cooperage company, Tonnellerie Vicard based in Cognac, has circulated recommendations (Anon. *c.*1989) to potential customers regarding the most appropriate type of oak wood and the corresponding firing level to use for various types of alcoholic beverages and the general characters to expect from these wood types. It points out that the recommendations are only valid for wood which is split, air–dried, and charred exclusively with an oak fire. The general guide was based on wood grain spacing. Tighter grain benefited from less firing and influenced the beverage more slowly and with more "finesse," and was more appropriate to lighter beverage styles such as dry white wine. Conversely, the more open grained wood benefited most from more severe firing and was most appropriately used for spirits, fortified wines or intensely flavoured dry red wines. The open grained Limousin wood could be expected to "perfume" and colour wines rapidly and with little "finesse," the medium grained Nevers and Bourgogne wood could be expected to give a vanilla flavour and balance to wines, and the tight grained Tronçais and Allier wood could be expected to release their "perfumes" slowly and with "finesse."

The grain nature of oak wood is correlated with tree growth rate. A faster growing tree will produce wood which has more widely spaced annual growth rings and a higher proportion of summer to spring wood (Singleton 1974). Slowly grown oak, which produces less summer wood and therefore is made up of a relatively high proportion of spring growth

wood, was found to be richer in extractable phenols than rapidly grown oak (Singleton 1974). The lower density wood of spring growth is intrinsically more permeable to liquids than the wood of summer growth due to larger cell size so this sort of wood may allow greater wine infiltration and increased extraction. Further, low density wood, by allowing greater infiltration of a wine into the wood cell spaces, may expose relatively high amounts of the cellulose and hemicellulose structures of the cell walls and relatively low amounts of the lignin structure which binds the cells. These structural components of wood are known to be the sources of different organoleptically important compounds.

To further complicate attempts at measuring variability in oak origin effects, there is some evidence of within–tree variability. Peng *et al.* (1991) and Viriot *et al.* (1994) have noted higher concentrations of soluble ellagitannins in the heartwood nearest the sapwood compared with that nearer to the pith. Maga (1989b) reported that coopers commonly select wood from various parts of the tree to make a more or less tannic barrel. Hemicellulose which provides structural integrity to wood can be present in high amounts when the tree is under stress (Maga 1989b). The fact that non–barrel oak (*e.g.* chips) may be obtained from tree sections structurally unsuitable for the construction of barrels may account for some of the perceived differences in effect between such oak and barrels.

Maga (1989b) stated that the mineral content of wood is dependent on soil conditions. Therefore and since mineral ions such as copper and iron are well known for their catalytic ability in oxidation reactions (Chang 1988 p. 915), soil mineral content may indirectly influence the potential for wine oxidation provided by oak wood. Litchev (1989) has reported the accumulation of dissolved iron in oak aged brandy over five years.

Finally, wood moisture and acid variability may have some influence on volatile compound formation during firing. Schmidt and Kerner–Gang (1986) have reported a macerated wood solution pH to be around pH 5. Ames (1990) has reported that pyrazine formation increases greatly in cooked foods with pH increases, especially above pH 5, and Baltes (1988) has suggested that lactic aldehyde, thought to be a precursor of the potently sweet and toasty cyclotene, is formed when D–fructose is cooked in an alkaline medium. These pH effects may be important in oak wood during the heating involved in coopering.

In addition to influencing the course of pH mediated reactions, wood moisture variability is likely to influence the degree of thermal degradation realised for a given quantity of heat applied. Some energy will be diverted from this course as the moisture is converted to steam. Largely a function of seasoning conditions, wood moisture variability may, thus, influence the potential aroma effect of oak wood.

Seasoning

The period of time between oak tree felling and coopering involves a drying process which is referred to as seasoning. This drying may be a natural process, involving open–air storage for a period commonly between one and three years, and/or an artificial process, involving controlled temperature and humidity conditions. The drying process is important to the resultant structural integrity of the barrels. It prevents the wood from shrinking after construction, thus ensuring a tight container. This aspect is, obviously, not important to non–barrel oak wood (*e.g.* chips). Any aroma and flavour effects of seasoning, however, should be equally important to all oak wood used in winemaking. The concentration of oak–derived flavour volatiles can change considerably as a result of the drying process and/or other factors during this period (Sefton *et al.* 1993a, Vivas 1993, Chatonnet *et al.* 1994b). Francis *et al.* (1992) found that seasoning oak wood for 12 months resulted in aroma changes from 'spicy' to those that are 'more distinct and intense.'

Puech (1987) stated that natural wood drying plays an important part in the flavour influence on the final product but that artificial drying does not. He claimed that natural drying affects the variety and concentration of flavour–impact compounds and that artificial drying only affects the moisture level. Despite this, the use of artificial drying of oak wood for cooperage is wide–spread. In the USA, all bourbon barrels are made with such wood, and much of the cooperage wood in France is at least partially dried artificially (Schahinger and Rankine 1992 pp. 23–34). Hoey and Codrington (1987) have reported the practice of a cooper from the Mâconais area of France who used hot water dipping (80 °C for 30 minutes) before firing, in combination with a relatively short seasoning period (two years). It was the opinion of Hoey and Codrington (1987) that this dipping may ameliorate any inadequacies of the seasoning.

Marche and Joseph (1975) reported that biochemical transformations can occur during oak wood seasoning. They suggested that enzymes which are naturally present or released from fungi are active catalysts in hydrolytic and oxidative reactions. These enzymes were not denatured by natural seasoning and were extracted into ethanol solutions. They suggested that the oxidases are particularly important to wine aroma, being active in lignin degradation and in the formation of vanillin. However, Chatonnet *et al.* (1994b) have argued that lignin–degrading fungi only develop in unusually wet conditions, and that a more important mechanism involves the hydrolysis and chemical oxidation of the terminal units of lignin.

Vivas (1993) also suggested that fungal enzymes can be important to the development of the flavour potential of seasoning oak wood staves but that their role is limited by the drying process. Some enzymes were also active in the condensation and polymerisation of wood tannins but kiln drying and firing during coopering denatured them (Marche and Joseph 1975). Chatonnet *et al.* (1994a) found that the fungal colonisation of staves less than five years old is only significant in the first few millimetres of the wood and that, as a result, the enzymatic modification of tannins seems only to be able to affect the external parts of the stave (0 – 4 mm). Further, any impact is minimised by the removal of surface wood by planing during the coopering process.

Chatonnet *et al.* (1994b) found only traces of the oak lactones in green (freshly harvested) oak, followed by increases during the seasoning period. These results are in agreement with those of Maga (1989a) who observed steady increases in these compounds in American oak seasoned under shelter for five years. By contrast, Sefton *et al.* (1993a) observed an irregular development, partially dependent on the conditions of seasoning (Australia versus France or the USA).

Open–air seasoning is an inconsistent treatment. The variability of temperature and moisture, in particular, probably have significant roles to play in determining oak wood composition. Sefton *et al.* (1993a) found that, generally, *cis*–oak lactone concentrations in the French woods were significantly lower in the France seasoned samples compared with those in the Australia seasoned samples. They also noted differences in seasoning location

on the loss of eugenol from the French oak. The same wood, seasoned for 12 months, showed that Australia–seasoning resulted in more intense aromas (Francis *et al.* 1992).

Presumably, such an effect can be explained in terms of natural conditions such as temperature and humidity. Whatever the physical conditions are that affect this variability, with data showing up to ten–fold differences between oak lactone levels in woods seasoned in various locations, when the levels at the green stage were similar (Sefton *et al.* 1993a), there seems to be no doubt that they are important.

Coopering

Once the barrel staves have been seasoned, they may be processed into barrels by machine–shaping, bending (usually with the aid of steam) and firing (toasting) to stabilise their bent shape. These processes result in compositional changes within the wood which can significantly influence wine flavour (Chatonnet *et al.* 1989). Francis *et al.* (1992) heated oak wood in an oven at 175 °C for two hours and demonstrated that this enhanced 'caramel,' 'nutty' and 'cedar' aromas in a model wine, while diminishing a 'raisin' aroma.

Variation of coopering 'toast' level — from 'low' to 'very high' — may be useful in causing optimal levels of oak lactones and volatile phenols (Chatonnet *et al.* 1991). The concentration of many other compounds could also be manipulated during coopering.

Progress in this regard has been impeded by the inconsistent nature of the coopering process. The extent of barrel heating is determined entirely empirically. Chatonnet and Boidron (1990) have reported wide differences in the heating conditions used by different coopers. Furthermore, barrels made by any one cooper can differ in the degree of heating even if some attempt is made at uniformity of treatment (Schahinger and Rankine 1992 p. 35).

Some researchers have reported conditions supposedly typical of those experienced by oak wood during coopering. In one experiment, Chatonnet and Boidron (1990) reported that, once the heating was complete, the temperature at the wood surface was between 200 and 230 °C. They suggested, however, that it is only with a closed barrel (heating with one head

in place), regulated by periodic humidification, that sufficient and homogenous thermodegradation is possible, and that it is only to a depth of 6 mm that the heat is sufficient for such degradation. Thus, the temperature experienced at the oak wood surface seems not to be the only variable of interest.

Schahinger and Rankine (1992) have provided a useful manual on the construction, maintenance and use of oak barrels which contains a suggestion that the application of a 'low fire' leads to the best results. Up to eighty or ninety minutes of this treatment was recommended for a 'heavy toast.' The low fire kept in place for an extended period is thought to cause significant heating below the wood surface, additional to that caused at the surface.

The carbohydrate–derived oak wood volatiles have been found only in low levels from non–heated oak but in high levels from toasted oak (Nishimura *et al.* 1983, Chatonnet *et al.* 1989, Sefton *et al.* 1990a). Reazin *et al.* (1976) noted that furfural increased with increasing toasting level. Chatonnet *et al.* (1991), however, reported that highest quantities of furans resulted from medium to high levels of firing, compared with low and very high levels. Although the thermal degradation of carbohydrates creates large amounts of furan aldehydes, these compounds are unstable and usually have little sensory impact in wines (Chatonnet and Boidron 1990) but may have some impact in spirits. This instability is apparently most notable if fermentation is conducted in the barrel (Chatonnet *et al.* 1991).

Also derived from carbohydrate degradation during coopering are maltol and cyclotene (Nishimura *et al.* 1983) but there are no reports on their variability under these conditions.

Maga (1985) observed pyrazines and pyridines in charred oak, and Sefton (1991) suggested that they are presumably formed from the thermal reaction of carbohydrates with amino acids (the Maillard reaction).

Lignin can be degraded by heat and hydrolysis (Puech 1981, Nishimura *et al.* 1983), and Reazin (1981) suggested that during charring some of the lignin is transformed into a form which is more reactive with ethanol, leading to the encouragement of the formation of

aromatic aldehydes. This degradation process involves the evolution of volatile phenols based on the guaiacyl or syringyl nucleus (Sefton *et al.* 1990a).

Nishimura *et al.* (1983) found that the aromatic aldehydes produced from toasting or charring oak chips increased as the heat treatment of the oak wood increased from 100 to 200 °C and then rapidly decreased when charring occurred. Chatonnet *et al.* (1991), similarly, found that highest levels of volatile phenols resulted from barrels which were subjected to high toast levels, compared with those which were subjected to low, medium and very high levels. Volatile phenols, phenyl ketones and phenolic aldehydes varied according to length of heating (Chatonnet and Boidron 1990).

Two of the volatile phenols, guaiacol and 4–methylguaiacol, which are likely to contribute to smoky aromas (Wittkowski *et al.* 1992), are only present in trace amounts in non–toasted oak wood (Sefton *et al.* 1990a) but they increase during coopering from 'low' through to 'high' toast (Chatonnet *et al.* 1989). Sefton *et al.* (1990a) recorded only trace amounts of these compounds in oven heated (175 °C, 2 hr) oak wood, and inferred that they are only formed at temperatures higher than 175 °C and that they are likely to be also derived from the smoke associated with firing barrels with oak wood off–cuts. Fiddler *et al.* (1967) have shown that guaiacol may be produced by heating ferulic acid to 240 – 260 °C.

Wittkowski *et al.* (1992) have reported that when abundant oxygen is available, wood combustion temperatures are generally higher than 400 °C, and that many important flavour reactions can occur at these temperatures while occurring insignificantly at the reported barrel surface temperatures of 200 – 230 °C. At temperatures between 230 and 260 °C, guaiacol, isopropylguaiacol, eugenol, isoeugenol, vanillin, acetovanillone, and methyl– , ethyl– , and vinyl–guaiacol were produced (Wittkowski *et al.* 1992). The vinylphenols also originated from guaiacol intermediates around these temperatures, and 4–ethylphenol was a major product of 4–ethylguaiacol at 390 – 420 °C (Connors *et al.* 1980). Thus, it seems likely that the combustion of oak wood fires over which barrels are 'toasted' — and, possibly, the combustion of the barrel inner–surface, if charring is practiced — may result in the production of appreciable amounts of these compounds, modifying the flavour potential of a barrel.

Vanillin is present in seasoned oak wood in moderate quantities (Chatonnet *et al.* 1994b) but coopering to 'low' or 'medium' 'toast' resulted in substantially increases (Chatonnet *et al.* 1989), and 'high toast' results in lower quantities.

There are conflicting reports regarding the effect of heating oak wood on the amounts of oak lactones subsequently extracted. Marsal and Sarre (1987) have reported that approximately half of the level of total oak lactones were extracted from a maceration of toasted oak compared with that extracted from a maceration of non–toasted oak. Similarly, Chatonnet *et al.* (1991), using a white wine stored in barrels for nine months, found that oak lactone levels decreased by about a half, as barrel toast levels increased from 'light,' through 'medium' and 'high,' to 'very high.' In contrast, Maga (1989a) showed, through a well controlled experiment, that a model alcoholic beverage extracted total oak lactones from charred oak samples at levels around three times those similarly extracted from non–charred samples. Further, Chatonnet *et al.* (1989) demonstrated in two experiments — one using barrels and the other using oak pieces in an oven — that both oak lactone isomers were present in higher amounts in either 'medium' or 'high' toasted wood, relative to non–toasted and 'low' toasted. 'Very high' toasting resulted in complete losses. The results of Conner *et al.* (1993), which show that the charred inside surface of a new bourbon barrel contained no oak lactones and that the highest concentrations were found at 5 mm (*cis* isomer) and 15 mm (*trans* isomer) below this surface, are in agreement with these observations.

Chatonnet and Boidron (1990) reported that heating oak wood resulted in steady reductions of the amounts of extractable hydrolysable tannins. This was presumed, by them, to have an important sensory effect in that the astringency would be reduced.

Conditioning

Following seasoning and coopering, conditioning of oak wood is sometimes practiced. This process can involve steam–, water–, caustic– or acid–washing, sulfur dioxide treatment or the practice of fermenting in new barrels, and is intended to modify the sensory effect that the oak will have on wine. Oak barrel users sometimes fill new barrels with water to check

for and prevent leaks (Schahinger and Rankine 1992 p. 40). This practice can also be considered a conditioning process with regard to the alteration of a barrel's potential influence on wine composition and flavour.

Hoey (1986) reported that it was most common for Californian winemakers to condition new oak barrels by fermentation, for Bordeaux winemakers to use hot water (80 – 90 °C) or steam for 3 to 6 minutes, and for Australian winemakers to use either of these techniques. Peynaud (1981) recommended that, additional to the use of boiling water or steam, soaking the wood "for some time" in a weak sulfite solution or storing a medium quality wine in the new barrel "for a few days" are techniques which may be suitable for conditioning barrels.

Towey and Waterhouse (1996) have suggested that gaseous sulfur dioxide, used to sanitise barrels prior to use or between uses, may increase the rate of oak lactone extraction from oak wood.

Tannins, probably the compounds most targeted by conditioning processes, are easily extracted from plant material with hot water (Deschamps 1989). Along with tannin losses, wash conditioning can also result in significant losses of desirable aroma compounds (Hoey and Codrington 1987). The wash temperature and duration that resulted in sufficient removal of compounds responsible for excessive astringency but that minimised the loss of desirable aroma compounds, for each barrel type, were determined by these authors. German oak required generally only half the conditioning time of Limousin oak.

Preparatory fermentation is sometimes used to condition barrels prior to their use in premium wine maturation, but this practice, for practical reasons, is usually not applied to oak chips. Oak chips used during fermentation are usually removed from the wine after only days or weeks of contact because it is convenient to do so, coincidentally with pressing or lees separation (*e.g.* racking). These 'used' chips, however, might have been improved by the process in the same way that a fermentation–conditioned barrel might have been.

Chatonnet *et al.* (1991) have reported that burnt oak wood contains considerable amounts of furan aldehydes and that these compounds are unstable during fermentation, being reduced by yeast to furan alcohols. They added that fermentation seemed not to affect the

extraction of the oak lactones. Thus, while fermentation–conditioning seems unlikely to affect the potential impact of the oak lactones, it seems likely that such conditioning can reduce the potential of charred oak wood to contribute compounds such as furfural and 5–methylfurfural to wines. The same is also probably true of vanillin (Wackerbauer *et al.* 1978).

The processes involved in oak wood conditioning during wine fermentation are not well understood and subject to some disagreement. Chatonnet *et al.* (1991) have reported the possibility of limiting the organoleptic impact of oak by fermenting in barrels since it could be expected to result in "transformation and adsorption of the aromatic and polyphenolic compounds on the yeast cell walls and on the glycoproteins resulting from their degradation." Extracted oak (hydrolysable) tannin compounds may not, however, be of any substantial sensory importance. Pocock *et al.* (1994) have reported data that suggest that the taste effect of oak wood–derived tannins in wine is, at most, only subtle at the levels commonly found.

The sensory effects of the presence of wood during wine fermentation may have little to do with the biochemical and physical impact of the microorganisms. Alternate influences, coincidental to the presence of microorganisms, include vigorous CO_2 sparging. Various low molecular weight compounds such as oak wood–derived acetic acid are likely to be partially removed by this gas flow.

The difference in the composition of the medium during the early stages (principally, the low ethanol concentration) may also exert an influence. Since ethanol can considerably inhibit the extraction of tannins from oak wood (Chatonnet *et al.* 1991), the low ethanol concentrations present during the early stages of fermentation may cause high rates of tannin extraction.

It would be interesting to test for differences between a fermentation and a water soaking of similar duration with corresponding increases in ethanol concentration and sparging with CO_2. The difference in effect between a water pre–treatment and a fermentation–conditioning might depend, primarily, on the difference in the duration of contact between the oak and the low ethanol solution.

Wine microbiology

Wine microorganisms are thought to play roles in modifying the aroma impact of oak wood in various ways. As discussed above, fermentation conditioning of barrels is practiced by some winemakers. They use grape juice destined to become wine of a quality category lower than that of the grape juice or wine to follow it into the barrel, on the assumption that the process removes some undesirable components from the wood. On the other hand, some winemakers use fermentation in new barrels for their best quality wines.

In 1986 there seemed to be strong opinion among some Australian winemakers that fermentation in barrel, as opposed to post–fermentation storage in barrel, resulted in different and better wines (Anon. 1986). Some of them were quoted as being of the opinion that fermentation in barrel resulted in a "superior integration of wood and wine," in a "more balanced sweet creamy oak character," in "distinctive aroma and flavour components," and that malolactic fermentation in barrel resulted in "quite distinctive smoky, clove–like characters."

There is evidence (Boidron *et al.* 1988, Marsal *et al.* 1988, Chatonnet *et al.* 1990) of fermentation in barrel modifying oak extractives, and of microbial activity after fermentation — during wine maturation in barrel — leading to further increases in compounds of microbial origin.

Marsal (1987) noted that furfuryl alcohol was "never a genuine oak substance," only being present after microbial activity, and Marsal *et al.* (1988) reported that the yeast *Saccharomyces cerevisiae* was able to reduce furfural to furfuryl alcohol, that this occurred naturally in new oak barrel wine fermentations, and that the reaction continued to occur after fermentation at a slower rate by the action of the yeast lees (presumably enzymatic activity).

Chatonnet *et al.* (1991) have measured the reduction in levels of furan aldehydes in a biomass–present, barrel matured wine and in a biomass–absent, barrel matured wine. They noted that concentrations decreased more rapidly in the wine with biomass present, and

conjectured that the enzymatic activity responsible for the reduction of the furan aldehydes was largely associated with the yeast cell walls, though some enzymes were probably released to the exocellular medium. They found that the reduction product of 5–hydroxymethylfurfural was not present after barrel fermentation in their experiments, although those of furfural and 5–methylfurfural were present. The furan aldehydes are not likely to have a major sensory effect since they have high flavour thresholds (Boidron *et al.* 1988).

As mentioned above, with regard to barrel conditioning, Chatonnet *et al.* (1991) have reported that the concentrations of the oak lactones seem not to be affected by fermentation.

Wines placed in barrels after the completion of fermentation have been found to contain higher levels of vanillin than wines fermented in barrels (Chatonnet *et al.* 1991). Steinke and Paulson (1964) reported that in beer the main metabolic product of vanillin is methylguaiacol, but Wackerbauer *et al.* (1978) have reported the total transformation in beer of vanillin to vanillyl alcohol. Omori *et al.* (1968) confirmed that yeast can demethoxylate vanillin to form *p*–hydroxybenzoic acid, *p*–hydroxybenzaldehyde and vanillic acid. Also, ferulic acid was converted to *p*–hydroxybenzoic acid and vanillic acid. They suggested that vanillin might be formed as an intermediate during the degradation of ferulic acid. The transformation products of vanillin in wine have been studied in detail by Chatonnet *et al.* (1992c).

Chatonnet *et al.* (1991) have shown that the reduction process for the phenolic and the furan aldehydes can be prevented by heating the medium (autoclaving for 10 minutes at 115 °C).

Chatonnet *et al.* (1992b, 1993, 1995) have described the mechanisms for the occurrence of the vinylphenols (4–vinylguaiacol and 4–vinylphenol) and the ethylphenols (4–ethylguaiacol and 4–ethylphenol) in white, rosé and red wines. As mentioned previously, the precursors for these generally–undesirable compounds are derived from oak wood only in small quantities relative to those derived from grapes (Miller *et al.* 1992). The two vinylphenols arise in white wines, in important concentrations, as products of *Saccharomyces cerevisiae*

during alcoholic fermentation (Chatonnet *et al.* 1993). The concentrations are partially dependent on precursor availability and yeast strain (Chatonnet *et al.* 1993). The vinylphenols are present in red wines in only small concentrations due to the presence of catechic tannins which inhibit the activity of the enzyme responsible for decarboxylation of the precursors (Chatonnet *et al.* 1993).

The occurrence of the ethylphenols (4–ethylguaiacol and 4–ethylphenol) requires the activity of particular microorganisms. Chatonnet *et al.* (1995) have demonstrated that yeast species belonging to the genus *Brettanomyces* and to its sporogenous form *Dekkera* are principally responsible for the synthesis of these compounds in wine. These microorganisms are rarely active in white wine. Consequently, the ethylphenols are found in significant concentrations usually only in red wines. Cavin *et al.* (1993) have shown that some *Lactobacillus* and *Pediococcus* (lactic acid bacteria) species can also transform hydroxycinnamic acids into ethylphenols but not to the extent achieved by *Brettanomyces/Dekkera* (yeast) species (Chatonnet *et al.* 1995).

Chatonnet *et al.* (1991) suggested that the greater richness of barrel fermented wines in soluble polysaccharides, compared with those in tank fermented / barrel stored wines, was due to the greater surface for exchange between yeast and medium that fermentation in barrel allowed. The longer contact time allowed between wine and yeast lees in barrels, due to the relatively more oxidative conditions in barrels compared with those in tanks (avoiding the occurrence of reduced sulfur compound aromas), may also contribute to the difference.

Apart from the possibility that enzyme systems may retain some activity after cell death and cause transformations of compounds into less aromatic or less 'woody' products, Chatonnet *et al.* (1991) suggested that there may also be bonding between yeast cell wall components, or mannoproteins released from cells, with some oak wood–derived compounds such as the volatile phenols or the oak lactones.

Lubbers *et al.* (1994) investigated the influence of mannoproteins, derived from yeast cell walls during alcoholic fermentation, on the volatility of some aroma substances in a model wine. They demonstrated that certain volatile compounds, particularly β–ionone and ethylhexanoate, were partially fixed to yeast–derived mannoproteins, and suggested that the

extent of interaction depended on the nature of the volatile compound and on the protein content of the macromolecule.

Kinsella (1990) has speculated that the nature of any affinity between proteins and volatile compounds is likely to be characterised by hydrogen bonds and hydrophobic interactions, and Calleja (1987) has argued that the same intermolecular forces are likely to participate in yeast cell flocculation.

Lubbers *et al.* (1994) also noted that some macromolecules can *increase* the volatility, and therefore the sensory impact, of some compounds. Sugar monomers or polysaccharides in aqueous systems were cited as capable of this effect. This might involve a modification of the coefficients of activity of the volatile compounds or the sugar molecules might compete for adsorption sites on the cells. Douglas (1987), in a review of yeast adhesion to surfaces, has cited medical research which demonstrated the inhibitory effect of some sugars on the adhesion of some non–wine yeast and bacteria to mammalian epithelial cells.

Calleja (1987) has noted the importance of the ionic composition of the medium to inter–cell adhesion. Calcium (Ca^{2+}) ions, in particular, have been implicated in brewer's yeast flocculation. It seems that the possibility of volatile compound adsorption to microbial cells, or to macromolecules released from them, might involve an interplay of the ionic and sugar composition of the medium, as well as, obviously, the structure and composition of the volatile compounds, the exocellular macromolecules and the cell wall components.

Wine composition and conditions

Ethanol concentration has been found to affect the extraction of certain oak wood compounds. Nykänen *et al.* (1985) found that various volatile and non–volatile oak wood constituents were extracted most efficiently in a 60 % aqueous ethanol solution. Total extract and total phenols were extracted at half this efficiency at 100 % and at three–quarters this efficiency at 10 %. Maga (1989a), in testing extractability of 0, 10, 20, 40 and 60 % ethanol solutions, found that oak lactones were extracted most completely in the 40 % solution. Chatonnet *et al.* (1991) reported that the extraction of ellagitannins decreased with increases in ethanol concentration.

Little has been reported regarding the effect of pH on oak wood extraction in alcoholic beverages. Maga (1989a) reported that the extraction of the oak lactones was more complete at pH 3.5 than at either a lower value (pH 2.5) or a higher one (pH 4.5). Phenol oxidation in wine is thought to proceed at a rate around nine times higher at pH 4.0 than at pH 3.0 (Singleton 1990). Thus the many oxidative reactions involving wood phenols are likely to decrease with decreasing wine pH.

The possibility of extracting various groups of flavour compounds from oak wood and/or variously affecting acid hydrolysis reactions under different pH conditions may have potential in a practical sense. Storing beverages at altered pH levels with later adjustment, preparing extracts for addition, or preconditioning oak wood with a solution of a particular pH are some examples. Indeed, Maga (1989b) has reported such applications in the brandy industry.

Baldwin *et al.* (1967) determined that lignin isolated from bourbon was structurally different to native oak lignin. They called it *ethanol–lignin*. Their proposition was that, under the acidic conditions of whisky, ethanol can react with the lignin of oak to form the ethanol–soluble form of lignin, and that, through oxidative reactions, many volatile compounds, such as sinapyl alcohol, coniferyl alcohol, syringaldehyde, vanillin and coniferaldehyde, can then be produced. It may be that pH variability can have some effect on the rate of these reactions.

The temperature of the medium in which extraction and transformation reactions occur could, obviously, also affect composition. Maga (1989b) has reviewed numerous articles that report the use of artificially raised beverage temperatures to accelerate brandy maturation. Reazin (1981) found that for whisky stored in barrels at 18, 19, and 23 °C, congener levels generally increased with increasing storage temperature. Nykänen *et al.* (1985) reported that around 2 to 3 % more of total extract was released from oak chips at 30 °C than at 20 °C (in 62 % alcohol over five months).

Beverage temperature increases will also influence the diffusion–evaporation of compounds from a barrel as the partial pressure of these compounds will rise as the temperature rises (Onishi *et al.* 1977).

Wood–wine contact duration

The accumulation of volatile oak compounds in wine during oak barrel maturation can depend on many factors. These include the size of the pool of volatiles and their precursors contained within the matrix of the wood, the rate of release of these compounds from the wood, the rate at which subsequent transformations take place, as well as the temperature and the duration of maturation.

Until recently, data on the rate of accumulation of oak wood–derived volatiles in wine were scarce. Puech (1987) has recorded the accumulation of vanillin in four individual barrels of Cabernet Sauvignon and Merlot. Chatonnet *et al.* (1990) have reported data (means of three barrels) for the evolution of several oak wood–derived volatiles in red wine stored in new oak barrels over a ten month period. They observed concentration increases for some volatile phenols and for the oak lactones but not for the furan aldehydes or alcohols.

More recently, Towey and Waterhouse (1996) have published a detailed study of the evolution of ten oak wood–derived volatiles in Chardonnay wines fermented and matured on lees for eight months in French, American or Hungarian oak wood barrels over three successive vintages. Their study focussed on the two oak lactone isomers, furan aldehydes and alcohols, and a group of volatile phenols, but did not include vanillin. The extractions were described as being 'diffusion–controlled' because the curves showed an exponential approach to a limit. The second year extraction rates were lower than the first year rates, particularly for the compounds arising most substantially from the coopering process. These compounds are presumably depleted most rapidly since they are initially concentrated at the wood surface. Interestingly, the oak lactones were extracted in higher quantities in the second year, compared with either years one or three. These authors suggested that this may have been caused by the action of sulfur dioxide gas, which was used for sanitation between fills, on precursors of the oak lactones.

In all of the above studies, the wines were subject to microbial activity which can influence the concentration of some, though not all oak volatiles. Thus, for some of these volatiles at least, accumulation profiles are not dependent solely on extraction rates, but are determined also by subsequent transformations.

Maga (1989a) reported that extraction of oak lactones from oak wood was nearly linear over a period of 32 months. In variously toasted barrels, however, there may be differences in the rate. Chatonnet *et al.* (1991) in comparing 'low,' 'medium,' 'high' and 'very high' 'toast' barrels, noted that oak lactones were initially extracted most quickly from the 'medium toast' barrels but more quickly from 'light toast' barrels as contact time progressed to two months. They presumed that higher levels of the oak lactones were created in a superficial layer on the 'medium toast' barrel through the action of greater intensity and duration of heat, and that these lactones were extracted quickly over the first four weeks.

This explanation is feasible. Conner *et al.* (1993) found that charring the inside surface of a bourbon barrel resulted in the complete loss of the oak lactones at that surface but also that concentration maxima for these compounds were found just below the surface. Of the 5 mm depth intervals tested, to 25 mm, the *cis*– and *trans*–isomers were most concentrated at a depth of 5 mm and 15 mm, respectively. These regions of high oak lactone concentration which were apparently created by the heat applied for charring would probably be found closer to the inside surface for a more moderately heated barrel.

Chatonnet *et al.* (1991) reported that the extraction of oak wood–derived volatile compounds is continuous throughout the whole wine maturation period and that the presence of biomass reduces the rate. In the longer maturation time of whisky, however, Reazin *et al.* (1976) found that the ethanol–lignin content increased over the first two years and then remained constant for the following two.

To achieve an adequate oak aroma effect, oak chips are often used in higher quantities, relative to that provided by the inner–surface of a barrel, and for shorter periods than barrels. It is convenient, for example, to ferment red wines in contact with loose oak chips

and then to remove them during pressing. Such practices, however, may influence the relative sensory effects of the two systems through differential rates of compound extraction.

It is likely that the disproportionality in extraction rates among aroma compounds could be increased with oak chip usage. Extraction rates for those compounds requiring action such as acid hydrolysis for release from barrels are likely to experience only small increases due to increased area of contact between oak wood and wine. Those compounds that are more easily extracted from barrels (*e.g.* by simple diffusion) are likely to experience more substantial increases since the most important limiting factor for them is probably the thoroughness of contact between oak and wine, and not the rate of chemical reaction.

Wine aeration

Singleton (1995) questioned the popular notion that atmospheric gasses pass into wine through wet barrel staves. While not refuting that dissolved oxygen levels in barrels usually are higher than those in tanks, he has speculated that these levels may result principally from aeration during topping or from passage past a loose bung or, if a relatively large ullage is allowed to develop, through the dry staves towards the top of the barrel. Whatever the mechanism of oxygen introduction to barrel–aging wine, its presence is certainly important. Non–coopered oak wood (*e.g.* chips) does not allow aeration except for the small amount of gases entrapped within the wood.

The greater oxygenation allowed by barrels has been considered by some winemakers to be slow or controlled in some way and, therefore, better for wine quality than any other method of oxygenation. With the appropriate care, however, it should be possible for tank wine to be aerated to the same oxygen concentration as is common for barrel matured wine. In such cases, the effects of oxygen, alone, should be similar to those occurring in barrel.

Ribéreau–Gayon and Glories (1987) suggested that tank–wine maturation could mimic barrel–wine maturation by dissolving oxygen in the wine during rackings, and Ribéreau–Gayon *et al.* (1983) have reported that intense aerations in tank do induce the same chemical reactions as the mild aerations associated with barrel storage. Hoey and

Codrington (1987) have reported that aerated tank wine was more similar, analytically and organoleptically, to barrel–matured wine than was non–aerated tank wine.

Where the oxidising effect allowed by oak *is* different to that allowed in aerated tanks is in the oxidation catalysis provided by oak constituents. These catalysts can just as easily come from oak wood chips as from barrels. Evidence for the catalytic role of some oak compounds in oxidation reactions was provided by Nishimura *et al.* (1983) in whisky and by Chatonnet *et al.* (1991) in wine. In response to the observation that sulfide odours arise less commonly and less intensely in barrel compared with those in tank fermentations, Chatonnet *et al.* (1991) showed that the total removal of selected reduced sulfur compounds from a model wine solution required a *combination* of oxygen with either oak tannin or gallic acid, an hydrolysis product of oak tannin. Additionally, Litchev (1989) has suggested that inorganic oxidation catalysts, released from the wood, may be important.

Further to the prevention or removal of sulfide odours, the oxidation allowed by oak may be important to the presence of many oak wood–derived aroma and flavour compounds or their precursors. Maga (1989b) has suggested that most of the oak wood lignin degradation reactions are oxidative, and he has reviewed various proposals involving the combination of immersed oak wood and oxygen for accelerated wood aging of brandy. Maarse and van den Berg (1989) have attributed the presence of vanillin, syringaldehyde, coniferaldehyde, sinapaldehyde, and the acids — gallic, protocatechuic, syringic, vanillic, ferulic, *p*–coumaric, *p*–hydroxybenzoic, cinnamic and ellagic to oxidative reactions. However, the oxidative generation of these compounds may be limited in the relatively less oxidative environment of most barrel–aging wines.

Ribéreau–Gayon and Glories (1987) suggested that it was possible to modify the oak wood–derived compound oxidation reactions by either adjusting the dissolved oxygen level or the sulfite level — decreases in the dissolved oxygen level or increases in the sulfite level were reported by them to slow the reactions.

The vinylphenols (4–vinylguaiacol and 4–vinylphenol), which often arise in white wines during the course of alcoholic fermentation (Chatonnet *et al.* 1993), have been shown by Nicolini *et al.* (1991) to oxidatively degrade during maturation. This degradation occurred

more rapidly in barrel–stored wine compared with tank–stored wine. Thus, the greater aeration allowed by barrel–storage can more rapidly diminish the 'phenolic' or 'pharmaceutic' odours of these compounds. Dugelay *et al.* (1995) have shown that the vinylphenols can also be depleted in wine by a slow acid catalysed addition of ethanol, forming 4–(1–ethoxyethyl)–guaiacol and 4–(1–ethoxyethyl)–phenol.

Other barrel structure effects

The structure of barrels can influence wine aroma and flavour in ways other than by aeration. Wine components can be lost to the atmosphere, due to passage through barrel staves, and some may be entrapped in the wood after oxidative polymerisation. The differential nature of these effects can cause substantial changes in wines — there may be significant losses of some compounds, and concentration of others.

Onishi *et al.* (1977) have described the oak stave as a diffusion barrier across which ethanol, water and other compounds can move. The factors affecting these diffusions are the partial pressure differences across the barrier and the resistance offered by the barrier. The vapour pressures of ethanol and the other volatile beverage compounds in the atmosphere are negligible so their movement is not affected by atmospheric humidity. These compounds, then, are affected only by diffusion resistance across the barrier. Onishi *et al.* (1977) summarised the factors affecting loss by diffusion–evaporation as temperature, container size (surface area to volume ratio), relative humidity, molecular size and stave thickness. The variable natural porosity of wood and the presence of impeding layers, are also likely to influence the resistance to diffusion.

Yoshizawa *et al.* (1981) have reported widely different proportional losses of flavour compounds through barrel staves from a whisky model system. After one year, 32 % of the acetaldehyde but only 13 % of the ethanol had been lost despite their similarity in molecular size (their molecular weights are 44 and 46, respectively). (It is size rather than weight that is of most importance but molecular weight is to be used, here, as a rough guide to size.) The relative polarities of these compounds (acetaldehyde < ethanol) may have been important. Only 1 % of a third compound, acetic acid, was lost from this system, perhaps due to the diffusion resistance caused by the high polarity of this acid when in dissociated

form. Additional diffusion resistance may have resulted from the greater molecular size of acetic acid (molecular weight = 60).

Whether any of the oak wood–derived aroma compounds are small enough and lack polarity sufficiently to pass through barrel staves has not been reported. The twenty oak derived or associated aroma compounds discussed in this thesis range in molecular weights from 96 to 182 — all higher than acetic acid which was 99 % retained by barrel staves over one year. However, molecular polarity differences may be of some importance in this comparison.

The nine compounds measured by Yoshizawa *et al.* (1981) can be grouped according to the functional groups present in the molecules, conferring some idea of the molecular polarity. Within each of these groups, the losses were greatest for the compounds of lowest molecular weight. This is true of the alcohols (four compounds) and the esters (three compounds).

If the 20 oak wood–derived or associated aroma compounds discussed in this thesis do pass from wine to atmosphere, those which pass most quickly probably do so at a slower rate than acetaldehyde which was 68 % retained over one year. All of these compounds contain only hydrogen, carbon and oxygen atoms and many contain aldehyde or alcohol functional groups so the atomic affects on molecular polarity can be expected to be similar among the compounds. The molecular polarity effects of the other functional groups listed must also be considered — some will increase and some will decrease the molecular polarity, dependent on the particular functional group and its position within the molecule. Notwithstanding these effects, molecular size is likely to be relatively important in determining the rates of passage through the barrel stave for these compounds. Consequently, losses greater than around 10 % over one year are unlikely for most of the compounds.

Techniques using oak chips do not incorporate this aspect of differential compound dilution and concentration found to be active during barrel maturation. Consequently, acetaldehyde and possibly some other wine components could be expected to be found in higher concentrations in aerated and oak chip treated tank wine than in barrel–aged wine. There is another factor to consider, however. Barrels appear to allow a general concentration of

aroma compounds caused by the loss of both ethanol and water through the staves. Thus, the relativity of tank and barrel concentrations will depend, at least partially, on the relative rates of these processes.

Barrels may also influence wine composition by entrapping large molecules within the matrix of the wood. This could occur following wine absorption, contact with the high concentrations of oxygen found in the wine–atmosphere interface region, and oxidative polymerisation. Considering that there is a portion of wood nearest the inside surface of the barrel which is saturated with wine, Singleton (1995) argues that there must be a region of contact between wine and atmosphere within the stave at which some oxidation reactions may be expected to occur more substantially than elsewhere in the barrel. This would not be of any particular interest, here, if all of the oxidation products were returned to the main body of the wine. The occurrence of a brown stain (probably oxidised phenolic compounds) in this region, however, suggests that some of the polymers resulting from reactions in this region may be entrapped and do not return. Singleton (1995) reported that examples of this sort of stain have been seen in wood from old wine casks — presumably after cutting the staves perpendicularly to the plane of wine movement. This process, if it does occur, could contribute to perceived differences of effect between barrels and alternative oak wood such as chips.

Previous use and reconditioning of barrels

Oak barrels used for wine production can vary in the above–mentioned ways when new. When already used for this purpose, however, the additional factor of the history of previous use (mainly use–duration) contributes to the variability of oak influence. Compounds of sensory importance are depleted with use and the surface may become partially sealed with foreign matter or populated with flavour–altering microorganisms.

Hoey (1986) considered that the useful life of a barrel, with regard to its potential to contribute organoleptic qualities to table wines was between four and six years. Similarly, but with regard to the contribution of phenolic extractives, Rous and Alderson (1983) suggested that the useful life of a barrel was four years. Barrels at Robert Mondavi Winery in California, however, are reputedly used for seven years but with at least one shaving

(Hoey 1986). Thus, the effective life of barrels can be extended by removing the 'heads' (barrel ends) and shaving the inner surface of the barrel staves to remove foreign material and expose wood which is less exhausted of flavouring material. The 'heads' can be replaced with new wood at the same time. Various additions such as oak planks or chips can also be made to the barrels, as they are to tanks. The use of oak chips in old barrels, a technique which may have some merit, is used by some winemakers (Schahinger and Rankine 1992 p. 101).

In addition to the loss of extractable compounds from the wood it seems that the decline in usefulness of barrels may also be due to increases in the microorganism population in the wood. Chatonnet *et al.* (1990) have observed that the levels of 4–ethylguaiacol and 4–ethylphenol formed in barrel–stored wines increases with increasing barrel use. As discussed above, these compounds, generally regarded as undesirable in wines, are formed by the microbial conversion of ferulic acid and *p*–coumaric acid. Chatonnet *et al.* (1990) suggested that shaving and scrubbing used barrels can significantly reduce the levels of these compounds in wines subsequently matured in such barrels.

Loss of hydrolysable tannins from barrels with time has been shown to play an important part in the decline in barrel usefulness (Chatonnet *et al.* 1991). It seems that the depletion of hydrolysable tannins, which act as oxidation catalysts, slows the course of colour stabilisation, palate 'softening' (tannin polymerisation), and impacts upon the course of various flavour–altering reactions that normally proceed during barrel maturation.

Another factor affecting changes in barrel character over the duration of its use may be that deposits coming from the beverages can form a barrier between wood and beverage, impeding the movement of compounds from one to the other. Thus, rejuvenation involving shaving, scrubbing or similar processes may be effective in removing such a barrier, removing excessive quantities of absorbed compounds, and exposing a fresh layer of wood which can contribute higher amounts of organoleptically important compounds.

1.5 Experimental design

The research reported in this thesis forms part of a project which was instigated by the wine industry of Australia. The importance of oak wood as a component of many Australian wines and the variability in its character and quality have provided the impetus.

Aim

Some aspects of oak selection and handling in the wine industry have been poorly understood and controlled. Thus, unintended aroma variations are often found among barrels. The aim of the project was to characterise some of the variability of oak wood–derived wine aroma commonly encountered by Australian winemakers. By describing this variability and relating it to natural and cultural variables (the treatment effects and the composition variability underlying them), opportunities for improving the wine aroma outcome of oak wood selection and processing might be identified.

One of the fundamental choices available to winemakers is that of the geographical origin of the oak. However, oak wood selection, based only on origin, bundles numerous natural variables (genetic and environmental) into one. Such choices are relatively simple but, unfortunately, they can lead to unreliable results. The identification of compositional parameters that may be indicative of the aroma potential of any oak wood, as it has naturally developed, may allow a more efficient approach.

Treatment imposition

Since the second world war, Australian winemakers have predominantly used either American or French oak in the production of red and white table wines (Schahinger and Rankine 1992 p.3). The American oak wood has usually been traded as a generic product while the French oak wood has usually been traded as a regionally identified product. To reflect this, one American oak source and three French sources were included in the experiment. The oak used for the barrels was harvested from Ohio in the USA, and from the Tronçais forest and the Vosges and Limousin regions of France (Appx. A).

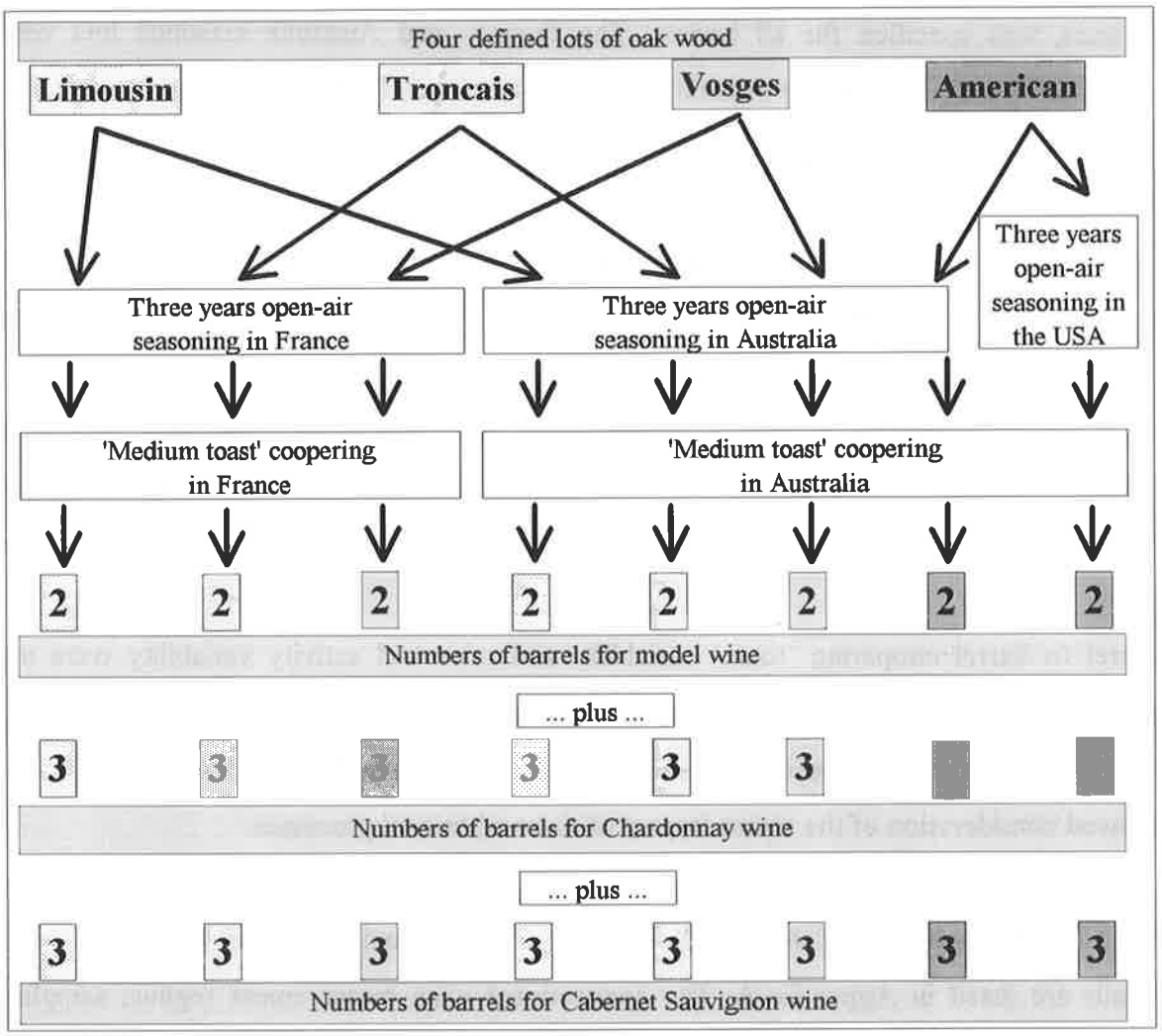


Figure 1.2. Oak wood source, seasoning, coopering and usage.

The seasoning processes for the Australian wine industry are usually carried out either in America, France or Australia, so after the wood within each of the four lots was mixed, half of each was seasoned in the country of origin while the other half was shipped to and seasoned in Australia (Appx. A). All lots were open–air seasoned for three years (Fig. 1.2).

A 'medium toast' coopering, consisting of 45 minutes of slow toasting over a fire of oak off–cuts, was specified for all barrels. The France– and Australia–seasoned lots were coopered by a French and an Australian cooper, respectively. The Australian cooper also coopered the America–seasoned oak.

Since each of the four oak wood origin treatments was subject to two different seasoning locations, a total of eight treatments resulted. Eight 300 L barrels were constructed from each of these eight oak lots (total of 64 barrels), three to be used for a Chardonnay, three for a Cabernet Sauvignon and two for a model wine (Fig. 1.2). Five other Australian wineries received a similar set of barrels for evaluation but the resultant wines were not subject to the detailed analysis described in this thesis so they are not discussed.

Barrel–to–barrel coopering 'toast' variability and microbial activity variability were not intended. However, during the course of the experiment, compounds indicative of the extent of heating or of the degree of microbial activity in wine varied significantly, and allowed consideration of the aroma impact of these additional processes.

The Chardonnay and the Cabernet Sauvignon vinification, and the model wine concoction, details are listed in Appendix A. The conventional wine measurement regime, sampling times, bottling and storage details are also listed there.

1.6 Conclusion

The project upon which his thesis is based commenced around a decade ago, and early research focussed mainly on changes occurring in the wood during the seasoning period (Sefton *et al.* 1990b & 1993a, Francis *et al.* 1992, Pocock *et al.* 1994). The oak origin and seasoning location treatments imposed during the first few years were subjected to commercial winery processes (*i.e.* barrel fermentation, wine type and storage duration treatments), and the resultant wines became the focus of the latter stages of the project. This thesis primarily describes the aroma and volatile composition effects and the underlying variabilities which arose from these treatments.

Chapter 2

The volatile composition

Chapter outline

2.1 Introduction

Why expend considerable effort quantifying the volatile compounds that are extracted from various oak wood treatments by wine when it is the aroma effect that is of most interest? It is the high efficacy and precision of the volatile composition analysis, relative to those of the sensory analysis, that has recommended its inclusion in the study. Provided that the target–compounds either contribute to the aromas or that they are quantitatively representative of those that do, composition analysis can yield useful data. Once aroma patterns are associated with compositional patterns, a relatively quick and easy compositional analysis of a particular oak wood may be able to indicate some likely wine aroma consequences of its use. Thus, cooperage and winery quality assurance programs could be optimised.

2.2 Volatile composition analysis

Compositional analysis of the 20 target–compounds in the Chardonnay, Cabernet Sauvignon and model wines was carried out using gas chromatography–mass spectrometry (GC–MS) after continuous liquid–liquid extraction with Freon F11 (similar to that described in Wilson *et al.* 1984). Details of the analytical materials and methods employed are in Appendix B. The extraction method chosen was one which allowed the quantification of all 20 compounds in a single analysis. It was a compromise from using a variety of analyses which may have yielded more accurate data but would not have allowed the nearly 2½ thousand quantifications performed in the time available.

Vanillin was initially analysed along with the other 19 compounds using this method. While the analysis for this compound was acceptably precise for the model wines, it was not so for the 'real' (Chardonnay and Cabernet Sauvignon) wines. Recoveries of vanillin, relative to the internal standard, were low (often less than 20 %) and variable. It is possible that losses occur due to acetal formation with wine glycols during the extraction of wines with organic solvents and during the subsequent concentration of these organic extracts. Such reactions would not take place with aqueous ethanol extracts which do not contain such glycols.

Problems in measuring vanillin in wines are implied by the omission of data on this compound from several key studies of oak wood–derived wine volatiles (Boidron *et al.* 1988, Chatonnet *et al.* 1990, Towey and Waterhouse 1996). As a result of the poor accuracy and precision experienced, a new method, involving stable isotope dilution analysis and GC–MS, was developed by Pollnitz and others in this laboratory, and then used to quantify vanillin in the wines (Spillman *et al.* 1997). The advantage of this method is that the internal standard is virtually identical chemically to the target–compound, and therefore the accuracy and precision of the analysis are not reduced by inefficiency in isolation or by analyte decomposition.

2.3 Quantification methods, confidence intervals and limits of detection

Raw quantification data

Each wine extract analysed by GC–MS resulted from a process including an internal standard addition to the wine sample prior to liquid/liquid (wine/Freon) extraction, subsequent evaporation of the Freon and its replacement with methylene chloride, and concentration of the methylene chloride solution to approximately 1 mL (Appx. B.1). A second internal standard (for the Cabernet Sauvignon wine only) was added to this concentrated solution when, after the Chardonnay and model wine analyses were complete, this methodological improvement was identified (Appx. B.1). A 'standards mix' (methylene chloride solution of weighed quantities of purified compounds, Appx. B.2) was also subject to GC–MS on each day of analysis.

The apparent concentration of most of the 20 target–compounds in each wine extract (methylene chloride solution) was calculated with reference to four chromatogram peak areas of specific fragment ions (Appx. Tab. B.2): one for each the internal standard (Appx. B.1) and the target–compound in both the wine extract and the corresponding day's 'standards mix.' The ratio of the target–compound fragment ion peak area to the internal standard fragment ion peak area in the 'standards mix' yielded an estimate of the relative responsiveness of the analytical system to each fragment ion, dependent on small GC–MS system condition variations from day to day. The corresponding ratio in each extract, along

with the known relative concentrations corresponding to three of the four peaks, allowed the calculation of the apparent concentration associated with the fourth peak. The apparent concentration was later transformed to an actual concentration with reference to standard recovery experiments (described below).

Whenever a compound was not present in a particular 'standards mix,' the apparent quantity was calculated with reference to a random selection of the GC–MS responses achieved for that compound in other 'standards mixes' or samples on different occasions, and then the quantification was completed with reference to the standard recovery data. Some compounds (cyclotene, maltol, furfural, 5–methylfurfural, furfuryl alcohol, vanillyl alcohol and vanillyl ethyl ether) were not available for the initial sample analyses but were later available for the standard recovery experiments.

For the compounds that never appeared in a 'standards mix' (furfuryl ethyl ether, 5–methylfurfuryl ethyl ether, 5–methylfurfuryl alcohol, 4–ethylguaiacol and 4–vinylphenol), a one–to–one response ratio between the total ion area for each compound and the internal standard was assumed. Thus, although relative concentrations were detected, there may have been systematic over– or under–estimations of the absolute values.

Data transformation according to standard recoveries

Separate to the process described for the acquisition of the raw (apparent concentration) data, above, standard recovery experiments were carried out to determine the recovery efficiency of each compound, relative to that of the internal standard. Purified compounds were added to non–oak stored wines, in a range of concentrations (Tabs. 2.1, 2.4 & 2.6).

Since each apparent concentration was determined from the analysis of a pair of extracts, the standard addition regression analyses were based on means of pairs of standard additions. Thus, ranges of standard addition pairs of each compound were made to the stainless steel–stored Chardonnay (control) wine, the stainless steel–stored Cabernet Sauvignon (control) wine and to a freshly concocted model wine, and these were extracted and analysed identically to the experimental samples. A regression line and estimates of accuracy and precision were calculated for each, following Miller and Miller (1988 pp. 109–

115). Tables 2.1, 2.4 and 2.6 show these details for the Chardonnay, the Cabernet Sauvignon and the model wines, respectively. Each *apparent* concentration was transformed to an actual concentration by reference to these equations. Thus, the standard recovery experiments allowed the accuracy of the quantifications to be optimised. They also allowed an estimation of precision for each compound (95 % confidence intervals, described below).

Standard recovery experiments were performed for 16 of the 20 target–compounds (5–methylfurfuryl alcohol, furfuryl ethyl ether, 5–methylfurfuryl ethyl ether and 4–vinylphenol were not initially available as purified standards). These experiments were conducted in all three wines for 14 of the compounds. A similar experiment was conducted for vanillin (stable isotope dilution method) only in the Cabernet Sauvignon wine, and for 4–ethylguaiacol only in the model wine.

The concentrations of some of the compounds found in the barrel–stored Chardonnay wines (*i.e.* 4–ethylphenol, Tab. 2.1) or model wines (*i.e.* vanillyl alcohol, vanillyl ethyl ether, 4–vinylguaiacol and 4–ethylphenol, Tab. 2.6) were very low and did not correspond to the standard addition ranges. Only those compounds found in a range corresponding approximately to the range of concentrations used in the standard recovery experiments were quantified with reference to the regression line equations. The remainder were quantified directly from the chromatogram peak areas, assuming an equivalent extraction recovery of target–compound to internal standard. The accuracy of these determinations is unknown but the precision implied by the consistency of the duplicate determinations (data not shown) suggests that the quantities may be used to compare samples within this study.

Table 2.1. Chardonnay wine standard addition recovery experiment data, quantification information, confidence intervals and limits of detection.

Compound	Addit[n] range (µg/L^a)	*n*	r^2	Slope	*y*–int.[1] (µg/L^a)	Conc. range[2] (µg/L^a)	N°. conc by extrap.[3]	95 % CI[4] (µg/L^a) barrel mean	barrel range	cont. at 55(11)wks	Limit of detect.[5] (µg/L^a)
cis–oak lactone[†]	0–580	11	0.970	1.49	-8	42–415	0	106	105–109	26(60)	58
trans–oak lactone[†]	0–398	11	0.966	1.92	-29	21–190	0	79	77–81	21(50)	50
eugenol	0–26	10	0.971	1.64	1[§]	7–26	0	6	6–6	2(6)	3
guaiacol	0–27	10	0.979	2.41	-2	3–28	1	4	4–5	1(3)	3
4–methylguaiacol	0–12	10	0.972	2.30	-1	1–10	0	2	2–3	1(1)	1
vanillin[‡]	50–1000	7	0.999	1.00	248[‡]	51–388	0	33	31–35		n.dn.
[vanillin–Freon[‡‡]*	0–1.0*	12	0.784	0.15	0.0*	0.1–1.6*	2	0.5*	0.5–0.7*	0.1(0.3)*	0.3*]
cyclotene	0–246	14	0.874	1.89	56[§]	6–246	0	101	98–103	37(102)	103
maltol	0–200	11	0.973	1.43	-3	19–139	0	35	34–35	15(36)	35
furfural*	0–5.0*	7	0.969	1.23	0.0*[§]	0.1–8.7*	2	1.6*	1.6–2.1*	0.7(1.6)*	1.6*
'est ext furfural'*[Σ]						1.8–14.7*[Σ]		3.8*[Σ]	3.7–4.1*[Σ]	0.8(1.9)*	1.8*[Σ]
5–methylfurfural*	0–1.21*	10	0.957	1.80	-0.10*	0.06–0.61*	0	0.29*	0.29–0.30*	0.08(0.18)*	0.19*
furfuryl alcohol*	0–10.5*	9	0.976	1.19	-0.3*	1.3–13.4*	3	2.1*	2.1–2.5*	0.1(0.3)*	0.2*
5–methylfurf.alc.[#]						17–41		n.dn.	n.dn.	n.dn.	n.dn.
vanillyl alcohol	0–119	7	0.959	0.14	0	1–162	2	41	40–48	19(41)	43
furfuryl ethyl ether						25–164		n.dn.	n.dn.	n.dn.	n.dn.
5–methylfurf.e.e.[#]						0–58		n.dn.	n.dn.	n.dn.	n.dn.
vanillyl ethyl ether	0–283	7	0.999	0.98	3[§]	5–23	0	16	16–16	7(16)	15
4–vinylguaiacol	0–180	7	0.991	0.85	32[§]	5–146	0	27	26–27	16(26)	30
4–ethylguaiacol						0–5		n.dn.	n.dn.	n.dn.	n.dn.
4–vinylphenol						7–844		n.dn.	n.dn.	n.dn.	n.dn.
4–ethylphenol	0–636	7	0.990	1.41	0	0–1		n.dn.	n.dn.	n.dn.	n.dn.

[1] *y*–intercept.

[2] The range of concentrations found in the barrel–stored Chardonnay wines (33 determinations: 9 at 11 weeks and 24 at 55 weeks). Zero values do not imply any absolute absence, nor do they imply that no concentration was detected; they are simply products of rounding.

[3] The number of concentration determinations based on extrapolation rather than interpolation of standard recovery data (out of totals of 33).

[4] 95 % confidence intervals of each quantification were calculated following Miller and Miller (1988 pp. 112–115). The mean and the range of 33 CIs, calculated for each compound in the barrel–stored wines, are quoted. Additionally, the 95 % confidence intervals for the control wine at 55 weeks are listed since they were determined using a different method (the method of standard additions, Miller and Miller 1988 pp. 117–120), and the values differ substantially from those of the barrel–stored wines. The 95 % confidence intervals for the control wine at 11 weeks are also listed (in parentheses).

[5] Limits of detection (93 %) (LODs) were calculated following Miller and Miller (1988 pp. 115–117). These are the concentrations at which the probabilities of erroneously concluding either compound presence or absence both equal 7 %. Each compound with a suitable standard addition subset range was subject to LOD calculations using that subset (*n* minus the 3 or 4 highest additions). Each of these range–subsets corresponds, approximately, to the lowest half of the addition range for the compound (lowest 20 % of range for furfuryl alcohol). The LOD for furfural, vanillyl alcohol, vanillyl ethyl ether and 4–vinylguaiacol were calculated using all seven standard additions.

* Vanillin (Freon extraction method), furfural, 'estimated extracted furfural' (furfural + furfuryl alcohol), 5–methylfurfural and furfuryl alcohol concentration–related values are in mg/L.

[†] The oak lactones (*cis*– and *trans*–β–methyl–γ–octalactone) were added as a racemic mixture. The relative amounts of the two isomers were determined with reference to the relative chromatogram peak areas.

[‡] For the stable isotope dilution analysis method, standard additions of vanillin were made to a barrel–stored Cabernet Sauvignon wine. Thus, for quantification of the Chardonnay wines using this method, the *y*–intercept was taken to be zero.

[‡‡] The method using Freon extraction for vanillin determination in the barrel wines was abandoned, and replaced by a stable isotope dilution analysis method. The control wine was not analysed using the new method.

[Σ] Values obtained by addition.

[#] 5–methylfurf.alc. = 5–methylfurfuryl alcohol; and 5–methylfurf.e.e. = 5–methylfurfuryl ethyl ether.

: Concentration values not based on a standard recovery experiment. Such an experiment was either not performed or the standard addition range was not suitable.

n.dn. = not determined.

[§] The quantity in the control wine (*y*–intercept/slope) was added in the calculation of the barrel wine concentrations, *i.e. y*–intercept values above zero are attributed to the presence of the compound in the control wine. When the control wine quantity was added in this way, the variance contributed by the control wine was incorporated into the confidence interval calculation.

Table 2.2. Chardonnay wine volatile composition at 55 weeks.

Barrel code	cis[1] (µg/L)	trans (µg/L)	eug (µg/L)	guai (µg/L)	4mg (µg/L)	van (µg/L)	cyc (µg/L)	malt (µg/L)	furf (mg/L)	eef (mg/L)	5mf (mg/L)	falc (mg/L)	5mfa (µg/L)	valc (µg/L)	fee (µg/L)	5mfee (µg/L)	vee (µg/L)	4vg (µg/L)	4eg (µg/L)	4vp (µg/L)	4ep (µg/L)
control	24	4	1	0	0	8	29	5	0.0	0.1	0.00	0.0	17	0	2	0	3	37	0	156	0
AU4	42	21	10	7	3	303	13	73	0.1	8.5	0.08	8.4	21	1	70	58	6	8	1	18	0
AU6	84	24	13	9	3	264	13	80	0.4	6.6	0.08	6.2	22	1	132	2	5	8	1	15	0
AU70	113	46	13	8	3	268	53	74	0.1	6.1	0.06	6.0	20	1	89	17	6	8	1	13	0
AA10	106	32	11	7	3	359	98	109	1.6	5.9	0.20	4.3	30	19	131	31	9	9	1	22	0
AA11	73	30	12	15	5	388	137	139	6.6	11.3	0.61	4.7	26	1	112	18	12	10	2	23	0
AA22	219	46	19	6	2	298	157	85	0.2	4.4	0.08	4.2	28	1	85	35	10	12	0	28	0
FL3	212	154	22	6	4	298	55	45	0.6	2.1	0.09	1.4	20	1	43	4	8	10	2	19	0
FL4	180	150	19	11	6	314	28	58	4.1	8.8	0.41	4.7	29	1	155	25	13	20	3	60	0
FL5	82	69	16	7	7	328	16	52	3.1	6.2	0.40	3.0	24	1	102	1	16	23	1	62	0
LA27	212	129	11	4	2	232	173	43	1.3	3.5	0.15	2.2	36	1	124	1	15	22	0	37	0
LA34	148	80	16	9	5	348	119	60	3.6	6.6	0.32	3.0	28	1	120	0	17	15	1	34	0
LA41	178	190	14	4	3	322	246	38	1.2	5.1	0.15	3.8	36	1	164	28	18	22	0	51	0
FT3	170	174	17	11	5	236	11	71	2.6	5.3	0.23	2.8	23	1	78	0	5	10	2	23	0
FT4	166	133	22	8	5	296	13	40	2.8	5.8	0.33	3.0	24	1	76	0	12	20	2	53	0
FT5	219	131	24	6	4	229	18	53	0.1	3.9	0.07	3.7	27	1	62	19	7	11	1	21	0
TA23	212	87	15	28	8	327	17	102	8.7	12.1	0.51	3.4	27	1	89	1	6	5	5	7	0
TA31	305	127	16	3	2	263	6	46	1.6	4.5	0.16	2.9	23	1	70	0	8	12	0	26	0
TA46	307	128	18	10	5	350	43	58	4.1	7.2	0.53	3.2	21	1	86	2	11	11	1	16	1
FV3	288	119	21	8	5	293	54	36	1.2	4.4	0.15	3.2	29	1	97	13	10	15	2	37	0
FV4	285	117	23	8	5	249	67	58	2.0	4.9	0.20	2.9	24	1	91	0	16	28	2	76	0
FV5	204	115	20	10	6	255	77	43	2.4	5.0	0.38	2.6	25	1	75	1	12	26	3	76	0
VA32	342	138	22	5	4	331	153	56	1.7	4.9	0.19	3.1	41	1	140	3	23	30	0	71	0
VA38	415	127	26	7	4	301	91	63	2.6	6.1	0.29	3.5	30	1	112	0	15	28	1	76	0
VA39	355	128	18	3	1	198	105	26	0.5	1.8	0.08	1.3	26	1	46	0	11	27	0	65	0

For barrel code meaning, see Appendix Table A.2.

[1] Abbreviations of compound names: cis=*cis*–oak lactone; trans=*trans*–oak lactone; eug=eugenol; guai=guaiacol; 4mg=4–methylguaiacol; van=vanillin; cyc=cyclotene; malt=maltol; furf=furfural; eef='estimated extracted furfural' (furfural + furfuryl alcohol); 5mf=5–methylfurfural; falc=furfuryl alcohol; 5mfa=5-methylfurfuryl alcohol; valc= vanillyl alcohol; fee=furfuryl ethyl ether; 5mfee=5–methylfurfuryl ethyl ether; vee=vanillyl ethyl ether; 4vg=4–vinylguaiacol; 4eg=4–ethylguaiacol; 4vp=4–vinylphenol; and 4ep=4–ethylphenol.

All figures have been rounded. Zero values do not imply any absolute absence, nor do they imply that no concentration was detected; they are simply products of rounding. The limit of detection has not been considered, and the precision implied by the units or the number of significant figures chosen is occasionally exaggerated: The appropriate rounding was performed only after further analysis. Refer to Table 2.1 for 95 % confidence intervals and limits of detection.

Table 2.3. Chardonnay wine volatile composition at 11 weeks.

Barrel code	cis[1] (µg/L)	trans (µg/L)	eug (µg/L)	guai (µg/L)	4mg (µg/L)	van (µg/L)	cyc (µg/L)	malt (µg/L)	furf (mg/L)	eef (mg/L)	5mf (mg/L)	falc (mg/L)	5mfa (µg/L)	valc (µg/L)	fee (µg/L)	5mfee (µg/L)	vee (µg/L)	4vg (µg/L)	4eg (µg/L)	4vp (µg/L)	4ep (µg/L)
control	21	4	1	0	0	51	2	4	0.0	0.0	0.00	0.0	6	1	0	0	1	188	0	992	0
AA10	87	30	9	8	4	102	29	135	1.8	10.7	0.48	8.9	32	162	41	32	14	101	1	651	0
AA11	55	27	7	12	7	103	23	108	2.1	12.3	0.43	10.2	21	44	38	27	9	82	2	386	0
AA22	126	33	10	3	2	84	12	24	1.1	9.4	0.27	8.3	19	43	34	26	8	90	0	433	0
FL3	133	87	13	7	6	106	23	29	1.9	7.3	0.33	5.4	17	71	25	20	8	146	3	716	0
FL4	102	77	9	9	6	86	15	19	1.4	12.2	0.47	10.8	24	41	49	40	9	103	2	562	0
FL5	52	42	7	6	6	99	25	19	1.2	8.7	0.36	7.6	22	74	40	30	11	94	2	509	0
FT3	138	123	12	12	7	51	14	40	1.2	14.7	0.34	13.4	21	24	59	52	9	111	4	844	2
FT4	144	101	16	13	10	81	27	132	1.1	11.4	0.36	10.3	35	152	48	36	14	62	5	640	0
FT5	152	92	15	9	6	60	33	22	0.7	12.2	0.28	11.5	22	87	47	45	9	108	2	747	0

For barrel code meaning, see Appendix Table A.2.

[1] See Table 2.2 for abbreviations of compound names and other notes.

Refer to Table 2.1 for 95 % confidence intervals and limits of detection.

Table 2.4. Cabernet Sauvignon wine standard addition recovery experiment data, quantification information, confidence intervals and limits of detection.

Compound	Addit^n range (µg/L*)	*n*	r^2	Slope	*y*–int.[1] (µg/L*)	Conc. range[2] (µg/L*)	N°. conc by extrap.[3]	95 % CI[4] (µg/L*) barrel mean	barrel range	cont.	Limit of detect.[5] (µg/L*)
cis–oak lactone[†]	0–580	13	0.999	0.80	3[§]	94–793	0	17	16–19	6	8
trans–oak lactone[†]	0–398	13	0.998	1.01	-1	20–381	0	16	16–17	6	1
eugenol	0–26	13	0.998	0.85	1[§]	17–52	15	2	1–2	1	2
guaiacol	0–27	13	0.997	1.03	5[§]	7–44	3	2	2–2	1	1
4–methylguaiacol	0–12.2	13	0.996	1.04	0.1[§]	0.8–15.5	5	0.8	0.8–0.9	0.3	0.4
vanillin[‡]	50–1000	7	0.999	1.00	248[‡]	121–369	0	32	31–34		n.dn.
[vanillin–Freon[‡‡]*	0–1.0*	13	0.958	0.12	0.0*	0.1–0.4*	0	0.2*	0.2–0.2*	0.1*	0.1*]
cyclotene	0–228	13	0.993	0.73	15[§]	2–59	0	21	21–21	8	10
maltol	0–200	13	0.995	0.54	32[§]	80–295	2	16	16–18	7	10
furfural*	0–10.01*	13	0.999	0.71	0.06*[§]	0.04–0.22*	0	0.34*	0.34–0.34*	0.11*	0.19*
'est ext furfural'*[Σ]						1.9–15.1*[Σ]		1.7*[Σ]	1.7–1.7*[Σ]	0.6*	0.8*[Σ]
5–methylfurfural	0–1210	13	0.997	0.65	0	9–217	0	71	70–71	25	14
furfuryl alcohol*	0–21.1*	13	0.996	0.59	0.1*[§]	1.8–14.9*	0	1.3*	1.3–1.3*	0.4*	0.6*
5–methylfurf.alc.[#]						7–19		n.dn.	n.dn.	n.dn.	n.dn.
vanillyl alcohol	0–238	13	0.962	0.13	2[§]	0–85	0	52	51–52	18	31
furfuryl ethyl ether						32–228		n.dn.	n.dn.	n.dn.	n.dn.
5–methylfurf.e.e.[#]						0–1		n.dn.	n.dn.	n.dn.	n.dn.
vanillyl ethyl ether	0–566	13	0.999	0.80	-1	3–5	0	18	18–18	6	2
4–vinylguaiacol	0–360	13	1.000	0.75	2[§]	1–3	0	7	7–7	2	3
4–ethylguaiacol						22–88		n.dn.	n.dn.	n.dn.	n.dn.
4–vinylphenol						1–5		n.dn.	n.dn.	n.dn.	n.dn.
4–ethylphenol*	0–1.27*	13	0.997	0.89	1.03*[§]	0.63–1.04*	24	0.10*	0.10–0.10*	0.06*	n.dn.

[1] *y*–intercept.

[2] The range of concentrations found in the barrel–stored Cabernet Sauvignon wines (24 determinations). Zero values do not imply any absolute absence, nor do they imply that no concentration was detected; they are simply products of rounding.

[3] The number of concentration determinations based on extrapolation rather than interpolation of standard recovery data (out of totals of 24).

[4] 95 % confidence intervals of each quantification were calculated following Miller and Miller (1988 pp. 112–115). The mean and the range of 24 CIs, calculated for each compound in the barrel–stored wines, are quoted. Additionally, the 95 % confidence intervals for the control wine are listed since they were determined using a different method (the method of standard additions, Miller and Miller 1988 pp. 117–120), and the values differ substantially from those of the barrel–stored wines.

[5] Limits of detection (93 %) (LODs) were calculated following Miller and Miller (1988 pp. 115–117). These are the concentrations at which the probabilities of erroneously concluding either compound presence or absence both equal 7 %. Those compounds with seven standard addition points below or near the lower end of the barrel–stored Cabernet Sauvignon wine concentration range were subject to LOD calculations using these seven standard additions. Each of these range–subsets corresponds, approximately, to the lowest 5 % of the addition range for the compound.

* Vanillin (Freon extraction method), furfural, 'estimated extracted furfural' (furfural + furfuryl alcohol), furfuryl alcohol and 4–ethylphenol concentration–related values are in mg/L.

[†] The oak lactones (*cis*– and *trans*–β–methyl–γ–octalactone) were added as a racemic mixture. The relative amounts of the two isomers were determined with reference to the relative chromatogram peak areas.

[‡] For the stable isotope dilution analysis method, standard additions of vanillin were made to a ***barrel–stored*** Cabernet Sauvignon wine. Thus, for quantification of the Cabernet Sauvignon wines using this method, the *y*–intercept was taken to be zero.

[‡‡] The method using Freon extraction for vanillin determination in the barrel wines was abandoned, and replaced by a stable isotope dilution analysis method. The control wine was not analysed using the new method.

[Σ] Values obtained by addition.

[#] 5–methylfurf.alc. = 5–methylfurfuryl alcohol; and 5–methylfurf.e.e. = 5–methylfurfuryl ethyl ether.

: Concentration values not based on a standard recovery experiment. Such an experiment was not performed for these compounds.

n.dn. = not determined.

[§] The quantity in the control wine (*y*–intercept/slope) was added in the calculation of the barrel wine concentrations, *i.e.* *y*–intercept values above zero are attributed to the presence of the compound in the control wine. When the control wine quantity was added in this way, the variance contributed by the control wine was incorporated into the confidence interval calculation.

Table 2.5. Cabernet Sauvignon wine volatile composition at 93 weeks.

Barrel code	cis[1] (μg/L)	trans (μg/L)	cug (μg/L)	guai (μg/L)	4mg (μg/L)	van–f[#] (μg/L)	van–c[#] (μg/L)	cyc (μg/L)	malt (μg/L)	furf (mg/L)	eef (mg/L)	5mf (μg/L)	falc (mg/L)	5mfa (μg/L)	valc (μg/L)	fee (μg/L)	5mfee (μg/L)	vee (μg/L)	4vg (μg/L)	4eg (μg/L)	4vp (μg/L)	4ep (mg/L)
control	4	0	3	4	0.1	29		21	60	0.08	0.2	0	0.2	5	13	2	0	0	3	23	3	1.16
AU7	265	49	28	18	4.4	267	148	25	150	0.12	6.9	54	6.8	8	14	72	0	4	2	34	2	0.69
AU8	109	20	20	7	0.8	121	80	25	124	0.06	1.9	14	1.8	13	0	41	0	3	2	69	5	0.98
AU9	157	39	20	8	1.3	193	95	2	80	0.04	2.5	9	2.5	7	0	32	0	4	1	30	2	0.84
AA36	215	51	17	8	1.5	206	113	2	109	0.06	2.9	20	2.8	17	85	81	0	4	3	67	4	0.84
AA40	236	55	24	16	4.5	280	183	14	172	0.04	5.0	24	4.9	10	21	81	1	5	2	54	3	0.77
AA48	186	46	24	14	3.7	269	157	49	175	0.04	7.3	16	7.3	7	0	57	0	4	2	26	2	0.83
NL6	175	136	28	12	8.2	251	126	15	193	0.09	3.9	42	3.8	8	0	52	0	4	1	28	1	0.63
NL7	119	50	24	23	9.7	265	115	38	179	0.09	5.2	65	5.1	11	0	67	0	4	1	31	1	0.89
NL8	94	76	20	26	11.8	321	148	39	154	0.18	8.9	97	8.7	11	30	89	0	4	1	43	2	0.96
LA23	328	199	21	10	5.2	324	159	9	98	0.08	4.0	37	3.9	12	19	67	0	3	2	53	1	0.69
LA30	274	219	29	16	8.1	291	212	17	148	0.10	7.3	32	7.2	13	0	105	0	4	2	52	2	0.71
LA38	128	90	25	16	9.9	369	177	16	106	0.09	8.1	33	8.0	7	0	83	0	4	1	28	1	0.63
NT6	421	381	41	20	11.5	331	154	33	179	0.06	6.0	44	5.9	12	36	93	0	4	2	69	3	0.92
NT7	330	307	52	20	7.7	242	177	20	187	0.08	3.8	49	3.7	8	25	37	0	4	1	27	2	0.79
NT8	302	262	40	32	12.4	246	131	38	168	0.14	7.0	74	6.9	9	0	68	0	4	2	46	2	1.04
TA8	484	197	46	19	6.3	318	143	16	132	0.13	9.0	217	8.9	8	0	97	1	4	2	22	2	0.69
TA25	681	221	38	19	6.0	340	169	14	128	0.21	8.9	205	8.7	8	8	88	0	3	1	29	1	0.70
TA39	330	171	33	44	12.6	308	154	56	256	0.18	12.4	128	12.2	8	0	109	0	4	1	24	1	0.69
NV6	615	262	46	14	5.2	192	91	22	160	0.04	2.6	46	2.6	19	13	75	0	3	2	88	4	0.93
NV7	453	276	44	23	13.0	290	125	27	159	0.09	5.7	143	5.6	7	25	83	0	4	2	37	1	0.80
NV8	336	233	46	27	12.5	253	173	24	173	0.09	5.2	54	5.1	13	49	76	1	4	2	69	4	1.02
VA12	483	308	33	35	15.5	354	178	59	295	0.22	15.1	76	14.9	10	4	228	0	4	1	43	2	0.66
VA21	793	199	46	17	6.7	303	197	18	187	0.13	7.1	52	7.0	9	0	73	0	3	2	30	2	0.76
VA27	694	177	42	12	6.8	315	161	23	151	0.13	7.6	62	7.5	8	16	66	0	4	2	38	2	0.74

For barrel code meaning, see Appendix Table A.4.

[1] See Table 2.2 for abbreviations of compound names and other notes.
Refer to Table 2.4 for 95% confidence intervals and limits of detection.

[#]: *van–f*=vanillin from freezer–stored samples (-10 °C for 3 years since barrel sampling); *van–c*=vanillin from cellar–stored samples (~20 °C for 1 year from barrel sampling, then sterilised with DMDC and stored for a further 2 years at ~20 °C). The sensory analyses were performed on the cellar–stored samples approximately 1 year after sterilisation.

Table 2.6. Model wine standard addition recovery experiment data, quantification information, confidence intervals and limits of detection.

Compound	Addit^n range (µg/L^)	*n*	r^2	Slope	*y*–int.[1] (µg/L^)	Conc. range[2] (µg/L^)	N°. conc by extrap.[3]	95 % CI[4] (µg/L^) barrel mean	95 % CI[4] (µg/L^) barrel range	Limit of detect.[5] (µg/L^)
cis–oak lactone[†]	0–278	8	0.987	1.29	-6	30–544	15	70	69–79	8
trans–oak lactone[†]	0–190	8	0.980	1.59	-5	8–299	5	70	69–72	4
eugenol	0–63	8	0.994	1.21	-1	5–43	0	5	5–5	3
guaiacol	0–64	7	0.969	1.25	-2	2–47	0	14	14–15	4
4–methylguaiacol	0–24	8	0.974	1.12	0	0–24	0	5	5–5	1
vanillin[‡]	50–1000	7	0.999	1.00	248[‡]	77–856	0	32	30–34	n.dn.
[vanillin–Freon[‡‡]*	0–1.1*	8	0.984	1.39	0.0*	0.0–0.6*	0	0.1*	0.1–0.2*	0.2*]
cyclotene	0–26	8	0.969	1.36	-1	1–169	39	11	5–29	2
maltol	0–102	8	0.953	1.42	-9	10–154	11	25	22–35	4
furfural*	0–6.1*	8	0.908	1.22	0.0*	0.9–21.7*	29	2.7*	2.0–6.6*	0.3*
'est ext furfural'*[Σ]						0.9–23.2*[Σ]		3.7*[Σ]	3.0–7.6*[Σ]	0.8*[Σ]
5–methylfurfural*	0–0.70*	8	0.944	1.27	-0.01*	0.06–2.16*	40	0.25*	0.18–0.50*	0.03*
furfuryl alcohol*	0–2.2*	8	0.866	1.05	0.0*	0.0–7.2*	7	1.1*	1.0–2.7*	0.5*
5–methylfurf.alc.[#]						0–9		n.dn.	n.dn.	n.dn.
vanillyl alcohol	0–222	4	0.997	0.65	-3	0–12		n.dn.	n.dn.	n.dn.
furfuryl ethyl ether						0–129		n.dn.	n.dn.	n.dn.
5–methylfurf.e.e.[#]						0–27		n.dn.	n.dn.	n.dn.
vanillyl ethyl ether	0–564	8	0.980	1.29	-10	0–10		n.dn.	n.dn.	n.dn.
4–vinylguaiacol	0–356	8	0.979	1.05	-18	0–5		n.dn.	n.dn.	n.dn.
4–ethylguaiacol	0–22	5	0.991	0.90	0	0–5	0	4	4–4	3
4–vinylphenol						0–0		n.dn.	n.dn.	n.dn.
4–ethylphenol	0–1264	5	0.997	1.10	-6	0–5		n.dn.	n.dn.	n.dn.

[1] *y*–intercept.
[2] The range of concentrations found in the barrel–stored model wines (58 determinations or 72 for vanillin). Zero values do not imply any absolute absence, nor do they imply that no concentration was detected; they are simply products of rounding.
[3] The number of concentration determinations based on extrapolation rather than interpolation of standard recovery data (out of totals of 58 or 72 for vanillin).
[4] 95 % confidence intervals of each quantification were calculated following Miller and Miller (1988 pp. 112–115). The mean and the range of 58 CIs, calculated for each compound, are quoted.
[5] Limits of detection (93 %) (LODs) were calculated following Miller and Miller (1988 pp. 115–117). These are the concentrations at which the probabilities of erroneously concluding either compound presence or absence both equal 7 %. Those compounds with four standard addition points below or near the lower end of the model wine concentration range were subject to LOD calculations using these four standard additions. Each of these range–subsets corresponds, approximately, to the lowest 10 % of the addition range for each compound. The LOD for 4–ethylguaiacol was calculated using all five standard additions.
* Vanillin (Freon extraction method), furfural, 'estimated extracted furfural' (furfural + furfuryl alcohol), 5–methylfurfural and furfuryl alcohol concentration–related values are in mg/L.
[†] The oak lactones (*cis*– and *trans*–β–methyl–γ–octalactone) were added as a racemic mixture. The relative amounts of the two isomers were determined with reference to the relative chromatogram peak areas.
[‡] For the stable isotope dilution analysis method, standard additions of vanillin were made to a barrel–stored Cabernet Sauvignon wine. Thus, for quantification of the model wines using this method, the *y*–intercept was taken to be zero.
[‡‡] The method using Freon extraction for vanillin determination was abandoned due to poor precision when analysing the Chardonnay and the Cabernet Sauvignon wines. The precision was acceptable when analysing the model wines. Nevertheless, the new method, involving stable isotope dilution analysis, was applied to the model wines, also.
[Σ] Values obtained by addition.
[#] 5–methylfurf.alc. = 5–methylfurfuryl alcohol; and 5–methylfurf.e.e. = 5–methylfurfuryl ethyl ether.
: Concentration values not based on a standard recovery experiment. Such an experiment was either not performed or the standard addition range was not suitable.
n.dn. = not determined.

Table 2.7. Model wine volatile composition at five sampling times from 6 to 93 weeks — (a) to (u): each of the 20 compounds and 'estimated extracted furfural.'

For barrel code meaning, see Appendix Table A.10.
All figures have been rounded. Zero values do not imply any absolute absence, nor do they imply that no concentration was detected; they are simply products of rounding. Compounds in the model wine control were quantified only at 11 weeks. No amounts were detected. The limit of detection has not been considered, and the precision implied by the units or the number of significant figures chosen is occasionally exaggerated: The appropriate rounding was performed only after further analysis.
Refer to Table 2.6 for 95 % confidence intervals and limits of detection.

(a)	*cis*–oak lactone (µg/L)					(b)	*trans*–oak lactone (µg/L)				
barrel	*6 wks*	*11 wks*	*32 wks*	*55 wks*	*93 wks*	*barrel*	*6 wks*	*11 wks*	*32 wks*	*55 wks*	*93 wks*
AU2	30	32	55	76	80	*AU2*	13	14	25	35	38
AU3	40	42	85	111	167	*AU3*	10	10	17	22	30
AA34	50	46	82	116	137	*AA34*	17	16	23	33	38
AA47	34	38	57	81	90	*AA47*	8	8	11	15	16
NL1	43	90	117	151	187	*NL1*	16	29	38	52	64
NL2	52	119	184	166	184	*NL2*	23	39	57	52	59
LA33	116	93	185	244	257	*LA33*	79	63	128	165	181
LA42	117	114	213	281	304	*LA42*	61	58	110	141	158
NT1		177		355	386	*NT1*		40		81	91
NT2		139		325	348	*NT2*		107		248	299
TA9				205	227	*TA9*				139	157
TA10				360	400	*TA10*				203	255
NV1				398	428	*NV1*				96	109
NV2				272	312	*NV2*				177	216
VA2				382	453	*VA2*				154	178
VA28				479	544	*VA28*				112	134
(c)	**eugenol (µg/L)**					**(d)**	**guaiacol (µg/L)**				
barrel	*6 wks*	*11 wks*	*32 wks*	*55 wks*	*93 wks*	*barrel*	*6 wks*	*11 wks*	*32 wks*	*55 wks*	*93 wks*
AU2	7	7	12	15	17	*AU2*	6	7	11	15	15
AU3	8	7	14	16	23	*AU3*	8	9	16	19	23
AA34	6	5	7	11	12	*AA34*	3	2	2	5	6
AA47	7	9	13	17	21	*AA47*	11	11	17	27	33
NL1	6	11	13	19	23	*NL1*	9	13	15	14	19
NL2	7	14	20	20	22	*NL2*	8	14	11	9	10
LA33	10	8	14	17	17	*LA33*	11	9	17	17	20
LA42	10	9	14	20	21	*LA42*	6	6	8	10	13
NT1		18		37	39	*NT1*		18		35	38
NT2		15		32	36	*NT2*		10		17	17
TA9				24	27	*TA9*				19	25
TA10				29	32	*TA10*				19	23
NV1				31	39	*NV1*				17	21
NV2				39	43	*NV2*				23	30
VA2				25	28	*VA2*				33	47
VA28				29	32	*VA28*				15	23

Table 2.7 (continued)

(e)	4-methylguaiacol (µg/L)					(f)	vanillin (µg/L)				
barrel	*6 wks*	*11 wks*	*32 wks*	*55 wks*	*93 wks*	*barrel*	*6 wks*	*11 wks*	*32 wks*	*55 wks*	*93 wks*
AU2	5	5	9	12	6	*AU2*	198	284	258	433	661
AU3	5	5	12	12	8	*AU3*	225	269	290	476	694
AA34	1	1	0	1	1	*AA34*	77	113	107	158	237
AA47	6	6	8	16	12	*AA47*	230	308	309	410	711
NL1	7	10	11	11	12	*NL1*	135	207	178	311	511
NL2	10	16	16	10	10	*NL2*	189	289	311	348	548
LA33	11	7	15	11	12	*LA33*	348	432	504	719	803
LA42	8	6	8	8	10	*LA42*	307	358	438	513	738
NT1		13		24	19	*NT1*	201	239		378	606
NT2		10		16	12	*NT2*	174	217		355	582
TA9				11	13	*TA9*	276	356		567	748
TA10				11	15	*TA10*	298	359		551	802
NV1				14	14	*NV1*	196	233		415	629
NV2				19	18	*NV2*	162	209		355	590
VA2				20	24	*VA2*	307	389		598	856
VA28				11	14	*VA28*	275	340		533	765

(g)	cyclotene (µg/L)					(h)	maltol (µg/L)				
barrel	*6 wks*	*11 wks*	*32 wks*	*55 wks*	*93 wks*	*barrel*	*6 wks*	*11 wks*	*32 wks*	*55 wks*	*93 wks*
AU2	15	17	38	67	94	*AU2*	41	52	72	109	101
AU3	19	25	57	94	138	*AU3*	76	52	97	117	138
AA34	6	1	2	17	29	*AA34*	15	10	14	34	40
AA47	21	12	33	90	118	*AA47*	61	51	96	136	133
NL1	16	8	39	55	103	*NL1*	34	45	54	79	79
NL2	23	9	47	51	93	*NL2*	38	78	75	68	63
LA33	34	25	48	59	105	*LA33*	68	59	82	102	84
LA42	17	23	40	52	90	*LA42*	64	38	61	94	69
NT1		17		94	88	*NT1*		51		100	92
NT2		16		81	62	*NT2*		36		78	61
TA9				60	158	*TA9*				93	93
TA10				78	124	*TA10*				115	89
NV1				85	138	*NV1*				79	82
NV2				87	128	*NV2*				103	86
VA2				68	169	*VA2*				143	154
VA28				81	121	*VA28*				130	107

(i)	furfural (mg/L)					(j)	'estimated extracted furfural'* (mg/L)				
barrel	*6 wks*	*11 wks*	*32 wks*	*55 wks*	*93 wks*	*barrel*	*6 wks*	*11 wks*	*32 wks*	*55 wks*	*93 wks*
AU2	6.3	5.4	8.1	10.1	10.4	*AU2*	6.3	5.4	8.2	10.2	10.4
AU3	6.0	9.5	9.3	7.9	16.4	*AU3*	6.1	9.5	9.4	8.0	16.5
AA34	2.1	0.9	0.9	0.9	1.2	*AA34*	2.1	0.9	0.9	0.9	2.0
AA47	5.1	8.2	6.1	7.7	13.7	*AA47*	5.1	8.2	6.2	7.7	13.7
NL1	4.2	6.6	4.7	3.1	3.3	*NL1*	4.2	7.6	9.1	4.9	6.0
NL2	4.4	8.2	3.2	2.3	2.3	*NL2*	4.4	8.6	6.6	4.1	4.1
LA33	8.9	5.7	10.6	5.3	4.8	*LA33*	8.9	5.7	12.9	6.4	12.0
LA42	3.8	8.1	4.7	4.2	8.1	*LA42*	3.8	8.2	5.1	4.5	8.5
NT1		6.0		9.5	10.6	*NT1*		6.0		9.6	13.1
NT2		2.7		4.5	6.5	*NT2*		2.7		4.5	6.6
TA9				8.2	15.5	*TA9*				8.3	17.1
TA10				7.0	10.0	*TA10*				7.1	10.4
NV1				5.7	9.6	*NV1*				5.7	10.4
NV2				5.2	11.3	*NV2*				5.2	11.9
VA2				10.8	21.7	*VA2*				11.0	23.2
VA28				5.1	9.0	*VA28*				5.2	12.3

*: 'estimated extracted furfural' = furfural + furfuryl alcohol

Table 2.7 (continued)

(k)	5–methylfurfural (mg/L)					(l)	furfuryl alcohol (mg/L)				
barrel	*6 wks*	*11 wks*	*32 wks*	*55 wks*	*93 wks*	*barrel*	*6 wks*	*11 wks*	*32 wks*	*55 wks*	*93 wks*
AU2	0.78	0.60	0.89	1.09	1.07	*AU2*	0.0	0.0	0.1	0.1	0.1
AU3	0.50	1.33	1.23	1.29	1.66	*AU3*	0.0	0.0	0.1	0.1	0.1
AA34	0.14	0.06	0.06	0.06	0.08	*AA34*	0.0	0.0	0.0	0.0	0.8
AA47	0.91	0.78	0.91	1.06	1.54	*AA47*	0.0	0.0	0.0	0.0	0.1
NL1	0.66	0.72	0.94	0.67	0.74	*NL1*	0.0	1.0	4.4	1.8	2.6
NL2	0.83	0.89	0.73	0.45	0.47	*NL2*	0.0	0.4	3.3	1.8	1.7
LA33	1.41	0.67	1.42	0.84	1.06	*LA33*	0.0	0.1	2.3	1.1	7.2
LA42	0.50	0.90	0.67	0.57	0.87	*LA42*	0.0	0.0	0.4	0.3	0.4
NT1		0.57		1.22	1.37	*NT1*		0.0		0.1	2.5
NT2		0.29		0.68	0.74	*NT2*		0.1		0.1	0.1
TA9				1.00	1.41	*TA9*				0.1	1.7
TA10				1.18	1.39	*TA10*				0.1	0.3
NV1				0.95	1.17	*NV1*				0.0	0.9
NV2				1.03	1.56	*NV2*				0.0	0.5
VA2				1.47	2.16	*VA2*				0.2	1.5
VA28				0.90	1.34	*VA28*				0.2	3.3

(m)	5–methylfurfuryl alcohol (µg/L)					(n)	vanillyl alcohol (µg/L)				
barrel	*6 wks*	*11 wks*	*32 wks*	*55 wks*	*93 wks*	*barrel*	*6 wks*	*11 wks*	*32 wks*	*55 wks*	*93 wks*
AU2	1	1	2	5	8	*AU2*	0	1	0	0	1
AU3	1	2	2	3	9	*AU3*	0	0	0	0	1
AA34	1	0	0	1	4	*AA34*	0	0	0	0	12
AA47	1	1	1	3	4	*AA47*	0	0	1	0	0
NL1	1	1	2	2	4	*NL1*	0	0	1	0	1
NL2	1	1	2	2	3	*NL2*	0	0	0	0	2
LA33	1	1	1	2	5	*LA33*	0	0	0	0	1
LA42	1	2	2	3	5	*LA42*	0	0	0	0	0
NT1		1		4	4	*NT1*		0		0	0
NT2		9		3	8	*NT2*		0		0	1
TA9				3	5	*TA9*				0	0
TA10				2	8	*TA10*				0	1
NV1				3	5	*NV1*				0	0
NV2				3	6	*NV2*				0	0
VA2				4	8	*VA2*				0	0
VA28				2	5	*VA28*				0	0

(o)	furfuryl ethyl ether (µg/L)					(p)	5–methylfurfuryl ethyl ether (µg/L)				
barrel	*6 wks*	*11 wks*	*32 wks*	*55 wks*	*93 wks*	*barrel*	*6 wks*	*11 wks*	*32 wks*	*55 wks*	*93 wks*
AU2	0	0	1	0	5	*AU2*	0	0	0	0	0
AU3	0	0	1	1	6	*AU3*	0	0	0	0	0
AA34	0	0	0	1	6	*AA34*	0	0	0	0	1
AA47	0	0	1	1	1	*AA47*	0	0	0	0	0
NL1	1	22	34	43	66	*NL1*	0	0	0	0	6
NL2	1	8	31	57	63	*NL2*	0	0	1	0	2
LA33	0	0	22	23	129	*LA33*	0	0	0	0	5
LA42	0	0	3	6	15	*LA42*	0	0	0	0	1
NT1		0		2	18	*NT1*		0		0	5
NT2		0		2	6	*NT2*		0		0	0
TA9				0	15	*TA9*				0	2
TA10				1	14	*TA10*				0	1
NV1				0	5	*NV1*				0	6
NV2				0	3	*NV2*				0	3
VA2				1	14	*VA2*				0	2
VA28				3	22	*VA28*				0	27

Table 2.7 (continued)

(q)	vanillyl ethyl ether (µg/L)				
barrel	*6 wks*	*11 wks*	*32 wks*	*55 wks*	*93 wks*
AU2	1	1	2	4	6
AU3	1	2	3	5	8
AA34	1	0	0	4	6
AA47	1	0	3	3	5
NL1	0	0	3	5	8
NL2	0	0	6	5	7
LA33	2	1	3	6	10
LA42	1	2	3	5	4
NT1		0		3	8
NT2		0		2	3
TA9				4	4
TA10				4	5
NV1				3	5
NV2				3	5
VA2				3	4
VA28				4	7

(r)	4–vinylguaiacol (µg/L)				
barrel	*6 wks*	*11 wks*	*32 wks*	*55 wks*	*93 wks*
AU2	1	0	0	0	0
AU3	0	1	0	0	1
AA34	0	0	0	2	0
AA47	1	0	1	0	0
NL1	1	0	5	1	1
NL2	0	0	2	2	1
LA33	0	1	3	1	1
LA42	1	1	1	1	0
NT1		0		0	0
NT2		1		0	0
TA9				0	0
TA10				1	1
NV1				0	0
NV2				0	0
VA2				0	0
VA28				1	0

(s)	4–ethylguaiacol (µg/L)				
barrel	*6 wks*	*11 wks*	*32 wks*	*55 wks*	*93 wks*
AU2	1	1	1	1	2
AU3	1	1	2	2	3
AA34	0	0	0	1	1
AA47	1	1	2	3	3
NL1	2	3	3	3	2
NL2	2	4	3	2	2
LA33	1	1	2	2	2
LA42	1	1	1	1	1
NT1		3		4	3
NT2		2		3	4
TA9				2	2
TA10				3	5
NV1				2	2
NV2				5	4
VA2				5	4
VA28				2	2

(t)	4–vinylphenol (µg/L)				
barrel	*6 wks*	*11 wks*	*32 wks*	*55 wks*	*93 wks*
AU2	0	0	0	0	0
AU3	0	0	0	0	0
AA34	0	0	0	0	0
AA47	0	0	0	0	0
NL1	0	0	0	0	0
NL2	0	0	0	0	0
LA33	0	0	0	0	0
LA42	0	0	0	0	0
NT1		0		0	0
NT2		0		0	0
TA9				0	0
TA10				0	0
NV1				0	0
NV2				0	0
VA2				0	0
VA28				0	0

(u)	4–ethylphenol (µg/L)				
barrel	*6 wks*	*11 wks*	*32 wks*	*55 wks*	*93 wks*
AU2	0	0	0	0	1
AU3	0	0	0	0	0
AA34	0	0	0	0	1
AA47	0	0	0	0	0
NL1	0	0	0	0	0
NL2	0	0	0	0	0
LA33	0	0	0	0	4
LA42	0	0	0	1	0
NT1		0		0	0
NT2		0		1	1
TA9				0	0
TA10				0	0
NV1				0	0
NV2				0	0
VA2				0	5
VA28				0	0

Regression line slopes

The slope of the regression line is indicative of the relative recovery of the target–compound standard, relative to the internal standard. Thus, it appears that vanillin (Freon method) and vanillyl alcohol were recovered in very low quantities from the Chardonnay wine, while guaiacol and 4–methylguaiacol were recovered in very high quantities, relative to the recovery of the internal standard, BHT (Tab. 2.1). These recovery efficiencies are accounted for in the quantification calculations (Miller and Miller 1988 pp. 102–104) so they do not affect the accuracy of the quantifications. However, vanillin (Freon method) was quantified with very low precision and the same factors that affected the poor recovery are likely to have also affected the low precision.

In the Cabernet Sauvignon wine, none of the compounds exhibited a slope substantially above 1.00 (Tab. 2.4). Thus, the internal standard, DMP, added after liquid/liquid extraction and concentration, was more thoroughly recovered compared with the internal standard, BHT, which was added prior to liquid/liquid extraction in the Chardonnay and the model wines.

In the model wines, the slopes of the regression lines were all greater than 0.64, indicating acceptable recovery of all of the target compound standards, relative to the internal standard (Tab. 2.6). Thus, the poor recoveries of vanillin (Freon method) and vanillyl alcohol in the Chardonnay and Cabernet Sauvignon wines were affected, at least partially, by components of 'real' wines which were absent from the model wines.

Regression line y–intercepts

For the model wines, any deviation from zero for the *y*–intercept was ascribed to systematic influences. This assumption can not be made for the regression analyses based on standard additions to the Chardonnay and the Cabernet Sauvignon wines. A *y*–intercept value above zero may have resulted from the presence of the target–compound, prior to any addition, in these wines. 4–Vinylguaiacol, for example, is known to be generated from ferulic acid in hundreds of micrograms per litre during primary fermentation (Chatonnet *et al.* 1993) and

then to oxidatively degrade (Nicolini *et al.* 1991) or to slowly react with ethanol (Dugelay *et al.* 1995) during storage so the Chardonnay wine *y*–intercept of 32 µg/L (Tab. 2.1) is best interpreted as an indicator of the compound's presence, and not of systematic error. Therefore, the quantification of the compounds that showed a positive *y*–intercept in the regression analysis, involved the addition of the *y*–intercept in the calculation. Thus, the control wine concentration was added to the *apparent* barrel wine concentration in the calculation. This simplified to taking the *y*–intercept as zero but the variance contributed by both the control wine and the barrel wine quantification was incorporated into the confidence interval calculations (Miller and Miller 1988 pp. 46–47). Negative *y*–intercept values were treated as systematic errors and incorporated into the quantification calculations. This also applied to the Cabernet Sauvignon wines.

95 % Confidence intervals

Individual 95 % confidence intervals were calculated for each concentration value using the standard error, based on the *y*–residuals from the regression analysis (Miller and Miller 1988 pp. 112–115). These individual confidence intervals were often very similar for each compound. Therefore, instead of listing each of them, only the mean and range for each are quoted (Tabs. 2.1, 2.4 & 2.6).

The measurement precision for the Chardonnay wine concentrations was generally the lowest of the three wines (Tab. 2.1) since the model wines required less preparation and their chromatograms were less affected by interfering compounds, and since the Cabernet Sauvignon wines were quantified, at a later stage, using a better internal standard. This internal standard (2,5–dimethylphenol, abbreviated to DMP) was included following the analysis of the Chardonnay and model wines. Results for the Cabernet Sauvignon standard recovery experiments showed that DMP gave better quantification precision (Tab. 2.4) than did the internal standard used for the Chardonnay and model wines (2,6–di–*tert*–butyl–4–methylphenol, *i.e.* butylated hydroxytoluene, abbreviated to BHT).

The method of calculating confidence intervals assumes homoscedasticity (equal variance over a range of values) (Miller and Miller 1988 p. 104), a quality not usually met with this type of experiment. Instead, the variance tends to start smaller than the average variance at low concentrations, and to increase as the concentration increases. Weighted regression analysis estimates the variance more realistically but the calculations are more complex and an estimation of the variance at each point along the scale is required (Miller and Miller 1988 pp. 124–128). The method chosen for this study is adequate but some of the confidence intervals may be unrealistically high for the lower concentrations, and they may be unrealistically low for the higher concentrations.

Some quantification by extrapolation of the regression lines

In some cases, the concentration determined fell outside of the range covered by the standard recovery experiment so quantification by extrapolation was necessary (Tabs. 2.1, 2.4 & 2.6). In the case of 4–ethylphenol in the Cabernet Sauvignon wines for example (Tab. 2.4), the highest concentration was found in the control wine, and since the standard additions were made to this wine, the range of concentrations in the standard recovery experiment were all higher than the concentrations in the 24 barrel wines. Since linearity cannot be guaranteed, and homoscedasticity is unlikely, beyond the range of the standard additions, the confidence intervals for these extrapolation–based concentrations must be considered underestimations. The majority of the concentrations, however, have been determined by interpolation.

Quantification in the 'control' wines

The concentrations in the Chardonnay control wine at 55 weeks and the Cabernet Sauvignon control wine were determined by the method of standard additions (Miller and Miller 1988 pp. 117–120) since these wines were used as the bases for the standard recovery experiments. The Chardonnay control wine at 11 weeks was quantified in the same way as were the barrel wines.

Limits of detection

Limits of detection (LODs) were determined only where sufficient standard additions had been made in the range close to the lower concentrations measured. Consequently, for example, no LOD was calculated for vanillin (stable isotope dilution analysis). The standard recovery experiment for this compound was conducted in a *barrel–stored* Cabernet Sauvignon which contained vanillin at a concentration of 248 µg/L before any additions (Tab. 2.4). Thus, the concentrations measured in the standard recovery experiment were well above the lowest quantities measured in the wines, and an LOD based on these standard recovery data would be unrealistically high. The LODs were calculated following Miller and Miller (1988 pp. 115–117) and may be defined as the concentration at which the probabilities of erroneously reporting either compound presence or absence both equal 7 %.

2.4 Volatile composition results

The concentrations of the 20 target–compounds in the 24 Chardonnay barrel wines and the Chardonnay control wine at 55 weeks are listed in Table 2.2. The corresponding data for the subset of nine barrel wines and the control wine that were sampled at 11 weeks (Appx. Tab. A.2) are listed in Table 2.3. The concentrations for the Cabernet Sauvignon barrel wines and the Cabernet Sauvignon control wine at 93 weeks are listed in Table 2.5, while those for the 16 model wines at various times (Appx. Tab. A.10) are listed in Table 2.7.

2.5 The multivariate nature of the volatile composition

Many of the volatile compounds quantified in the wines were correlated with one another. Principal components analysis was used to explore these patterns. It is not surprising that such associations should exist considering that many oak wood–derived volatile compounds have common sources (*e.g.* those arising from thermal degradation during coopering).

These compositional principal components (PCs) are used as summaries of groups of correlated compounds, in the expectation that they may be indicative of underlying natural or cultural variables. The 'coopering heat products' PC, for example, is used as an indicator

of the unmeasured heating variability that apparently occurred during the coopering process (Chapter 6).

Interpretation in the Chardonnay wines (Fig. 2.1)

Three of the 20 compounds were excluded from the Chardonnay wine composition principal components analysis (Appx. C.1): 4–ethylphenol was rarely detected; vanillyl alcohol was only found in small quantities and, at these quantities, chromatogram peak assignment was doubtful; and cyclotene was subject to very low precision (Tabs. 2.1 & 2.2).

74 % of the Chardonnay wine composition variance could be explained by three PCs (Appx. Fig. C.1). The first PC describes the 28 % of the variance that was typified by increasing eugenol, oak lactones, 4–vinylguaiacol and 4–vinylphenol but decreasing furfuryl alcohol, maltol and 5–methylfurfuryl ethyl ether (Fig. 2.1 a & b).

The variable occurrence of malolactic fermentation (MLF) among the Chardonnay barrel wines during maturation, resulting in a disproportionate number of MLF–affected barrels among the treatments, should be considered when interpreting the meaning of the PCs. The American oak barrels were considerably more affected than the French oak barrels (Fig. 7.5). Thus, oak origin and MLF effects may overlap, and the first PC may have been affected by both of these factors. The PC describes, on one hand, the co–variation among compounds that were present in the oak wood prior to coopering. These compounds were shown to vary according to oak origin in this study (Chapter 5). On the other hand, it also describes variation, in the opposite direction, among furfuryl alcohol, 5–methylfurfuryl ethyl ether and maltol. The first two compounds are microbially derived and were present in higher amounts among the barrels containing low quantities of the oak lactones and eugenol (the American oak barrels), probably due to the greater extent of MLF among these barrels. Maltol was also found in significantly higher quantities among the American oak barrel Chardonnay wines. This particular oak origin trend was not repeated in the model or the Cabernet Sauvignon wines. Its occurrence in the Chardonnay wines is probably indicative of some systematic error.

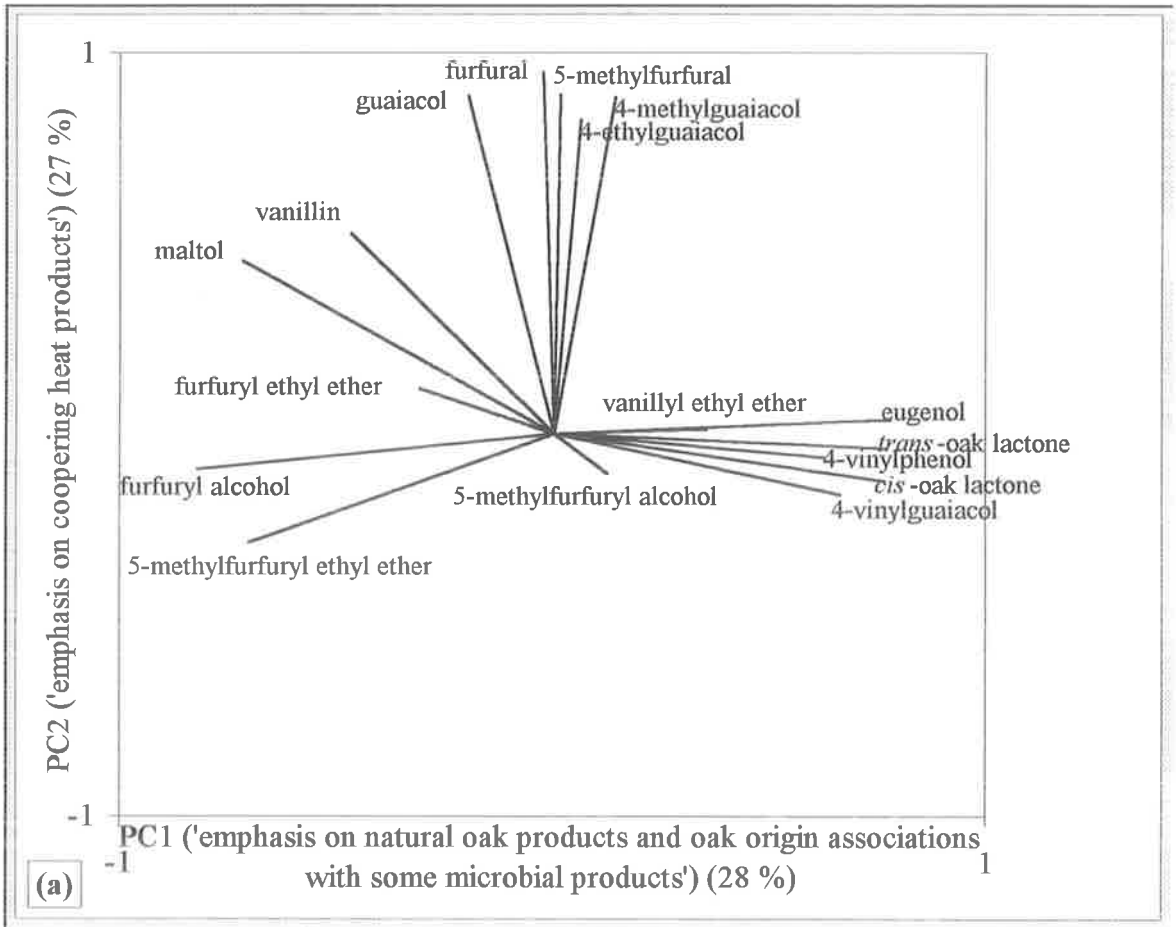
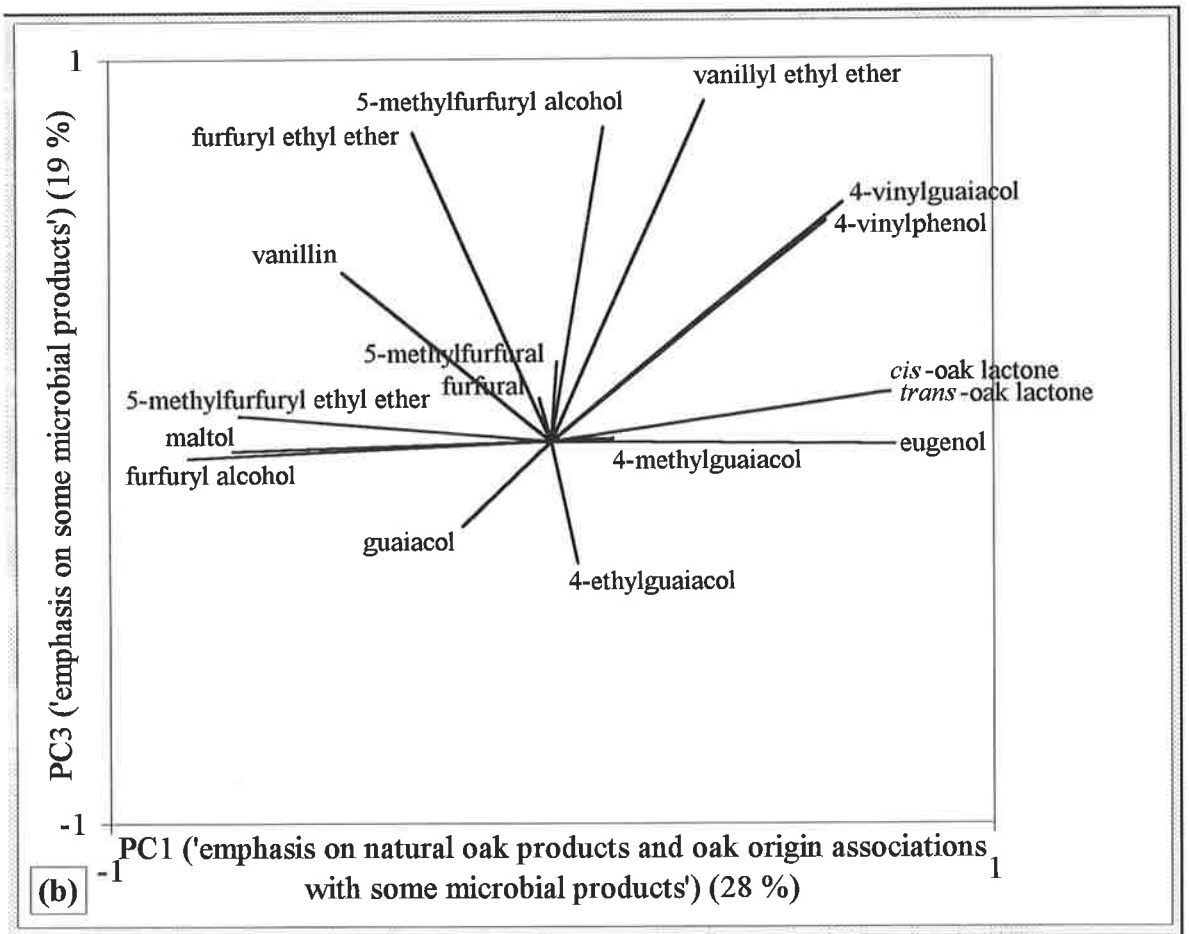
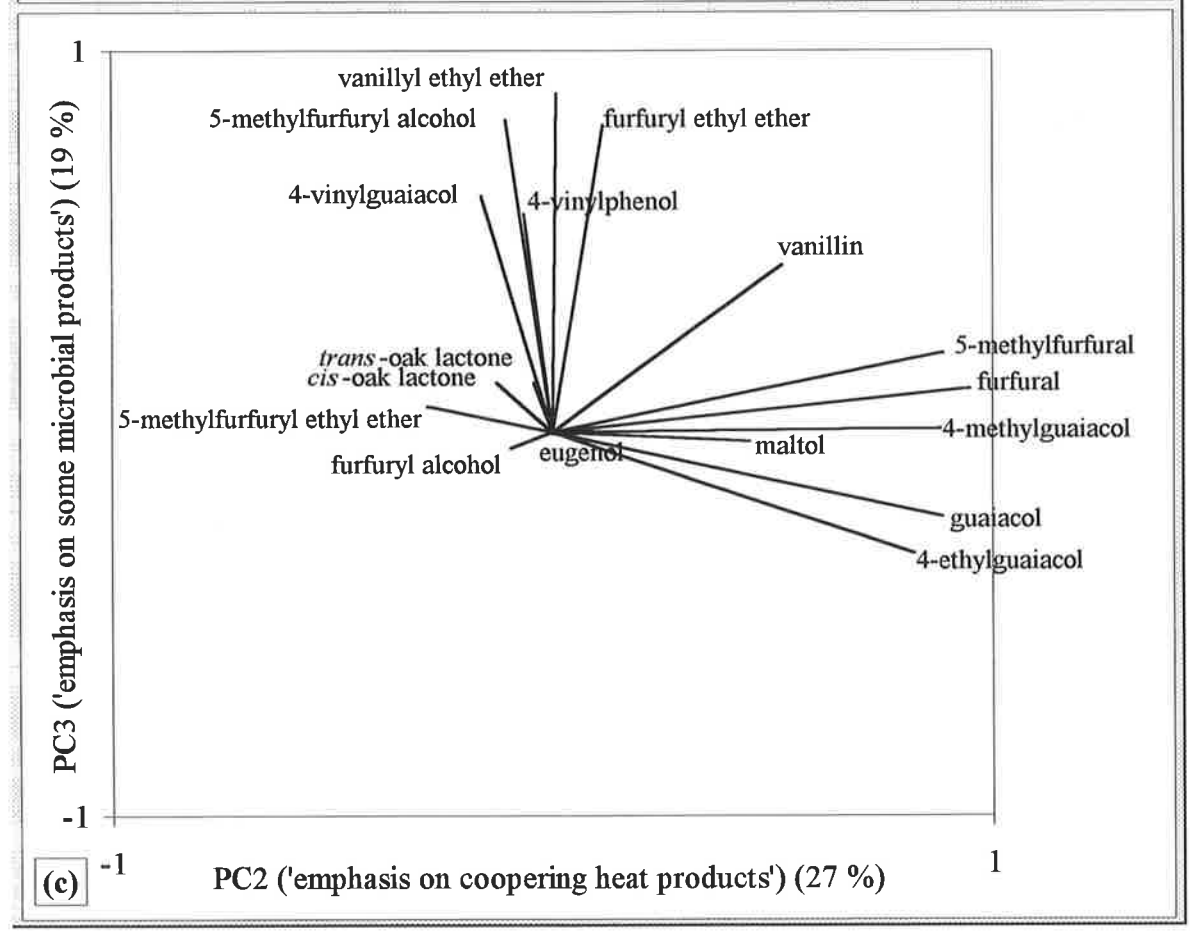


Figure 2.1. Projection of the Chardonnay wine volatile composition on rotated principal components.

(a), (b) and (c): PC1 versus PC2, PC1 versus PC3, and PC2 versus PC3, respectively. See Appendix C for analysis details.


1
-1
PC3 ('emphasis on some microbial products') (19 %)
vanillyl ethyl ether
5-methylfurfuryl alcohol
furfuryl ethyl ether
4-vinylguaiacol
4-vinylphenol
vanillin
5-methylfurfural
furfural
cis-oak lactone
trans-oak lactone
5-methylfurfuryl ethyl ether
maltol
eugenol
furfuryl alcohol
4-methylguaiacol
guaiacol
4-ethylguaiacol
-1
1
PC1 ('emphasis on natural oak products and oak origin associations with some microbial products') (28 %)
(b)


1
-1
PC3 ('emphasis on some microbial products') (19 %)
vanillyl ethyl ether
5-methylfurfuryl alcohol
furfuryl ethyl ether
4-vinylguaiacol
4-vinylphenol
vanillin
trans-oak lactone
cis-oak lactone
5-methylfurfural
furfural
5-methylfurfuryl ethyl ether
4-methylguaiacol
maltol
eugenol
furfuryl alcohol
guaiacol
4-ethylguaiacol
-1
1
PC2 ('emphasis on coopering heat products') (27 %)
(c)

The second PC describes the 27 % of the variance that was typified by increasing furfural, 5–methylfurfural, guaiacol, 4–methylguaiacol, 4–ethylguaiacol and vanillin (Fig. 2.1 a & c). This variation has arisen from the coopering process. Chatonnet *et al.* (1992b) have reported that 4–ethylguaiacol arises most substantially from the activity of microorganisms in red wines (0 – 1561 μg/L, n = 83), and is usually found only in small quantities in white wines (0 – 7 μg/L, n = 54). Its presence among the Chardonnay wines (0 – 5 μg/L) is consistent with these observations but its association with the coopering heat derived products suggests that it may also be formed, in small amounts, during coopering. This may not be so surprising since guaiacol and 4–methylguaiacol, compounds possessing similar structures to 4–ethylguaiacol, are also formed during coopering.

The third PC describes the 19 % of the variance that was typified by increasing vanillyl ethyl ether, 5–methylfurfuryl alcohol, furfuryl ethyl ether, 4–vinylguaiacol and 4–vinylphenol (Fig. 2.1 b & c). This variation has arisen directly or indirectly from microbial activity.

Interpretation in the Cabernet Sauvignon wines (Fig. 2.2)

Three of the 20 compounds were excluded from the Cabernet Sauvignon wine composition principal components analysis (Appx. C.2): 5–methylfurfuryl ethyl ether was rarely detected and, at the low levels found, chromatogram peak assignment is doubtful; vanillyl alcohol was often not detected and, when detected, there was some doubt about chromatogram peak assignment; and vanillyl ethyl ether was measured at low quantities and only with low precision (Tabs. 2.4 & 2.5).

In the Cabernet Sauvignon wines, the vast majority of the extracted furfural and 5–methylfurfural was transformed to other products. Around 98 % of the furfural was present as furfuryl alcohol at 93 weeks, and 5–methylfurfural was present at only 5 % of the quantity measured in the model wines after the same 93 week period. 5–Methylfurfuryl alcohol degrades quickly in wine (work of Sefton, in Spillman *et al.* 1998) so it cannot be used to estimate the quantity of 5–methylfurfural extracted. Furfuryl alcohol, on the other hand, is sufficiently stable to allow such an estimation (work of Sefton, in Spillman *et al.* 1998). The ethyl ethers appear to exist in equilibria with their corresponding furan alcohols

(work of Sefton, in Spillman *et al.* 1998). Consequently, only furfuryl ethyl ether was found in any significant quantities. These transformations must be considered when interpreting the meaning of the PCs. The chemical behaviour of these furan derivatives were not studied as part of the work described in this thesis, and therefore, further detailed discussion is omitted here.

72 % of the Cabernet Sauvignon wine composition variance could be explained by three PCs (Appx. Fig. C.2). The first PC describes the 30 % of the variance that was typified by increasing guaiacol, cyclotene, maltol, 4–methylguaiacol, furfuryl alcohol, furfuryl ethyl ether and furfural (Fig. 2.2 a & b). This variation has arisen from the coopering process. Furfuryl alcohol and furfuryl ethyl ether, although derived from microbial activity, are included probably because their quantities were determined by the initial quantity of furfural present since the biochemical reductions were almost complete.

The second PC describes the 25 % of the variance that was typified by increasing 4–ethylguaiacol, 4–vinylphenol, 5–methylfurfuryl alcohol, 4–ethylphenol and 4–vinylguaiacol but decreasing vanillin (Fig. 2.2 a & c). This PC describes variation among five of the compounds arising from microbial activity during barrel maturation and, in the opposite direction, it describes the variation in vanillin. This bi–directional PC suggests that microorganisms may have been active in vanillin degradation in the Cabernet Sauvignon wines (Chapter 7).

The third PC describes the 17 % of the variance that was typified by increasing oak lactones, eugenol and 5–methylfurfural (Fig. 2.2 b & c). This PC describes variation among the three compounds that were present in the oak wood prior to coopering (the oak lactones and eugenol). 5–Methylfurfural probably accompanies these compounds by chance, and it was present only at low concentration.

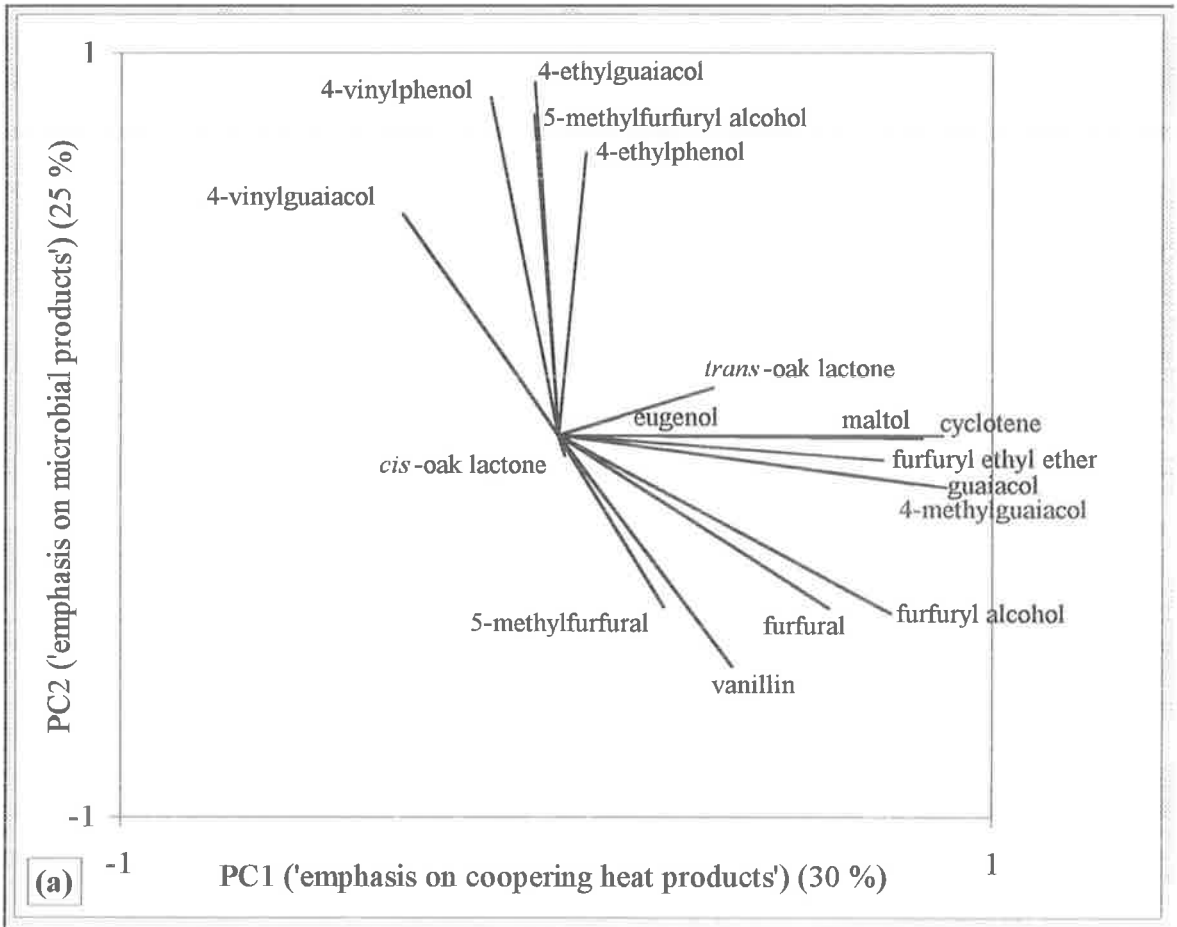
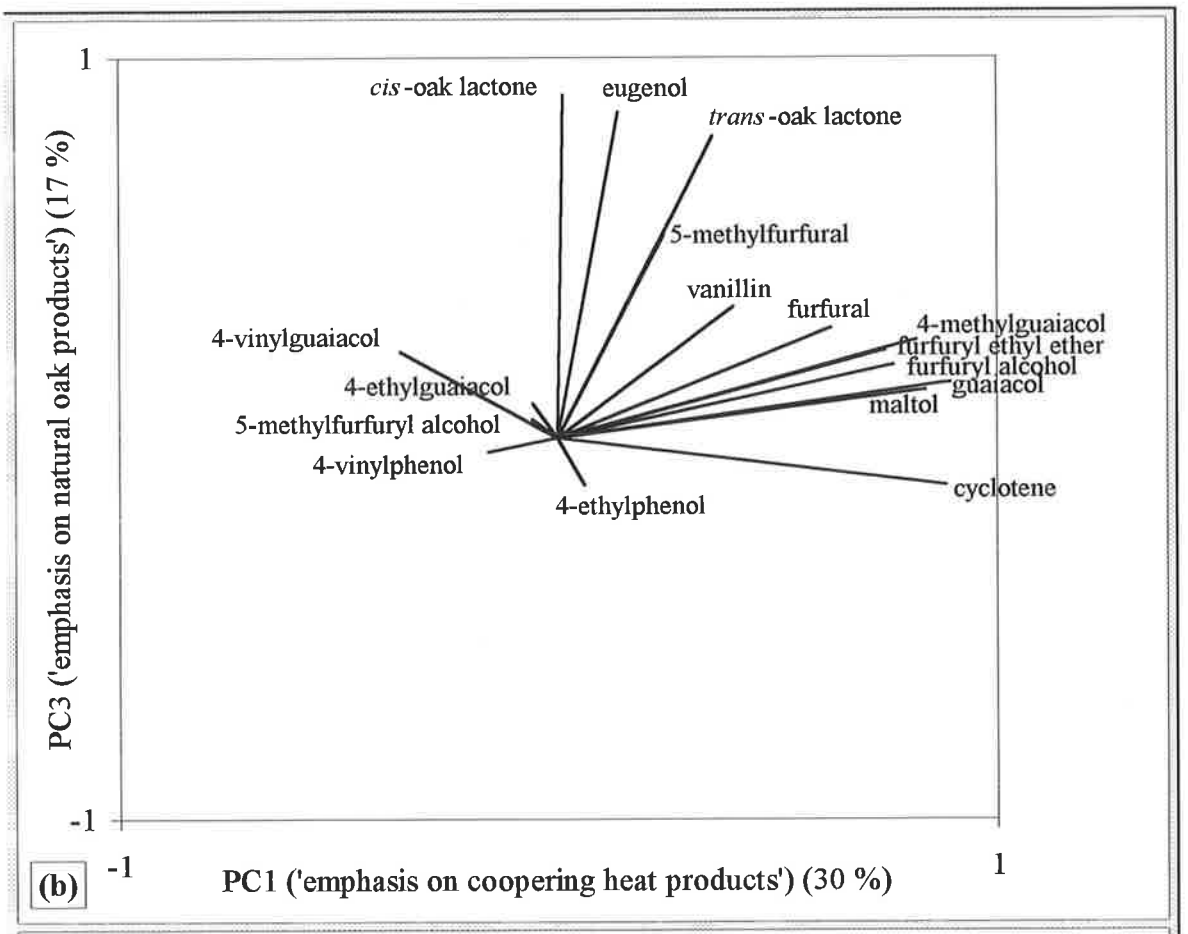
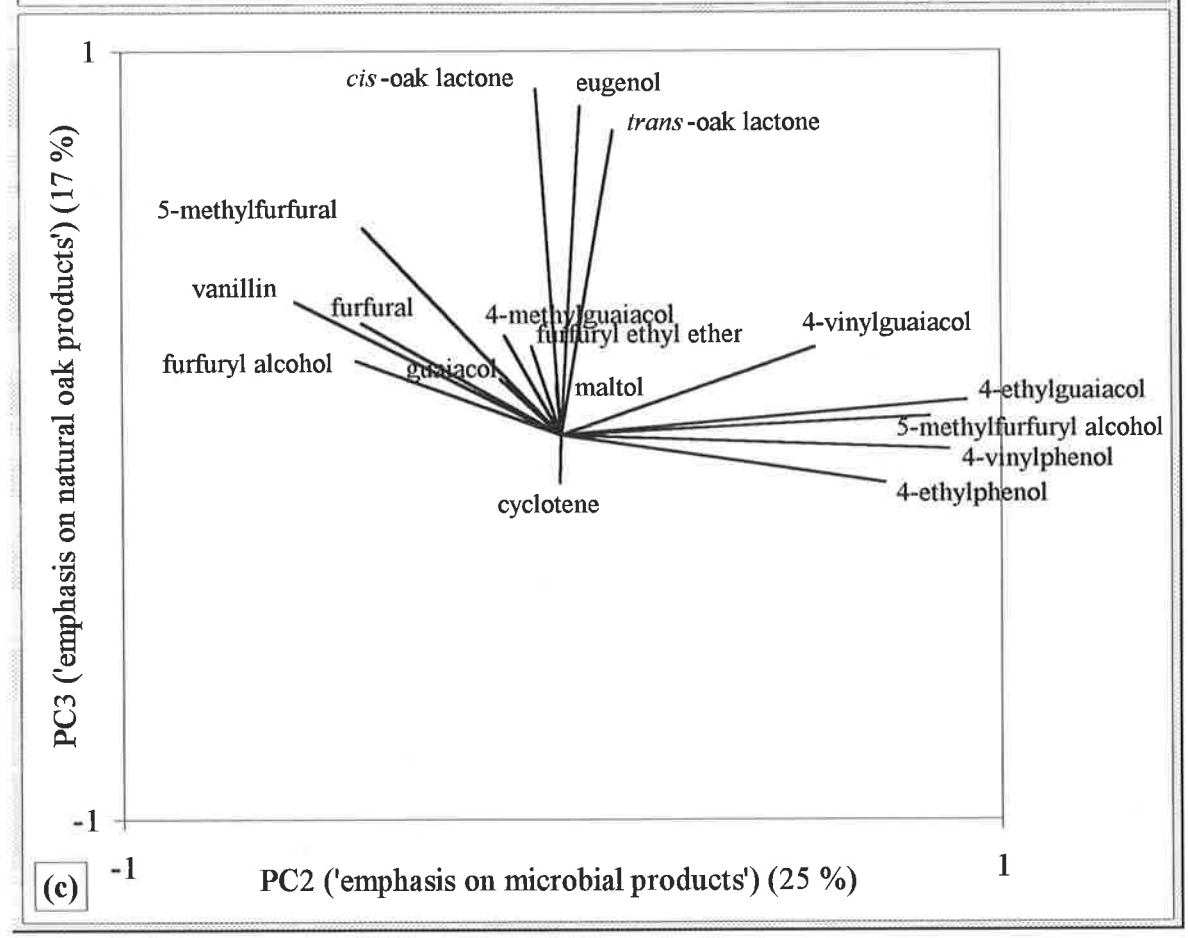


Figure 2.2. Projection of the Cabernet Sauvignon wine volatile composition on rotated principal components.

(a), (b) and (c): PC1 versus PC2, PC1 versus PC3, and PC2 versus PC3, respectively. See Appendix C for analysis details.


1
-1
PC3 ('emphasis on natural oak products') (17 %)
cis-oak lactone
eugenol
trans-oak lactone
5-methylfurfural
vanillin
furfural
4-methylguaiacol
furfuryl ethyl ether
furfuryl alcohol
guaiacol
maltol
4-vinylguaiacol
4-ethylguaiacol
5-methylfurfuryl alcohol
4-vinylphenol
4-ethylphenol
cyclotene
(b)
-1
PC1 ('emphasis on coopering heat products') (30 %)
1


1
-1
PC3 ('emphasis on natural oak products') (17 %)
cis-oak lactone
eugenol
trans-oak lactone
5-methylfurfural
vanillin
furfural
4-methylguaiacol
furfuryl ethyl ether
4-vinylguaiacol
furfuryl alcohol
guaiacol
maltol
4-ethylguaiacol
5-methylfurfuryl alcohol
4-vinylphenol
4-ethylphenol
cyclotene
(c)
-1
PC2 ('emphasis on microbial products') (25 %)
1

Interpretation in the model wines (Fig. 2.3)

Seven of the 20 compounds — vanillyl alcohol, vanillyl ethyl ether, 5–methylfurfuryl alcohol, 5–methylfurfuryl ethyl ether, 4–vinylguaiacol, 4–vinylphenol and 4–ethylphenol — were excluded from the composition principal components analysis of the model wines at 93 weeks (Appx. C.3). All are microbially derived in wine and they were either not detected or only found in low quantities, partially as a result of the sanitation imposed on the model wines. At these low quantities, quantification precision was also low. The data for these compounds were excluded from the analysis because they contained no useful information and because they may have 'clouded' the information in the remaining data.

Furfuryl alcohol, furfuryl ethyl ether and 4–ethylguaiacol, three compounds also microbially derived in wine, were included because they were measured in higher quantities or with greater relative precision (Tabs. 2.6 & 2.7 l, o & s). Additionally, since the sum of furfural and furfuryl alcohol is likely to be a reasonably good estimate of the initial amount of furfural extracted from the oak wood (work of Sefton, in Spillman *et al.* 1998), this summation was also included, and is referred to as 'estimated extracted furfural.'

84 % of the compositional variance could be explained by three PCs (Appx. Fig. C.3). The first PC accounted for 44 % of the variance and was typified by increases in the compounds known to arise from coopering ('estimated extracted furfural,' maltol, 5–methylfurfural, furfural, cyclotene, guaiacol, vanillin and 4–methylguaiacol) (Fig. 2.3 a & b).

The second PC accounted for 24 % of the variance and was typified by increases in the compounds known to be present in oak wood prior to coopering (the oak lactones and eugenol). 4–Methylguaiacol and 4–ethylguaiacol also contributed to the second PC (Fig. 2.3 a & c). The reason for the contribution of these latter two compounds to this PC is unknown.

The third PC accounted for 16 % of the variance and was typified by increases in two compounds of microbial derivation (furfuryl alcohol and, consequently, furfuryl ethyl ether) (Fig. 2.3 b & c).

Commonality among the wines

Notwithstanding the peculiarities of each wine type, the principal components analyses have partitioned the volatile composition variance among the barrel wines into three main groups of compounds. These groups represent variations occurring at three main stages, one affected mostly by natural variables and two affected mostly by cultural practices. The natural variability found in oak wood is added to by the variability of heating applied during coopering and by the variability in microbial activity allowed to occur during barrel maturation. Three stages of oak wood handling — procurement, barrel construction and wine maturation — are implicated, and opportunities for understanding and manipulating the majority of volatile oak wood composition is likely to be found in an exploration of these three areas.

2.6 Summary and conclusion

The methodology applied to the volatile composition analysis and to the calculation of accuracy and precision measures for 20 compounds quantified at various times in 64 barrel–stored wines is presented in this chapter and in Appendix B. The results are tabulated, and the variability of each compound, in relation to the variability of the other compounds from the same barrels, is explored through PC analysis.

Having described the variability of the volatile compounds under study, the next chapter describes the aroma variability which was found to occur among the same wines, and the following chapters consider the two sets of data, together. The composition–PCs are used as summaries of natural or cultural variables in correlation analyses with the aroma data, *e.g.* the 'emphasis on coopering heat products'–PC is used as an estimation of the unmeasured coopering heat applied to each barrel.

The factors responsible for the variation in the volatile composition are discussed in detail in Chapters 5, 6 and 7.

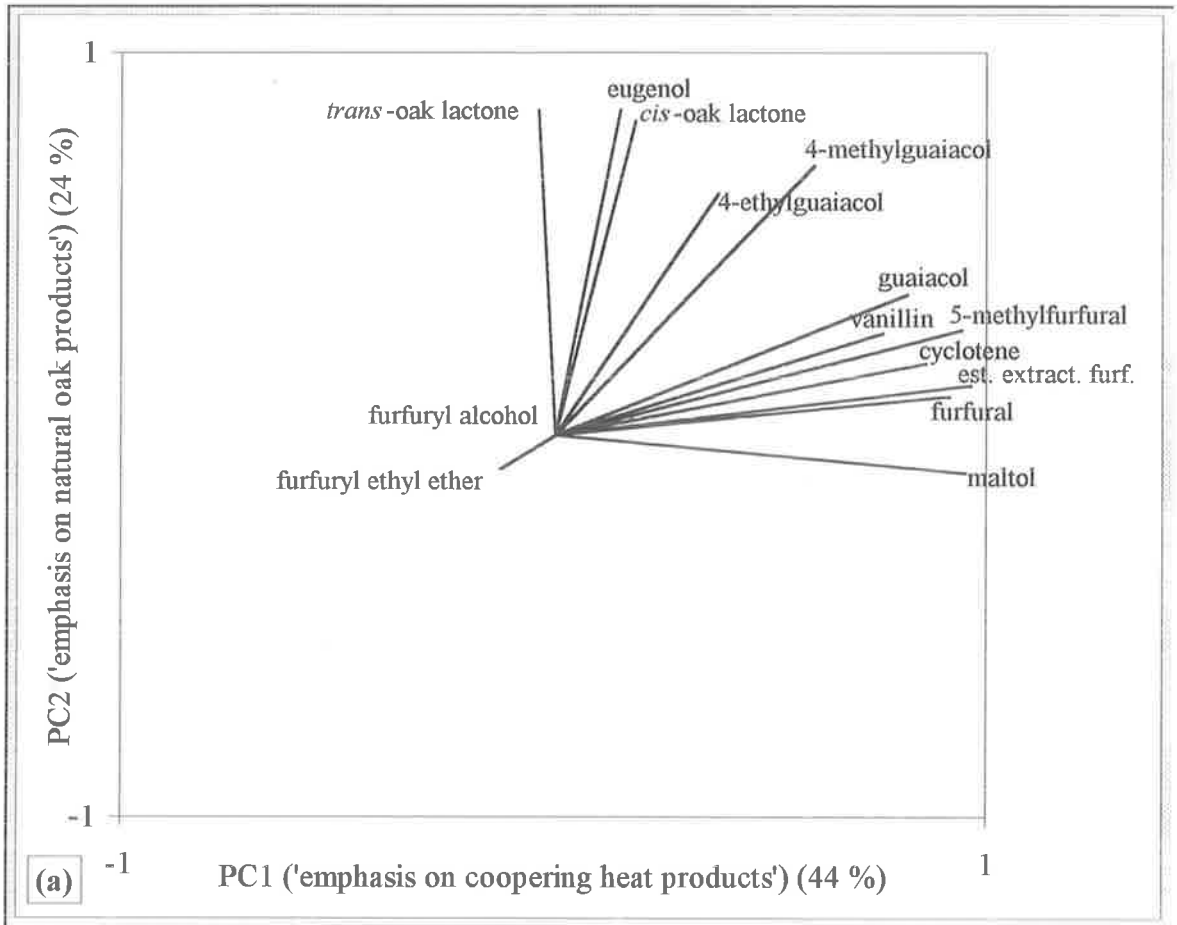
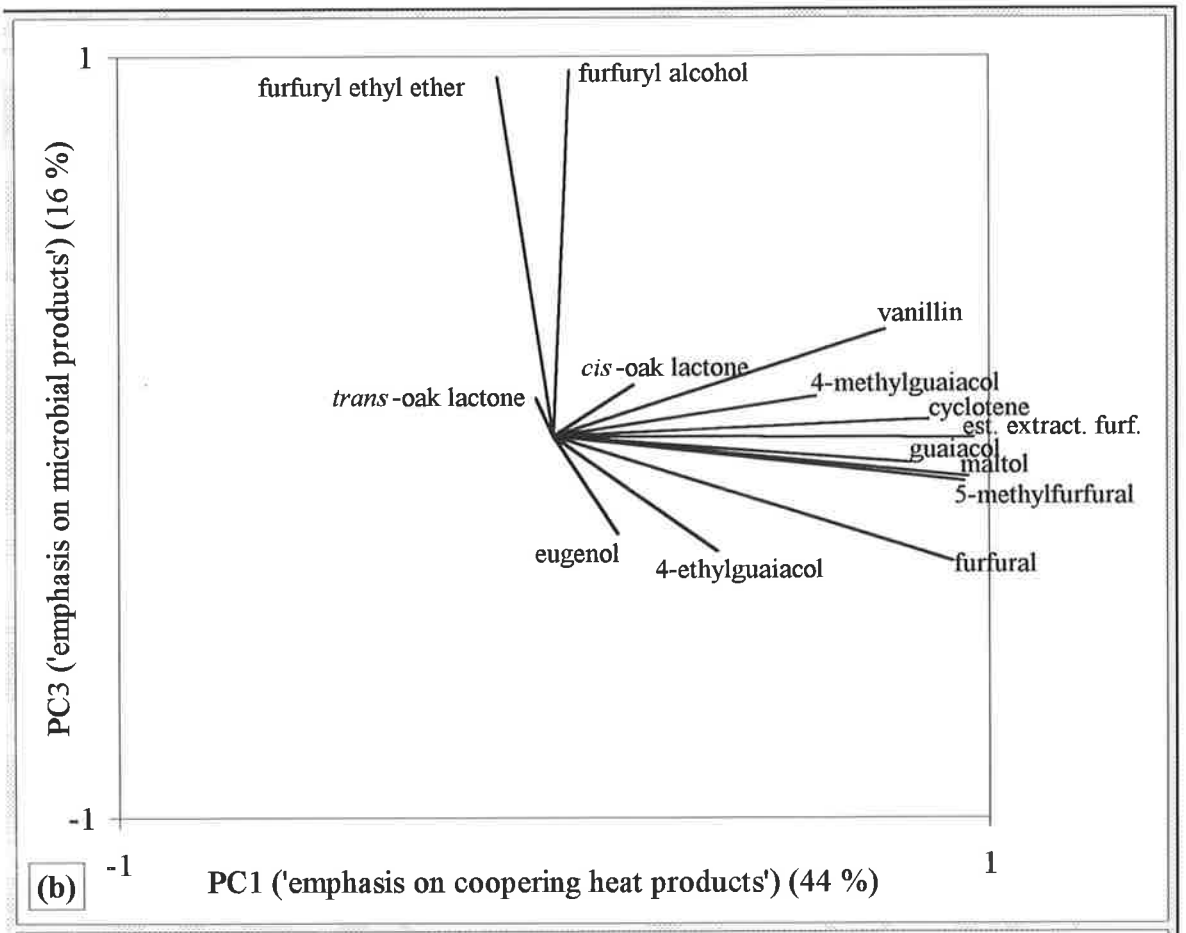
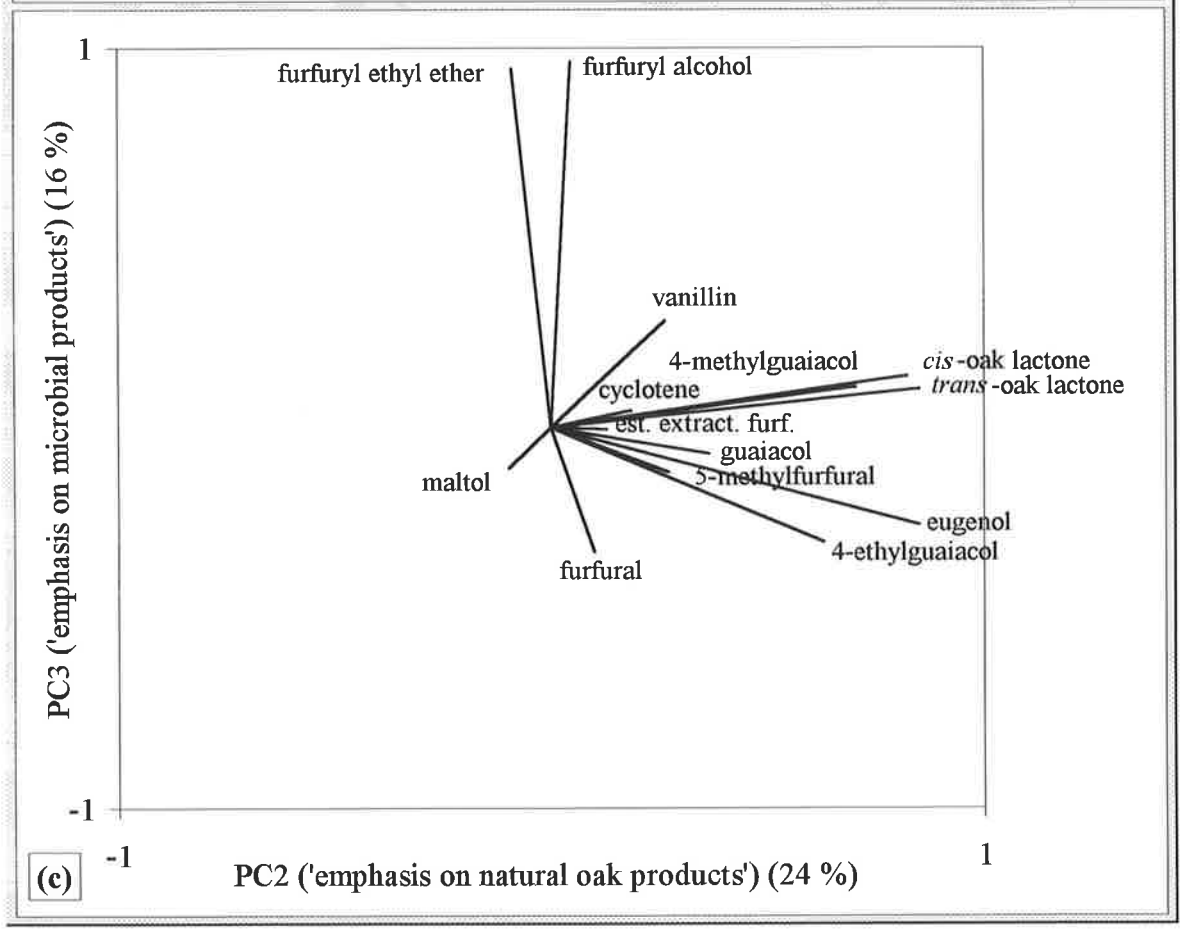


Figure 2.3. Projection of the 93 week model wine volatile composition on rotated principal components.

(a), (b) and (c): PC1 versus PC2, PC1 versus PC3 and PC2 versus PC3, respectively.
See Appendix C for analysis details.

est. extract. furf. = 'estimated extracted furfural'


1
-1
-1
1
PC3 ('emphasis on microbial products') (16 %)
PC1 ('emphasis on coopering heat products') (44 %)
furfuryl ethyl ether
furfuryl alcohol
vanillin
cis-oak lactone
trans-oak lactone
4-methylguaiacol
cyclotene
est. extract. furf.
guaiacol
maltol
5-methylfurfural
eugenol
4-ethylguaiacol
furfural
(b)


1
-1
-1
1
PC3 ('emphasis on microbial products') (16 %)
PC2 ('emphasis on natural oak products') (24 %)
furfuryl ethyl ether
furfuryl alcohol
vanillin
4-methylguaiacol
cis-oak lactone
trans-oak lactone
cyclotene
est. extract. furf.
guaiacol
5-methylfurfural
maltol
eugenol
4-ethylguaiacol
furfural
(c)

Chapter 3

The aroma

Chapter outline

3.1 Introduction

Having set a foundation based on the work of others (Chapter 1) and on the volatile composition results (Chapter 2), the present chapter introduces the sensory data which become the focus of enquiry throughout the remainder of the thesis.

Within the boundaries of the experimental treatments and within the limits of the capabilities of the sensory method, the description of the oak wood contribution to wine aroma, in this chapter, is complete. This is probably not so for the volatile composition description given in Chapter 2 because, as previously discussed, it is likely that some important compounds were not quantified. Thus, the sensory data stand as a complete picture of the oak wood effect in this chapter, and the volatile composition data are used to elucidate the mechanisms leading to this effect (Chapters 4 to 9).

3.2 Wine aroma description by ranking

Wine sensory analysis is often based on measurement scales that are presumed to be interval or ratio in type. There are definite advantages in working with such data. In particular, they are amenable to parametric analysis. Some of the corresponding non–parametric data analyses, which can be used on rank data for example, either do not exist or are not commonly available in statistical software packages. While some researchers may be enticed by these considerations, the ability of even the most thoroughly trained individual or panel to measure subtle aromas, typical of those in wine, using a category or ratio scale in an accurate manner is highly questionable. This is particularly so when each wine is analysed in isolation to points of reference (*e.g.* ranges of standards) over numerous occasions. In such cases, changes in physiological or psychological inclination can cause substantial variation. Further, some rating scales (*i.e.* categoric) may fail to resolve subtle sensory variations between samples due to the fact that each point on the categoric scale can represent a broad segment of the sensory continuum.

A more realistic measurement of the subtle aromas in wine can be achieved using ranking because the samples act as their own points of reference and because the task is relatively simple. The adequacy of ranking as an alternative to rating becomes more apparent with

increases in the number of samples, taken from a defined population, to be analysed. As the sample number increases, the real intervals to be estimated tend to decrease in size and variance, becoming more rank–like (Appx. D.1), and the simplicity of the ranking process is suited to the resolution of these small intervals.

The wines for which aroma descriptions were performed in this study numbered two sets of 25. A ranking procedure was recommended by these large sample numbers and by the fact that subtle aroma differences among the wines were the focus of the study.

3.3 A summary of the sensory descriptive analysis method

In general, the method used is similar to that described by Meilgaard *et al.* (1991, pp. 138–142, 117–119 & 262–264). Panelist aptitude tests, training, and descriptor– and standard–generation by a combination of individual introspection and group discussion were followed by the use of standards and separate rankings of each descriptor (arranged as a balanced incomplete block design). Significant aroma differentiations were detected using a Friedman–type statistical analysis and a non–parametric analogue to Fisher's least significant difference (LSD). Although not a requirement of the method, the repeatability of the ranking was tested by Spearman's rank correlation analysis. Details of the materials and methods are shown in Appendix D.

Further analysis of the data was facilitated by Fisher–Yates rank transformations (Fisher and Yates 1963 pp. 31, 94) (Appx. E). A variety of parametric statistical methods — Pearson's correlation coefficient, analysis of variance, and principal components analysis — were then applied to the transformed data.

The 25 Chardonnay and 25 Cabernet Sauvignon wines (24 barrels plus 1 stainless steel drum, each) were subject to aroma descriptive analysis and to aroma 'preference' analysis. For the Chardonnay wines, seven half–hour training sessions on seven different days were followed by 17 quarter–hour individual ranking sessions on 17 different days. A similar procedure, using many of the same panelists, was followed for the Cabernet Sauvignon wines.

3.4 The Chardonnay wine aroma differentiation

Significant differentiation among the 25 Chardonnay wines was noted for all of the aromas and for 'preference.' These results, the rank sums and significant differentiations are shown in Table 3.1. Those wines not joined by a vertical line — the LSD (5%) — were significantly different ($p<0.05$).

Table 3.2 shows a summary of the repeatability estimations derived from repetitions of the ranking procedure with less–than–full sets (*i.e.* 16 or 21 of the original 25 samples) due to depletion of stocks (Appx. D.5). These tests, being less–than–full repetitions, are questionable in their adequacy. Nevertheless, they are included for what information they provide. Five of the six sensory occasion pairs were positively correlated, indicating adequate ranking repeatability for 'coconut,' 'vanilla,' 'butter,' 'smoky' and 'green apple.' The lack of association between the two 'pencil shavings' occasions indicates poor reliability for that attribute. This lack of association might also introduce some doubt about the repeatability of the non–repeated attribute rankings, although the five attributes that were successfully repeated are more supportive of a generally adequate level of repeatability among these rankings. All aroma rankings were included in the analyses but the relative precision of each of the repeat rankings is borne in mind.

Table 3.2. Chardonnay wine aroma ranking repeatability estimates for six of the ten descriptors (expressed as the significance of the Spearman's rank correlation coefficient for the two sensory occasions).

Descriptor	*Correlation coefficient*	*Inter-occasion rank correlation significance*
'coconut'	0.477	$p<0.05$
'pencil shavings'	0.099	n.s. ($p>0.10$)
'vanilla'	0.499	$p<0.05$
'butter'	0.742	$p<0.001$
'smoky'	0.791	$p<0.001$
'green apple'	0.458	$p<0.05$

n.s.= not significant.

Table 3.1. Chardonnay wine aroma rank sums and significant differences.

Significant differences?		preference		coconut		pencil shavings		caramel		vanilla	
preference	$p<0.01$	*FV4*	69	*VA39*	74	*FL5*	69	*AA22*	72	*TA46*	69
coconut	$p<0.001$	*VA38*	67	*VA38*	68	*VA38*	68	*AU70*	68	*FL4*	65
pencil shavings	$p<0.001$	*FL4*	66	*VA32*	67	*FT3*	67	*AU4*	67	*LA41*	62
caramel	$p<0.05$	*VA39*	65	*AA22*	66	*TA46*	67	*AU6*	67	*LA34*	60
vanilla	$p<0.01$	*TA31*	62	*FL3*	64	*LA34*	65	*AA10*	59	*TA31*	60
butter	$p<0.001$	*TA46*	62	*TA31*	63	*FT5*	65	*AA11*	58	*AU4*	60
allspice	$p<0.001$	*LA34*	59	*FV3*	61	*FL4*	64	*FL4*	56	*AA11*	60
smoky	$p<0.001$	*AU4*	58	*TA46*	59	*FV5*	62	*LA27*	56	*FV4*	59
cashew nut	$p<0.01$	*FV3*	57	*LA34*	58	*TA23*	56	*LA34*	56	*FV3*	57
green apple	$p<0.001$	*VA32*	57	*FL4*	57	*FV4*	55	*FL3*	54	*VA32*	57
cinnamon	$p<0.01$	*AA10*	56	*LA41*	56	*VA32*	54	*FT5*	54	*VA39*	57
higher rank sum = 'more'		*AA11*	55	*TA23*	56	*LA27*	53	*TA31*	54	*FT3*	56
Wines joined by line		*FV5*	54	*LA27*	55	*FT4*	53	*FV4*	54	*AA10*	56
were n.s. diff. ($p<0.05$).		*FT5*	53	*FT4*	53	*VA39*	53	*FT4*	53	*AA22*	56
LSD (5%)=rank sum 17		*FT4*	52	*AU70*	53	*FL3*	52	*TA46*	52	*FL3*	55
Grouping by LSD (5%)		*TA23*	52	*AU4*	49	*TA31*	52	*TA23*	51	*FT4*	55
(control excluded)		*AU70*	51	*FV4*	48	*AA11*	51	*FV5*	51	*VA38*	53
: higher than		*FT3*	50	*FV5*	48	*FV3*	50	*LA41*	49	*AU70*	53
at least one other barrel		*LA41*	49	*FT5*	45	*AU6*	50	*FV3*	49	*FV5*	51
: neither higher		*AA22*	49	*AA10*	45	*AU70*	46	*VA38*	48	*AU6*	49
nor lower than others		*FL3*	47	*AU6*	44	*LA41*	45	*control*	47	*FT5*	45
: lower than		*FL5*	41	*AA11*	44	*AU4*	44	*VA32*	47	*TA23*	45
at least one other barrel		*LA27*	41	*FT3*	43	*AA10*	44	*FT3*	45	*LA27*	40
: higher & lower		*AU6*	39	*control*	40	*control*	33	*VA39*	44	*FL5*	39
than at least one other		*control*	39	*FL5*	34	*AA22*	32	*FL5*	39	*control*	31

butter		allspice		smoky		cashew nut		green apple		cinnamon	
AU6	67	*FL4*	71	*TA23*	82	*VA38*	69	*control*	73	*TA46*	65
AU70	66	*TA46*	71	*LA34*	74	*FV3*	66	*LA27*	70	*AU6*	61
LA41	65	*AA11*	69	*FT3*	73	*FL3*	65	*VA39*	69	*LA34*	60
TA31	65	*TA23*	65	*FL4*	65	*FL4*	64	*FL3*	67	*LA41*	60
AU4	65	*FV5*	61	*TA46*	63	*TA46*	63	*LA41*	62	*AA11*	60
AA22	64	*FV4*	59	*AA11*	63	*LA34*	61	*LA34*	59	*FL4*	59
LA34	63	*FV3*	58	*AU4*	62	*TA23*	59	*AU6*	59	*FT4*	59
VA39	60	*VA39*	57	*FV5*	57	*FL5*	58	*FT5*	58	*VA32*	59
FV3	58	*LA27*	55	*FL5*	56	*FV4*	58	*VA32*	57	*FL5*	58
FL5	57	*VA32*	55	*FT4*	55	*AA10*	58	*FL5*	55	*FT5*	58
TA46	56	*AA10*	55	*FT5*	55	*LA27*	57	*TA31*	55	*FL3*	57
VA32	56	*FL3*	54	*VA32*	54	*AU4*	56	*TA46*	55	*TA23*	57
AA10	54	*TA31*	54	*FV4*	53	*VA32*	55	*FV3*	55	*AU4*	57
FT5	53	*AU4*	54	*VA38*	53	*LA41*	54	*FV4*	55	*FV3*	56
FT4	52	*LA34*	53	*FV3*	49	*FT5*	54	*VA38*	54	*FV4*	56
AA11	52	*LA41*	53	*AA10*	49	*FV5*	52	*AA10*	51.5	*VA38*	55
VA38	51	*FL5*	52	*LA41*	47	*AA22*	51	*AA22*	51.5	*VA39*	53
FV4	48	*FT5*	52	*TA31*	47	*TA31*	50	*AU70*	51	*AA10*	52.5
TA23	47	*FT3*	49	*AU6*	47	*VA39*	50	*FT4*	46	*AA22*	52.5
FL3	45	*AU6*	49	*AU70*	47	*FT4*	45	*FV5*	45	*AU70*	52
LA27	45	*VA38*	48	*FL3*	45	*AU6*	45	*FT3*	44	*FV5*	51
FT3	45	*AU70*	46	*AA22*	44	*AA11*	44	*AU4*	41	*FT3*	50
FL4	44	*AA22*	43	*VA39*	40	*FT3*	43	*AA11*	41	*LA27*	44
FV5	41	*FT4*	40	*LA27*	38	*AU70*	42	*FL4*	39	*TA31*	33
control	31	*control*	27	*control*	32	*control*	31	*TA23*	37	*control*	25

The failure to repeat the 'pencil shavings' ranking may have been a function of the composition of the subset available for the repeat occasion. A significant amount of the differentiation established on the first occasion may have been due to samples that were unavailable for the second occasion.

3.5 The Cabernet Sauvignon wine aroma differentiation

Significant differentiation among the 25 Cabernet Sauvignon wines was noted for 'preference' and for all of the aromas except for 'earthy' ($0.10>p>0.05$) and for 'mint' ($0.20>p>0.10$). These results, the rank sums and significant differences are shown in Table 3.3. No LSD calculations were applied to 'earthy' and 'mint.' Despite the failure of the panel to differentiate the samples, according to 'earthy' and 'mint,' as measured by the Friedman–type statistic, the data for these aromas were subject to the same analyses as were the data for the other aromas. However, any discussion of the results for 'earthy' or 'mint' is presented with the statistical non–significance of the differentiation in mind.

Table 3.4 shows a summary of the repeatability estimations. The rankings across occasions were positively correlated, indicating adequate ranking precision for all of the attributes tested.

Table 3.4. Cabernet Sauvignon wine aroma ranking repeatability estimates for five of the twelve descriptors (expressed as the significance of the Spearman's rank correlation coefficient for the two sensory occasions).

Descriptor	*Correlation coefficient*	*Inter-occasion rank correlation significance*
'pencil shavings'	0.399	$p<0.05$
'berry'	0.791	$p<0.001$
'smoky'	0.351	$p<0.05$
'vanilla'	0.707	$p<0.001$
'coffee'	0.570	$p<0.01$

Table 3.3. Cabernet Sauvignon wine aroma rank sums and significant differences.

Significant differences?		preference		coconut		pencil shavings#		allspice		berry#		smoky#	
preference	$p<0.001$	LA38	69	NT7	72	VA27	139	VA21	72	TA25	138	LA23	153
coconut	$p<0.001$	TA8	67	TA25	71	NL7	126	TA39	71	NT7	134	LA38	135
pencil shavings	$p<0.001$'	TA25	66	NV8	70	NL6	125	LA30	66	NV8	134	VA12	129
allspice	$p<0.01$	NV7	65	NV7	68	NT8	123	NV7	65	AU7	129	TA39	128
berry	$p<0.001$'	TA39	64	VA21	68	LA38	119	LA38	61	VA21	128	VA21	117
smoky	$p<0.001$'	VA21	64	NV6	67	NT6	119	TA25	61	NV7	122	NL8	115
caramel	$p<0.001$	VA27	62	VA12	66	LA30	117	NL6	58	LA38	121	VA27	112
vanilla	$p<0.001$'	NT7	60	TA8	65	NV6	115	LA23	58	VA27	118	LA30	111
coffee	$p<0.001$'	VA12	60	VA27	57	TA8	114	NT7	58	LA30	112	AU8	109
dark chocolate	$p<0.05$	AU7	59	NT8	56	TA39	112	VA27	56	LA23	109	AA36	108
Band-aid	$p<0.01$	AA48	58	TA39	55	AA40	110	AU9	54	TA8	109	NL7	105
earthy	$0.10>p>0.05$	NT8	57	NT6	54	NV7	108	NL8	54	NV6	109	NT6	105
mint	$0.20>p>0.10$	NV6	54	AU7	53	VA21	106	TA8	54	NT6	108	AA48	104
higher rank sum = 'more'		NL8	52	LA30	53	AU8	105	NV8	52	AU8	105	NT8	104
Wines joined by line		LA30	51	AA40	50	NL8	105	VA12	52	VA12	101	TA8	101
were n.s. diff. ($p<0.05$).		NT6	51	LA23	50	VA12	104	NV6	51	AA40	100	TA25	100
LSD=rank sum 17 or 24'		AU9	48	LA38	49	LA23	101	AA48	50	AA48	99	control	99
Grouping by LSD (5%)		AA40	48	AA48	48	TA25	101	NL7	49	TA39	99	NV7	99
(control excluded)		AU8	47	AA36	46	AA36	99	AA36	48	AU9	98	AU9	98
	: > 1 or more	NV8	47	NL8	44	AA48	99	AA40	48	NT8	97	NV6	98
	: neither > nor <	NL6	44	AU8	43	AU7	98	AU7	44	NL6	95	AA40	97
	: < 1 or more	AA36	43	AU9	42	AU9	98	NT8	44	NL8	90	NL6	97
	: >&< 1 or more	LA23	43	NL7	40	NV8	95	AU8	43	AA36	89	AU7	95
' Based on sum of two occasion rank sums		NL7	38	NL6	39	NT7	84	control	41	NL7	80	NT7	93
		control	33	control	24	control	78	NT6	40	control	76	NV8	88

caramel		vanilla#		coffee#		dark chocolate		Band-aid		earthy		mint	
VA12	73	VA21	146	LA23	143	NT6	67	control	78	control	72	TA25	71
NV8	67	TA25	135	VA12	133	VA21	67	LA23	70	AA36	65	NL6	70
NT7	66	LA30	133	TA39	131	NV7	65	NL8	69	LA23	64	VA27	65
NV7	65	VA12	130	TA8	126	TA25	64	NT8	66	TA39	60	VA12	59
LA30	62	VA27	125	LA38	125	VA12	64	LA38	62	NL6	59	LA38	58
TA25	60	NV7	124	TA25	122	LA30	63	AA36	59	NL8	59	NV6	57
TA39	60	NV8	122	NT6	120	TA39	58	TA8	57	NT8	58	LA30	56
NL8	57	AU7	120	VA27	120	AU7	56	AA48	56	AU9	57	VA21	56
LA38	57	TA8	120	NV8	118	LA38	56	NL6	55	LA38	57	control	55
AA48	56	TA39	118	VA21	117	TA8	56	NL7	55	NV6	57	NL7	55
VA27	56	NT7	115	NL8	116	NV8	55	TA25	55	NL7	56	NL8	55
AU7	55	NT8	115	LA30	111	LA23	54	VA12	55	AA40	55	LA23	54
AA40	55	NV6	113	NV7	109	NV6	54	VA21	55	NV7	55	NV8	54
TA8	55	NT6	108	AA48	102	AA40	53	NT6	54	VA12	53	AU7	53
NV6	55	AA48	100	NT7	99	NL8	53	VA27	53	AA48	52	NT8	52
AU8	53	AU8	99	NL7	97	AU8	51	AU8	52	AU8	51	NT6	51
LA23	53	NL7	98	AA40	96	NT7	51	AU9	49	LA30	50	NT7	50
VA21	51	LA23	97	NT8	96	AA48	50	AA40	48	NV8	50	AU9	49
NT6	48	AA40	91	NL6	95	VA27	50	TA39	47	NT6	49	AA36	49
AU9	46	LA38	91	AU7	93	NL6	48	NV7	47	VA21	48	AA48	49
NL7	46	NL6	88	AU8	89	AU9	47	NV8	45	AU7	47	AU8	48
NT8	44	NL8	86	NV6	88	AA36	47	AU7	43	TA25	47	TA8	47
AA36	38	AU9	80	AA36	86	NL7	44	LA30	42	NT7	46	AA40	46
NL6	37	AA36	79	AU9	84	NT8	43	NT7	41	VA27	44	TA39	46
control	35	control	67	control	84	control	34	NV6	37	TA8	39	NV7	45

3.6 The multivariate nature of the wine aroma descriptions

Many of the descriptors assigned to the wines were correlated with one another. Principal components analysis was used to explore these patterns (Appx. F). Both the Chardonnay wine aroma (Fig. 3.1) and the Cabernet Sauvignon wine aroma (Fig. 3.2) varied most substantially in three 'directions.'

It is not surprising that associations should exist among some of the aromas. They may have arisen from a common process (*e.g.* coopering heat), and assigning aroma descriptions involved the combination of 15 personal perceptions, each individual bringing different aroma and semantic experiences to the task. A single stimulus could be described by different words, leading to sets of associated descriptors. Although extensive training was conducted in an attempt to minimise this effect (Appx. D.2), it is unlikely that every discrete stimulus was neatly assigned a discrete descriptor. Regardless of the success, *sets* of descriptors allow communication of the perceptions of the sometimes difficult to define aroma of wine to an audience of diverse aroma and semantic experience. The aroma principal components were occasionally used, as summaries of the general aroma perceptions, in the discussion, especially in Chapters 5 and 6.

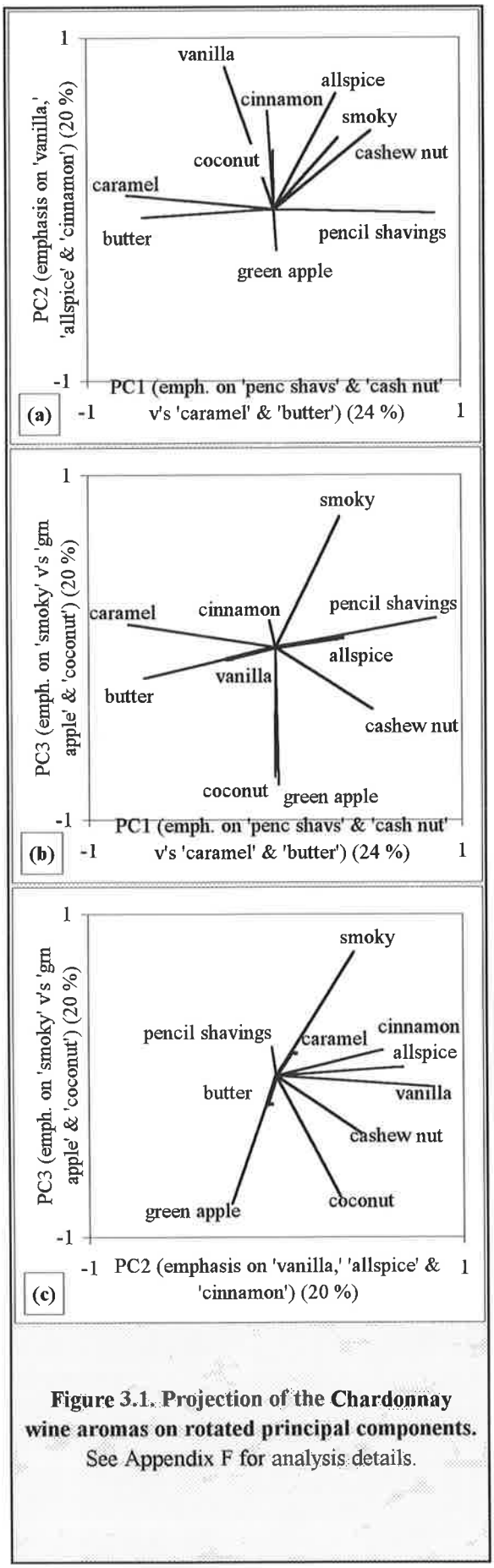


Figure 3.1. Projection of the Chardonnay wine aromas on rotated principal components.
See Appendix F for analysis details.

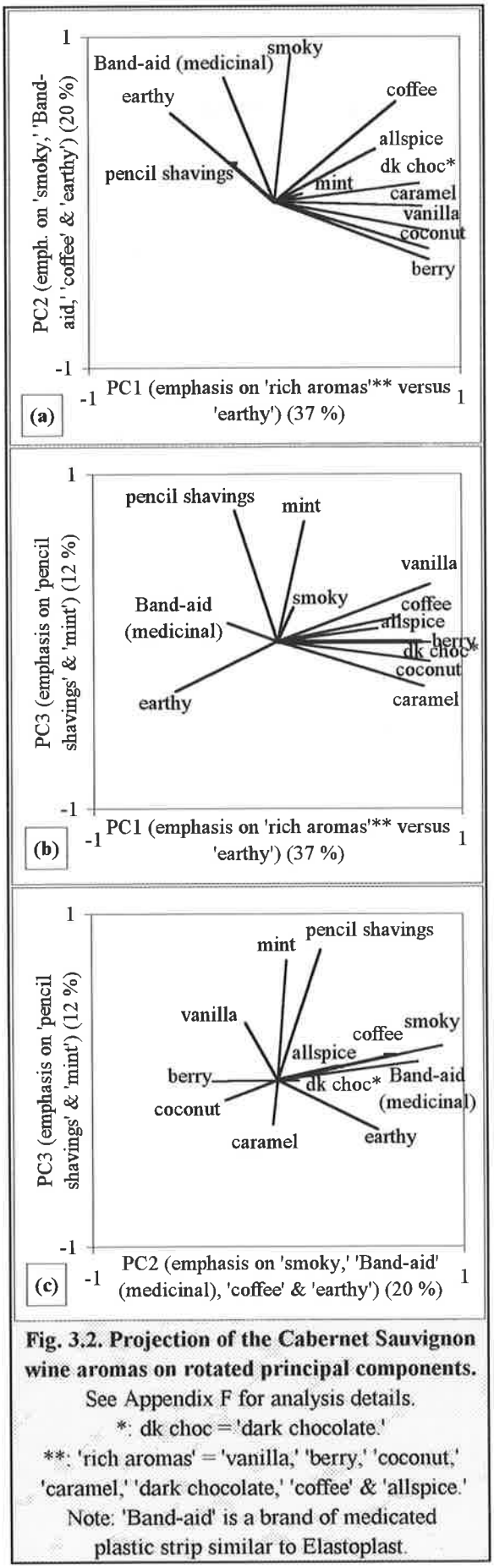


Fig. 3.2. Projection of the Cabernet Sauvignon wine aromas on rotated principal components.
See Appendix F for analysis details.
*: dk choc = 'dark chocolate.'
**: 'rich aromas' = 'vanilla,' 'berry,' 'coconut,' 'caramel,' 'dark chocolate,' 'coffee' & 'allspice.'
Note: 'Band-aid' is a brand of medicated plastic strip similar to Elastoplast.

3.7 Summary and conclusion

The sensory descriptive analysis method applied to the Chardonnay and the Cabernet Sauvignon wines is summarised in this chapter (and detailed in Appendix D), and the results are tabulated along with the repeatability estimates. Both the Chardonnay wine panel and the Cabernet Sauvignon wine panel could discriminate differences in ten aroma attributes and 'preference' among the barrels. Only 'earthy' and 'mint' in the Cabernet Sauvignon wines were not discriminated with statistical significance. Each panel also demonstrated that it could reproduce its assessment of the attribute intensities.

The variability of each aroma descriptor in relation to the variability of other aroma descriptors applied to the same barrel wines is explored through PC analysis.

With both the volatile composition variability (Chapter 2) and the aroma variability now thoroughly described, the remaining chapters are concerned with elucidating the nature of any relationships which might exist between these two sets of data. The aroma effects of the treatments are also considered.

Chapter 4

A protocol for elucidating the relationships between aroma and composition

Chapter outline

4.1 A limitation to the treatment–based experimentation, and an alternative approach

Now that the aroma and the composition variabilities among the wines have been illustrated (Chapters 2 & 3), the remainder of the thesis deals with an exploration of their natural or cultural causes. Chapters 5 and 6 are most particularly concerned with this. Chapter 5 deals with the treatment effects and the underlying variabilities that were apparently established most substantially in the oak wood prior to the coopering process. Chapter 6 deals with the effects and variabilities that were apparently established most substantially during the coopering process.

Treatment–based experiments are suited to studies where a successful experimental treatment can be reliably repeated on a large scale. However, the treatments imposed in the main experiment of this study (Section 1.5) can not be reliably repeated. The oak trees were not selected randomly from each defined location; they were harvested from relatively small areas within these locations. Therefore, a winemaker who orders barrels made from Ohio oak for a current vintage, may or may not receive barrels similar in aroma potential to the American oak barrels of this study. This is dependent on the variation in aroma potential within the population of Ohio oak trees, a parameter that could not be reliably estimated from the sample.

Wine aroma can be described, thoroughly, only with the use of sensory analysis. However, it is unrealistic for most oak wood suppliers and winemakers to use sensory descriptive analysis as a routine quality assurance tool due to the high human resource costs involved. Composition analysis, on the other hand, does not provide a complete aroma 'picture' because the aroma properties of many individual compounds and their interactions are not well understood and because some oak wood compounds of sensory importance are probably yet to be identified. Nevertheless, composition analysis is generally more precise than sensory descriptive analysis and it might provide an adequate summary of the aroma–effect. Consequently, exploring the underlying compositional cause of each aroma–effect arising from the barrels may be more valuable than exploring treatment effects.

Associations between compounds and aromas can suggest possible causal relationships or the possibility that both compound and aroma arose independently from the same source. Furthermore, chemical structure can suggest the probable genesis of the compound and, consequently, the possible genesis of the aroma. Once a cause or an indicator were identified, and it could be quantified with a relatively simple or affordable method, that method could be adopted to assure quality during oak wood selection and processing.

In each of the two following chapters, the aroma and then the composition treatment effects are considered separately for each of the experimental levels (*e.g.* oak origin). Then correlations between the aromas and the composition principal components (PCs) are considered. These treatments and PCs can reflect, generally, underlying natural or cultural variables. The significant differentiations for each aroma to have exhibited a treatment effect, or a significant correlation with the appropriate composition PC, were then allocated a likely, and general, natural or cultural genesis (*e.g.* coopering heat).

The possible compositional causes of these aromas are explored using the correlation data for the individual compounds. A novel analytical method was also used in the Cabernet Sauvignon wines for this purpose. This method involved the interpretation of patterns arising from specific aroma differentiations, in relation to compound concentration differences among the wines. Reported compound aroma–likenesses were also used in these considerations.

4.2 Aroma correlations with composition–PCs and volatile compounds

An interpretation of the volatile compound correlation and composition–PC analyses led to the conclusion that the main natural and cultural variables, reflected in the variance of the volatile compounds, were likely to number only a few, and that these variables could be summarised by the composition–PCs (Chapter 2). Two of these PCs were particularly useful. For each wine, the PCs that showed an 'emphasis on natural oak products' or an 'emphasis on coopering heat products' were used to help identify any aroma that could have owed its genesis, at least partially, to 'natural oak product' variation (Chapter 5) or to 'coopering heat product' variation (Chapter 6).

An aroma that was correlated with a composition–PC or that exhibited a treatment effect, associating it with a particular natural or cultural variable, was subject to further analyses, including correlation analysis with the individual volatile compounds, in an attempt to identify the likely compositional causes or indicators of each aroma (Appx. G & H). Some compounds may have directly caused or contributed to some of the aromas, and some may have only been related by having arisen from a common source or by having arisen due to chance.

The associations between aromas and composition–PCs or compounds were explored by both Spearman's rank order correlation, after ranking the composition values, and by Pearson's product–moment correlation, after converting the sensory rankings to Fisher–Yates rank transformations. Since the latter method preserved more information, it is the method referred to during discussions and it was used to generate the Figures (Appx. Figs. G.1 to G.12 for the Chardonnay wines, and Appx. Figs. H.1 to H.14 for the Cabernet Sauvignon wines).

Compositional discrepancies between 'composition' and 'sensory' samples

Since the sensory (aroma) data and the volatile compound data were to be compared for possible associations, it was important to ensure that the two different sets of samples remained compositionally as close as possible, between barrel–sampling and analysis. However, while all of the 'composition' samples were stored at approximately -10 °C in large, incompletely filled containers until analysis, the 'sensory' samples were stored at higher temperatures to prevent the small, near–full containers from exploding. This has led to some compositional discrepancies.

The Chardonnay wine 'sensory' samples were stored at approximately 2 °C prior to analysis (Appx. A.3) so the composition, at the time of the sensory analysis, is likely to have been similar to the composition of the different set of samples at the time of the composition analysis. However, the Cabernet Sauvignon wine 'sensory' samples were stored at approximately 20 °C (Appx. A.4) due to lack of freezer space. This approximately 30 °C

temperature–discrepancy, over a storage period of two years, has caused some compositional discrepancies between the two sets of Cabernet Sauvignon wine samples.

An analysis (carried out by others in this laboratory, using alternative analysis methods) of seven of the 20 volatile compounds in the Cabernet Sauvignon wine 'sensory' samples, one year after the sensory analysis, indicated that some concentration changes had occurred, relative to the 'composition' samples. Five of the seven compounds (*cis*– and *trans*–oak lactone, 4–methylguaiacol, furfural and 4–ethylphenol) changed little over the storage period (Sefton, unpublished data). Small decreases in the oak lactones and small increases in 4–methylguaiacol are likely to have resulted from methodological differences.

One compound, furfuryl alcohol, was degraded by approximately 75 %, over the three years cellar–storage period, and the degradation was consistent among the samples (Sefton, unpublished data). When estimating the concentration of furfuryl alcohol that was associated with significant aroma differentiations (explained in Section 4.5), an estimate of the concentration was made, based on a linear interpolation of the degradation–rate line. The concentrations likely to have been present at the time of the sensory analysis — after two years of cellar–storage — are approximately half those found in the freezer–stored samples (Tab. 2.5).

The concentrations of furfuryl ethyl ether, a compound that apparently exists in equilibrium with furfuryl alcohol, at consistent proportions (work of Sefton, in Spillman *et al.* 1998), were similarly estimated for this purpose.

Concentration changes, provided that they were consistent among the samples, would not have affected the correlation–based results. However, for one compound which was degraded inconsistently among the samples (vanillin, approximately 30 to 50 % degradation; Tab. 2.5), all of the analyses involving comparisons between the original composition data (from the freezer–stored samples) and the sensory data are dubious. To mitigate this problem, analysis of the (excess) cellar–stored samples was performed approximately one year after these samples were subjected to sensory analysis (Tab. 2.5). These composition data are likely to most closely resemble those at the time of the sensory analysis. Therefore, these vanillin data are used when exploring aroma associations with this compound.

The inconsistency of the degradation of vanillin among these Cabernet Sauvignon samples is likely to have resulted at least in part from microbial activity. Each ten–litre 'sensory' sample was stored in two five–litre containers for 41 weeks at approximately 20 °C prior to sterilisation, and vanillin may have been subject to microbial reduction in these wines (Chapter 7).

4.3 Difficulties in estimating the aroma effect of a single compound

While correlation analysis can be useful in identifying aromas associated with individual compounds or, more generally, with natural or cultural variability in oak wood, it is preferable to identify the actual compositional cause of each aroma. Methods involving compound purification and sensory description or sensory threshold analysis are applied for this purpose. This area of study produces unreliable results unless a good deal of care and effort is invested in the process.

Compound purification and its practical limitation

Compound purity is an important consideration when determining thresholds. Meilgaard (1989) suggested using successive purification steps with the threshold and character determined after each. Then, like constant melting point in chemical purification, *sensory purity* may be considered once successive steps cause no change in sensory type and threshold. However, this is seldom carried out due to the substantial effort required.

Some compounds are very difficult and/or expensive to extract from natural products in sufficient quantities or to synthesise from more readily available compounds for the purpose of sensory studies. In such cases, an estimation of the compound's aroma effect is either impossible, unlikely to be attempted, or a sample of questionable purity might be used.

An example of this sort of problem which is important to this study involves the isomers of the oak lactones. While there is only one naturally occurring *cis*– and one naturally occurring *trans*–enantiomer, samples of these enantiomers are difficult to obtain. The sample used in this study (Allied Flavours, whiskey lactone, isomeric mix of β–methyl–γ–

octalactone) was a racemic mixture of the two enantiomers of *cis*– and the two enantiomers of *trans*–β–methyl–γ–octalactone. Most of the sensory analyses reported for this compound in a beverage (*e.g.* Boidron *et al.* 1988) have used a racemic mixture similar to this sample so the description and the threshold data resulting from these studies may be deficient in some ways. While Boidron *et al.* (1988) described a racemic mixture of the oak lactones as coconut– and oak–like, Günther and Mosandl (1986) described each of the naturally occurring enantiomers in more detail. The naturally occurring *cis*–enantiomer (3S, 4S) possessed a "coconut, slightly musty and earthy" aroma with a "hay" note, while the naturally occurring *trans*–enantiomer (3S, 4R) possessed a "fragrant celery" note, with a "weak coconut" aroma and some "green walnut" character.

Compound description and its practical limitations

The concentration at which a compound is presented for aroma description can impact upon the result. Description of a compound's sensory effect must be performed at a concentration or over a range of concentrations typical of those found in the product of interest, and in a medium similar to the product. Chatonnet *et al.* (1991) presented two figures which describe the descriptive analysis of racemic β–methyl–γ–octalactone (*cis/trans* = 1) at various concentrations in a white wine. They generated descriptions of the isomeric mixture at concentrations of 50, 150, 300, 500, 800 and 1600 μg/L using the terms, 'intensity,' 'finesse,' 'fruity,' 'woody,' 'coconut,' 'varnish' and 'resin.' They found that 'intensity,' 'coconut' and 'varnish' increased over the range whilst 'finesse,' 'fruity,' 'woody' and 'resin' increased to a concentration around 500 μg/L and then decreased.

Unfortunately, even when care is taken with the compound concentration and the medium, the data may still be misleading since the effect of the purified compound in a natural product may depend on a variety of compounds acting in combination. Reazin (1981) has observed that oak lactones on their own appear to have a coconut–like aroma but when mixed with furfural this seems to be modified to a 'woody,' 'caramel' or vanilla–like aroma. This observation recommends the incorporation of mixtures of compounds into individual compound character studies.

Compound threshold analysis and its practical limitations

The potency of a compound is classically determined through threshold testing. Altner (1986) suggested that it is useful to consider two different thresholds, the detection– and the recognition–threshold. The detection threshold is the minimum concentration at which a certain substance can be *perceived* 50 % of the time, while the recognition threshold (usually higher) is the minimum concentration at which a substance can be *identified* 50 % of the time. Another useful threshold measurement is that known as the difference threshold. This is the minimum concentration difference, either higher or lower, from a given concentration above the detection threshold, at which a difference is perceived 50 % of the time.

The practical limitations, discussed above with reference to compound descriptions, also apply to threshold determinations. Even if the analyses of the individual compounds become comprehensive, they will never be really useful without a good understanding of the interactions among compounds.

Since each oak wood–derived volatile compound does not occur in isolation from the others in wine, and since their quantities tend to vary in groups, according to natural or cultural influences (*e.g.* coopering), the study of the aroma impact of the individual compounds is of limited use. The estimation of the impact of the correlated groups of compounds, in the presence of relatively low quantities of other oak wood–derived compounds, recognises the possible substantial contribution of unknown compounds and the possibility of interactive effects.

To overcome some of the limitations discussed above, the experiments described in Sections 4.4 and 4.5 were performed.

4.4 Potency of the overall, oak wood–derived, aroma–effect of selected individual barrel wines

Where it is desired to estimate the overall effect of compounds at known concentrations within an 'ingredient' of a food product, the threshold of the 'ingredient' may be determined

by mixing it with the food product in varying proportions. However, for this 'ingredient potency' to be of use in the area of oak wood research or wine quality assurance, the quantities of the compounds of interest must be correlated with one another across a wide range of oak wood samples, and other compounds within the 'ingredient' which are suspected to be aroma–active must be limited in their quantities.

This analysis can provide an estimate of the potency of the targeted compounds, in combination, limited to the point at which the most potent known or unknown compound or group of compounds, acting in concert, is perceived. Analyses of this type were performed on some of the Chardonnay barrel wines.

Three different barrel–stored Chardonnay wines were selected, each one to emphasise one of the three main 'directions' of compositional variability (Fig. 2.1). The stainless steel drum–stored Chardonnay (control) wine was used to dilute each one of the wines to varying degrees before presentation to a panel of 20 persons for 3–Alternative Forced Choice (3–AFC) difference testing against the control (Appx. I). In other words, each of these barrel–stored wines was diluted with the same but stainless steel–stored wine to determine the proportion of barrel wine required in the blend for the panel to just notice a difference.

In the main experiment of the study, the Chardonnay control wine differed to the barrel–stored Chardonnay wines according to the barrel–maturation stage. The control wine lacked the oak wood compounds absorbed by the latter (Tab. 2.2). There are likely to have been other compositional difference, due to the possible permeability of barrel staves to wine components and to air, and to the probable impact of oak wood–derived oxidation catalysts on the barrel–stored wines. Nevertheless, the control wine was considered to be a suitable base, to which selected individual barrel wines could be added, to estimate the overall aroma impact of particular aroma compound groups. Figure 4.1 illustrates the relative quantities of the oak wood–derived compounds for the three selected barrel wines.

'Natural oak product' potency

The first barrel wine (*VA39*), possessing the highest composition–PC1 score (*i.e.* an 'emphasis on natural oak products and oak origin associations with some microbial products') and the lowest PC2 score (*i.e.* a lack of 'emphasis on coopering heat products'), was used to estimate the aroma impact of the 'natural oak products' (*i.e. cis*– and *trans*–oak lactone and eugenol). This wine is a useful selection for the purpose of the experiment except that 4–vinylguaiacol and 4–vinylphenol were also associated with the 'natural oak products.' These two volatile phenols were only present in very small quantities in the Cabernet Sauvignon and model wines (< 6 µg/L) and were not correlated with the oak lactone or eugenol concentrations so they are not considered an integral part of the PC1 group. Since these compounds are known to be present in large quantities directly after primary fermentation and then to degrade during storage (Nicolini *et al.* 1991, Dugelay *et al.* 1995), they may have been coincidentally associated with the oak lactones and eugenol due to variable degrees of oxidation catalysis provided by the different oak woods. However, the two compounds may not have substantially influenced the threshold estimations since they were present at less than one–tenth of their reported thresholds in a white wine (Tabs. 1.1 & 2.2). Indeed, the compounds were present at less than one half of one percent of their reported thresholds after *VA39* was diluted to the group 'best estimate threshold' (BET, Meilgaard *et al.* 1991 pp. 124–128).

Appendix I details the analysis and the results, and Table 4.1 shows the group BETs, *i.e.* the estimated group detection thresholds, each as a percentage of barrel wine *VA39* in the control wine. It also shows the concentration of the 'featured' compounds when diluted to this level, and the degree to which the published individual compound thresholds differ from these concentrations. The results suggest that the concentrations at which the compounds, acting as groups, begin to have a significant impact on aroma differentiation among wines is much lower than that indicated by the published individual compound thresholds. Consequently, compounds present at concentrations below their published threshold cannot be overlooked when exploring the possible causes of aroma variation.

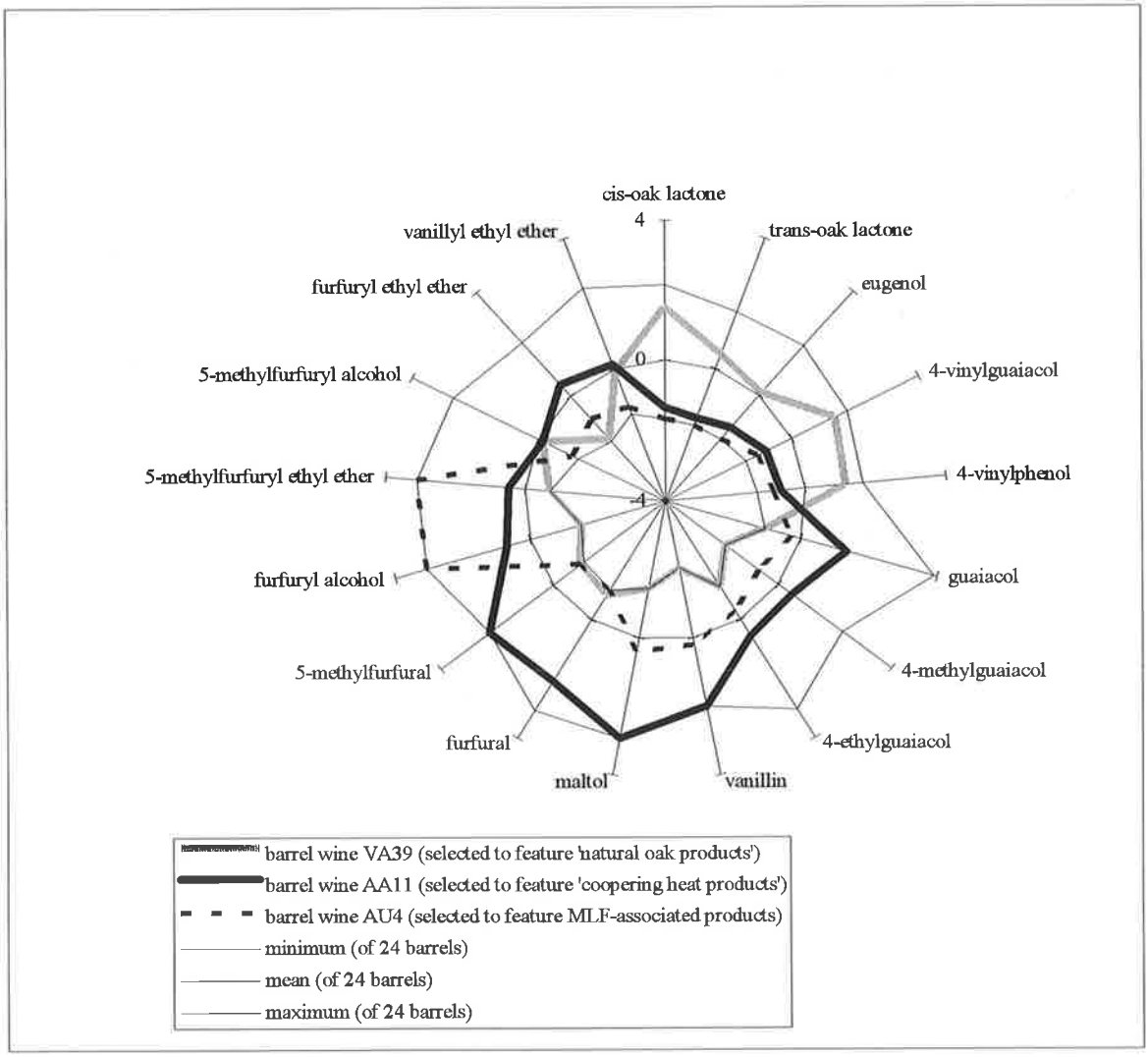


Figure 4.1. Relative composition (as *z*-scores) of the three Chardonnay barrel wines selected for the 'ingredient potency' tests.
Three compounds (cyclotene, vanillyl alcohol and 4-ethylphenol) have been omitted due to imprecision of measurement, *etc.*, as discussed in Chapter 2.

The *cis*–oak lactone was present at a concentration closest to its threshold concentration, but with a disagreement factor of four, it would need to have been present at four times its concentration to have reached the published threshold concentration of 92 µg/L (Chatonnet *et al.* 1992c).

'Coopering heat product' potency

A second barrel wine (*AA11*), possessing the second highest composition–PC2 score (*i.e.* an 'emphasis on coopering heat products') and the second lowest PC1 score (*i.e.* a lack of 'emphasis on natural oak products and oak origin associations with some microbial products') was used to estimate the aroma impact of the 'coopering heat products.' This barrel was probably most suited to the analysis. All seven of the 'coopering heat products' were strongly correlated with one another, and they were present at relatively high concentrations, against a relatively low background of the other compounds (Fig. 4.1). Despite this, none of the seven compound concentrations at the BETs were near the published thresholds (Tab. 4.1). Perhaps these compounds are particularly inclined to impress themselves on the olfactory senses in a concerted manner.

'Malolactic fermentation product' potency

A third barrel wine (*AU4*) was included since it had experienced near complete MLF (88 % malate consumption) and since it possessed a medium composition–PC2 score (*i.e.* a moderate 'emphasis on coopering heat products') and the lowest PC1 score (*i.e.* a lack of 'emphasis on natural oak products and oak origin associations with some microbial products'). Despite an almost complete MLF, however, the compounds known to arise from microbial activity were not all correlated. Some constituted PC3 (*i.e.* 'emphasis on some microbial products') and some were incorporated negatively into PC1. Thus, *AU4* contained relatively high quantities of only two of the ten compounds known to arise from microbial activity (Fig. 4.1) and it was, perhaps, not particularly useful in this analysis. Nevertheless, it is included for the information it provides.

Table 4.1 The extent of disagreement between published detection thresholds and the concentrations at which the concerted oak wood aroma–effect in a Chardonnay wine reached detection threshold.

Group BET[††] of selected individual barrel wines (as % dilution in control wine)	Compounds 'featured' in the selected barrels	Conc. at Group BET (µg/L*)	Published detection threshold (wh. wine) (µg/L*)[†]	Disagreement factor[‡] for the 'featured' compounds		
				'Natural oak products'	'Coopering heat products'	MLF–associated products
barrel wine *VA39* (selected to feature 'natural oak products') diluted to 6.4 %	*cis*–oak lactone	23	92	4		
	trans–oak lactone	8	460	58		
	eugenol	1	100	100		
	4–vinylguaiacol	2	440	220		
	4–vinylphenol	4	770	192		
barrel wine *AA11* (selected to feature 'coopering heat products') diluted to 6.4 %	guaiacol	1	95		95	
	4–methylguaiacol	0.3	65		217	
	4–ethylguaiacol	0.1	70		700	
	vanillin	25	400		16	
	maltol	9	unknown		unknown	
	furfural*	0.42*	65*		155	
	5–methylfurfural*	0.04*	52*		1300	
barrel wine *AU4* (selected to feature MLF–associated products) diluted to 7.4 %	furfuryl alcohol*	0.62*	35*			56
	5-methylfurfuryl ethyl ether	4	unknown			unknown

[††] BET: Best estimate threshold.

* Furfural, 5–methylfurfural and furfuryl alcohol concentration values are in mg/L.

[†] Boidron *et al.* (1988); Chatonnet *et al.* (1992c). Determined in a white wine. See Table 1.1 for more information.

[‡] Disagreement factor = published threshold / group BET for 'featured' compounds, *e.g.* the *cis*–oak lactone concentration in barrel wine *VA39*, diluted to the group BET of 6.4 %, was 4 times lower than the published threshold. The disagreement factor for those compounds not 'featured' in each selected barrel wine was at least 20.

Conclusion

The fact that all of the compound concentrations at the group BETs are lower (at least four times lower) than the published threshold values suggests that additivity of aroma effect, at least, is likely to have occurred in the system. Guadagni *et al.* (1963), by experimenting with mixtures of sub–threshold concentrations of odorous materials, demonstrated that aroma compounds were capable of this effect. For example, odour was perceived for a mix of ten compounds, each at 10 % of its individual threshold. Keith and Powers (1968), however, concluded from experimentation that sub–threshold additive effects are not common. In support, Salo *et al.* (1972) concluded that, in a whisky model system, the aromas perceived were more characterised by suppression and synergism than additivity. The acid fraction was typified by suppression (antagonistic effect), while some of the carbonyl and ester mixes were typified by apparent synergism. Additivity seemed to be typical only of mixtures composed of few components.

There are some alternative explanations including the possibility that an undetected compound may have been present at a concentration above its detection threshold. It is also possible that the dilution process could have caused some chemical changes (*e.g.* slight oxidation) which have not been detected.

Whatever might have been the case in the Chardonnay wines, it seems very unlikely that the compounds exerted influences on the aroma, independently. Consequently, a more holistic approach would seem to be appropriate, and the approach taken in this thesis may be more valuable than a study of compounds in isolation.

4.5 A novel data analysis method, involving the interpretation of patterns arising from specific aroma differentiations in relation to compound concentration differences

This method makes use of the same raw data as those used in the aroma–composition correlation analyses (Section 4.2, Appx. G & H). Thus, it is limited to the illustration of patterns of association and, like correlation analysis, a pattern of association generated by this method may or may not have arisen from a causal relationship. Nevertheless, since the

method uses only the statistically significant aroma differences and since it applies novel tests to the data, it can be a useful *additional* tool in studies which attempt to elucidate the relationship among aromas and compounds. This novel data analysis method was applied in this thesis to the Cabernet Sauvignon wines only.

The method first required the calculation of the concentration differences for each compound among the 24 barrel wines. The 276 concentration differences (among 24) were ranked from smallest to largest and then ten roughly equal sized groups (deciles) of 28 were formed (Appx. Tab. J.1). For each of the aromas, the proportion of the 28–odd comparisons in each decile, that was significantly different (Tab. 3.3) in the same direction as the compound concentration, was determined. Similarly, the proportion that was significantly different in the opposite direction as the compound concentration was determined. The pattern that emerged was a reflection of the association between the statistically significant differentiations for a specific aroma and the compound concentration differences within samples of a product which are concurrently subject to many other compound concentration variations, as are typical of a real product.

One of the 276 concentration differences for each of the compounds was present before a panelist when he or she considered a specific aroma difference between two glasses of wine, and attempted to rank one higher or lower than the other (Chapter 3). If a compound were active in affecting any specific aroma differentiation between the two glasses, two aspects of the result are likely. The panel is likely to have successfully differentiated between the two wines, according to the specified aroma, and the direction of the aroma difference (*e.g.* A>B) is likely to have been the same as the direction of the concentration difference (*i.e.* A>B). Alternatively, the direction of the concentration difference may have been opposite (*i.e.* B>A) to the direction of the aroma difference, in which case the relationship may have been characterised by masking. A test, discussed below, was developed to allow these considerations to be applied to the data with some degree of formality.

Specific aroma 'impact–pattern conformity' (IPC) test

There were 2 x 276 ways (given no nil concentration differences) of a successful aroma differentiation to be consistent with the possibility that a compound had been aroma–active; the contribution may have been a positive one or a negative one (*e.g.* masking). A pattern was overlayed on this background by tallying the number of significant aroma differentiations to have been achieved in each of the bi–directional composition deciles. The percentage of comparisons found to be significantly different in each was calculated for each aroma (Appx. Figs. J.1 – J.11). As an example, Figure 4.2 (same as Appx. Fig. J.2a) shows the pattern for 'coconut' and the *cis*–oak lactone.

If a compound had contributed positively to an aroma differentiation, a sigmoidal pattern, which passes from low at the negative–decile (left–hand) side of the Figure to high at the positive–decile (right–hand) side, should be present. Figure 4.2 is consistent with this pattern except that it failed to approach 100 % significant differentiation. However, given higher concentration differences, the curve would presumably 'flatten out' near 100 % and complete a sigmoidal pattern. The inflection of the curve should be on the side to which the curve is rising (*i.e.* the positive side in Figure 4.2). Further, the curve should be relatively 'smooth,' at least 50 % of the comparisons in one or more of the deciles should be significantly different, and deciles of larger concentration difference should also exceed the 50 % line. If a compound had contributed negatively to an aroma differentiation (*e.g.* by masking), a reverse of this pattern should be present. Non–conformity to such a shape suggests that the compound is likely to have had no significant effect on the aroma; conformity provides evidence to suggests that it may have.

The specific aroma 'impact–pattern conformity' (IPC) test is so–named because it deals with the premise that, if the variation in the concentration of a compound were to have impacted upon the significant differentiation of a specified aroma (or other sensory signal), the pattern involving a comparison of the compound concentration differences and the proportion of significant aroma differentiation should conform to that described, above.

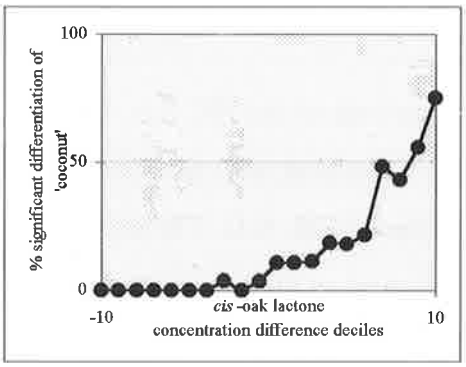


Figure 4.2. 'Coconut' aroma 'impact-pattern conformity' (IPC) test for *cis*-oak lactone in the Cabernet Sauvignon wines.

Table 4.2. Naturally occurring specific aroma 'differentiation potencies or accompaniments' (DPAs) in a Cabernet Sauvignon wine.

Each is the approximate concentration above which at least 50 % of every decile of comparisons (n ~28 per decile) was differentiated according to the specified aroma (p <0.05). 276 Comparisons among 24 barrels of a 93 week barrel-stored Coonawarra Cabernet Sauvignon wine were involved. Each value is the minimum, in the range of absolute values, of the first decile to exceed 50 % significant differentiation. Deciles of larger concentration differences had to also exceed 50 %, and a 'smooth' curve was required. See Appendix Figures J.1 to J.11. 'Earthy' & 'mint' were excluded since no significant differentiation was achieved (Tab. 3.3).

	DPAs (expressed to 1 significant figure, in mg/L)												
	'Natural oak products'			*'Coopering heat products'*					*'Microbial activity products'*				
Aroma	***cis***	***trans***	***eug***	***guaiac***	***4mg***	***van-c#***	***cyc***	***malt***	***falc***	***5mfalc***	***fee***	***4eg***	***4ep***
preference													
coconut	0.4	0.2	0.02										
pencil shavings													
allspice													
berry	0.5		0.03										
smoky				0.02					4*		0.03*		
caramel					0.01			0.1	4*		0.03*		
vanilla	0.4		0.02		0.01	0.08							
coffee					0.01	0.05			3*		0.02*		
dark chocolate													
Band-aid													

cis=*cis*-oak lactone, trans=*trans*-oak lactone, eug=eugenol, guaiac=guaiacol, 4mg=4-methylguaiacol, van=vanillin, cyc=cyclotene, malt=maltol, falc=furfuryl alcohol, 5mfalc=5-methylfurfuryl alcohol, fee=furfuryl ethyl ether, 4eg=4-ethylguaiacol, 4ep=4-ethylphenol.

van-c =vanillin from cellar-stored samples (~20 degC for 1 year from barrel sampling, then sterilised with DMDC and stored for a further 2 years at ~20 degC). The sensory analysis was performed on the cellar-stored samples approximately 1 year after DMDC sterilisation. The vanillin data from the freezer-stored samples have been omitted since the data from the cellar-stored samples more closely approximate the vanillin concentrations at the time of the sensory analysis.

*: Values for furfuryl alcohol and furfuryl ethyl ether have been adjusted to account for discrepancies between the 'sensory' samples and the 'composition' samples (*i.e.* concentration changes occurred during cellar-storage of 'sensory' sample bottles, relative to the freezer-stored - 'composition' - sample bottles). See Section 4.2 for details.

Three compounds (vanillyl alcohol, 5-methylfurfuryl ethyl ether and vanillyl ethyl ether) have been omitted, as discussed in Section 2.4. 'Estimated extracted furfural' has been omitted since it does not exist as a unit in the wine. 4-Vinylguaiacol and 4-vinylphenol have been omitted since the repetitively low concentrations made roughly equal decile groupings impractical. Furfural and 5-methylfurfural have been omitted since they were present in very low quantities relative to their detection thresholds (Tabs. 1.1 & 2.5).

A naturally occurring, specific aroma 'differentiation potency or accompaniment' (DPA)

For each compound that did conform to the IPC test, for a particular aroma, an estimation of the concentration difference associated with the panel's 50 % recognition of the specific aroma difference could be obtained (Tab. 4.2). The point at which the curve crosses the 50 % line (*e.g.* Fig. 4.2) was estimated as the minimum concentration difference value within the first decile to exceed the 50 % line (provided that the curve remained above the line once it had been crossed). This concentration difference value was rounded to one significant figure (but not below 0.01 mg/L), given the questionable usefulness of precise compound potency data (Altner 1986), and is referred to as the specific aroma (*e.g.* the 'coconut'–) 'differentiation potency or accompaniment' (DPA) for the compound. The 'coconut'–DPA for the *cis*–oak lactone was 0.4 mg/L (Tab. 4.2). Thus, within this experiment and among all of the variation expressed by the other compounds, the *cis*–oak lactone concentration difference above which the 'coconut' aroma was differentiated in the correct direction and with statistical significance on more than half of the occasions it was presented was 0.4 mg/L. The DPA is similar, in very general terms, to a difference threshold value but there are some important differences.

Whilst a difference threshold is based on the variation of a single *purified* compound, *isolated* from any other compound variation, the DPA is based on the variation of a single *pure* compound among a large variety of compound variations typical of those to be found in the natural system of interest. Therefore, an aroma effect suggested by a DPA value might have arisen from a known or unknown associated compound or from a combination of compounds. This is why the concentration difference value is referred to as being *either* a potency for causing aroma differentiation *or* as being merely an accompaniment of the aroma differentiation which was caused by another agent.

Another point of difference is that the aroma differentiations used in these analyses were based on differentiation of specific aromas, whereas classical threshold values are usually based on non–specific differentiation.

Nevertheless, these differences may be improvements, for some practical purposes, on classical threshold determinations. The method considers a *specific* aroma and a *pure*

compound in a *whole* natural system, and the concentration difference that might *cause or accompany* a specific aroma differentiation is estimated.

The DPAs estimated for those compounds that passed the IPC tests (Tab. 4.2) are incorporated into discussions throughout the following chapters.

4.6 Summary and conclusion

Given the limitations of the treatment–based experimentation, an alternative approach was required to extract the maximum information from the raw data described in Chapters 2 and 3. This approach, involving the exploration of associations between aroma and composition data and the development of a novel data analysis method, was explained in the current chapter. The intention of this approach was to elucidate possible causal relationships between specific compounds and aromas.

The limitations of classical purified compound characterisation techniques (*e.g.* threshold determination) was discussed with supporting data from a novel ('ingredient potency') test.

Another novel data analysis method was developed to further explore the possibility of causal relationships between volatile compound concentration variation and a specific aroma effect: The IPC test and the associated DPA (which is similar, in very general terms, to a difference threshold value) have been described.

Having, thus, developed a protocol for elucidating the relationships between aroma and composition, a full discussion of the experimental treatment effects and the underlying variations can proceed.

Chapter 5

The contribution of the oak compounds that were present in the wood prior to coopering

Chapter outline

5.1 The boundaries of the variation under consideration

This chapter focuses on the wine aroma treatment effects that are likely to have arisen, at least partially, from the oak compounds known to be present in wood in significant quantities prior to coopering. The treatment effects for these compounds — *cis*– and *trans*–oak lactone and eugenol — were also explored. The experimental levels imposed at this stage were oak origin and seasoning location. There may have also been significant aroma variation independent of any treatment or error, so the aroma data for the Chardonnay and the Cabernet Sauvignon wine were explored by correlation analysis with the 'natural oak products' principal components (PCs). Any aroma that showed a significant treatment effect or correlation from these analyses was explored, further, by correlation analysis with individual compounds and (for the Cabernet Sauvignon wine only) by the 'impact–pattern conformity' test (Section 4.5).

The oak wood was obtained from four geographical locations (Section 1.5), and is referred to as American, Limousin, Tronçais and Vosges. The selection of these four sources was not random — they were selected because they are commercially important. Further, the selection of trees within each location was not random (*i.e.* trees from specific areas within each location were harvested). Consequently, conclusions are drawn with reference only to the samples, and their predictive quality is limited to the extent that the samples were representative of the oak tree populations of the defined locations (something which was impossible to estimate from the samples). The results of future experiments, involving samples from the same broadly designated regions, may not be the same as those found in this study.

The American oak wood sample, in particular, is least likely to have estimated the variability within the population of American oak trees since it was restricted to one area of one state in the USA. Indeed, many researchers have found American oak to contain higher quantities of the oak lactones than French oak (*e.g.* Marco *et al.* 1994, Masson *et al.* 1995), a trend opposite to that found in this study. Waterhouse and Towey (1994) concluded that, due to the large variance among their American oak samples, oak lactone quantities would be a poor indicator of wood source. Obviously, the term 'American oak' is not specific enough

to be of any descriptive value in terms of many of the apparently aroma–important volatile compounds.

Even the relatively well defined and small oak wood sources of France show large variances in oak lactone quantities (*e.g.* Masson *et al.* 1995). It seems likely that the sampling protocols for many oak wood origin experiments may have been inadequate to allow an accurate estimation of the volatile compound variance to be found within these populations.

The French oak seasoning location treatments were subject to coopering by different coopering companies (tonnelleries). Therefore, any of the (apparent) seasoning location effects may have been confounded by a cooper effect. A 'medium toast' level was specified for all barrels but some of the compounds derived from coopering heat varied according to whether the French oak barrels were coopered in France or in Australia. Thus, different heating conditions may have been inadvertently applied by the different coopers and/or the seasoning conditions may have had an impact on the response of the wood to the heating conditions. The moisture content of the wood, for example, could absorb some of the heat applied, therefore reducing the overall impact of the heating. The overall variation among the barrels, regardless of the coopering company, is discussed in Chapter 6.

Despite the concurrence of both the seasoning and cooper treatments for the French oak — and to facilitate the discussion — effects for the oak lactones and eugenol, which were present in substantial amounts prior to coopering, are discussed as seasoning effects, while those for the 'coopering heat products' are discussed as coopering heat effects (Chapter 6). Chatonnet *et al.* (1989) have reported that the oak lactones and eugenol can be affected by coopering heat variation but the range of heating involved in their experiments was apparently much greater than the range imposed in this study. Within the coopering heat variation encountered in this study, the oak lactones and eugenol were apparently not affected (*i.e.* there was no association between the 'natural oak products' and the 'coopering heat products' — Figs. 2.1, 2.2 & 2.3; Appx. Tabs. C.1, C.6 & C.11).

The American oak was seasoned in two different locations before being coopered by one cooper. Thus, seasoning location effects were isolated from cooper effects for this oak.

5.2 Oak origin effects

The data for the French oak barrels made in both France and Australia were analysed in fully crossed, factorial analyses of variance (ANOVAs) (fixed factors). However, when comparing the American oak with each of the French oaks, only half of all of the barrels could be included, namely those that had been subjected to the same seasoning and coopering treatment (in Australia) (Fig.1.2).

The wines (Chardonnay and Cabernet Sauvignon for aroma — Chardonnay, Cabernet Sauvignon and model wine for composition) were tested for treatment effects together, before any of the wines were tested singly. Only those aromas or compounds showing a significant interaction in the combined wines ANOVAs were considered in the separate wine analyses. This rule was overlooked once (for 'vanilla') when, despite the absence of any significant analytical interaction, an effect seems to have differed between the wines (Appx. Tabs. K.1, K.2 & K.3). Full details of all of the ANOVAs are shown in Appendix K, and these should be consulted when considering the treatment effect Figures.

In Figures 5.1 to 5.7 the combined wines analyses are shown in sub–Figure (a). Those aromas or compounds excluded from this sub–Figure (due to significant analytical interaction) are shown for the different wine analyses in the other sub–Figures. Treatment effects for the PCs are also illustrated in these Figures, and are discussed when they offer a useful summary of the individual aroma or composition effects.

Differences among the French oak woods

No significant aroma differences in either individual aromas or aroma–PCs were found among the three French oak wood origin treatments for the Chardonnay wine (Fig. 5.1 a, b & c). By contrast, for the Cabernet Sauvignon wine, 'coconut' and 'vanilla' were higher in the Vosges and Tronçais barrels than in the Limousin (Fig. 5.1d).

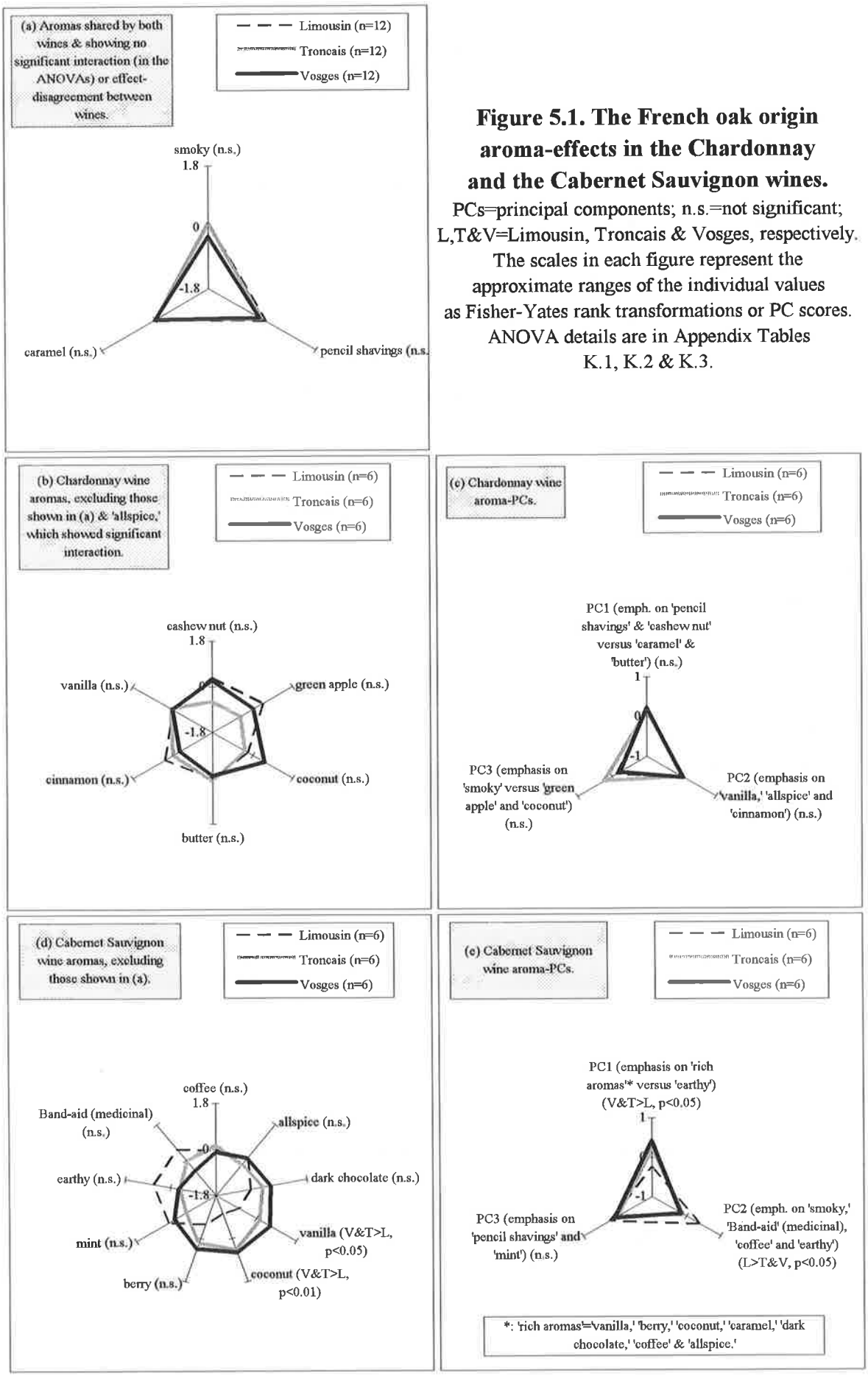


Figure 5.1. The French oak origin aroma-effects in the Chardonnay and the Cabernet Sauvignon wines.
PCs=principal components; n.s.=not significant; L,T&V=Limousin, Troncais & Vosges, respectively. The scales in each figure represent the approximate ranges of the individual values as Fisher-Yates rank transformations or PC scores. ANOVA details are in Appendix Tables K.1, K.2 & K.3.

Two of the Cabernet Sauvignon wine PCs showed significant differences that were consistent with the 'coconut' and 'vanilla' differences. The aroma–PC1, involving an emphasis on 'rich aromas' (including 'coconut' and 'vanilla') versus 'earthy,' was higher in the Vosges and Tronçais barrel wines than in the Limousin (Fig. 5.1e). Aroma–PC2, involving an emphasis on 'smoky,' 'Band–aid' (medicinal), 'coffee' and 'earthy,' was higher in the Limousin barrel wines than in the Vosges and Tronçais (Fig. 5.1e).

Numerous composition effects were also found among the treatments. For the combined wines analysis, guaiacol was found to be higher in the Tronçais barrel wines than in the Limousin (Fig. 5.2a). Since this compound arises most substantially from the influence of coopering heat, this oak origin effect is curious.

The compositional effects for the separate wines are illustrated in Figure 5.2 (b, d & f) but the effects for the composition–PCs (Fig. 5.2 c, e & g) offer useful summaries. In each of the three wines, the PC involving an 'emphasis on natural oak products' was higher for the Vosges barrels than it was for the Limousin. There was a consistent trend for the *cis*–oak lactone and eugenol among all three wines — Vosges was higher than Tronçais, which was higher than Limousin — although not all of the differences were statistically significant. This trend is consistent with that found in samples of the same oak wood taken prior to coopering (Sefton *et al.* 1993a). The *trans*–oak lactone did not vary significantly, according to oak origin treatment.

There was also a significant effect, in the Chardonnay wine, for the PC involving an 'emphasis on some microbial products,' the Vosges and Limousin barrels being higher than the Tronçais (Fig. 5.2c). No explanation is apparent for this effect.

An explanation for the lack of any significant aroma effect in the Chardonnay wine, according to the origin of the French oak, despite the fact that significant composition effects were found among all three wines, may involve confounding from coopering heat and malolactic fermentation (MLF) variation. The Chardonnay wine barrel–storage period, which was around half of the duration of that for the Cabernet Sauvignon wine, resulting in lower compound concentrations, may also be a factor.

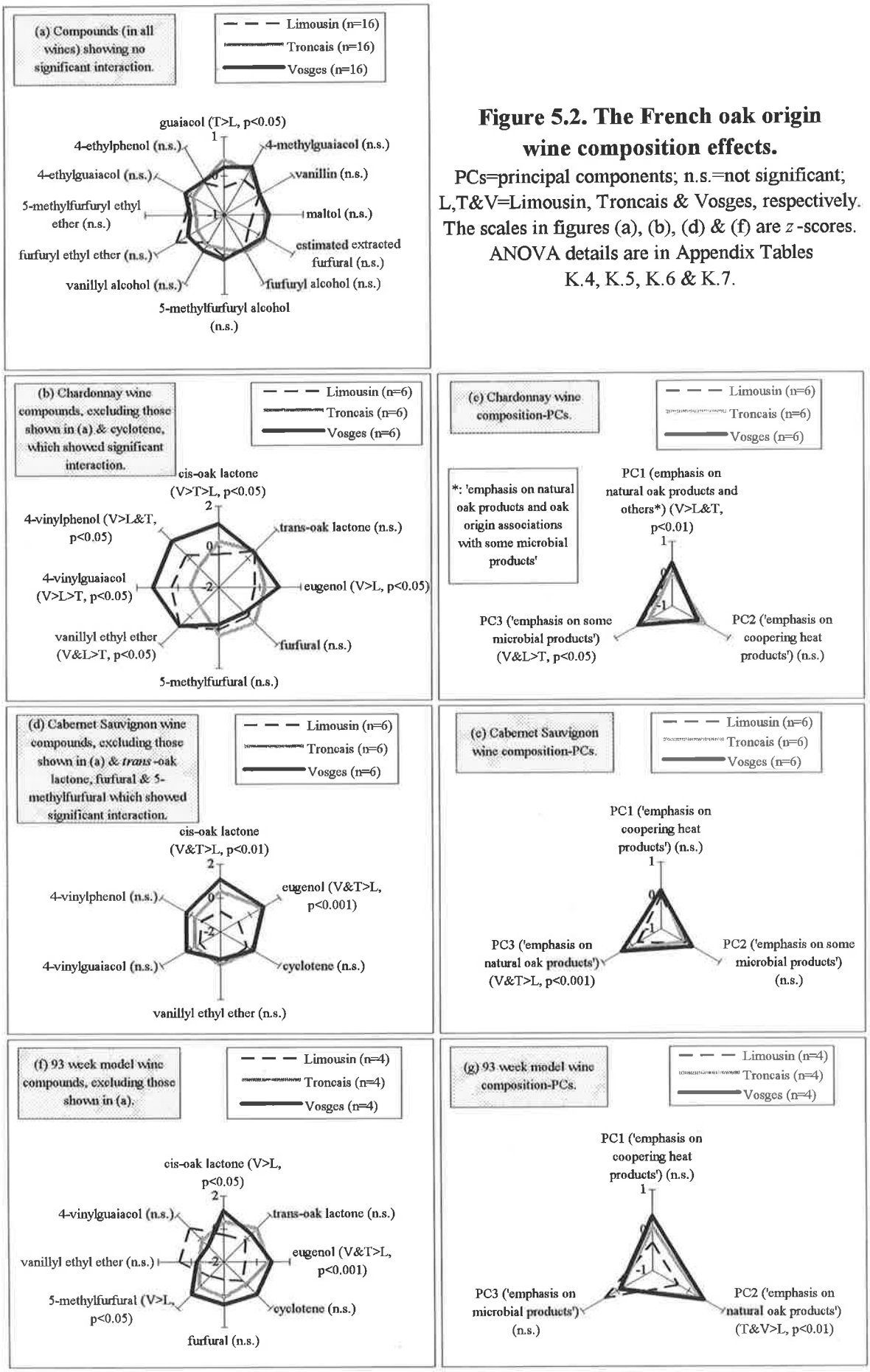


Figure 5.2. The French oak origin wine composition effects.

PCs=principal components; n.s.=not significant; L,T&V=Limousin, Troncais & Vosges, respectively. The scales in figures (a), (b), (d) & (f) are *z*-scores. ANOVA details are in Appendix Tables K.4, K.5, K.6 & K.7.

The Limousin barrel Cabernet Sauvignon wines did not contain significantly higher concentrations of any of the volatile compounds; they contained significantly lower concentrations of the *cis*–oak lactone and eugenol (Fig. 5.2d). Consequently, the significantly higher aroma–PC2, involving an emphasis on 'smoky,' 'Band–aid' (medicinal), 'coffee' and 'earthy' for these Limousin barrel wines, might have arisen due to compounds not quantified. Alternately or additionally, it might have arisen due to a relative lack of masking which may result from high concentrations of the *cis*–oak lactone and/or eugenol.

The American oak compared with each of the French oaks

For the analyses involving data from both the Chardonnay and the Cabernet Sauvignon wines, the American barrel wines were lower in 'pencil shavings' than the French, and they were lower in 'coconut' than the Tronçais and Vosges (Fig. 5.3a). For the Chardonnay analyses, the American barrel wines were higher in 'caramel' than the French, and they were lower in 'green apple' than the Limousin (Fig. 5.3b).

The 'caramel' effect in the Chardonnay wine may have resulted from the fact that a disproportionate number of the American barrel wines had been affected by MLF during barrel–storage (Fig.7.5). 'Caramel' was associated with malate consumption (Fig. 7.4b), and, being similar in some ways to the aroma character of 'butter,' it may have been a descriptor applied to some of the stimuli also giving rise to 'butter' variation (these descriptors varied in similar ways among the Chardonnay wines, Fig.3.1). Furthermore, the oak origin treatment trend for 'caramel' in the Cabernet Sauvignon wine (Fig. 5.3d), although not significant, was opposite to that in the Chardonnay.

Consistent with the effects for 'coconut' and 'pencil shavings' shown in Figure 5.3a, the Cabernet Sauvignon wine aroma–PC1, involving an emphasis on 'rich aromas' versus 'earthy,' and the aroma–PC3, involving an emphasis on 'pencil shavings' and 'mint,' were lower in the American barrel wines than in the French (Fig. 5.3e).

These results are similar to those obtained by Francis *et al.* (1992) for a model wine extract of the same wood, two years prior to coopering: The American oak treatment resulted in generally less intense wine aromas than the French oak treatments.

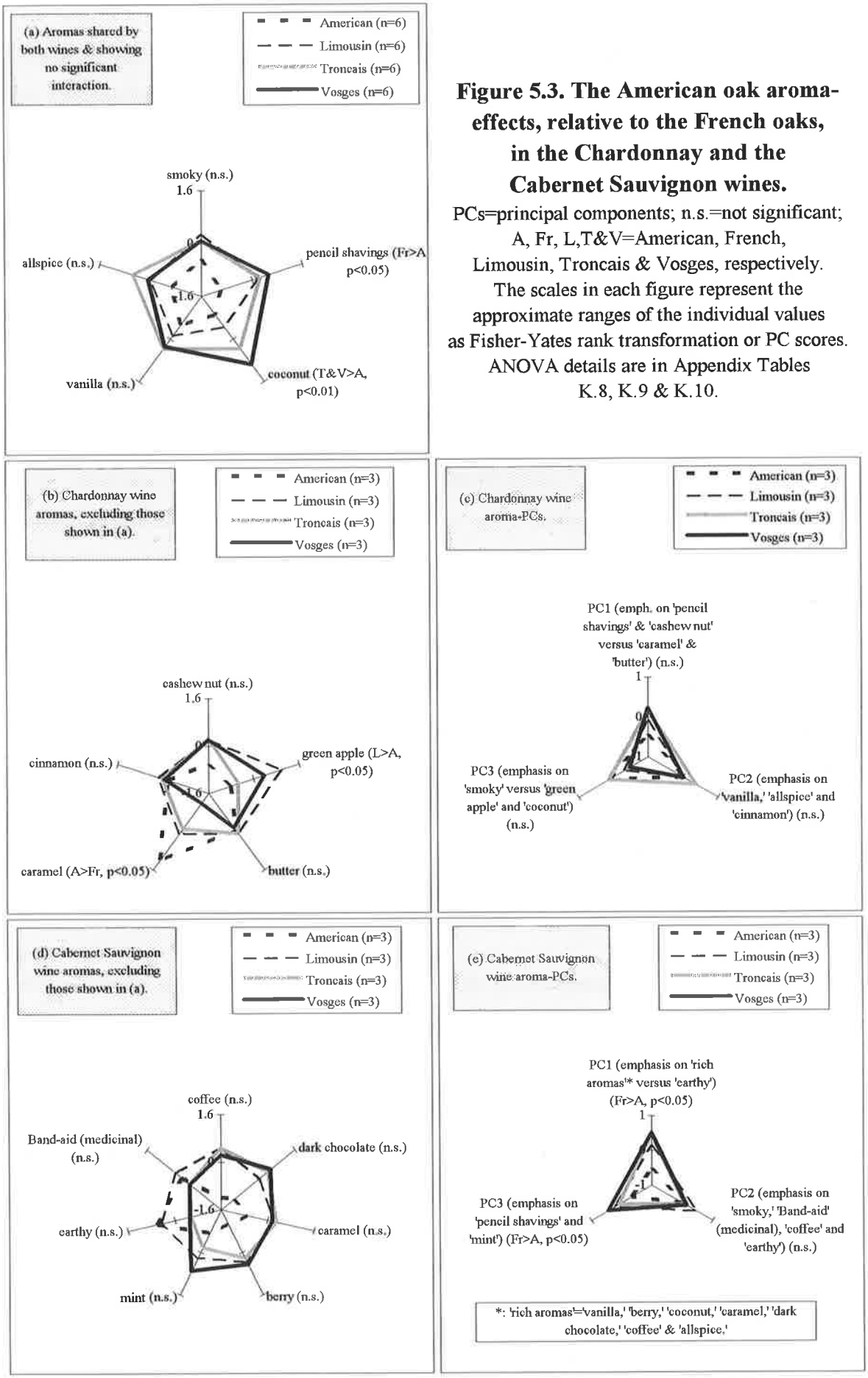


Figure 5.3. The American oak aroma-effects, relative to the French oaks, in the Chardonnay and the Cabernet Sauvignon wines.

PCs=principal components; n.s.=not significant; A, Fr, L,T&V=American, French, Limousin, Troncais & Vosges, respectively. The scales in each figure represent the approximate ranges of the individual values as Fisher-Yates rank transformation or PC scores. ANOVA details are in Appendix Tables K.8, K.9 & K.10.

Numerous composition effects were also found between the American and the French oak treatments. For the combined analysis of the three wines, the oak lactones and eugenol were lower in the American barrel wines than in the Tronçais and Vosges (Fig. 5.4a). The *trans*–oak lactone was also lower in the American barrel wines than in the Limousin. These results are consistent with those obtained from samples of the same oak wood taken prior to coopering (Sefton *et al.* 1993a). The composition–PCs, involving emphases on 'natural oak products' (*i.e.* the oak lactones and eugenol), reflected the effects found, individually, for the oak lactones and eugenol (Fig. 5.4 c & e).

Figure 5.4b shows that the American oak–stored Chardonnay wines contained higher amounts of cyclotene and 5–methylfurfuryl ethyl ether and lower amounts of 4–vinylguaiacol and 4–vinylphenol than one or more of the French oak treatments containing the same wine. Cyclotene was measured with low precision in the Chardonnay wine (Tab. 2.1) so this treatment effect may not be meaningful. The disproportionate occurrence of MLF in the American barrel wines (Fig. 7.5) may have been responsible for the treatment effect for 5–methylfurfuryl ethyl ether since this compound forms an equilibrium with 5–methylfurfuryl alcohol which is a product of MLF (Chapter 7). The low quantity of 5–methylfurfural in the American barrel wines (Fig. 5.4a) is consistent with this conclusion. The effect for the two volatile phenols may involve variable degrees of oxidation allowed by the different oaks. Oxidative degradation has been suggested as a possible mechanism for the loss of these compounds during storage (Nicolini *et al.* 1991).

The Cabernet Sauvignon composition–PC2, involving an 'emphasis on some microbial products,' showed an oak origin effect (Fig. 5.4e). It was higher in the American barrel wines than in those of the Vosges and Tronçais barrels. Of the microbial products constituting this PC, 4–ethylphenol which was higher in the American barrel wines than in any of the three French oak treatment wines (Fig. 5.4d), may be the most important, both for its aroma contribution and since it suggests the activity of *Brettanomyces/Dekkera* species (Chatonnet *et al.* 1992b).

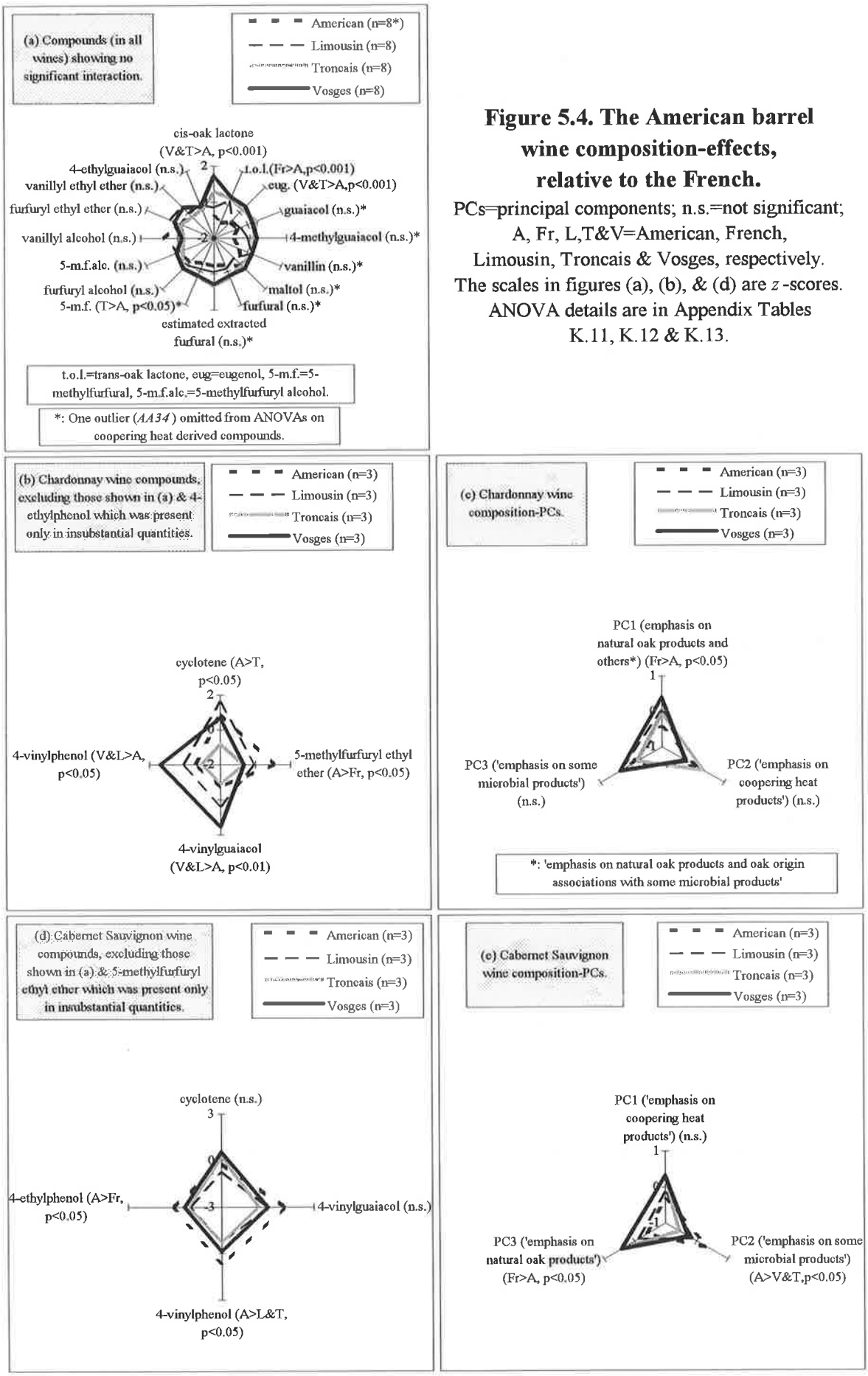


Figure 5.4. The American barrel wine composition-effects, relative to the French.

PCs=principal components; n.s.=not significant; A, Fr, L,T&V=American, French, Limousin, Troncais & Vosges, respectively. The scales in figures (a), (b), & (d) are *z*-scores. ANOVA details are in Appendix Tables K.11, K.12 & K.13.

Among the American oak Cabernet Sauvignon wines, the low concentrations of furfural and 5–methylfurfural, relative to some of the French oak origin treatments (Appx. Tab. K.13), may have resulted from higher microbial activity occurring in the American oak barrel wines (Chapter 7). It is interesting that microbial activity of different types (MLF in the Chardonnay wine and *Brettanomyces/Dekkera* activity in the Cabernet Sauvignon wine) occurred to a greater degree in the American oak barrels for both of the wines. Explanations for these observations include the possibility that there may have been more rapid depletion of sulfite in the American oak barrel–stored wines, and the possibility that lower quantities of a microbial inhibitor may have been extracted from the American oak.

5.3 Seasoning location effects

Contrary to the approach taken elsewhere in this thesis, the composition effects in this section are considered before the aroma effects for the French oak wood seasoning/cooper treatments. This has been necessary since the effects of these dual treatments are discussed in different chapters, and it is only the composition effects that can be easily assigned to one chapter or the other, *i.e.* to the discussion of seasoning effects (here) or cooper effects (Chapter 6).

The oak lactones and eugenol were present in substantial amounts prior to coopering (Sefton *et al.* 1993a) and were not associated with the 'coopering heat products' (Appx. Tabs. C.1, C.6 & C.11). Consequently, these compounds are considered, here, for possible seasoning location effects. Following this, the aromas that were most strongly associated with the oak lactones and eugenol are also considered for possible seasoning location effects. These aroma effects are likely to have depended, at least partially, on seasoning variables.

Since the 'coopering heat product' effects were more numerous than the 'natural oak product' effects, full illustrations of the aroma and composition effects of the French oak seasoning location / cooper treatments are presented in Chapter 6 (Figs. 6.1 & 6.2). Only the effects likely to have been impacted upon by seasoning influences are shown, here.

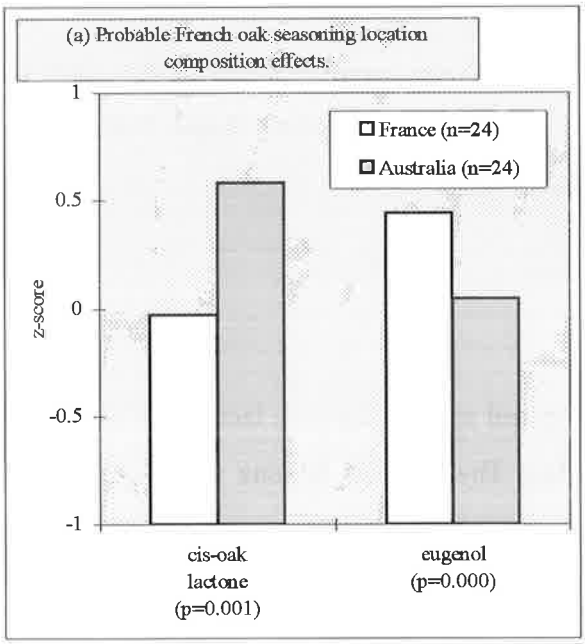
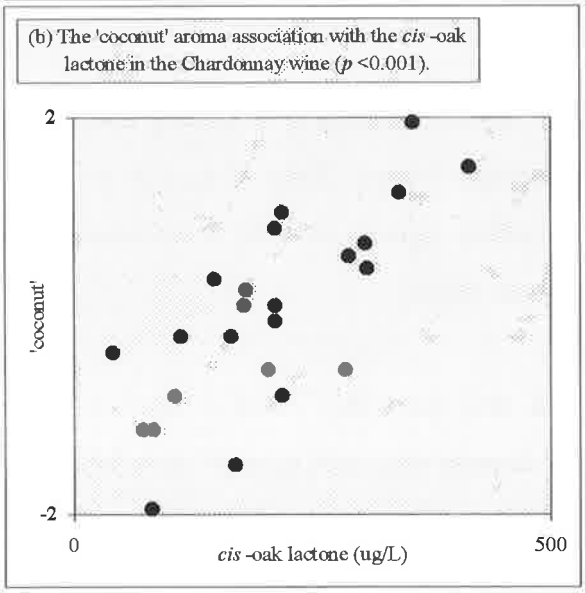
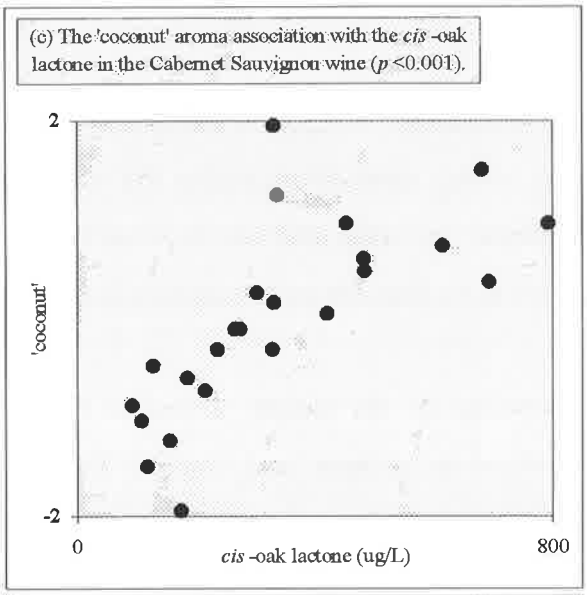
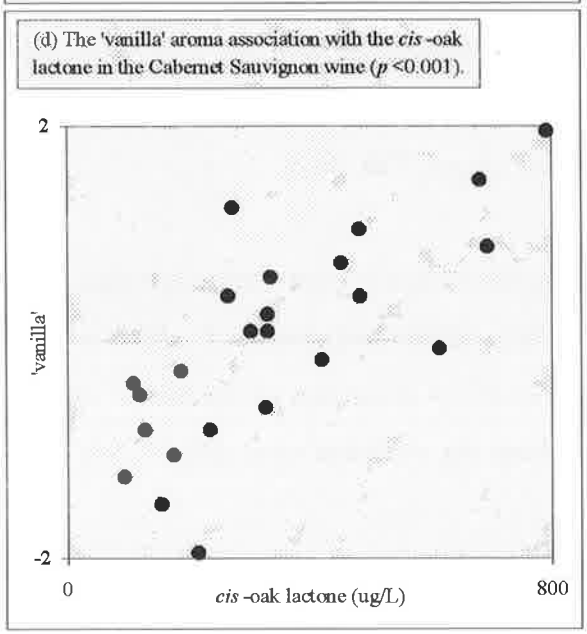


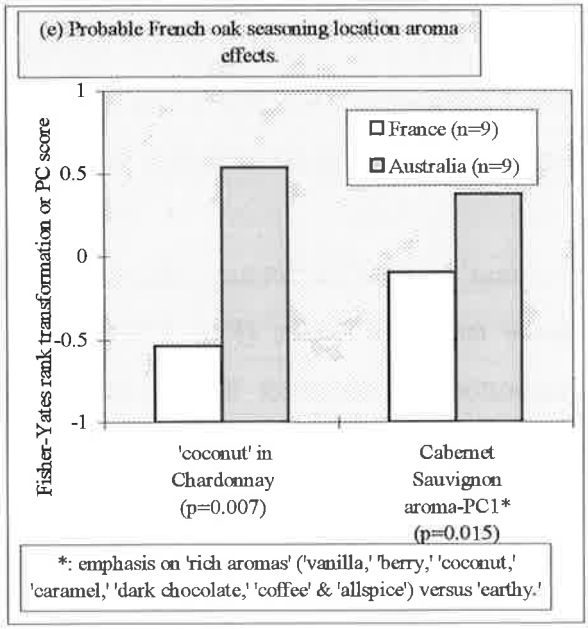
Figure 5.5. Probable French oak seasoning location effects.

The effects from these treatments may have arisen from seasoning and/or cooper causes. The effects ascribed to seasoning causes, here, are those for the 'natural oak products' (eugenol and the oak lactones) and those for the aromas associated with the 'natural oak products.' The other effects involved or were associated with 'coopering heat products' so are discussed in Chapter 6 (Figs. 6.1 & 6.2). ANOVA details are in Appendix Tables K.2, K.3 & K.4.









The French oak seasoning location / cooper effects were explored concurrently with the French oak origin effect explorations, discussed above (same ANOVAs, Appx. Tabs. K.1 to K.7). Details of all of the ANOVAs are in Appendix K.

French oak wood

Highly significant differences were found for eugenol and the *cis*–oak lactone, according to seasoning location of the French oak (Fig. 5.5a). The *cis*–oak lactone was higher in the Australia seasoned and coopered French oak (p=0.001). Conversely, eugenol was higher in the oak that was seasoned and coopered in France (p=0.000). These results concur with those found for the wood prior to coopering (Sefton *et al.* 1993a).

The substantial variation in effect between these two compounds is surprising considering their strong association across the wider experiment (Appx. Tabs. C.1, C.6 & C.11). However, variation attributable to an oak origin effect appears to have overshadowed the variation attributable to a seasoning location / cooper effect.

Discussion of the aroma effects of the French oak seasoning location treatments are restricted to 'coconut' and 'vanilla.' The *cis*–oak lactone was most strongly associated with 'coconut' (p<0.001) in the Chardonnay wine (Fig. 5.5b, Appx. Tab. G.2), and with 'coconut' (p<0.001) and 'vanilla' (p<0.001) in the Cabernet Sauvignon wine (Fig. 5.5 c & d, Appx. Tab. H.2). Eugenol did not show any strong association with any aroma descriptor in the Chardonnay wine, and it was most strongly associated with 'coconut' (p<0.001) and 'vanilla' (p<0.001) in the Cabernet Sauvignon wine (Appx. Tab. H.2).

'Coconut' in the Chardonnay wine was higher in the Australia treatment wines than in the France treatment wines (Fig. 5.5e), and a group of aromas, most of which partially constituted the Cabernet Sauvignon wine aroma–PC1, involving an emphasis on 'rich aromas' versus 'earthy,' were also higher in the Australia treatment wines (Fig. 5.5e).

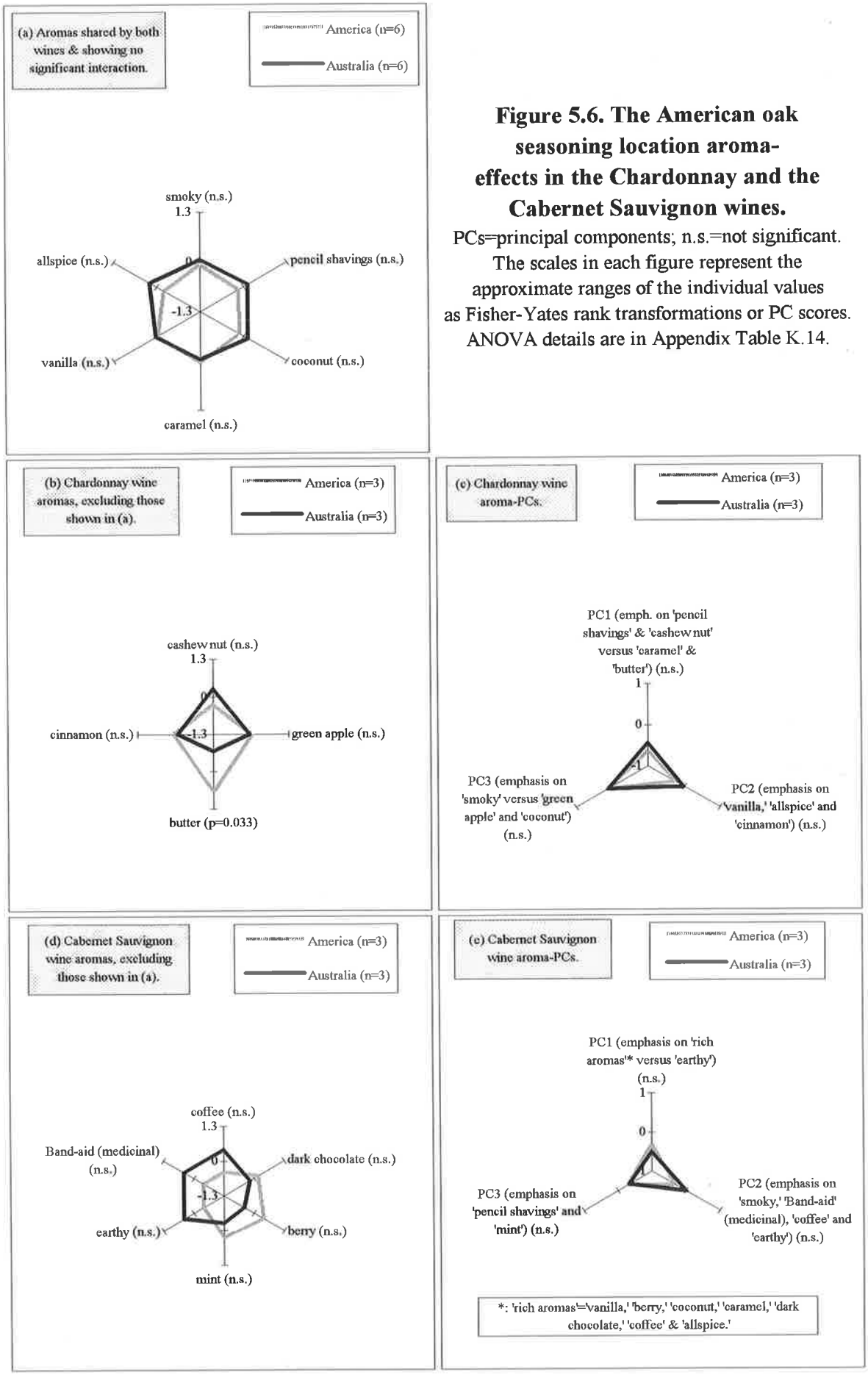


Figure 5.6. The American oak seasoning location aroma-effects in the Chardonnay and the Cabernet Sauvignon wines.

PCs=principal components; n.s.=not significant. The scales in each figure represent the approximate ranges of the individual values as Fisher-Yates rank transformations or PC scores. ANOVA details are in Appendix Table K.14.

Francis *et al.* (1992) found that a 'vanilla' aroma in model wines reached higher intensities using Australia–seasoned French oak than when using the same oak which was seasoned in France. Their observation is consistent with the apparent seasoning location effect in the Cabernet Sauvignon wine.

Some of the aromas constituting the Cabernet Sauvignon wine aroma–PC1 may have been influenced by both the 'natural oak products' and the 'coopering heat products.' 'Vanilla' in the Cabernet Sauvignon wine, for example, was associated with both product groups (Appx. Tab. H.2).

To determine whether the effects were more likely results of seasoning conditions, coopering or a combination of these variables, a new experiment, involving tighter control over coopering conditions, is required.

American oak wood

Of all of the aromas in both of the wines, only 'butter,' in the Chardonnay wine, showed a significant American oak seasoning location effect (Fig. 5.6) but this is likely to have been a result of the disproportionate extent of MLF experienced by the two treatments (Fig.7.5). Francis *et al.* (1992) found that the Australia–seasoned American oak, when sampled after 12 months of seasoning, imparted higher 'vanilla,' 'caramel' and 'spicy' aromas to a model wine than did the corresponding America–seasoned oak. At this time, the oak lactone concentration differences between the two samples were at their greatest. However, after three years seasoning, *i.e.* just prior to coopering, seasoning effects had evened out these differences (Sefton *et al.* 1993a), and this may well be the reason for the disagreement between these authors' findings and those in Figure 5.6a.

The American oak seasoning location composition effects are shown in Figure 5.7. The combined wines effect for 4–methylguaiacol (Fig. 5.7a) was significant but involved only a very small difference (1 µg/L) between treatment means (Appx. Tab. K.15).

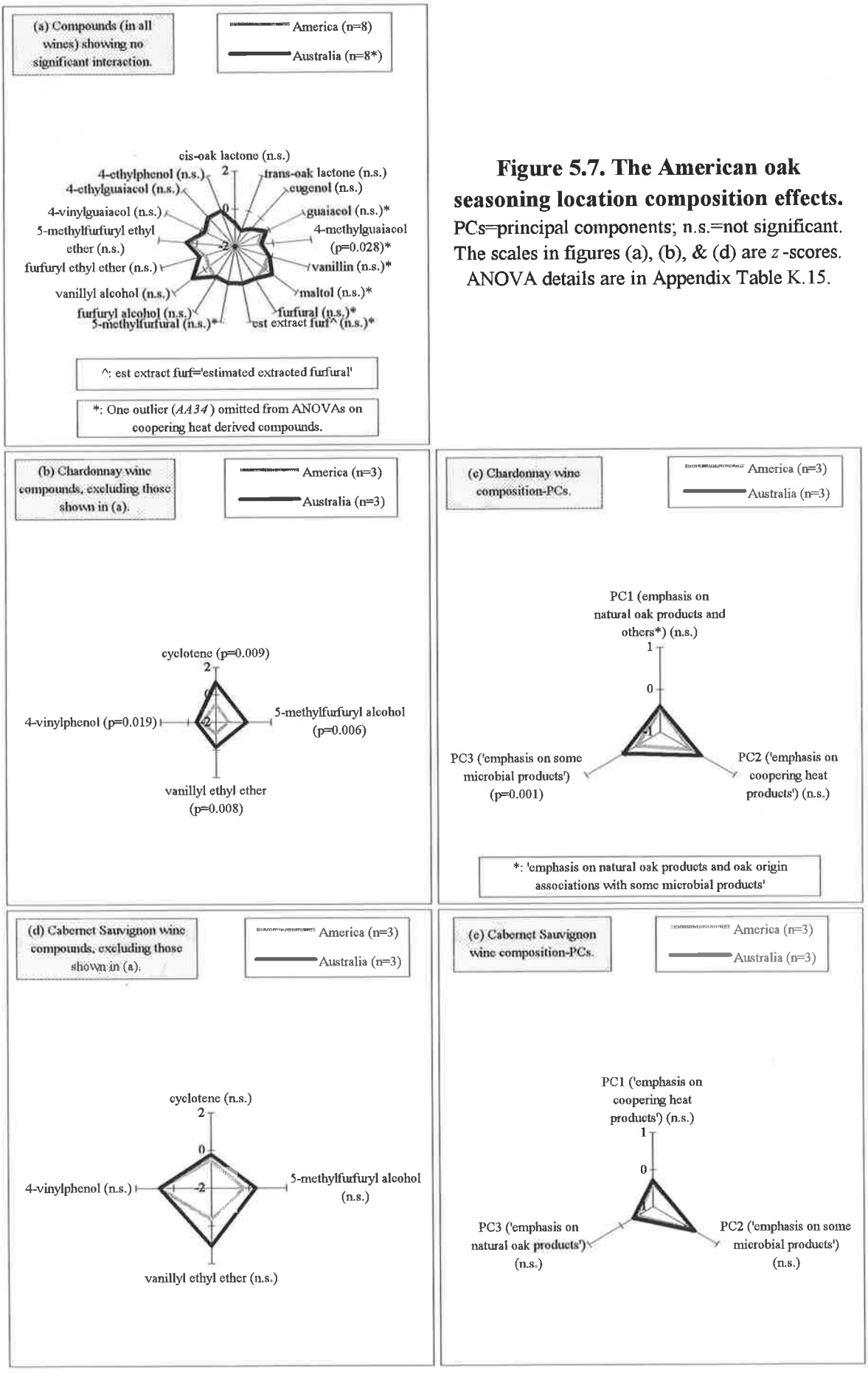


Figure 5.7. The American oak seasoning location composition effects. PCs=principal components; n.s.=not significant. The scales in figures (a), (b), & (d) are *z*-scores. ANOVA details are in Appendix Table K.15.

Most of the composition effects for the Chardonnay wine (Fig. 5.7b) can be summarised by the composition–PC3, involving an 'emphasis on some microbial products' (Fig. 5.7c). The Australia treatment experienced less complete malate consumption yet showed higher composition–PC3 values. However, most of the compounds that were affected are not associated with MLF but arise most substantially due to yeast activity (Chapter 7).

There were no significant differences among the American oak barrel wines for eugenol or the oak lactones (Fig. 5.7a). This is consistent with the composition of the wood prior to coopering (Sefton *et al.* 1993a). These authors found that, although the seasoning location had a notable affect on how oak lactone concentrations changed during the seasoning period, the levels in the wood at the end of this period were similar. It is interesting that the French oak barrel wines showed seasoning treatment effects for these compounds while the American oak did not. Perhaps the difference in the seasoning conditions between France and Australia is important, or perhaps the French oak wood may have been predisposed, chemically, to respond to seasoning influences in a different way.

5.4 Aromas associated with 'natural oak product' variation (estimated by correlation with the 'emphasis on natural oak products' principal component)

The preceding sections have shown that oak wood selection and handling, prior to coopering, can result in substantial aroma and composition effects. The unpredictability of these treatment effects, however (Section 5.1), has led to explorations of the underlying aroma variations and the possible compositional causes of these aroma variations.

As discussed earlier (Chapter 2), both the Chardonnay wine and the Cabernet Sauvignon wine composition principal components analysis (PCA) identified the compositional variance of the 'natural oak products,' the oak lactones and eugenol, along with the variance of some coincidentally associated compounds, as one of the three most substantial variance 'directions' (principal components) within the composition data (Appx. C).

The 'natural oak product' PC variations (PC1 for the Chardonnay and PC3 for the Cabernet Sauvignon wine) were compared with the wine aromas by correlation analysis (Appx. Tabs. G.2 & H.2). This allowed the identification of aromas that may have been, at least partially,

affected by 'natural oak product' variability. Following this discussion, associations and patterns among aromas and individual compounds are explored (Section 5.5).

Chardonnay wine

For the 17 compounds submitted to PCA for the Chardonnay wines, 28 % of the variance (PC1) was accounted for by the 'natural oak products,' *i.e.* the oak lactones and eugenol (along with 4–vinylguaiacol and 4–vinylphenol in the same direction, and furfuryl alcohol, 5–methylfurfuryl ethyl ether and maltol in the opposite direction) (Appx. C).

This PC has been most affected by oak origin variables. The emphasis is on high concentrations of the oak lactones and eugenol, compounds found to differ significantly among the oak origin treatments (Figs. 5.2 & 5.4). The contributions of 4–vinylguaiacol and 4–vinylphenol probably arose from differential rates of oxidative degradation associated with the different oak woods. These compounds arise most substantially from the action of microorganisms on hydroxycinnamic acids during primary fermentation, and concentrations decrease substantially during barrel storage (Nicolini *et al.* 1991).

The Chardonnay composition–PC1 was also affected by the spontaneous and variable MLF which occurred among the barrels. Four of the six American oak barrels but only one of the 18 French oak barrels experienced more than 50 % depletion of malic acid (Fig.7.5). The coincidence of low oak lactones, eugenol, 4–vinylguaiacol and 4–vinylphenol with high MLF–associated products caused PC1 to be affected by compound variations in two directions. Maltol also participated in the negative direction due to a possible oak origin effect (Appx. Tab. K.12). However, this is unlikely to have been a robust oak origin effect since a similar trend was not observed among the Cabernet Sauvignon (Appx. Tab. K.13) or model wines (Tab. 2.7h). At least two variables have contributed to the composition–PC1, making the assignment of some of the probable aroma associations to one or more of these variables difficult. Nevertheless, the associations have been identified, and are discussed in the next section.

The Chardonnay wine composition–PC1, with an 'emphasis on natural oak products and oak origin associations with some microbial products,' was associated positively with

'pencil shavings,' 'coconut' and 'green apple' ($p<0.05$), and negatively with 'caramel' and 'butter' ($p<0.001$ and $p<0.05$, respectively) (Appx. Tab. G.2 & Appx. Fig. G.12). Possible causes or indicators of these effects are discussed below.

Cabernet Sauvignon wine

For the 17 compounds submitted to PCA for the Cabernet Sauvignon wines, 17 % of the variance (PC3) was accounted for by the 'natural oak products,' *i.e.* the oak lactones and eugenol (along with 5–methylfurfural which was present in low quantities and was, therefore, of little interest) (Appx. C).

The Cabernet Sauvignon wine composition–PC3, with an 'emphasis on natural oak products,' was associated positively with 'coconut,' 'vanilla,' 'dark chocolate,' 'coffee,' 'berry' and 'caramel' ($p<0.001$, $p<0.001$, $p<0.01$, $p<0.01$, $p<0.01$, and $p<0.05$, respectively), and negatively with 'earthy' ($p<0.05$) (Appx. Tab. H.2 & Appx. Fig. H.14).

5.5 Possible compositional causes or indicators of these aroma effects and variations

Having identified the aromas that varied according to oak origin or seasoning location treatment or, more generally, according to the 'natural oak products' principal component for each of the two wines, this section considers the relationships between these aromas and the volatile compounds. This was already considered, partially, in Section 5.3 to facilitate the discussion there. It is now pursued more fully. Correlation analysis (and the specific aroma 'impact–pattern conformity' tests for the Cabernet Sauvignon wine only; Chapter 4) are used for this purpose.

The five Chardonnay wine aromas identified as being associated with 'natural oak product' variation, in the preceding section (5.4), were the same as those exhibiting oak origin or seasoning location treatment effects (Sections 5.2 & 5.3). Of these five, the negative correlations involving 'butter' and 'caramel' are likely to have arisen due to the variable MLF (Section 7.5). Consequently, the 'natural oak product' associations with 'pencil shavings,' 'coconut' and 'green apple,' only, are discussed here.

The seven Cabernet Sauvignon wine aromas identified as being associated with 'natural oak product' variation, in the preceding section (5.4), and 'pencil shavings,' which exhibited an oak origin treatment effect (Fig. 5.3a) are also discussed here.

The compounds responsible for these Chardonnay or Cabernet Sauvignon wine aromas may owe their genesis principally to natural processes that occur in the wood prior to coopering. By considering each compound's aroma associations individually it may be possible to identify those compounds which are more likely than the others to have impacted upon each aroma. These compounds may cause or contribute to an aroma or they may indicate the presence of another cause. Alternatively, they may inhibit or contribute to the inhibition of an aroma or they may indicate the presence of another inhibitor. Whatever the case, the associations may be useful as indicators of likely aroma effects in wine.

The specific aroma 'impact–pattern conformity' test for the Cabernet Sauvignon wines (Appx. J) was used to test for any association between compound and aroma variation which is consistent with the existence of a causal relationship (Section 4.5). The Chardonnay wine data were not subject to this analysis.

Compounds other than the oak lactones and eugenol were present in possibly significant quantities prior to coopering. For example, Sefton *et al.* (1990b) have identified 31 volatile norisoprenoid compounds in a model wine extract of non–heated oak wood. While acknowledging the possibility of aroma effects of other compounds, only the oak lactones and eugenol are considered, here.

Chardonnay wine

'Coconut,' 'pencil shavings' and 'green apple' may have arisen in the Chardonnay wine, at least partially, as a result of 'natural oak product' variation. What specific compounds could have contributed to each of these aromas?

'Coconut' was associated only with the *cis*–oak lactone ($p<0.001$) in the Chardonnay wine (Appx. Tab. G.2 & Fig. 5.5b). Günther and Mosandl (1986) have described an optically

pure sample of this compound as possessing a "coconut, slightly musty and earthy" aroma with a "hay" note. Therefore the association between 'coconut' and the *cis*–oak lactone is not surprising and it is possible that a causal relationship may have been active.

Günther and Mosandl (1986) have described an optically pure sample of the naturally occurring *trans*–oak lactone as possessing a "fragrant celery" note, with a "weak coconut" aroma and some "green walnut" character. This compound, along with eugenol, showed a significant positive correlation only with 'pencil shavings' ($p<0.05$). Eugenol possesses a clove–like aroma (Boidron *et al.* 1988). The perception of 'pencil shavings' may have arisen from a combination of these two compounds.

'Pencil shavings' was also associated with some of the 'coopering heat products' (4–methylguaiacol, furfural and 5–methylfurfural; $p<0.01$, $p<0.05$ and $p<0.05$, respectively) but it was negatively correlated with 5–methylfurfuryl ethyl ether and furfuryl alcohol ($p<0.01$ and $p<0.05$, respectively) (Appx. Tab. G.2). Consequently, relatively high concentrations of 'coopering heat products' and/or relatively low concentrations of MLF–associated products may have also contributed to this aroma.

The association between the 'natural oak products' composition–PC and 'green apple' was not accompanied by associations with the oak lactones or eugenol. Instead, 'green apple' may have been most affected by inhibition by 'coopering heat products' (composition–PC2) (Appx. Tab. G.2).

Cabernet Sauvignon wine

What specific compounds could have contributed to 'pencil shavings,' 'coconut,' 'vanilla,' 'dark chocolate,' 'coffee,' 'berry,' 'caramel' and 'earthy' in the Cabernet Sauvignon wine?

In this wine, none of the 'natural oak products,' *i.e.* the oak lactones and eugenol, was associated with 'pencil shavings' or 'caramel,' and the *trans*–oak lactone was the only one of these compounds to be correlated with 'coffee' ($p<0.05$) (Appx. Tab. H.2). 'Coffee' seems to have been affected mostly by 'coopering heat products' and is discussed in Section 6.4.

The specific aroma 'impact–pattern conformity' (IPC) tests (Appx. J) show that, of the five Cabernet Sauvignon wine aromas remaining for discussion, here, 'coconut,' 'berry' and 'vanilla' have exhibited patterns which are consistent with the possibility of the existence of causal relationships with the oak lactones and/or eugenol ('vanilla' in combination with some 'coopering heat products'). Consequently, these three aromas are discussed most fully, below.

'Coconut' was associated with both isomers of the oak lactone and eugenol ($p<0.001$) (Appx. Tab. H.2 & Appx. Fig. H.2), and the 'coconut' IPC tests (Fig. 5.8) support the possibility that one or more of these compounds could have been active in contributing to the aroma. The 'differentiation potency or accompaniment' (DPA) values shown in Figure 5.8 show that the *cis*–oak lactone at 0.4 mg/L, the *trans*–oak lactone at 0.2 mg/L and eugenol at 0.02 mg/L were estimated as the concentration differences, within the range of the samples in this experiment, above which at least 50 % of every decile of comparisons (n~28 per decile) was differentiated according to 'coconut' ($p<0.05$) (Tab. 4.2).

In view of the known sensory properties of the oak lactones and eugenol (Günther and Mosandl 1986, Boidron *et al.* 1988), it is most likely that the *cis*–oak lactone has contributed most substantially to the differentiation of 'coconut' among these wines. As previously discussed, conformity to the IPC test may have resulted from the compound impacting upon the aroma or from the compound being associated with one that did. Thus, in the case of 'coconut,' eugenol may have exhibited conformity simply because of its strong association with the *cis*–oak lactone.

'Berry' was associated most strongly with the *cis*–oak lactone and eugenol ($p<0.01$) (Appx. Tab. H.2 & Appx. Fig. H.5), and the 'berry' IPC tests (Fig. 5.9) support the possibility that one or both of these compounds could have been active in contributing to the aroma. The 'berry'–DPAs for the *cis*–oak lactone and eugenol were estimated to be 0.5 mg/L and 0.03 mg/L, respectively (Tab. 4.2).

Given that lactones similar to the oak lactones are known to be aroma–active in many fruits (Gatfield and Sommer 1993), the *cis*–oak lactone association with 'berry' is not surprising.

Further, given the known clove–like aroma of eugenol (Boidron *et al.* 1988), it is most likely that the *cis*–oak lactone has contributed most substantially to the differentiation of 'berry' among these wines.

'Vanilla' was associated most strongly with the *cis*–oak lactone and eugenol ($p<0.001$) (Appx. Tab. H.2 & Appx. Fig. H.8) but it was also associated with some 'coopering heat products,' including vanillin ($p<0.01$). It is likely that this aroma has been influenced by both 'natural oak products' and 'coopering heat products.' The contribution of the 'coopering heat products' is discussed in Section 6.4.

The 'vanilla' IPC tests for the *cis*–oak lactone and eugenol (Fig. 5.10) support the possibility that one or both of these 'natural oak products' could have been active in contributing to the 'vanilla' aroma. This was also the case for the 'coopering heat products,' 4–methylguaiacol and vanillin. The 'vanilla'–DPAs for the *cis*–oak lactone, eugenol, 4–methylguaiacol and vanillin were estimated to be 0.4 mg/L, 0.02 mg/L, 0.01 mg/L and 0.08 mg/L, respectively (Tab. 4.2).

In view of the known sensory properties of the oak lactones, eugenol, 4–methylguaiacol and vanillin (Günther and Mosandl 1986, Boidron *et al.* 1988), it would seem that vanillin has contributed most substantially to the differentiation of 'vanilla' among these wines. There is, however, some disagreement in the literature regarding the importance of vanillin to wine flavour. Chatonnet *et al.* (1991, 1992c) have concluded that vanillin plays a significant role in the flavour of barrel–aged wines, although this role is much diminished when wines are fermented in barrel and stored on yeast lees. On the other hand, Dubois (1989) citing lower amounts of vanillin in barrel–aged red and white wines and a higher sensory threshold, concluded that vanillin plays no role in the flavour of barrel–aged wines. Dubois considered the perception of the so–called 'vanilla–oak' character in wines to be due to the influence of oak components other than vanillin. Indeed, the evidence from this study (Appx. Tab. H.2 & Fig. 5.10) is more strongly in favour of a 'vanilla' effect from the *cis*–oak lactone or eugenol than from vanillin. It seems most likely that a 'vanilla' effect should arise from a combination of these compounds, particularly the *cis*–oak lactone and vanillin.

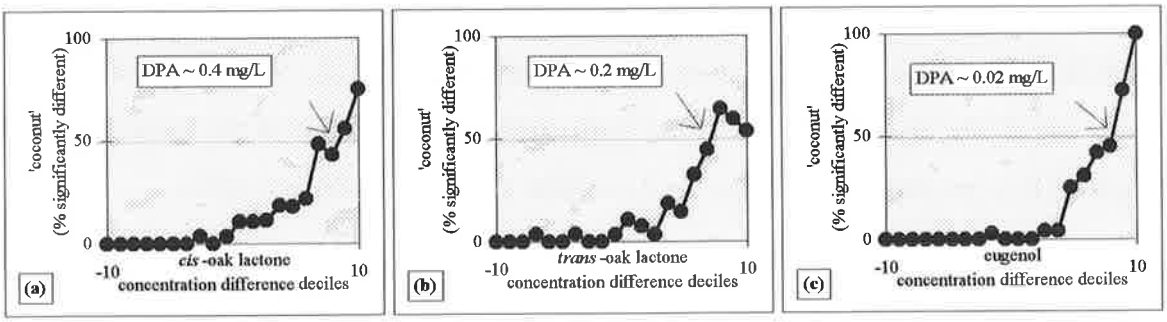


Figure 5.8. 'Coconut' aroma 'impact-pattern conformity' (IPC) test for those compounds that passed the test (Cabernet Sauvignon wines).
The specific aroma 'differentiation potencies or accompaniments' (DPAs) are also shown.
See Section 4.5 and Appendix J for details.

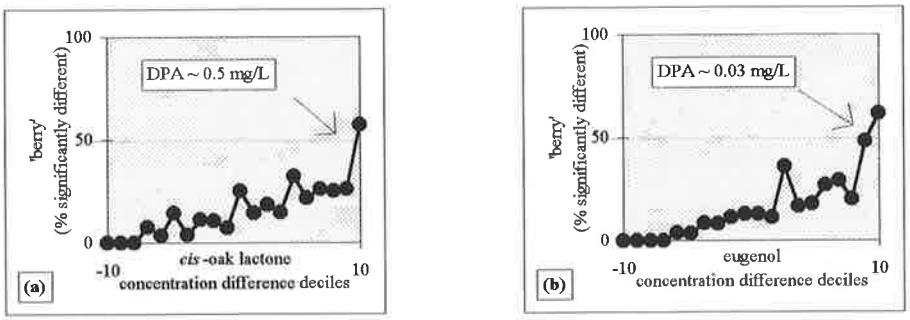


Figure 5.9. 'Berry' aroma 'impact-pattern conformity' (IPC) test for those compounds that passed the test (Cabernet Sauvignon wines).
The specific aroma 'differentiation potencies or accompaniments' (DPAs) are also shown.
See Section 4.5 and Appendix J for details.

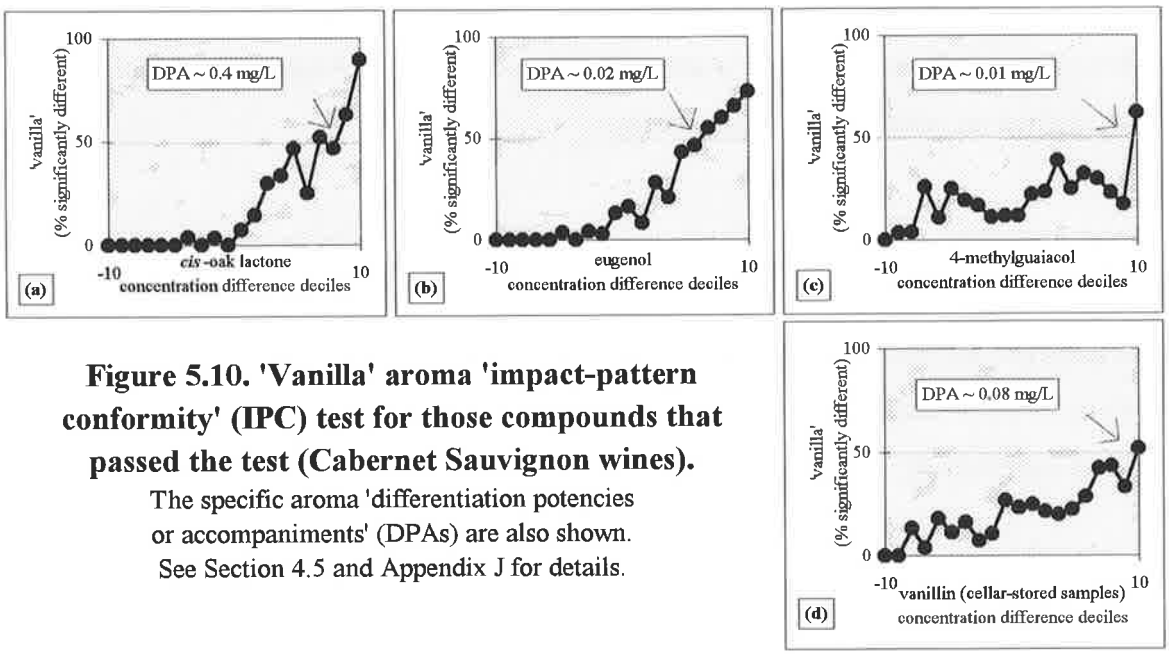


Figure 5.10. 'Vanilla' aroma 'impact-pattern conformity' (IPC) test for those compounds that passed the test (Cabernet Sauvignon wines).
The specific aroma 'differentiation potencies or accompaniments' (DPAs) are also shown.
See Section 4.5 and Appendix J for details.

'Dark chocolate' was associated with the oak lactones but the IPC tests do not support the possibility that these compounds were active in contributing to the variation in this aroma (Appx. Fig. J.10). Associations between 'dark chocolate' and some of the natural oak and coopering heat products (Appx. Tab. H.2) suggest a possible combined effect.

'Earthy' was not correlated with any coopering heat or microbial products; it was negatively correlated with the *cis*–oak lactone and eugenol ($p<0.05$ and $p<0.01$, respectively). Consequently, this aroma is likely to have arisen from unknown compounds. However, the *cis*–oak lactone and eugenol may have contributed to the inhibition of 'earthy,' allowing it to be perceived more when the compounds were at low concentration.

5.6 Summary and conclusion

Notwithstanding the limitations of the sampling protocol (discussed in Section 5.1), some of the strongest oak origin treatment effects are summarised: The *cis*–oak lactone and eugenol concentrations were highest in the Vosges oak, next highest in that from Tronçais, and lowest in the Limousin and American oak. The *trans*–oak lactone concentrations were similar among the French oaks but lower in the American oak.

The *cis*–oak lactone and eugenol also showed strong seasoning location effects in the French oak but it is also possible that these effects were due to the cooper treatment (imposed concurrently). Australia seasoning and coopering of the French oak resulted in higher *cis*–oak lactone and lower eugenol concentrations. The American oak, on the other hand, showed no seasoning location effects.

Greater microbial activity appears to have occurred in the American oak–stored wines, leading to oak origin effects for some microbial activity products and aroma descriptors. This occurred for both the Chardonnay and the Cabernet Sauvignon wines and, therefore, appears to have been a somewhat robust effect, at least within the sanitation regime imposed in this study.

Treatment effects were also found for some of the aroma descriptors. Possible compositional causes for these effects were explored.

There is evidence that most of the potential of the oak wood to affect 'coconut,' 'vanilla,' 'berry' and 'pencil shavings' in one or both of the wines was established before coopering. The oak lactones and eugenol exhibited compositional patterns which are consistent with the possibility that these compounds could have been active in contributing to these aromas.

Since it has been demonstrated that the oak lactones and eugenol can vary significantly according to some oak wood origin and seasoning variables, the results confirm the importance of the selection and seasoning stages. Further, and since the oak lactones and eugenol were highly correlated with one another, the results suggest that estimating the richness of oak wood in the *cis*–oak lactone, which was present in the largest quantities and may be the most important of the three compounds, could aid quality assurance in the selection process.

Chapter 6

The contribution of proprietary and unintended heating variation around 'medium toast' coopering

Chapter outline

6.1 The boundaries of the variation under consideration

As previously discussed, seven of the 20 volatile compounds under study are known to arise most substantially from the heat imposed during coopering, and another six arise from the degradation of some of these compounds (Sections 1.2 & 1.3). Thus, 13 of the 20 compounds owe their existence in wine, originally, to the need for setting the barrel stave curves after bending (the heat of a fire rapidly dries the staves and sets the bent shape), and not to any need for wine aroma effects. However, the perpetuation and development of the 'toasting' process in coopering would have been partially dependent on recognition of the positive sensory outcomes of the process.

It is interesting to note that many of the products of coopering heat can be derived from other sources. Thus, part of the wine aroma that is currently described as 'oak aroma' by many consumers could, conceivably, be derived from a source other than oak. Grape marc (the solid residue of grapes left after pressing), for example, is capable of yielding at least five of the seven 'coopering heat products' when subjected to heating conditions typical of those imposed during coopering (Appx. L).

Within this study, a 'medium toast,' consisting of heating each barrel over a fire of oak wood off–cuts for approximately 45 minutes was specified. However, the concentration of compounds known to arise in differing amounts according to the extent of heating was found to vary substantially (*e.g.* Fig. 6.4). Furthermore, correlations among compounds known to arise from the thermal degradation of unrelated precursors (lignin and carbohydrate), suggest that the concentrations for each of these compounds were affected most substantially by coopering heat. These observations suggest that, despite the specification, coopering heat was not a constant in the experiment, and also that coopering toast level consistency is difficult to achieve.

As discussed in the preceding chapter, the concurrent imposition of seasoning location and cooper treatments on the French oak wood has meant that the seasoning and coopering effects could not be separated. Nevertheless, the lack of association between the 'coopering heat products' and each of the oak lactones and eugenol (Appx. Tabs. C.1, C.6 & C.11) has suggested the participation of seasoning influences on the effects for these latter

compounds. Consequently, the effects for these three 'natural oak products' and the associated aroma effects were discussed in Chapter 5. The remaining seasoning location and/or cooper effects, involving coopering heat–derived compounds and some microbial activity–derived compounds, are discussed here.

Most of the reported coopering heat experimentation has involved the imposition of categoric treatments — *e.g.* 'light,' 'medium' and 'heavy' 'toast' levels (Chatonnet *et al.* 1989) — and the precision of these treatment impositions, in many cases, is likely to have been low. When determining 'toast' levels, coopers often base their decisions on a combination of the perceived intensity of the fire, the estimated duration of the firing and the appearance (shade of brown or black) of the inside surface of the barrel. Obtaining optimal results from this sort of experimentation requires exercising tight control over the firing process. An alternative, however, is to estimate the heating experienced by each barrel by quantifying compounds known to vary with heating.

For the barrels in this study, the relative coopering heat levels were estimated by considering some or all of seven compounds — guaiacol, 4–methylguaiacol, 5–methylfurfural, cyclotene, maltol, vanillin and furfural (or 'estimated extracted furfural': furfural plus its degradation product, furfuryl alcohol). These compounds are known to arise most substantially, if not entirely, from coopering heat, and their quantities are dependent on the level of coopering heat applied. Also, apart from occasional interferences by microbial activity or measurement imprecision, they were correlated with one another. Consequently, the principal component describing their variation in each set of wines has been a convenient *de facto* estimate of the relative degree of coopering heat imposed on each barrel.

6.2 The differences in effect resulting from the environmental and proprietary peculiarities of open–air seasoning and 'medium toast' coopering associated with or imposed by an Australian and a French cooper

The oak wood used for the eight Limousin, eight Tronçais and eight Vosges barrels coopered in France was open–air seasoned for three years in France and then coopered to 'medium toast' by a French cooper. Randomly sampled portions from the same lots of

wood were shipped to Australia to be open–air seasoned for the same period and then coopered to 'medium toast' by an Australian cooper.

The data were analysed as described in Section 5.2. Full details of all of the ANOVAs are shown in Appendix K, and these should be consulted when considering the treatment effect Figures.

Wine aroma

Of the aromas shared by the two wines, 'vanilla' was the only one to show an effect without interaction between treatment levels (Appx. Tab. K.1). The Australia–treatment barrel wines were higher in 'vanilla' than the France–treatment barrel wines but the effect was stronger in the Cabernet Sauvignon wine than in the Chardonnay wine (Appx. Tabs. K.2 & K.3). Since the effect was not significant in the Chardonnay wine, the effect for 'vanilla' is considered individually for each of the wines (Fig. 6.1 b & d).

The only Chardonnay wine aroma that was differentiated according to the seasoning/cooper treatment was 'coconut.' The Australia–treatment was higher than the France–treatment (p=0.007) (Fig. 6.1b). However, since this aroma was associated only with the *cis*–oak lactone (p<0.001), a compound associated mostly with natural oak wood variability, the effect was discussed in Chapter 5.

Four of the twelve Cabernet Sauvignon wine aromas were differentiated according to the seasoning/cooper treatment. The Australia–treatment was higher than the France–treatment in 'smoky,' 'coffee,' 'vanilla' and 'allspice' (p=0.003, p=0.003, p=0.015 and p=0.030, respectively) (Fig. 6.1d). 'Vanilla' was associated with the oak lactones and eugenol, as well as with some of the 'coopering heat products' (Appx. Tab. H.2). Thus, the effect may have arisen due to a combination of seasoning and cooper influences. The three other aromas, 'smoky,' 'coffee' and 'allspice,' however, were associated mostly with the 'coopering heat products.' The possible compositional causes of these four aromas are discussed in Section 6.4.

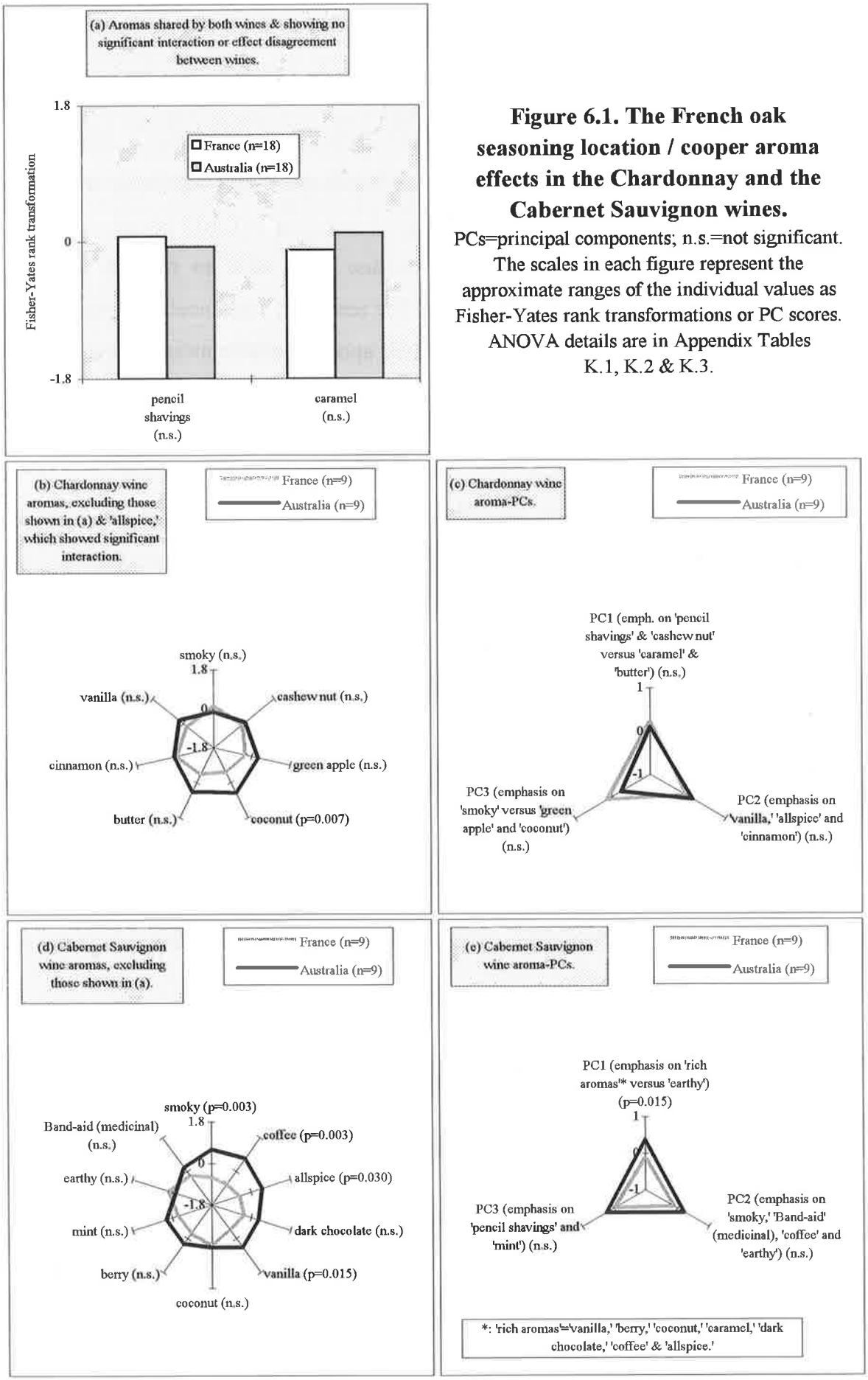


Figure 6.1. The French oak seasoning location / cooper aroma effects in the Chardonnay and the Cabernet Sauvignon wines.

PCs=principal components; n.s.=not significant. The scales in each figure represent the approximate ranges of the individual values as Fisher-Yates rank transformations or PC scores. ANOVA details are in Appendix Tables K.1, K.2 & K.3.

Wine composition

Treatment effects

Figure 6.2 shows the composition effects of the French oak seasoning/cooper treatments. Twelve of the 21 compounds (including 'estimated extracted furfural') are presented as a combination of the three wines (Fig. 6.2a) because there were no relevant significant interactions in the ANOVAs (Appx. Tab. K.4). The remaining compounds are presented on some of the Figures (Fig. 6.2 b, d & f), dependent upon acceptable measurement precision and an absence of relevant significant interaction for each of the wines (Appx. Tabs. K.5, K.6 & K.7).

Of the 12 compounds presented in Figure 6.2a, three were significantly different according to the location of seasoning and cooper. The eugenol and *cis*–oak lactone effects were discussed in Chapter 5 since seasoning influences are likely. However, the effect for 'estimated extracted furfural' (highest in the Australia–treatment) is likely to have involved coopering influences so is discussed below.

In the Cabernet Sauvignon wines, over 95 % of the 'estimated extracted furfural' was present as furfuryl alcohol, *i.e.* nearly all of the furfural had been reduced. Consequently, the quantities of furfuryl alcohol were determined more by the initial quantity of furfural present than by the influence of reducing agents, *e.g.* microorganisms. Thus, the cooper effect for furfuryl alcohol (Fig. 6.2d) can be considered equivalent to the effect for 'estimated extracted furfural' in Figure 6.2a.

The significant effect for vanillin seen in the Cabernet Sauvignon and model wines (Fig. 6.2 d & f) was a robust effect, only absent from the Chardonnay wine (Fig. 6.2b) due to the nullifying effect exerted by the alcoholic fermentation which took place in barrel for this wine (Chapter 7). Figure 6.3 shows that the effect was established within the first six weeks of maturation in the model wine. Thus, the yeast activity in the Chardonnay wine during these first weeks could erase any vanillin effect that may have been present due to cooper variation. The implications of the apparent cooper effect for vanillin in the Cabernet Sauvignon and model wines are discussed below.

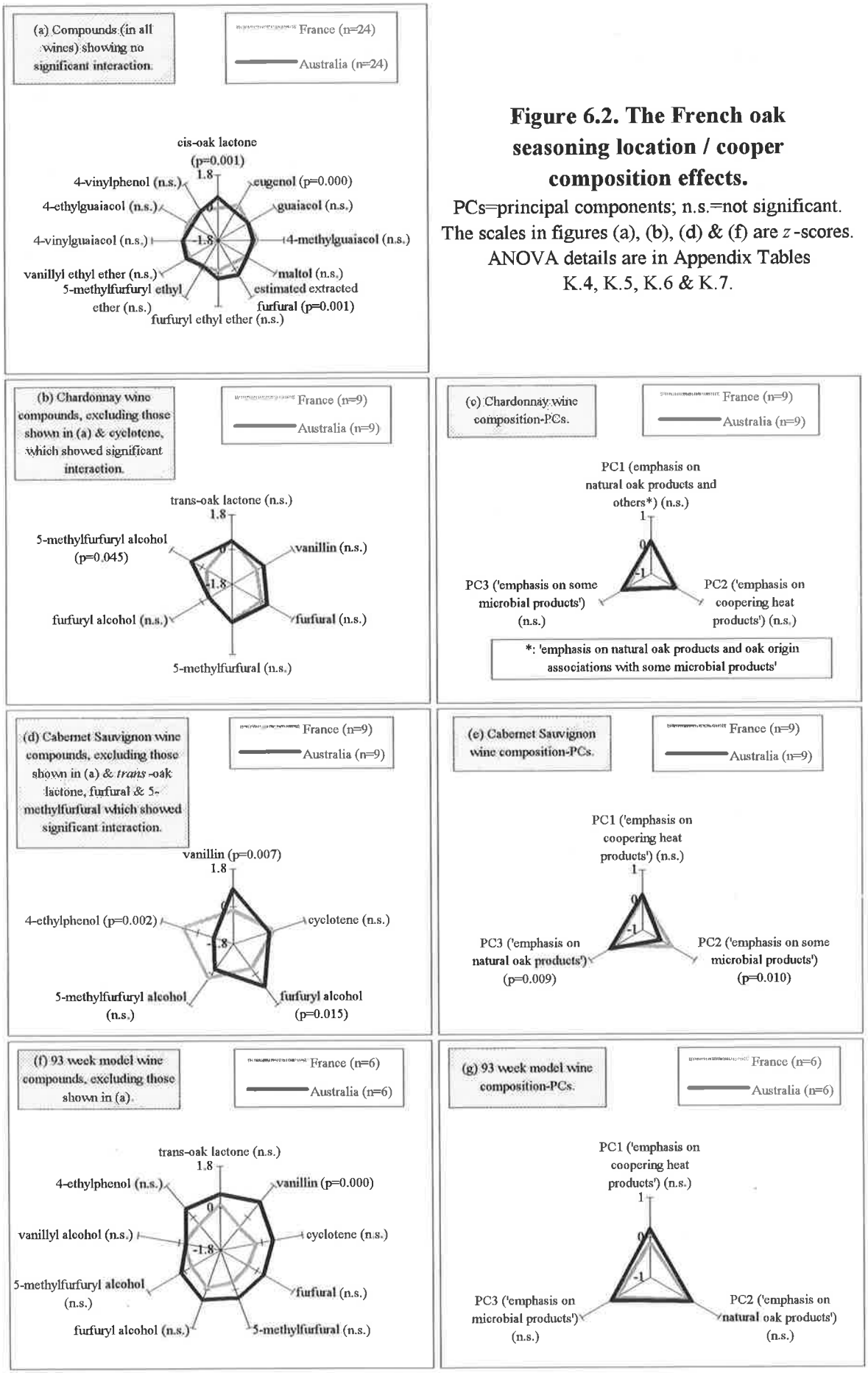


Figure 6.2. The French oak seasoning location / cooper composition effects.

PCs=principal components; n.s.=not significant. The scales in figures (a), (b), (d) & (f) are *z*-scores. ANOVA details are in Appendix Tables K.4, K.5, K.6 & K.7.

4–Ethylphenol was only present in appreciable quantities in the Cabernet Sauvignon wine so it was only possible to see an effect in that wine. The reason for the higher concentration in the France–treatment barrel wines (a mean of 0.89 mg/L versus a mean of 0.70 mg/L in the Australia–treatment barrel wines) is unknown. The compound usually arises from the activity of *Brettanomyces/Dekkera* species (Chatonnet *et al.* 1992b) so it seems likely that this activity varied between the treatments (discussed in Chapter 7). It may be that coopering caused variation in compounds that can encourage (*e.g.* by transforming sulfite) or inhibit yeast activity. Or it may be that environmental conditions during shipping encouraged the development of populations of these yeast in the France–treatment barrels prior to wine storage.

The reason for the significant effect for 5–methylfurfuryl alcohol in the Chardonnay wine (Fig. 6.2b) is unknown. However, the treatment mean difference of 5 µg/L may be insubstantial in relation to the precision of the quantification (not determined; Tab. 2.1). Further, the effect was not seen in the Cabernet Sauvignon or the model wines (Fig. 6.2 d & f).

Apparent relative quantities of surface and sub–surface heat absorbed by the oak wood

It is interesting that effects were observed for 'estimated extracted furfural' and vanillin but not for any of the other 'coopering heat products.' What do these results indicate about the proprietary differences between the two coopers?

Chatonnet *et al.* (1989) have reported that, for 'medium' and 'heavy' toasted barrels, furfural was formed in higher quantities beyond approximately one millimetre below the wood surface than at the surface. Therefore, the quantity of furfural extracted from each barrel by wine, determined as furfural plus its degradation product furfuryl alcohol ('estimated extracted furfural'), may indicate the extent of thermal degradation which has occurred below the wood surface. Guaiacol, on the other hand, has been found to be most concentrated at the surface (Chatonnet *et al.* 1989) so the amount extracted from each barrel by wine may indicate the extent to which the inside surface of the barrel (to approximately one millimetre) had been thermally degraded.

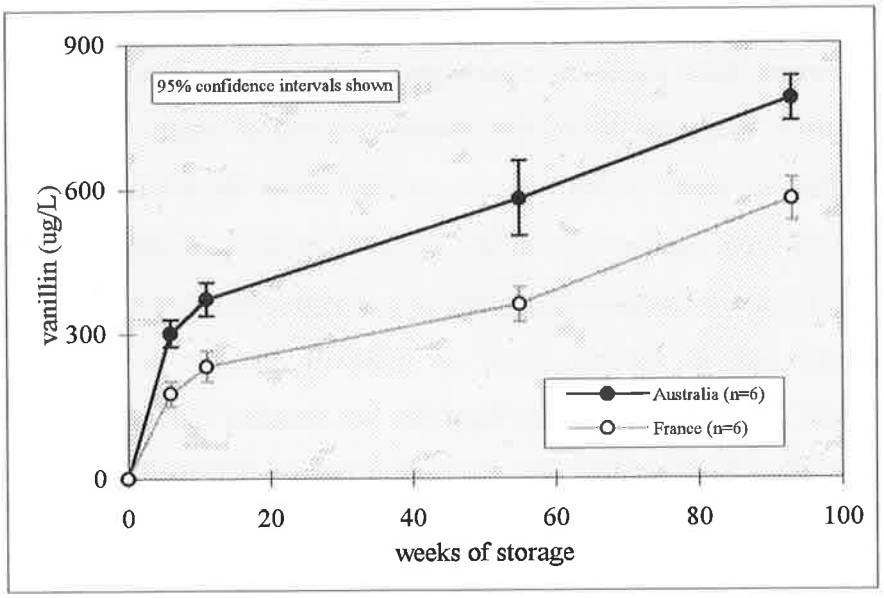


Figure 6.3. The accumulation of vanillin in a model wine stored in French oak wood barrels which were seasoned and coopered either in Australia or in France.

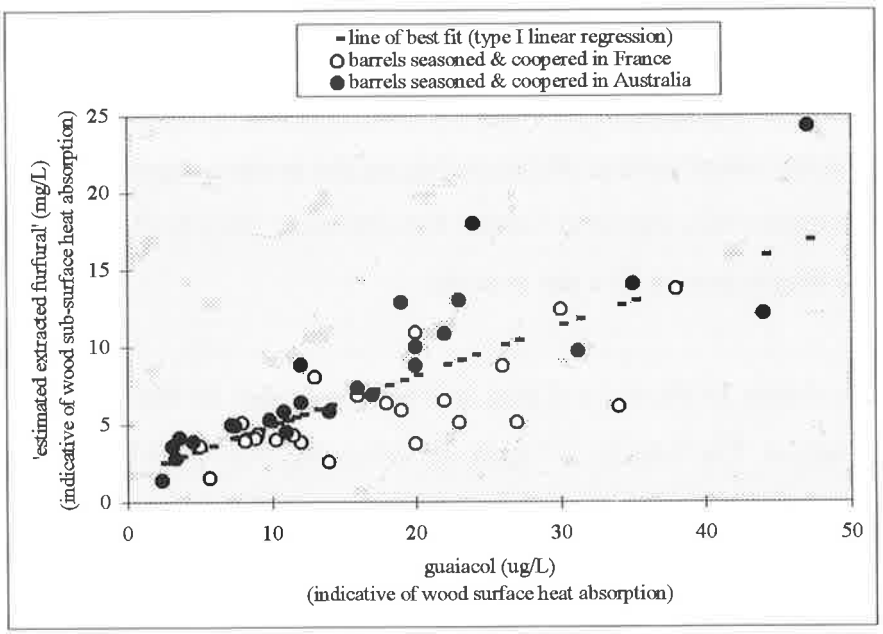


Figure 6.4. A comparison between the two coopers, based on the apparent relative quantities of surface and sub-surface heat absorbed by oak wood during coopering.

Each cooper constructed 24 barrels from the same three lots of wood (eight from Limousin oak, eight from Troncais and eight from Vosges), after three years open-air seasoning at the coopers' premises (Fig. 1.2). 'Medium toast' was specified for all. The compounds were extracted from the wood by the Chardonnay, Cabernet Sauvignon and model wines stored in these barrels. Relative to the line of best fit, the barrels seasoned and coopered in Australia were separated from those seasoned and coopered in France ($p<0.001$, single-factor ANOVA of y-residuals).

It is interesting to consider the relationship between these compounds, and to speculate about the coopering heat variables which may influence it. Figure 6.4 illustrates the association between them for all of the French oak barrel wines. When the data were grouped, according to which of the two coopers had made the barrels, a pattern emerged, most of the barrels from each cooper falling on different sides of the line of best fit. There was no difference between coopers according to guaiacol concentration (estimate of surface heat degradation) but, at various levels of guaiacol, the concentration of 'estimated extracted furfural' differed consistently between the coopers. This suggests that, for any given level of surface heat applied, the Australian cooper achieved more substantial heat penetration of the wood.

The heat absorbed at two and three millimetres below the wood surface depends, among other things, on the heat absorbed at the surface. Any deviation in sub–surface heat absorption from that typical of the surface heat absorption (estimated by the line of best fit) should reflect, principally, variations in the heat conductivity of the wood. Important associated variables include the temperature and duration of heating. These deviations appear to offer some measure of the depth of heat penetration, relative to the amount of heat absorbed at the wood surface. Points falling on the positive (upper–left) side of the line of best fit have higher than expected values. Consequently, they have been designated, as a group, as more deeply heated than the average.

The deviations shown in Figure 6.4 may not be simply due to differences in proprietary coopering technique. The location of open–air seasoning also differed. Thus, the moisture content of the wood may have influenced its ability to absorb heat. The France seasoned and coopered wood is likely to have been moister and, therefore, more able to absorb heat before significant thermal degradation occurred.

Compound accumulation curve effects

The first– and second–year compound accumulation rates in the model wines, stored in the France and Australia treated French oak barrels, were also compared. An unbiased comparison required that the concentrations be standardised by conversion to a percentage of the maximum concentration reached for each compound in each barrel wine (limited to

the 55 and 93 week sampling times). This was required because the absolute quantities of each compound available for extraction differed among the barrels. A comparison of accumulation curve *shapes* among the barrels, requiring a standardisation of the relative *size* of each of the curves, is discussed more fully in Section 8.2.

Accumulation rates, according to compound concentration, were also compared. Table 6.1 shows the accumulation rates, both as percentages and as concentrations. Details of the ANOVAs are in Appendix Tables K.16 and K.17. Of the seven compounds which arise most substantially from the coopering process, five showed a significant seasoning location / cooper treatment effect. The oak lactones and eugenol, compounds arising most substantially prior to coopering, showed no effect.

An effect between the Australia– and the France–seasoned and coopered French oak barrels, denoted by significant *F*–ratios for the mean accumulation rates of both periods (as a percentage of the maximum concentration reached), can be visualised as two accumulation lines commencing at 0 %, rising at different rates in the first year, then reversing the direction of the difference in the second year to finish at or near 100 %. The effect is, thus, an accumulation 'curve shape' difference between the two treatments. These standardised curve shapes are not shown; the concentration accumulation curves are shown in Figure 6.5. Two different effects were observed and explanations are proposed below.

Effect A (for guaiacol, 4–methylguaiacol, 'estimated extracted furfural' and 5–methylfurfural): The curve shape difference between the two treatments, for these compounds, has resulted from a *second* year extraction rate difference, in concentration terms. The accumulation rate occurring in the France–treatment dropped, relative to that in the Australia–treatment. The Australia–treatment barrels were less rapidly depleted of the compounds, perhaps due to deeper heating.

Effect B (for vanillin): The curve shape difference between the two treatments, for vanillin, has resulted from a *first* year extraction rate difference, in concentration terms. The Australia–treatment barrels yielded vanillin at a higher rate in the first year than did the France–treatment barrels, but thereafter the rates were similar.

Whatever has been responsible for the Australia–treatment's greater accumulation of vanillin during the first year may also be implicated in the lower second–year depletion rates for the other compounds, marked 'Effect A,' in these barrels. Perhaps vanillin is formed more thoroughly at the moderate temperatures likely to be applied for long, deep heating. Alternatively, drier wood (as is likely for the Australia–treatment) may result in both greater vanillin production and more thorough heat penetration into the wood. Compound accumulation rates, independent of cooper treatment, are discussed in Chapter 8.

Table 6.1. Seasoning location / cooper effects on oak wood–derived volatile compound accumulation 'curve shapes' arising from a model wine stored in 12 new 'medium toast' French oak barrels for 93 weeks.

	First year: 0 to 55 weeks			Second year: 55 to 93 weeks			
	Signif. of	Accumulation/month[2]		Signif. of	Accumulation/month[2]		
Compound	*F*–ratio[1]	Australia	France	*F*–ratio[1]	Australia	France	Conclusion[3]
cis–oak lactone[‡]	n.s. (n.s.)	7.0 (25)	6.9 (22)	n.s. (n.s.)	1.1 (4.4)	1.2 (3.3)	No effect[4]
trans–oak lactone[‡]	n.s. (n.s.)	6.7 (12)	6.6 (9.1)	n.s. (n.s.)	1.5 (2.8)	1.7 (2.5)	No effect
eugenol	n.s. (*)	7.2 (1.9)	6.8 (2.3)	n.s. (n.s.)	0.9 (0.2)	1.4 (0.5)	No effect
guaiacol	* (n.s.)	5.9 (1.5)	6.6 (1.5)	* (n.s.[§])	2.7 (0.7)	1.6 (0.4)	Effect A
4–methylguaiacol	** (n.s.)	6.4 (0.9)	7.6 (1.2)	*** (***)	2.0 (0.3)	-0.8 (-0.2)	Effect A[4]
vanillin	* (**)	5.7 (45)	4.8 (28)	* (n.s.)	2.9 (23)	4.3 (25)	Effect B
cyclotene	* (n.s.)	4.2 (5.1)	5.7 (5.9)	* (*)	5.2 (6.9)	2.5 (3.0)	No effect[5]
maltol	n.s. (**)	7.7 (8.7)	7.7 (6.6)	n.s. (n.s.)	-1.5 (-1.5)	-0.9 (-0.8)	No effect
'est extract furfural'[†]	* (n.s.)	4.0 (0.55)	5.4 (0.44)	* (*)	5.4 (0.77)	3.4 (0.34)	Effect A
5–methylfurfural[†]	* (n.s.)	5.6 (0.08)	6.6 (0.06)	* (*)	3.1 (0.04)	1.6 (0.02)	Effect A

[1] Results are from data expressed as the percentage of the maximum concentration reached for each compound in each barrel, and also (in parentheses) from data expressed as concentration values. Significance of *F*–ratios: n.s. = not significant; *, **, *** = significant at $p<0.05$, $p<0.01$, $p<0.001$. See Appendix Tables K.16 & K.17 for ANOVA details.

[2] Mean accumulation rate (per month, *i.e.* 30 days) expressed as a percentage of the maximum concentration reached, and also (in parentheses) expressed as a concentration value (μg/L[†]).

[3] Two effects are described in the text: Effect A and B.

[4] Some significant interaction ($p<0.05$) but not important to the conclusion.

[5] No effect, due to significant interaction ($p<0.05$).

[‡] Oak lactones = *cis*– and *trans*–β–methyl–γ–octalactone.

[†] Concentrations in mg/L for 'estimated extracted furfural' (furfural + furfuryl alcohol) and 5–methylfurfural.

[§] Significance of guaiacol's second–year concentration accumulation rate difference: $p=0.081$.

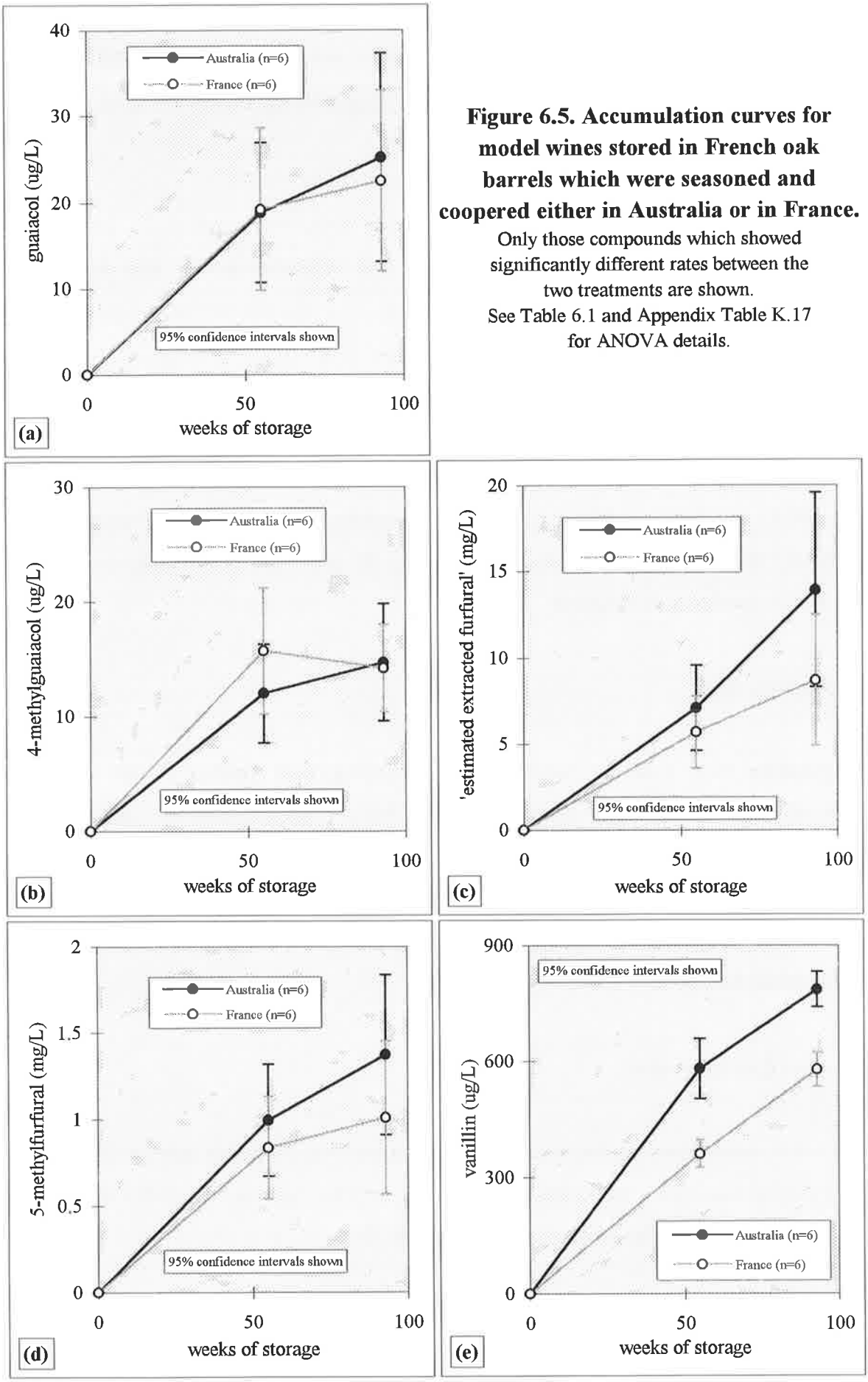


Figure 6.5. Accumulation curves for model wines stored in French oak barrels which were seasoned and coopered either in Australia or in France.
Only those compounds which showed significantly different rates between the two treatments are shown.
See Table 6.1 and Appendix Table K.17 for ANOVA details.

6.3 Aromas associated with unintended heating variation around 'medium toast' coopering (estimated by correlation with the 'emphasis on coopering heat products' principal component)

The preceding section has shown that proprietary coopering peculiarities can result in significant wine aroma and composition effects. Also of interest are the underlying aroma and composition variations.

Both the Chardonnay wine and the Cabernet Sauvignon wine composition principal components analyses (Appx. C) showed that a substantial quantity of the variance among the compounds (27 % for the Chardonnay wine PC2, and 30 % for the Cabernet Sauvignon wine PC1) could be explained by the PC describing the variation in 'coopering heat products.' Some aromas were associated with this PC and, therefore, may have arisen as a result of coopering heat variation.

Chardonnay wine

Composition–PC2, with an 'emphasis on coopering heat products,' was associated positively with 'smoky' and 'allspice' ($p<0.001$ and $p<0.05$, respectively), and negatively with 'green apple' and 'butter' ($p<0.001$ and $p<0.05$, respectively) (Appx. Tab. G.2). The two strongest associations, occurring in opposite directions to one another, are shown in Figure 6.6. The smokiness generated by higher coopering heats may have obscured the fruit aroma of the Chardonnay wine.

Cabernet Sauvignon wine

The aroma most strongly associated with the 'emphasis on coopering heat products' PC in the Cabernet Sauvignon wines was 'coffee' (Fig. 6.7) but 'caramel' ($p<0.01$) and 'vanilla' ($p<0.05$) exhibited similar associations (Appx. Tab. H.2).

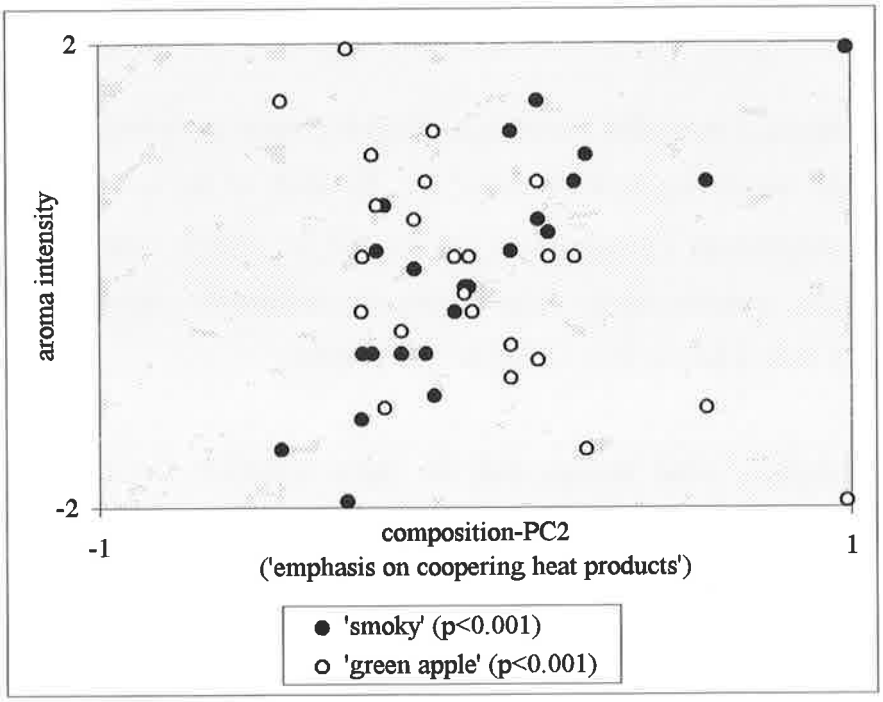


Figure 6.6. The 'smoky' and the 'green apple' associations with composition-PC2 ('emphasis on coopering heat products') in the Chardonnay wine.

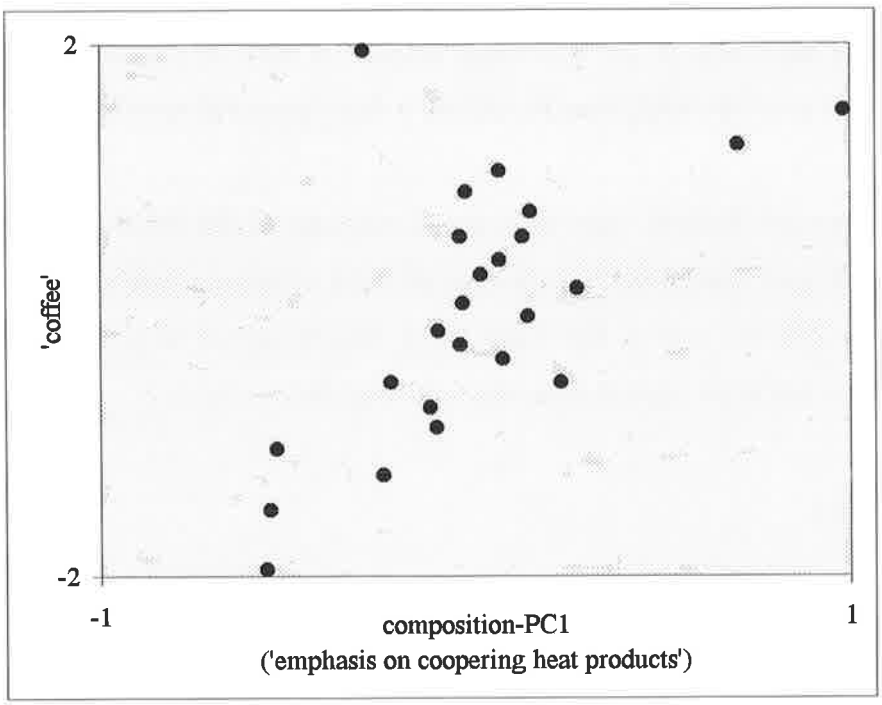


Figure 6.7. The 'coffee' aroma association with composition-PC1 ('emphasis on coopering heat products') in the Cabernet Sauvignon wine (p <0.01).

6.4 Possible compositional causes or indicators of these aroma effects and variations

Having identified the aromas that varied according to cooper treatment or, more generally, according to the 'coopering heat products' PC for each of the two wines, this section considers the relationships between these aromas and the volatile compounds. Correlation analysis (and the specific aroma 'impact–pattern conformity' tests for the Cabernet Sauvignon wine only; Chapter 4) are used for this purpose.

The four Chardonnay wine aromas and the three Cabernet Sauvignon wine aromas identified as being associated with 'coopering heat product' variation, in the preceding section, are discussed here. 'Smoky' and 'allspice,' in the Cabernet Sauvignon wines, which also exhibited a seasoning location / cooper effect (Fig. 6.1d), are also discussed. 'Coconut,' in the Chardonnay wines, which exhibited a seasoning location / cooper effect (Fig. 6.1b) was discussed in Chapter 5.

The compounds responsible for these Chardonnay or Cabernet Sauvignon wine aromas may owe their genesis principally to the coopering heat absorbed by the oak wood during coopering. By considering each compound's aroma associations individually, in the same manner as that discussed in the preceding chapter, it may be possible to identify those compounds that are more likely than the others to have impacted upon each aroma.

'Estimated extracted furfural' may be a useful estimate of the initial quantity of furfural extracted and it may, therefore, be indicative of some coopering heat variables, but it does not exist as a discrete unit in the wines so it cannot impact directly upon the aroma. Consequently, it has been omitted from the following discussion.

Chardonnay wine

'Smoky,' 'allspice,' 'green apple' and 'butter' each may have been influenced by 'coopering heat product' variation in the Chardonnay wine. What specific compounds are likely to have impacted upon each of these aromas?

The compounds most strongly associated with 'smoky' were guaiacol, 4–methylguaiacol, 4–ethylguaiacol, furfural, and 5–methylfurfural ($p<0.001$) (Appx. Tab. G.2). Those most strongly correlated (negatively) with 'green apple' were guaiacol and 4–ethylguaiacol ($p<0.001$). Guaiacol and 4–methylguaiacol, known to possess smoke–like aromas (Wittkowski *et al.* 1992), are most likely to have been, at least partially, responsible for the 'smoky' variation. Whether they have been directly active in this manner or not, it is likely that 'smoky' arose from, and 'green apple' was inhibited by, the application of relatively high 'medium toast' coopering heat.

'Allspice' was associated with furfural and 5–methylfurfural ($p<0.05$). Thus, the variation in this aroma may have arisen due to variation in coopering heat. However, these compounds are known to possess almond– or grilled almond–like aromas (Boidron *et al.* 1988).

'Butter' was positively correlated with furfuryl alcohol ($p<0.05$), a product associated with microbial activity, including malolactic fermentation (MLF). This is not surprising given the association of MLF with butter–like aromas (Henick–Kling *et al.* 1993). There may have been other influences on 'butter,' however, since there were other associations. A negative correlation existed between 'butter' and the *trans*–oak lactone ($p<0.05$), probably due to the higher occurrence of MLF in the American oak barrel Chardonnay wines (Fig. 7.5) along with the coincidentally lower concentration of oak lactones (Chapter 5).

The other negative correlations all involved coopering heat products — 4–ethylguaiacol (considered a coopering heat product only in small amounts relative to that which can be present due to microbial degradation of ferulic acid) ($p<0.01$), 4–methylguaiacol, furfural and 5–methylfurfural ($p<0.05$) — so it may be that 'butter' was inhibited to some degree by relatively high 'medium toast' coopering heat. It is, however, more likely that 'butter' varied only according to MLF, and that the negative correlations with furfural and 5–methylfurfural merely reflect the fact that these compounds are reduced as consequences of the MLF (Chapter 7). It is also possible that compounds extracted from the relatively heavily 'toasted' barrels could have inhibited the MLF.

Other Chardonnay wine aromas possibly affected by the 'coopering heat products'

'Pencil shavings,' 'caramel' and 'cinnamon' in the Chardonnay wine also showed some associations with the 'coopering heat products.' Of these compounds, 4–methylguaiacol was the most strongly associated with 'pencil shavings' ($p<0.01$), and furfural and 5–methylfurfural were also correlated with it ($p<0.05$). 'Pencil shavings' was also associated with two 'natural oak products,' eugenol and *trans*–oak lactone ($p<0.05$), and was negatively correlated with two 'microbial activity products,' 5–methylfurfuryl ethyl ether ($p<0.01$) and furfuryl alcohol ($p<0.05$). Consequently, 'pencil shavings' may have been affected by a combination of the three main processes.

'Caramel' was associated with maltol ($p<0.05$) but also with two microbial activity products, 5–methylfurfuryl ethyl ether and furfuryl alcohol ($p<0.01$), and was negatively correlated with the oak lactones (*cis:* $p<0.05$, and *trans:* $p<0.01$), 4–vinylguaiacol and 4–vinylphenol ($p<0.01$). As discussed in Section 5.2, this aroma has probably varied most substantially due to MLF influences, especially if 'caramel' was used to describe some of the same stimuli which gave rise to 'butter,' an aroma which can often result from MLF (Henick–Kling *et al.* 1993) and which may be considered similar in character, in some ways, to 'caramel.' These two aromas varied in similar ways in the Chardonnay wine (Fig. 3.1).

Vanillin, a product of coopering heat and subject to microbial degradation, was the only compound associated with 'cinnamon' ($p<0.01$) but, surprisingly, it was not associated with the aroma, 'vanilla' (Appx. Tab. G.2 & Appx. Fig. G.5f). Thus, if there has been an aroma impact caused by this compound in the Chardonnay wine, it seems that it may have been restricted to 'cinnamon.' It should be noted, however, that due to barrel fermentation, the range of vanillin concentrations was restricted (198 – 388 µg/L, Tab. 2.2). This compound might have contributed more to the differentiation of 'vanilla' if barrel fermentation had not been carried out.

Cabernet Sauvignon wine

What specific compounds are likely to have contributed to 'coffee,' 'caramel,' 'vanilla,' 'smoky' and 'allspice' in the Cabernet Sauvignon wine?

Two different sets of vanillin concentration values are available for use in considering the compound's possible aroma effects. The freezer–stored sample values are most indicative of the values at the completion of the barrel storage period, while the cellar–stored sample values are lower. Neither set is precisely representative of that which was in the samples used for the aroma ranking but, as previously discussed (Section 4.2), the cellar–stored sample values are likely to best approximate them. Consequently, the cellar–stored sample values are used in the discussion.

Since furfural, in the Cabernet Sauvignon wines, was more than 95 % transformed to furfuryl alcohol, and since furfuryl ethyl ether existed in equilibrium with furfuryl alcohol (work of Sefton, in Spillman *et al.* 1998), the quantities of both of these transformation products were determined mostly by the initial quantity of furfural present, rather than by any agent associated with the transformations. Consequently, they reflect coopering heat influences rather than microbial activity influences. Both of these compounds are implicated, below, as possible contributors to 'coffee,' 'caramel,' 'smoky' and 'dark chocolate.'

'Coffee,' the aroma most strongly associated with the 'emphasis on coopering heat products' principal component, may have been moderately influenced by compounds other than those arising from coopering. For this aroma, there was an association with the *trans*–oak lactone ($p<0.05$) and negative correlations with two 'microbial activity products,' 4–vinylphenol and 4–ethylphenol. Nevertheless, the strongest associations were with the 'coopering heat products' or associated compounds: furfuryl alcohol ($p<0.001$), vanillin, furfural, furfuryl ethyl ether and 4–methylguaiacol ($p<0.01$).

The 'coffee' 'impact–pattern conformity' (IPC) tests (Fig. 6.8) support the possibility that one or more of these compounds could have been active in contributing to the aroma. The 'differentiation potency or accompaniment' (DPA) values shown in Figure 6.8 show that 4–methylguaiacol at 0.01 mg/L, vanillin at 0.05 mg/L, furfuryl alcohol at 3 mg/L and furfuryl ethyl ether at 0.02 mg/L were estimated as the concentration differences, within the range of the samples in the experiment, above which at least 50 % of every decile of comparisons (n~28 per decile) was differentiated according to 'coffee' (p<0.05) (Tab. 4.2).

In view of the aroma likenesses of the compounds found to be associated with 'coffee,' it seems most likely that 4–methylguaiacol (musty–, smoke– and caramel–like, Wittkowski *et al.* 1992) and vanillin (vanilla–like, Boidron *et al.* 1988) could have contributed to the differentiation of this aroma among these wines. The fact that 'coffee' was not chosen to differentiate among the Chardonnay wines suggests that Cabernet Sauvignon grape–derived compounds are also likely to participate in this aroma.

'Caramel' was not correlated with any 'natural oak products' or 'microbial activity products.' It was most strongly associated with furfuryl alcohol and vanillin (p<0.01) but was also associated with guaiacol, 4–methylguaiacol, furfural and furfuryl ethyl ether (p<0.05). The 'caramel' IPC tests for 4–methylguaiacol, maltol, furfuryl alcohol and furfuryl ethyl ether (Fig. 6.9) support the possibility that one or more of these compounds may have been active in contributing to the aroma. The 'caramel'–DPAs for 4–methylguaiacol, maltol, furfuryl alcohol and furfuryl ethyl ether were estimated to be 0.01 mg/L, 0.1 mg/L, 4 mg/L and 0.03 mg/L, respectively (Tab. 4.2).

Neither cyclotene nor maltol were associated with any aroma in the Cabernet Sauvignon wines (Appx. Tab. H.2), but the 'caramel' IPC test for maltol supports the possibility that this compound, possessing a 'fragrant, caramel' aroma (Hodge 1967), may have also contributed to 'caramel.'

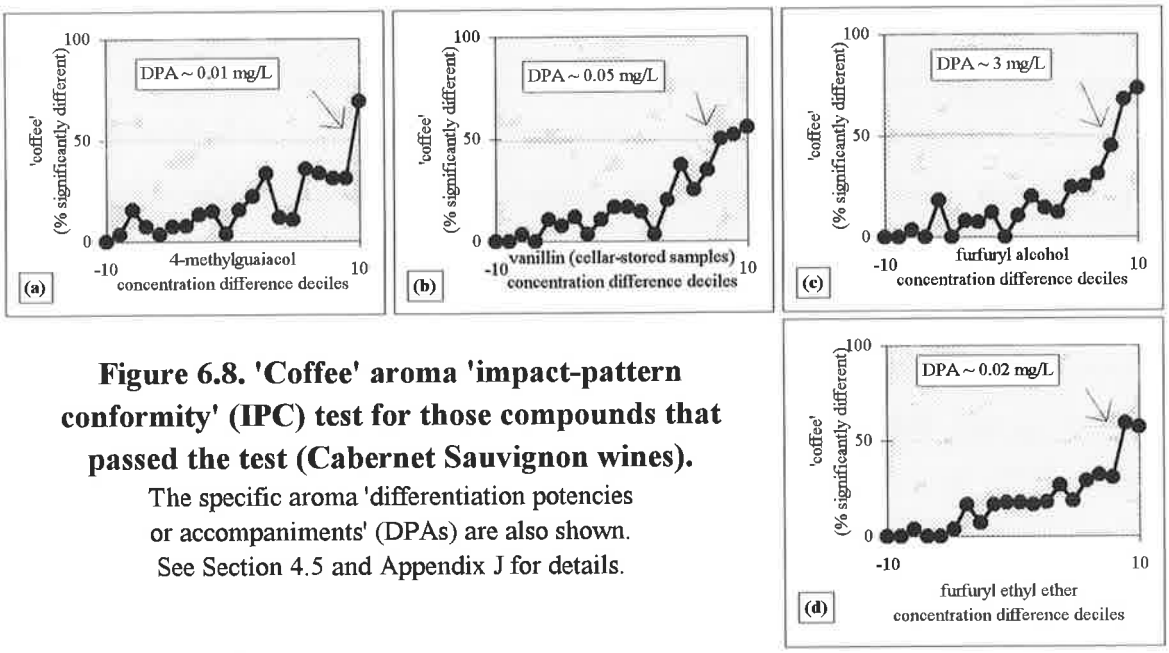


Figure 6.8. 'Coffee' aroma 'impact-pattern conformity' (IPC) test for those compounds that passed the test (Cabernet Sauvignon wines).
The specific aroma 'differentiation potencies or accompaniments' (DPAs) are also shown.
See Section 4.5 and Appendix J for details.

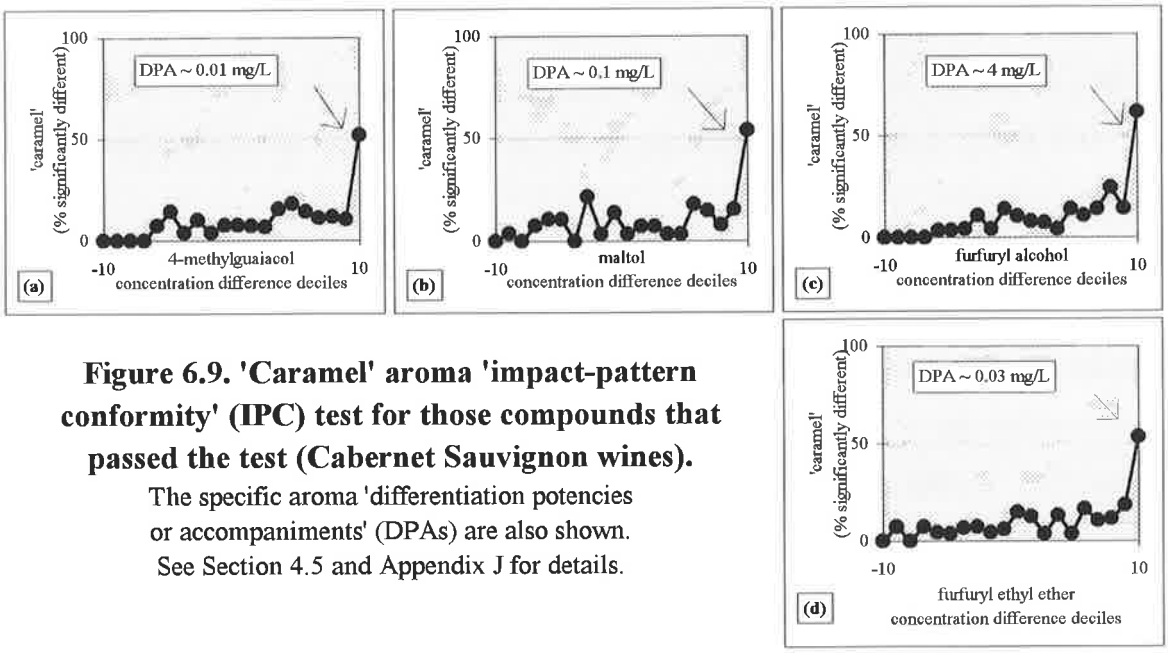


Figure 6.9. 'Caramel' aroma 'impact-pattern conformity' (IPC) test for those compounds that passed the test (Cabernet Sauvignon wines).
The specific aroma 'differentiation potencies or accompaniments' (DPAs) are also shown.
See Section 4.5 and Appendix J for details.

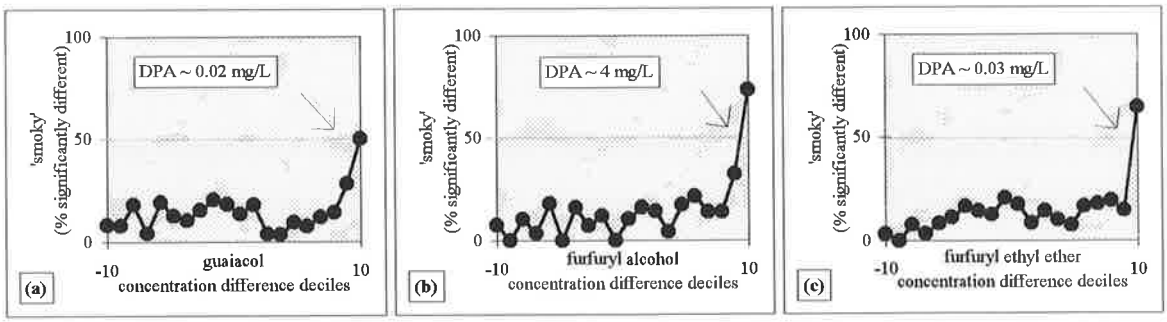


Figure 6.10. 'Smoky' aroma 'impact-pattern conformity' (IPC) test for those compounds that passed the test (Cabernet Sauvignon wines).
The specific aroma 'differentiation potencies or accompaniments' (DPAs) are also shown.
See Section 4.5 and Appendix J for details.

'Vanilla' was also associated with some of the 'coopering heat products' but was more strongly associated with the 'natural oak products' (*cis*–oak lactone and eugenol: $p<0.001$, and *trans*–oak lactone: $p<0.01$). It may be that compounds from each of these groups could have impacted upon 'vanilla' in these wines (Section 5.5). Vanillin ($p<0.01$), 5–methylfurfural, furfural and furfuryl alcohol ($p<0.05$) were each associated with 'vanilla.' The 'vanilla' IPC tests for 4–methylguaiacol and vanillin (Fig. 5.10) support the possibility that one or both of these 'coopering heat products' may have been active, in combination with the *cis*–oak lactone and eugenol (Chapter 5), in contributing to the aroma. The 'vanilla'–DPAs for 4–methylguaiacol and vanillin were estimated to be 0.01 mg/L and 0.08 mg/L, respectively (Tab. 4.2).

In view of the well–known vanilla–like aroma of the compound, vanillin (Boidron *et al.* 1988), it was thought most likely that this compound would have largely caused the 'vanilla' aroma. As discussed in Section 5.5, however, the evidence from this study (Appx. Tab. H.2 & Fig. 5.10) is more strongly in favour of a 'vanilla' effect from the *cis*–oak lactone.

'Smoky' was only associated with vanillin and furfuryl alcohol ($p<0.05$). The 'smoky' IPC tests for guaiacol, furfuryl alcohol and furfuryl ethyl ether, however (Fig. 6.10), support the possibility that one or more of these compounds may have been active in contributing to the aroma. The 'smoky'–DPAs for guaiacol, furfuryl alcohol and furfuryl ethyl ether were estimated to be 0.02 mg/L, 4 mg/L and 0.03 mg/L, respectively (Tab. 4.2). The fact that guaiacol has been identified as one of the dominant smoke flavour compounds of smoked foods (Wittkowski *et al.* 1992) supports the possibility that this compound could have contributed to 'smoky' among the wines, yet there was no significant correlation.

Figure 6.10a shows that 'smoky' differentiation among the wines was significant 50 % of the time only when the samples were separated by the largest concentration differences. Perhaps the IPC test is sensitive to detecting a pattern between an aroma and a compound in a situation where only those samples with the largest concentration differences might be identified as possessing different aromas, while correlation analysis might reveal no association.

'Allspice' was associated with one of the 'coopering heat products,' vanillin, but the 'allspice' IPC tests do not support the possibility that vanillin or any of the other compounds could have been active in contributing to the 'allspice' aroma variation (Appx. Fig. J.4). It is reasonable to conclude that vanillin concentration differences are unlikely to have contributed to the 'allspice' aroma differences since less than half of the samples that were separated by the largest vanillin concentration differences were identified as significantly different in 'allspice.' The association with vanillin ($p<0.05$), and the negative correlations with three 'microbial activity products,' 4–vinylphenol, 4–ethylphenol and 4–ethylguaiacol ($p<0.01$, 0.01 and 0.05, respectively), suggest that microbial activity may have contributed to the variation of 'allspice' in these wines (Chapter 7).

Other Cabernet Sauvignon wine aromas possibly affected by the 'coopering heat products'

'Coconut,' 'berry' and 'dark chocolate' were associated with some of the 'coopering heat products' but the IPC tests do not support the possibility that these compounds could have been active in contributing to the variation within each of these aromas. 'Coconut' and 'berry' were most strongly associated with the 'natural oak products,' and the IPC tests (Appx. Figs. J.2 & J.5) support the possibility that these compounds could have been active in contributing to the variation within these aromas (Chapter 5).

The 'dark chocolate' IPC tests do not suggest the participation of any of the compounds (Appx. Fig. J.10). Associations between 'dark chocolate' and the oak lactones (*cis:* $p<0.01$, and *trans:* $p<0.05$), vanillin, furfuryl alcohol and furfuryl ethyl ether ($p<0.05$) suggest that the cause of the aroma might involve a combination of 'natural oak products' and coopering heat or associated products.

6.5 Summary and conclusion

For the French oak, vanillin and 'estimated extracted furfural' were found in higher concentrations in those barrels coopered in Australia. It appears that, for any given level of surface heat applied, the Australian cooper achieved more substantial heat penetration of the wood. Seasoning location (treatment imposed concurrently) may also have impacted on

this effect, *e.g.* by variation in the wood's moisture content which could absorb some of the heat applied.

The vanillin effect was established within the first couple of months of storage; thereafter this compound continued to accumulate but the rates for each treatment were similar. The accumulation profile for 'estimated extracted furfural,' on the other hand, suggests that a treatment effect for this compound may change with the duration of storage.

Guaiacol, 4–methylguaiacol and 5–methylfurfural exhibited similar patterns to that of 'estimated extracted furfural' among the model wines. The accumulation rate occurring in the France–treatment barrels (seasoned and coopered in France) dropped in the second year of storage, relative to that in the Australia–treatment barrels. These latter barrels were less rapidly depleted of the compounds, perhaps due to deeper heating.

Greater 4–ethylphenol concentrations found in the France–treatment Cabernet Sauvignon wines suggest that shipping conditions could have impacted on microbial populations within the barrels, or that the coopering applied to the treatments could have affected compounds within the wood that can either encourage or inhibit yeast activity.

There is evidence that some of the aroma descriptors applied to the Chardonnay wine, particularly 'smoky' and 'green apple' (negatively), were affected by coopering heat variation. This is also true of some of the Cabernet Sauvignon wine aroma descriptors, particularly 'coffee,' 'caramel,' 'vanilla' and 'smoky.' Suggestions regarding likely compositional contributors to these aromas are made.

The aroma impact of coopering technique variations within and between coopering companies was significant. Thus, there appears to be considerable scope for the optimisation of this process in relation to wine aroma outcomes.

Chapter 7

The modifying contribution of wine microorganisms

Chapter outline

7.1 The boundaries of the variation under consideration

This chapter represents a minor part of the study. The experiments were exploratory and were often based on extreme treatments (*e.g.* the effects of fermentation relative to no fermentation). Nevertheless, they have yielded some results worthy of inclusion.

'Natural oak products' and 'coopering heat products' determine a barrel's aroma potential, but yeast and lactic acid bacteria (LAB) activities during wine fermentation and maturation can transform some of these products, leading to a modification of the aroma–effect.

The effects of alcoholic (primary) fermentation on the oak wood–derived compounds were explored via a comparison of a selection of the Chardonnay and the model wines taken at 11 weeks (corresponding to the time of racking the Chardonnay wines off their yeast lees).

The effects of malolactic fermentation (MLF) were explored in two ways. Malate degradation occurred spontaneously in some of the Chardonnay barrel wines between weeks 11 and 55, resulting in a 0 to 95 % consumption of the 2.0 g/L malate originally present. Correlations between the extent of the observed malate consumption and other compositional or aroma variations could suggest possible MLF effects. Subsequently, a newly vinified Pinot Noir wine was subjected to various treatments in order to better understand these effects.

The physical effects (*i.e.* non–enzyme activity related effects) of microbial cells were also explored in a separate experiment. A wine was autoclaved to sterilise it and to denature any residual grape or microbial enzymes. The possibility of physical removal of volatile compounds by bonding to settling microbial cells was tested using activated and denatured yeast and LAB cells.

The Cabernet Sauvignon wine was transferred to barrels after both the alcoholic and the malolactic fermentations were completed. Nevertheless, this wine was apparently subject to further microbial activity during maturation in barrel, as evidenced by the near–complete reduction of furfural to furfuryl alcohol. The variable final concentration of the microbial product, 4–ethylphenol (0.63 to 1.04 mg/L), among the 24 barrel wines is indicative of

variation in the activity of *Brettanomyces/Dekkera* species (Chatonnet *et al.* 1992b) or, possibly, of *Lactobacillus* or *Pediococcus* (LAB) species (Cavin *et al.* 1993). Correlations between this compound and other compounds or aromas could suggest possible effects for these species.

7.2 Alcoholic fermentation effects

The alcoholic fermentation experiment was a part of the main experiment. It involved a comparison between a selection of the Chardonnay barrel wines (alcoholic fermentation in barrel) and the corresponding replicate barrels of the model wine (same period in barrels but without any fermentation).

The Chardonnay wine was transferred to barrels half way through primary fermentation (at 6 °Baumé). The model wines were concocted and placed in barrel at the same time. After 11 weeks the wines were sampled. No malate degradation had occurred at this stage (Appx. Tab. A.1).

Three of the eight main experiment treatments (Fig. 1.2) — the Australia seasoned and coopered American oak barrels and the France seasoned and coopered Limousin and Tronçais oak barrels — were examined. This comprised nine Chardonnay wine barrels (three of each treatment) and six model wine barrels (two of each treatment). The volatile composition data were explored for treatment effects (Appx. Tab. M.1).

Barrel wine *AA34*, previously identified as an outlier for the coopering heat–derived compounds (Chapter 6), was involved in the experiment so a second set of analyses was performed for these compounds after the outlier had been removed. This second set is referred to throughout the following discussion.

Figure 7.1 shows the relative concentrations of the 20 target–compounds for the two treatments. 'Estimated extracted furfural' is excluded since it is of little interest in this case and since the analysis showed significant interaction.

The three aldehydes, vanillin, furfural and 5–methylfurfural, were all found in higher quantities in the model wine, and the corresponding alcohols and ethyl ethers were found in higher quantities in the Chardonnay wine. These differences were all highly significant (p=0.000 or 0.001) and consistent with what has been reported by others (*e.g.* Chatonnet *et al.* 1992c and references therein).

The significantly higher quantities of 4–vinylguaiacol and 4–vinylphenol were also found in the Chardonnay wine (p=0.000) but the precursors seem to have been mostly grape– rather than oak–derived. The stainless steel–stored Chardonnay (control) wine contained even higher quantities of these compounds than did the barrel–stored Chardonnay wines (Appx. Tab. M.1). Ferulic and *p*–coumaric acid are known to be subject to decarboxylation by the activity of many microorganisms, including yeast, to form 4–vinylguaiacol and 4–vinylphenol, respectively (*e.g.* Chatonnet *et al.* 1993 and references therein). The subsequent transformations to 4–ethylguaiacol and 4–ethylphenol usually proceed only in red wines (Chatonnet *et al.* 1995).

There were three other significant differences. The quantification of cyclotene in the Chardonnay wines was problematic since the GC peak appeared to be affected (broadened) by a wine component in the Chardonnay wine extract. The model wine was not affected in this way so a comparison between the wines may be invalid.

The significant differences for *trans*–oak lactone and 4–methylguaiacol are difficult to explain. Chatonnet *et al.* (1992c) have suggested that 4–methylguaiacol may be a microbial degradation product of vanillin. For the data to support this, however, the direction of the difference would have to have been opposite to that observed, *i.e.* 4–methylguaiacol would have to have been in higher quantities in the Chardonnay wine.

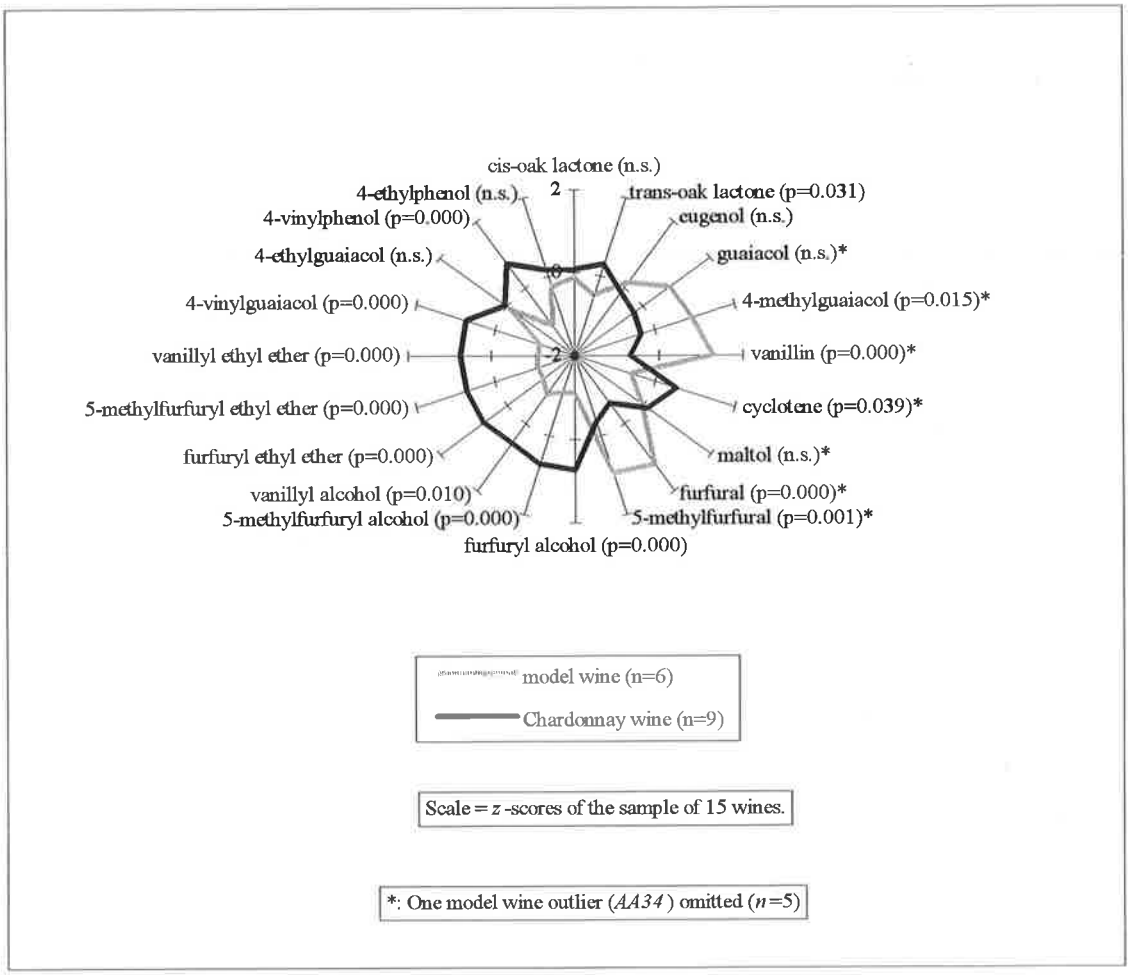


Figure 7.1. Alcoholic fermentation effects: Differences between the Chardonnay and the model wine at 11 weeks.

Furfuryl ethyl ether showed significant interaction (p=0.049) but is included since the interaction does not seem to have been important to the conclusion. No other compounds showed significant interaction (p<0.05)

Table 7.1. Malolactic fermentation experiment treatment summary.

Sample number:	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Initial treatment:	*No treatment*			*Sterilised (DMDC)*				*Denatured (boiled)*				
'Standards mix' added?:			Yes		Yes	Yes	Yes		Yes	Yes	Yes	Yes
MLF induced?:		Yes				Yes	Yes				Yes	Yes
Final malate (g/L)*:	4.2	0.1	4.2	4.1	4.3	0.0	0.1	4.9	4.4	4.5	0.1	0.1

*: Malate prior to MLF: No treatment = 4.4 g/L; Sterilised (DMDC) = 4.6 g/L; Denatured (boiled) = 4.6 g/L.

DMDC = dimethyldicarbonate.

7.3 Malolactic fermentation effects

A separate experiment was conducted to explore the compositional effects of MLF using a Pinot Noir wine which had been variously treated to isolate any possible effects of induced MLF from those of coincidental microbial metabolism or residual exocellular grape or microbial enzymes. Table 7.1 shows a representation of this experimental scheme, and Appendix M.2 details the materials and methods.

The three treatments imposed on the wine prior to any addition of the oak compounds ('standards mix,' Tab. 7.2) or LAB cells were (1) non–treated wine, (2) dimethyldicarbonate (DMDC) sterilised wine and (3) boiled (enzyme denatured) wine (Tab. 7.1). Treatment (1) was sanitised with 100 mg/L of SO_2 but was not sterilised so it allowed the possibility of microbial metabolism and activity from residual exocellular grape or microbial enzymes in the wine. Treatment (2) did not allow the possibility of any microbial metabolism because it was sterilised but it allowed the possibility of activity from residual exocellular grape or microbial enzymes. None of these possibilities was allowed by treatment (3) which was boiled to sterilise and to denature the medium. Consequently, treatment (3) isolated any MLF effects most thoroughly.

The three samples not to have received any 'standards mix' and not to have undergone MLF (sample numbers 1, 4 & 8; Tab. 7.1) were included as controls for these three treatments. Only cyclotene and 5–methylfurfuryl alcohol may have been affected differently by one of the treatments (Appx. Tab. M.2). The heat–treatment may have caused an increase in the concentrations, presumably from non–oak derived wine components.

The sample not to have received any sterilisation–treatment or 'standards mix' addition but to have undergone induced MLF (sample number 2; Tab. 7.1) was included to check for the possibility that some of the compounds may have been produced from grape–derived precursors during MLF. This was found not to be the case (Appx. Tab. M.2). The precursors of 4–vinylguaiacol and 4–vinylphenol (ferulic and *p*–coumaric acid, respectively), known to be degraded by yeast during alcoholic fermentation in white wine

(Chatonnet *et al.* 1993), appear to have been unaffected by the lactic acid bacteria activity in the Pinot Noir wine. This is consistent with the observations of Chatonnet *et al.* (1995).

Table 7.2 shows the effects of the three MLF experiment treatments, relative to their control wines. The data are expressed as percentage variations from each control wine and also as absolute concentration variations (in parentheses). A possible effect was highlighted (in Tab. 7.2) wherever a compound had varied from the control wine concentration by more than the 95 % confidence interval of the quantification (mean of the 12 confidence intervals calculated for this experiment). However, it is important to note that this experiment has not been sufficiently replicated to conclude any effect unless a variation was very large.

The possible effects for *cis*–oak lactone, eugenol and maltol (Tab. 7.2) are all small (within 20 % variation from each control) so they are not discussed. However, the possible effects for furfural, 5–methylfurfural and the corresponding alcohols and ethers are worthy of discussion.

The MLF effects on furfural and 5–methylfurfural for the sterilised wine were similar to those for the denatured wine: both of these furan aldehydes were 100 % degraded with MLF (Tab. 7.2). Thus, residual exocellular grape or microbial enzymes do not seem to have been important to these effects; lactic acid bacteria (LAB) metabolism appears to have been the agent.

Furfural and 5–methylfurfural were affected similarly during alcoholic fermentation (Fig. 7.1). Vanillin, on the other hand, while behaving similarly to the furan aldehydes during alcoholic fermentation, seemed not to be affected by LAB metabolism. However, this compound, along with vanillyl alcohol, was subject to very low quantification precision (Freon extraction, Tab. 2.4), so a treatment effect below the sensitivity of the quantification method may have existed. The presence of a possible MLF effect for vanillyl ethyl ether (Tab. 7.2), is curious because the direction of the effect is opposite to that which would imply any MLF affected vanillin reduction.

The patterns in the transformation products of furfural and 5–methylfurfural (Tab. 7.2) were not entirely consistent with those found to accompany the alcoholic fermentation (*i.e.*, increases in both of the alcohols and the ethyl ethers were not found). Furfuryl alcohol quantities were higher in the MLF–affected wines but there appears to have been little difference in furfuryl ethyl ether quantities. On the other hand, 5–methylfurfuryl alcohol appears to have shown little difference between treatments while 5–methylfurfuryl ethyl ether was found in higher quantities in the MLF–affected wines.

These observations are explained by the kinetic studies of Sefton (Spillman *et al.* 1998). Thus, furfuryl ethyl ether is formed slowly from furfuryl alcohol so the MLF effect on furfuryl alcohol (Tab. 7.2) was preserved. Given longer storage time, however, an effect is likely to have been seen for furfuryl ethyl ether, also.

On the other hand, 5–methylfurfuryl ethyl ether is formed very rapidly from its alcohol and is then degraded rapidly (work of Sefton, in Spillman *et al.* 1998). The MLF effect for 5–methylfurfuryl ethyl ether discussed in this chapter suggests, therefore, that most of the 5–methylfurfuryl alcohol that was formed by the reduction of 5–methylfurfural had been transformed to 5–methylfurfuryl ethyl ether by the time of the analysis. Given longer storage time, however, it is likely that the effect seen for 5–methylfurfuryl ethyl ether would have disappeared, too.

The relatively slow degradation of furfuryl alcohol has allowed an estimation of the effect of MLF on furfural in the Chardonnay wines (main experiment). Any effects on 5–methylfurfural was not well illustrated in these wines due to the rapid degradation of the alcohol. This was also true of vanillin because vanillyl alcohol, despite being stable in model wine (work of Sefton, in Spillman *et al.* 1998), was apparently rapidly degraded in the Chardonnay wine.

Figure 7.2 illustrates the association between the consumption of malate and the reduction of furfural, independent of the original furfural concentration, which was determined by coopering heat. MLF was accompanied by furfural reduction, the first half of which occurred even with limited MLF (*i.e.* less than 25 % malate consumption).

Table 7.2. Malolactic fermentation effects on oak wood-derived or associated volatile compounds in a Pinot Noir wine.

		% Variation from the relevant control wine (concentration variation in parentheses, ug/L)*			
Compound	*Addition (ug/L*)*	*Non-sterilised, non-MLF wine*[1]	*Sterilised, MLF wine*[2]	*Denatured, MLF wine*[3]	*Mean of the 95% confidence intervals (ug/L*)*
cis -oak lactone	391**	+2 (5)	+16 (32)	+7 (15)	16
trans -oak lactone	**	+1 (2)	+9 (13)	+4 (6)	16
eugenol	10	0 (0)	+17 (2)	+4 (1)	1
guaiacol	11	-8 (1)	-4 (1)	+10 (1)	1
4-methylguaiacol	5	-2 (0.1)	-2 (0.1)	+8 (0.4)	0.8
vanillin (Freon extract)*	0.392	0 (0.0)	0 (0.0)	-33 (0.1)	0.2
cyclotene	91	-7 (7)	+1 (1)	+1 (2)	21
maltol	80	-16 (17)	+19 (21)	+16 (18)	16
furfural*	4.002	-95 (3.58)	-100 (3.77)	-100 (2.70)	0.34
5-methylfurfural	484	-37 (175)	-100 (478)	-100 (347)	70
furfuryl alcohol*	8.434	+29 (2.5)	+15 (1.3)	+65 (4.1)	1.3
5-methylfurfuryl alcohol		0 (0)	+25 (1)	-8 (1)	n.dn.
vanillyl alcohol	95	+84 (27)	+3 (1)	-9 (4)	52
furfuryl ethyl ether		-18 (3)	+18 (3)	+16 (2)	n.dn.
5-methylfurfuryl ethyl ether		+? (1)	+? (16)	+? (16)	n.dn.
vanillyl ethyl ether	226	+8 (11)	-26 (35)	-16 (27)	17
4-vinylguaiacol	144	0 (0)	+20 (1)	+33 (2)	7
4-ethylguaiacol		? (0)	? (0)	? (0)	n.dn.
4-vinylphenol		+? (1)	+? (1)	+? (1)	n.dn.
4-ethylphenol*	0.509	+2 (0.01)	-13 (0.06)	+5 (0.02)	0.11

*: mg/L for vanillin (Freon extraction method), furfural, furfuryl alcohol and 4-ethylphenol.

**: One addition (391 ug/L) of a racemic mixture of the oak lactones was made.

[1]: Sample 3 relative to sample 5; Tab. 7.1.

[2]: Mean of samples 6 & 7 relative to sample 5; Tab. 7.1.

[3]: Mean of samples 11 & 12 relative to mean of samples 9 & 10; Tab. 7.1.

: Variation from the relevant control was greater than that which could be explained by quantification error (*i.e.* greater than the mean of the 95 % confidence intervals).

: No compound added due to non-availability.

n.dn. = not determined.

?: Variation according to % could not be calculated because the denominator equalled zero.

The non–sterilised, non–MLF Pinot Noir wine (sample number 3; Tab. 7.1) had received 100 mg/L SO_2 immediately prior to crown sealing so the activity of microorganisms was likely to have been minimal. Nevertheless, furfural, and 5–methylfurfural to a lesser extent, decreased while furfuryl alcohol increased in concentration relative to the sterilised wine control (sample number 5; Tab. 7.1) (Tab. 7.2). The reduction is not likely to have been chemically induced since the sterilised wine control was not affected. Consequently, it seems that furfural is particularly susceptible to microbial reduction, requiring only very limited activity for its degradation (Fig. 7.2), while 5–methylfurfural seems to be less readily reduced. Figure 7.3 illustrates the variation in the extent of furfural reduction experienced for the three wines of the study, and it suggests that a winemaker could actively encourage either the preservation or the reduction of this compound.

7.4 Denatured microbial cell effects

To explore the possibility that the volatile compounds could be removed by adsorption to settling microbial cells, a separate experiment was conducted. Compounds ('standards mix,' similar to that shown in Tab. 7.2 for the MLF experiment) were added to the stainless steel–stored Chardonnay (control) wine, and a suspension of either activated ('activated cells' treatment) or denatured (autoclaved) ('denatured cells' treatment) yeast or lactic acid bacteria (LAB) (one strain of each) was imposed for five days before separation of the cells by centrifugation (Spillman 1995). See Appendix M.3 for the materials and methods and Appendix Table M.3 for details of the results.

Any intermolecular interactions among compounds and macromolecules, not separable by centrifugation, have not been addressed. Neither has the suggestion, by Chatonnet *et al.* (1991), that phenolic compounds may be affected.

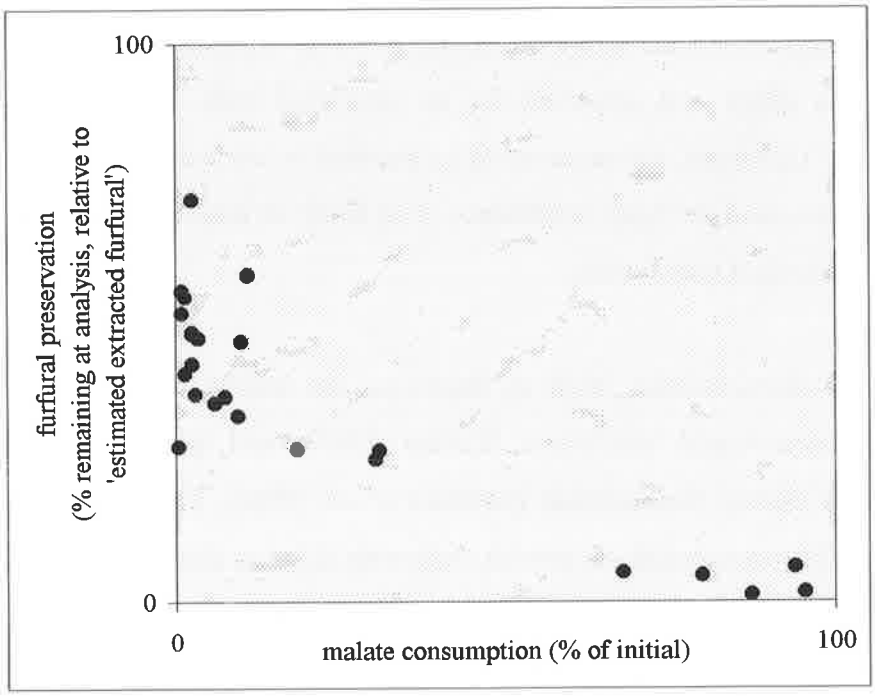


Figure 7.2. The preservation of furfural relative to the extent of MLF (as malate consumption) which proceeded in the Chardonnay wines.

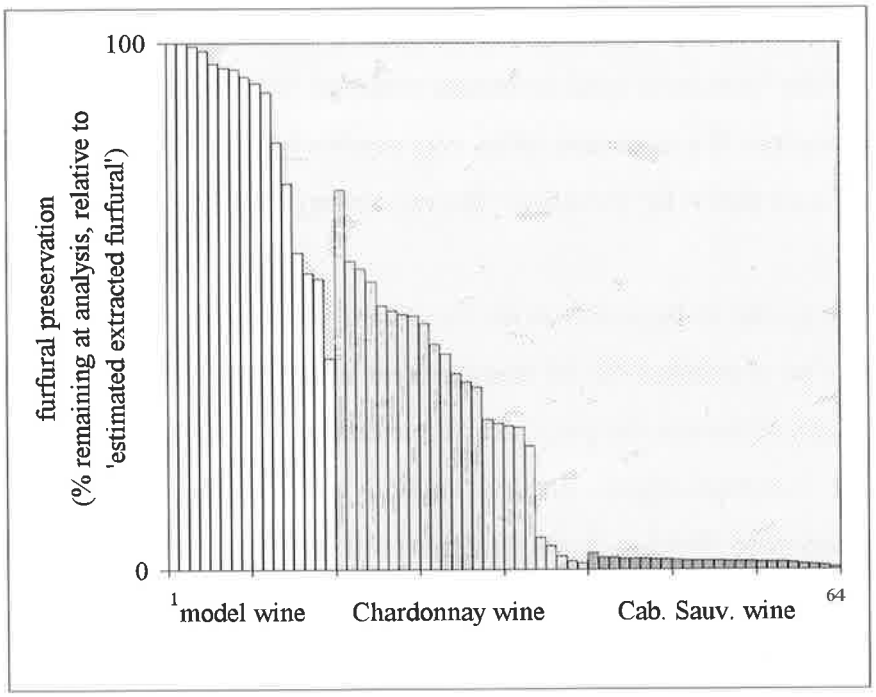


Figure 7.3. Furfural preservation in each of the 64 barrel wines.
Cab. Sauv. = Cabernet Sauvignon

It was necessary to include an enzyme–denaturing treatment, such as autoclaving, to separate the effects of enzyme activity from those of non–enzymic molecular interaction. If, for example, an effect was observed for an 'activated cells' treatment but not for a 'denatured cells' treatment, the cause could be ascribed to biochemical activity. However, if an effect was observed for both treatments, it is likely to have been caused by chemical rather than biochemical interactions.

Yeast cell wall characteristics, such as thickness, are known to change in response to changes in environmental conditions (Calleja 1987), and yeast continuously release macromolecules during fermentation (Lubbers *et al.* 1994). Thus, the inoculation of a finished wine with yeast would not provide cells with identical characteristics to those which have recently finished a fermentation, and it would not provide the medium with the same levels of exocellular macromolecules as might have been encountered in a wine matured on lees. Nevertheless, yeast cells do not lose their negative charge during fermentation (Calleja 1987) so any potential for adsorbing oak wood–derived compounds should have been adequately modelled by the imposed treatments.

Another thing to consider is that autoclaving can substantially alter the chemistry of many macromolecules either incorporated in or released from cell walls. However, the combination of the 'activated cells' treatment with the 'denatured cells' treatment allowed for this consideration. If a treatment effect was exhibited by the 'denatured cells' treatment but not by the 'activated cells' treatment, the autoclaving could have been responsible for it.

The treatments appear to have shown no significant variation from the control, outside of that which may be accounted for by measurement imprecision, for any of the compounds (Appx. Tab. M.3). However, the precision of quantification for some of the compounds was low. Furfural, 5–methylfurfural, furfuryl alcohol and, particularly, vanillin, have been imprecisely determined. Further, 4–ethylguaiacol was only present in very small amounts.

The suggestion by Chatonnet *et al.* (1991), that the oak lactones may be susceptible to removal by adsorption to yeast cells is not supported by the data. The variations in oak lactone concentration according to the comparisons of barrel–fermentation/barrel–storage with 'vat'–fermentation/barrel–storage, and of storage on fine lees with storage on total

lees, reported by these authors, are likely to be due to differences in barrel contact time and to natural barrel–to–barrel variations. Further, the depletions of 4–vinylguaiacol and 4–vinylphenol during barrel maturation on lees over nine months, noted by the same authors, are not supported by the data and are likely to be due to oxidation reactions (Nicolini *et al.* 1991) or to a slow, acid catalysed reaction with ethanol (Dugelay *et al.* 1995).

Of the compounds under consideration, the furan– and phenolic–aldehydes (furfural, 5–methylfurfural and vanillin) appear to be most affected during barrel maturation, even in the presence of small residues of microorganisms (Sections 7.2 & 7.3), and the data are consistent with the belief that depletions of these compounds are characterised by molecular conversions rather than physical separations.

Notwithstanding the limitations of the experiment, the data suggest that the oak wood–derived volatile compounds are not likely to be subject to physical removal from wine by settling yeast or lactic acid bacteria cells.

7.5 Aroma variations associated with compositional indicators of microbial activity

An exploration of the aroma effects of microbial activity in the Chardonnay and the Cabernet Sauvignon wines is difficult since no microbial treatments were imposed on these wines. The only possibility is to base an exploration on the analysis of correlations between wine aromas and the compounds known to arise from microbial activity. However, this can be problematic since the samples were affected by various natural and cultural treatments, and some of these compounds were affected by more than just microbial activity. Furfural, for example, was affected by microbial activity *and* coopering heat. However, sensory analyses were performed only on the wines of the main study so the correlation analyses have been explored for what information they provide and the discussion should be considered with the limitations in mind.

Alcoholic fermentation aroma effects could not be considered with the available data. The MLF aroma effects, however, could be explored by correlation with the extent of malate consumption.

The Chardonnay wine composition–PC3, with an 'emphasis on some microbial products' (vanillyl ethyl ether, 5–methylfurfuryl alcohol, furfuryl ethyl ether, 4–vinylguaiacol and 4–vinylphenol) might have also been used as an indicator of microbial activity but some of the compounds arose from yeast and some arose from LAB activity. In any case, there were no associations between this PC and any of the aromas (Appx. Tab. G.2). Similarly, most of the compounds incorporated in PC3, with the exception of 4–vinylguaiacol and 4–vinylphenol (which also contributed to composition PC1), were not associated individually with any of the aromas. It seems that this unidentified microbial activity in the Chardonnay wine has had little aroma effect and, therefore, is not discussed further.

Microbial activity variation in the Cabernet Sauvignon wines is explored using the composition–PC2 which incorporates emphases on the hydroxycinnamic acid degradation products, 4–vinylguaiacol, 4–ethylguaiacol, 4–vinylphenol and 4–ethylphenol. Also incorporated were 5–methylfurfuryl alcohol and vanillin (negatively). The activity indicated by this PC does not necessarily involve microorganisms associated with the alcoholic– or the malolactic–fermentation since these processes were completed in a stainless steel tank prior to barrel maturation. The activity, therefore, could have involved any microorganisms that could function in a sugar– and malate–depleted, low SO_2, moderate pH and moderate alcohol red wine (Appx. Tab. A.3). In particular, yeast species belonging to the genus *Brettanomyces* and to its sporogenous form *Dekkera* are likely to have participated since such yeast are implicated in 4–ethylphenol production (Chatonnet *et al.* 1992b). Some *Lactobacillus* or *Pediococcus* species (LAB) may also have been involved (Cavin *et al.* 1993).

Chardonnay wine

Four aromas were correlated with malate consumption in the Chardonnay wine (Fig. 7.4), 'butter' and 'caramel,' positively ($p<0.01$ and $p<0.001$, respectively), and 'pencil shavings' and 'allspice,' negatively ($p<0.01$ and $p<0.05$, respectively). Appendix M.4 illustrates all of the aroma associations with malate consumption among the 24 Chardonnay wines.

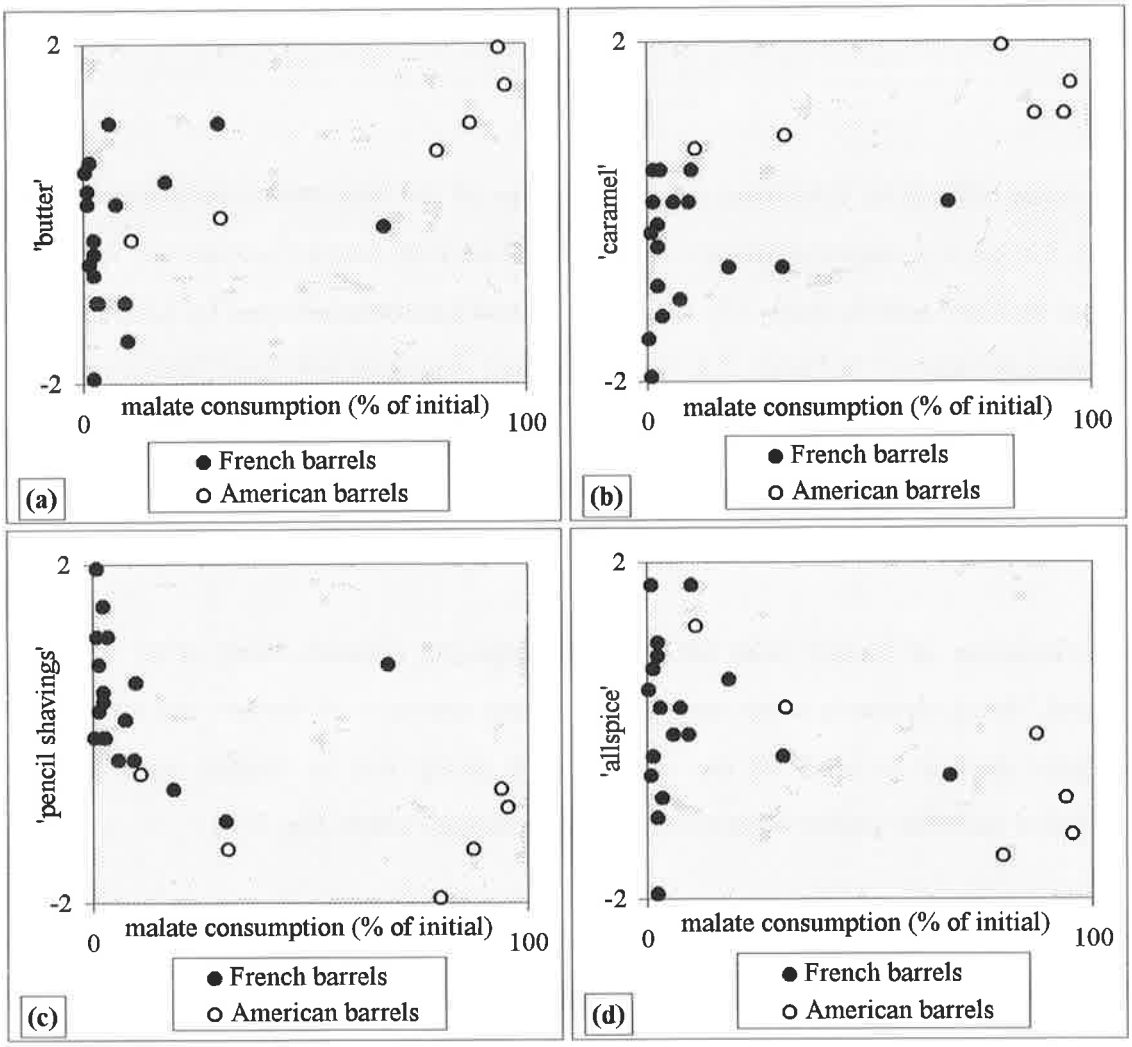


Figure 7.4. Significant associations between the Chardonnay wine aroma and the extent of malate consumption.

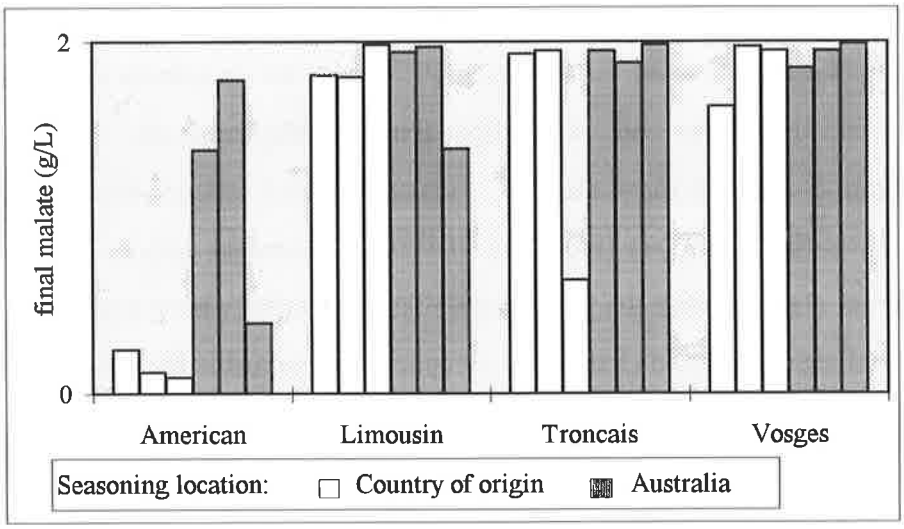


Figure 7.5. Final malate concentrations in 24 Chardonnay barrel-wines.

It has been difficult to determine the likely causes of the four aroma variations shown in Figure 7.4 since a disproportionate number of American–barrel Chardonnay wines were affected by LAB activity (Fig. 7.5). Were the aroma variations affected by LAB activity or oak origin influences? In Figure 7.4, the French and American oak wood barrel wines were separately identified since the American barrel wines experienced a disproportionate amount of malate consumption, and may have influenced the correlation in some unknown, oak origin–related way.

The association of 'butter' with MLF is not surprising (Henick–Kling *et al.* 1993), and 'caramel,' being similar in some ways to the aroma character of 'butter,' may have been a descriptor applied to some of the same stimuli giving rise to 'butter' variation (these descriptors varied in similar ways among the Chardonnay wines, Fig. 3.1).

The negative correlation involving 'pencil shavings' may have been caused by oak origin effects. 'Pencil shavings' was significantly lower in the American oak barrel wines than in the three French oak barrel treatment wines (Fig. 5.3a). The oak origin means for 'allspice' followed a similar trend, without being significantly different.

Cabernet Sauvignon wine

The Cabernet Sauvignon wine composition–PC2, with an 'emphasis on some microbial products' (*i.e.*, emphases on 4–vinylguaiacol, 4–ethylguaiacol, 4–vinylphenol, 4–ethylphenol and 5–methylfurfuryl alcohol ... versus vanillin) was negatively correlated with 'allspice' and 'coffee' ($p<0.01$) (Fig. 7.6). If indeed, microbial activity has affected these aromas, it is not clear whether the products of microbial activity have acted to mask them or if the microbial activity caused changes to compounds responsible for causing the aromas.

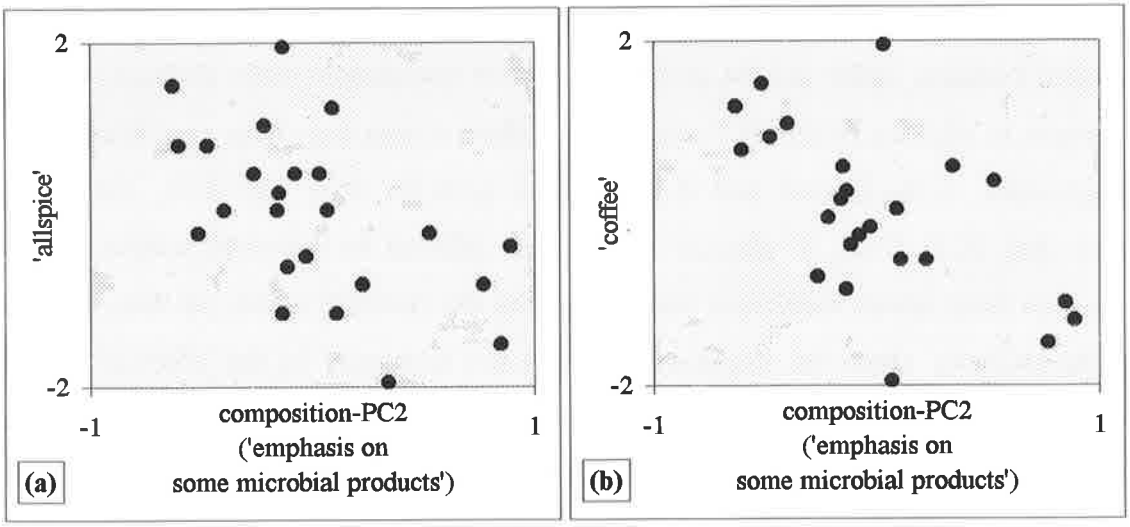


Figure 7.6. Significant aroma associations with the Cabernet Sauvignon wine composition-PC2 ('emphasis on some microbial products').

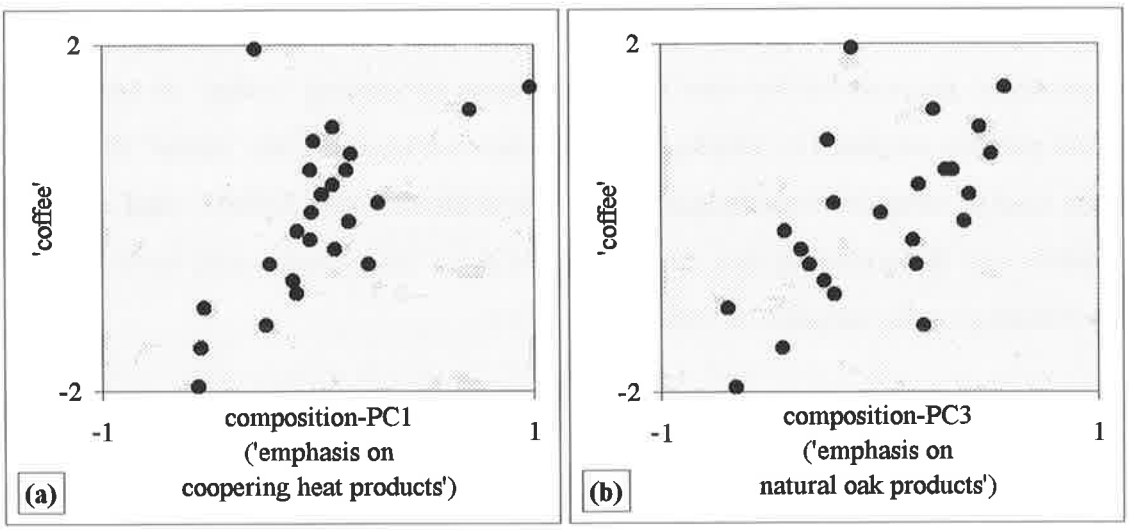


Figure 7.7. Composition-PC associations with 'coffee' in the Cabernet Sauvignon wine (additional to that in Fig. 7.6b).

There was only one positive association between 'allspice' and the wine compounds measured (vanillin, cellar sample, $p<0.05$); negative associations were stronger and more numerous. In addition to the PC2 association, 'allspice' was negatively correlated with 4–ethylguaiacol, 4–vinylphenol and 4–ethylphenol ($p<0.05$, 0.01 and 0.01, respectively) (Appx. Tab. H.2). Thus, if 'allspice' was indeed, affected by microbial activity, it seems likely that these aroma variations were caused by the masking effects of these microbial activity products. However, this possibility was not supported by the 'allspice' 'impact–pattern conformity' (IPC) tests (Appx. Fig. J.4 p, q & r). The patterns were not inconsistent with the suggested masking effect but they were not strong enough to support it.

The source of variation in 'coffee' appears to be complicated. In addition to being negatively correlated with composition–PC2, 'coffee' was positively associated with composition–PCs 1 and 3, with emphases on 'coopering heat products' and 'natural oak products,' respectively ($p<0.01$) (Fig. 7.7). Individually, the 'coopering heat products' or their degradation products, especially vanillin (cellar sample, $p<0.01$) and furfuryl alcohol ($p<0.001$), were most strongly and positively associated with 'coffee.' Indeed, 'coopering heat products' are probably the most likely candidates for causing 'coffee,' an aroma from a product partially produced by roasting. As discussed in Chapter 6, the 'coffee' IPC tests for vanillin (cellar sample), 4–methylguaiacol, furfuryl alcohol and furfuryl ethyl ether were consistent with the possibility that one or more of these compounds could have been active in contributing to the variation in 'coffee' (Fig. 6.8).

If 'coffee' was affected mostly by 'coopering heat products,' at least one of which (vanillin) is susceptible to degradation by microbial activity, it is not surprising that 'coffee' was seen to diminish along with apparent increases in microbial activity.

7.6 Summary and conclusion

Despite the exploratory nature of the experiments described in this chapter, it is clear that the activity of some microorganisms can have significant oak wood–derived or associated composition effects in wine. Alcoholic fermentation was accompanied by the near–complete transformation of three oak–derived aldehydes — vanillin, furfural and 5–methylfurfural — to the corresponding alcohols which exist in equilibrium with their ethyl ethers. These products are variously susceptible to chemical degradation during storage.

The effect of MLF on furfural and 5–methylfurfural were similar to those of the alcoholic fermentation but vanillin appears to have been unaffected by MLF. Furfural appears to be most readily reduced.

None of the volatile compounds tested appeared to be removed by adsorption to settling yeast or LAB cells.

Despite the various aroma effects of the other influences (*e.g.* oak origin), there may have been some microbial influences on the aromas, particularly a negative effect for 'allspice' and 'coffee' among the Cabernet Sauvignon barrel wines. A study focussing on the microbial effects on oak wood–derived wine aromas, while holding all the other variables identified in this thesis constant, would be useful in extending this enquiry.

Chapter 8

The contribution of the duration of contact between oak wood and wine

Chapter outline

8.1 The boundaries of the variation under consideration

In Australia, premium oak–affected white wines are commonly barrel–matured for between three and twelve months, and premium red wines for between one and two years. These maturation practices are intended to fulfil more than one objective. In addition to providing oak wood–derived aroma (and/or flavour) compounds, the wood may provide taste or tactile compounds and it may encourage grape– or microorganism–derived compound changes, and these considerations probably contribute to the maturation duration decided upon for each wine. This chapter deals only with volatile compound effects.

Does the aroma effect evolve due to a general increase in all of the oak wood–derived aroma compounds or is there some change in the relative quantities of each which occurs under various conditions? If accumulation rates, at various stages, differ among these compounds, it may be that, dependent on the duration of barrel maturation, different aroma–effects could be expected.

During barrel maturation, some winemakers encourage the activity of microorganisms, actively (barrel fermentation and malolactic fermentation) or coincidentally (*e.g.* minimal SO_2 concentrations for perceived taste development or consumer health benefits). These activities can alter the accumulation profile for some of the compounds and, therefore, the relative proportions among the compounds may vary in the final product. Thus, the contribution of the duration of contact between oak wood and wine to the aroma profile of the product is intimately connected with sanitation practices, some of the consequences of which were discussed in Chapter 7.

8.2 The accumulation of volatile 'natural oak products' and 'coopering heat products' in a periodically sterilised, American and Limousin barrel–stored model wine, over two years

This section describes the accumulation profiles, in the periodically sterilised model wine, of ten oak wood–derived volatile compounds extracted from four American and four Limousin barrels.

Accumulation profiles

The absolute quantity of each compound that was apparently available for extraction varied substantially among the barrels, and of most interest were the characteristics of the curve profiles independent of the absolute quantities available. Therefore, each concentration value was standardised by adjusting it to a percentage of the maximum concentration reached for each compound in each barrel. The mean of these percentage points at each sampling time was plotted.

The percentage points were also used to calculate the 95 % confidence intervals (CIs) around each mean. The error–estimate applied to each concentration value (95 % CIs) (Tab. 2.6) is different to those applied to the curve profiles (Figs. 8.1 to 8.5). The curve–profile error (95 % CIs) represents, principally, the sampling error among the eight barrels and, importantly, it is affected by the 'anchoring' of the eight curve profiles (to a point at which the compound reached maximum concentration, *i.e.* the 100 % point) and by the subsequent adjustment of each point in the curve to a percentage of that maximum. Therefore, the CIs around each curve refer to the curve profile as a whole, and not directly to the quantification precision of each point.

To consider when the accumulation in compound concentration between sampling times was becoming statistically insignificant, the absolute concentration data were analysed (Tab. 8.1). Details of these analyses are in Appendix N.

Eugenol and the oak lactones (Fig. 8.1)

With the exception of the six–week point, the accumulation profiles of eugenol and *cis*– and *trans*–oak lactone were essentially identical and asymptotic, *i.e.* each curve rose most steeply initially and then gradually approached a maximum as the curve became flatter with increasing *x*–values (equivalent to a first–order reaction curve in chemistry) (Snedecor and Cochran 1967 p. 448). Approximately 30 to 40 % of the final (93 week) concentration of these compounds was extracted during the first six weeks of storage. Compared with most

of the other oak volatiles (Figs. 8.2 to 8.5), eugenol and the oak lactones were extracted slowly during this six–week period. The ratio of the mean concentration of the *cis*– and *trans*–oak lactones was 2.12 at six weeks storage, and thereafter, from 11 to 93 weeks, remained constant at 2.40 +/- 0.02. The final concentration of the sensorially important *cis*–isomer ranged from 80 µg/L (in an American oak barrel) to 304 µg/L (in a Limousin oak barrel). The concentration of eugenol ranged from 12 to 23 µg/L after the 93 weeks of storage.

Guaiacol and 4–methylguaiacol (Fig. 8.2)

Guaiacol and 4–methylguaiacol are lignin decomposition products formed during barrel toasting. They are formed in wood at higher concentration with increasing toast levels and accumulate mainly in the first two millimetres of the inner surface of the barrel (Chatonnet *et al.* 1989). The maximum concentration of guaiacol in the model wines ranged from 6 to 33 µg/L, while that of 4–methylguaiacol ranged from 1 to 16 µg/L. Variation in concentration among the barrels is presumed to reflect variation in toast levels (Chatonnet *et al.* 1989).

Given that these compounds are largely located on, or close to, the innermost surface of the barrel, it might be expected that they would accumulate in wines relatively quickly in comparison with other volatile oak components. 4–Methylguaiacol was rapidly extracted during the first six weeks, and no significant change occurred in the mean concentration beyond week 32 (Tab. 8.1). The failure of Towey and Waterhouse (1996) to detect any 4–methylguaiacol in wines in second–fill barrels is consistent with total extraction during the first fill over eight months duration.

Guaiacol was also rapidly extracted during the first six weeks but, in contrast to the 4–methyl analogue, the concentration then continued to increase in a near–linear fashion.

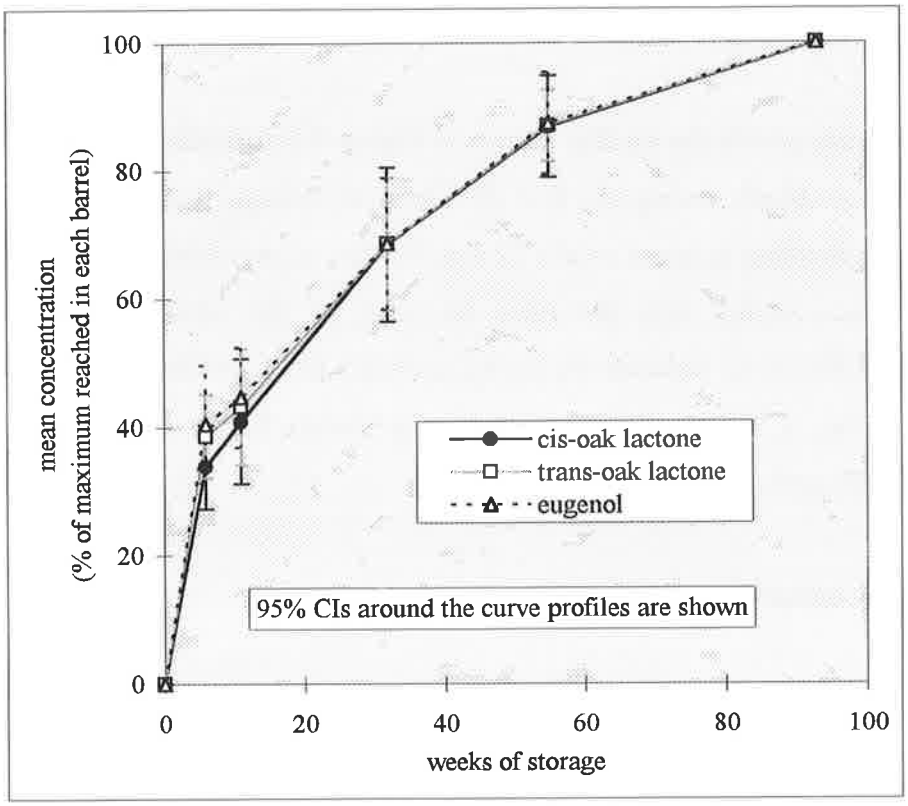


Figure 8.1. The accumulation of eugenol and the oak lactones in a periodically sterilised, American and Limousin barrel-stored model wine (n = 8).

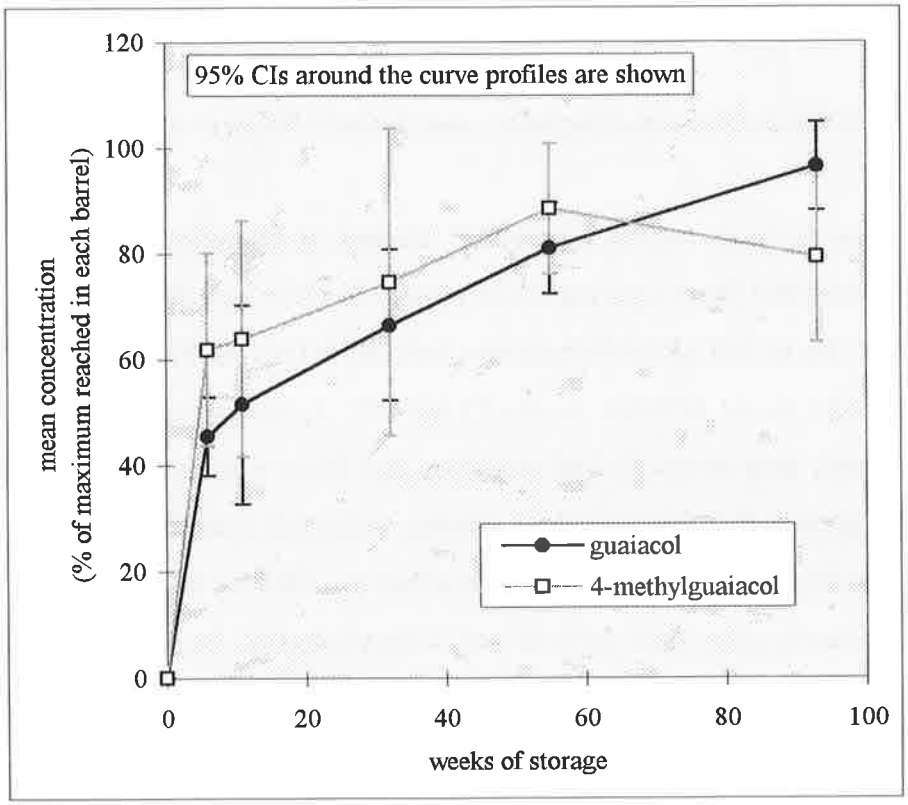


Figure 8.2. The accumulation of guaiacol and 4-methylguaiacol in a periodically sterilised, American and Limousin barrel-stored model wine (n = 8).

Vanillin (Fig. 8.3)

The accumulation profile for vanillin shown in Figure 8.3 indicates that the concentration increased asymptotically during the first 32 weeks of storage; thereafter it increased in a linear manner, doubling between weeks 32 and 93. The accumulation rate for vanillin in the second year was higher than the rates for most of the other compounds, and the concentration differences between successive sampling times continued to be significant up to 93 weeks (Tab. 8.1). The final concentration of vanillin in the model wines varied from 237 µg/L to 803 µg/L.

Cyclotene and maltol (Fig. 8.4)

Cyclotene and maltol were found, each at a concentration of up to 138 µg/L. The mean concentration of maltol increased up to the 55 week sampling, but not during the second year of storage (Tab. 8.1). The accumulation of cyclotene, on the other hand, was essentially linear throughout the storage period. Thus, the accumulation rate for cyclotene was the lowest of all of the oak volatiles in the first six weeks, and the highest towards the end of the storage period.

Furfural (as 'estimated extracted furfural') and 5–methylfurfural (Fig. 8.5)

The appearance in most barrels of furfuryl alcohol, a biological reduction product of furfural, indicated that there was microbial activity in these barrels for at least part of the storage period. Up to half of the furfural was reduced in two barrels between weeks 11 and 32, and in a third barrel between weeks 55 and 93. A comparison of the data for those model wines with little or no furfural reduction and those with significant reduction (data not shown) indicated that this microbial activity, occurring despite the sanitation imposed on the model wines, was not sufficient to have had any obvious effect on the accumulation rates of compounds other than furfural and furfuryl alcohol. In particular, there was no evidence of any significant transformation of vanillin in the model wines (Spillman *et al.* 1997). Furfural appears to be the most susceptible of the various oak wood volatile compounds to microbial transformations, being almost totally transformed during alcoholic and malolactic fermentation and during red wine maturation (Chapter 7).

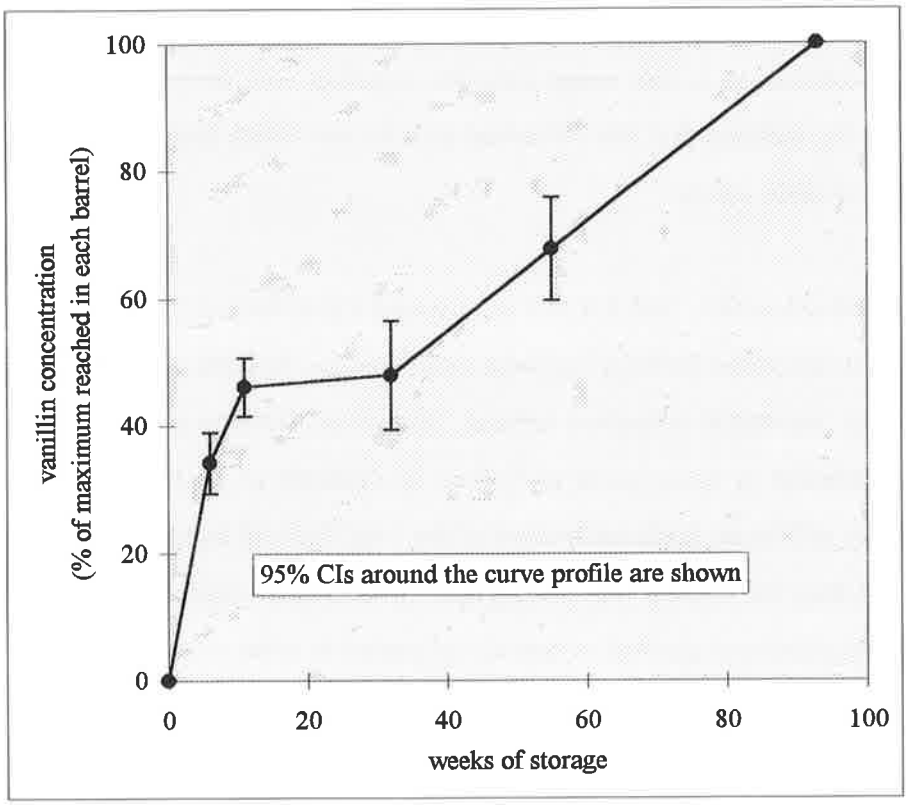


Figure 8.3. The accumulation of vanillin in a periodically sterilised, American and Limousin barrel-stored model wine (n = 8).

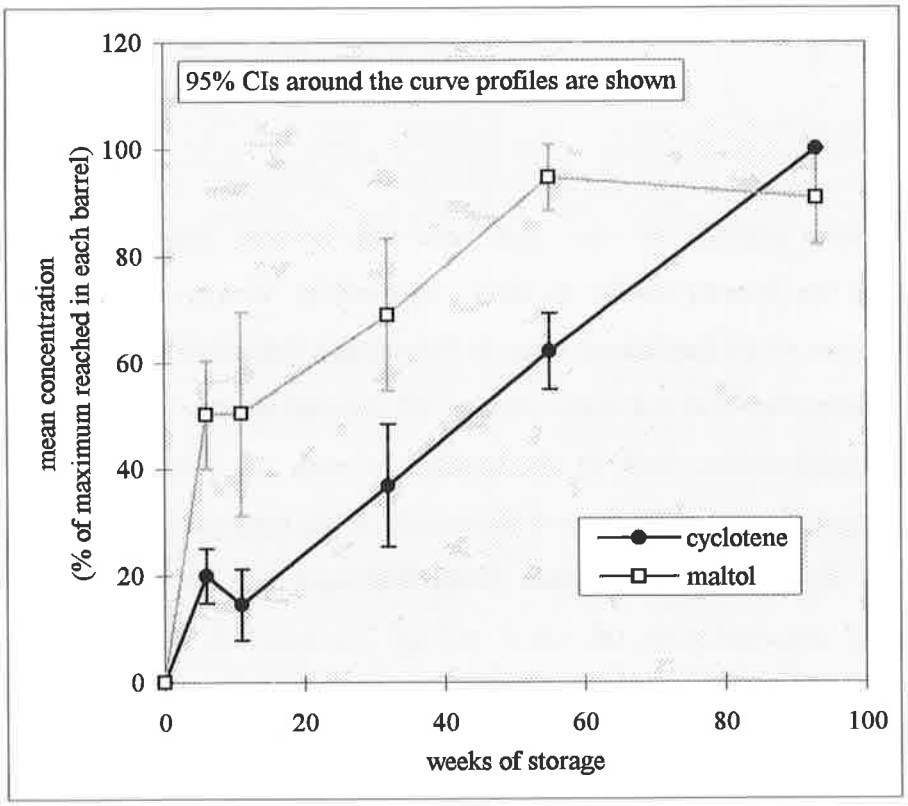


Figure 8.4. The accumulation of cyclotene and maltol in a periodically sterilised, American and Limousin barrel-stored model wine (n = 8).

While the model wines were therefore not free of microbial activity, the degree of the observed transformations of oak wood volatiles, together with the yeast and bacteria counts (Appx. Tab. A.9), indicate that this microbial activity was much less than normally occurs in barrel storage of table wines.

Since compound extraction, and not any subsequent transformation, is of primary interest in this chapter, the extracted furfural has been estimated as 'furfural plus furfuryl alcohol' and is referred to as 'estimated extracted furfural.' However, furfuryl alcohol is subject to slow chemical degradation in wine (work of Sefton, in Spillman *et al.* 1998), and therefore this summation may still be an underestimation of the total furfural extracted from the wood. 5–Methylfurfural may be slightly less susceptible to microbial degradation but its reduction product, 5–methylfurfuryl alcohol, is quickly degraded in wine (work of Sefton, in Spillman *et al.* 1998) so it has not been possible to estimate the extent of any degradation.

Given these considerations, it is not possible to determine from the data in Figure 8.5 whether the levelling–off in the accumulation curves at around the 6–11 week point for 'estimated extracted furfural' and 5–methylfurfural was due to a cessation of extraction from the wood. Consequently, the furan aldehydes are not discussed further.

Discussion

The accumulation profiles of *cis* and *trans* oak lactone, eugenol, guaiacol and 4–methylguaiacol are broadly similar to those observed by Towey and Waterhouse (1996) during maturation of a Chardonnay wine in French and American oak barrels, except that these authors reported that the concentration of eugenol in wine aged in new oak wood barrels decreased between week 21 and bottling at week 30. Towey and Waterhouse did not observe eugenol in wines in second–fill barrels. It has been postulated (Chatonnet *et al.* 1989 & 1990, Towey and Waterhouse 1996) that yeast lees are capable of fixing some volatile phenols extracted from oak wood, and that this may limit the accumulation of these compounds in some barrel aged wines. However, the data discussed in Section 7.4 suggest that this probably does not occur.

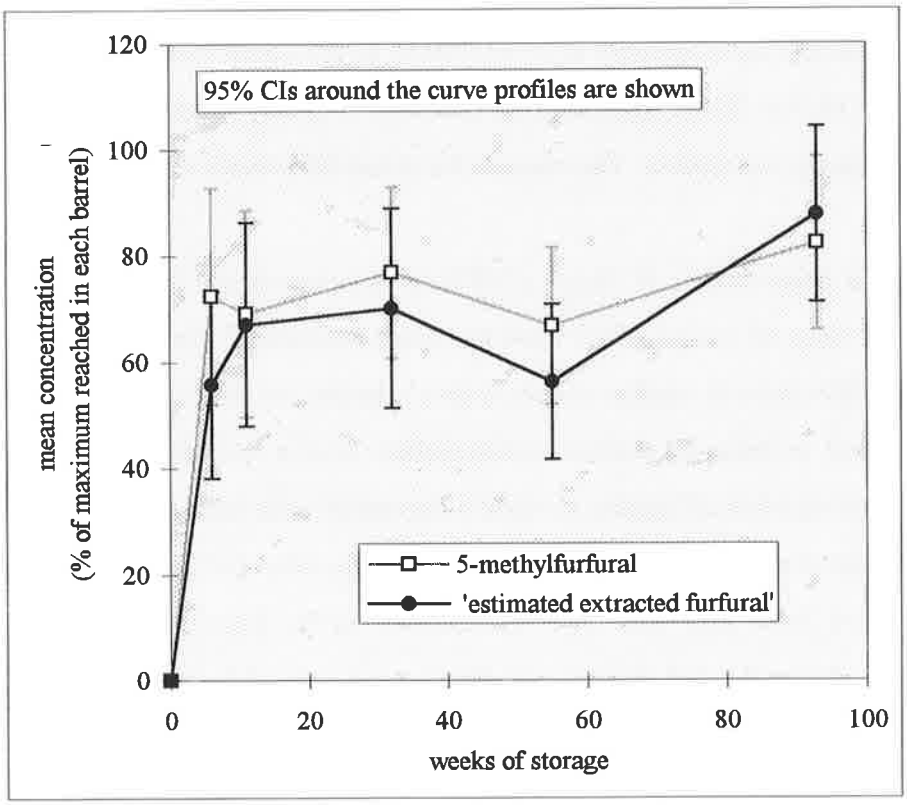


Figure 8.5. The accumulation of 'estimated extracted furfural' and 5-methylfurfural in a periodically sterilised, American and Limousin barrel-stored model wine (n = 8).

The accumulation data for *cis*– and *trans*–oak lactone, guaiacol and 4–methylguaiacol obtained by Chatonnet *et al.* (1990) for red wines aged in new oak wood barrels for 10 months are limited in that only three replicates were studied, not all compounds were analysed at each sampling, and apparently no statistical analyses were performed. Their data for guaiacol and 4–methylguaiacol are compatible with those presented here, but their data for the oak lactones contrasts with those shown in Figure 8.1 and with those obtained by Towey and Waterhouse (1996). Only a small proportion of the final concentration of oak lactones were shown by Chatonnet *et al.* (1990) to be extracted within the first six weeks of maturation (< 10 %). These authors also observed a steady increase in the *cis/trans* oak lactone ratio during maturation. The reasons for these differences are unclear.

Apart from the observations of Puech (1987), accumulation profiles of vanillin, cyclotene and maltol in wines or model wines have not been determined previously. Puech recorded considerable differences in vanillin accumulation between red wines in four barrels, with one showing a linear increase in vanillin concentration over a 24 month period, while in the others, the concentration of vanillin reached a maximum at an early stage. The accumulation of vanillin in barrel aging wines is greatly dependent on microbial processes within the wine (Chapter 7), and these may well have varied between the barrels. Similarly, accumulation data for furan aldehydes and alcohols recorded previously (Chatonnet *et al.* 1990, Towey and Waterhouse 1996) were obviously substantially affected by wine microbiology.

It is evident that some structurally related oak components thought to be formed in oak wood in similar manners (*e.g.* cyclotene and maltol) apparently had different accumulation profiles in the model wines (Fig. 8.4), suggesting either that they were actually formed in different ways, in different parts of the wood, or that they suffered different fates once extracted into the wine. Conversely, some structurally unrelated compounds gave similar curves, *e.g.* eugenol compared with the oak lactones.

Table 8.1. Accumulation duration: Oak wood–derived volatile compound concentrations over 93 weeks of maturation for a model wine stored in eight new 'medium toast' barrels.

Two barrels from each of four treatments, involving American and Limousin oak wood were used (Appx. Tab. A.10).

Compound	Significance of the *F*–ratio[1]		Concentration (µg/L[†]) at various sampling time (weeks from start)				
	Time[2]	Inter.[3]	6	11	32	55	93
cis–oak lactone[4‡]	***	n.s.	60[a]	72[a]	122[b]	153[c]	176[c]
trans–oak lactone[‡]	***	***					
- American/America	**		12[a]	12[a]	21[b]	28[bc]	34[c]
- American/Australia	n.s.		12	12	17	24	27
- Limousin/France	*		20[a]	34[ab]	48[bc]	52[c]	62[c]
- Limousin/Australia	***		70[a]	60[a]	119[b]	153[c]	170[d]
eugenol	***	n.s.	8[a]	9[a]	13[b]	17[c]	20[d]
guaiacol	***	n.s.	8[a]	9[ab]	12[bc]	14[cd]	17[d]
4–methylguaiacol	*	n.s.	7[a]	7[a]	10[b]	10[b]	9[ab]
vanillin	***	n.s.	214[a]	282[b]	299[b]	421[c]	613[d]
cyclotene	***	n.s.	19[a]	15[a]	38[b]	61[c]	96[d]
maltol	***	n.s.	50[a]	48[a]	69[b]	92[c]	88[c]
'estimated extracted furfural'[†]	*	n.s.	5.1[a]	6.8[a]	7.3[ab]	5.8[a]	9.2[b]
5–methylfurfural[†]	n.s.	n.s.	0.72	0.74	0.86	0.75	0.94

[†] Concentrations in mg/L for 'estimated extracted furfural' (furfural + furfuryl alcohol) and 5–methylfurfural.

[1] Significance of *F*–ratios: n.s. = not significant; *, **, *** = significant at $p<0.05$, $p<0.01$, $p<0.001$.

[2] Sampling time effect from two–factor, repeated measures, ANOVA, without replication (5 sampling times x 8 barrels) (Appx. Tab. N.1).

[3] Interaction effect from two–factor ANOVA, with replication (5 sampling times x 4 oak origin / seasoning locations; $n=2$) (Appx. Tab. N.1). Due to a significant interaction effect for *trans*–oak lactone, data subsets were analysed separately by two–factor, repeated measures, ANOVA, without replication (5 sampling times x 2 barrels) (Appx. Tab. N.1).

[4] For each compound, the sampling time concentration means separated by different superscripts were significantly different ($p<0.05$), according to Fisher's LSD.

[‡] Oak lactones = *cis*– and *trans*–β–methyl–γ–octalactone.

The profiles of the curves for eugenol and *cis*– and *trans*–oak lactone are entirely consistent with diffusion kinetics, *i.e.* once a portion of wood is wetted, dissolution of the compounds occurs rapidly, and then the accumulation rates (in the wine) are dependent on diffusion which is mediated by wood structure, compound structure, wine composition, temperature and physical agitation. Once the compounds dissolved from the wetted portion of the wood have diffused evenly throughout the wine (in the wine contained by the barrel, and in the wine within the wood spaces), the only new source of the compound is from newly wetted portions of the wood as the wine progresses more deeply into it. This progression is likely to be accompanied by increases in diffusion resistance as more of the wood matrix fills the distance between dissolution point and the wine contained by the barrel. Thus, the accumulation rate would slow gradually to a point of exhaustion.

Maltol, and possibly also 4–methylguaiacol, appear to have reached a maximum concentration before the end of the storage period, although given the quantification precision for 4–methylguaiacol — the final concentrations were around 9 µg/L with 95 % confidence intervals of 5 µg/L (+/-) (Tab. 2.6) — no firm conclusion can be drawn about this compound. The curves of these two compounds are consistent with their generation by toasting, their greater accumulation on the inner surface of the barrel, and hence their relatively rapid extraction. The failure of Towey and Waterhouse (1996) to detect any 4–methylguaiacol in wines in second–fill barrels is consistent with total extraction during the first fill (lasting eight months).

Guaiacol, on the other hand, accumulated over the whole storage period, with significant increases in concentration between 6 and 32, and then between 32 and 93 weeks (Tab. 8.1). This compound was not observed in extracts of non–heated oak wood, nor in those which were heated to 175 °C or less (Sefton *et al.* 1990a). It might therefore be expected to be found only in the first few millimetres of toasted barrel–staves (Chatonnet *et al.* 1989) and to be extracted quickly into wine, yet the profile for guaiacol shows that this was not the case. Guaiacol was extracted at a rate similar to the oak lactones, which are distributed evenly throughout barrel staves (Chatonnet *et al.* 1994b). Guaiacol might be more tightly bound to the oak matrix and therefore extracted more slowly than, for example, the oak lactones. Another possibility is that guaiacol is formed, at least in part, by acid hydrolysis of

lignin or lignin–like precursors. The near–linear profile of the accumulation curve beyond week 11 is consistent with either interpretation.

In a preliminary discussion of some of the data presented in this thesis (Sefton *et al.* 1993b), the concentration of guaiacol in the Chardonnay wine, after 55 weeks maturation, was reported to be essentially the same as that recorded following the first 11 weeks of the maturation period. This contrasts with the presently discussed data, obtained for the model wine. It is possible that guaiacol formation by acid hydrolysis took place faster in the model wines (mean pH of 3.12 at 55 weeks) than in the Chardonnay wines (mean pH 3.32 at the same time). It may be significant that, in their study of the evolution of oak wood–derived volatiles in Chardonnay wines fermented and matured on lees for eight months over three successive vintages, Towey and Waterhouse (1996) reported only a slight increase in mean guaiacol concentration (7 μg/L, equivalent to 13 % of concentration at three months) between the third and eighth month of the maturation period for the first–fill when the mean pH of the finished wines was 3.34, but observed a higher rate of increase over the same period (three and eight months) for the second–fill (15 μg/L) when the mean wine pH was 3.22. Nevertheless, the reason for these apparent discrepancies remains a matter of conjecture.

Two other oak volatiles, vanillin and cyclotene, which are normally associated with the heating process, also showed accumulation profiles which are consistent with their partial or total generation by hydrolytic mechanisms.

The accumulation profile for cyclotene was essentially linear throughout the storage period (Fig. 8.4). This suggests that, unlike maltol, cyclotene is formed in oak at only low concentration during toasting, but is then generated slowly by acid hydrolysis following the filling of the barrels. This is consistent with the findings of Johnson *et al.* (1969) who compared products of the pyrolysis of sucrose with those formed from sucrose by acid hydrolysis under reducing conditions. While maltol was formed from sucrose by pyrolysis only, cyclotene was formed from sucrose by both processes. Cyclotene was also formed from *glucose* as a major acid hydrolysis product.

Although the final step to cyclotene formation is probably hydrolytic, coopering heat also appears to be important to the generation of the intermediates between oak carbohydrates and this compound. The final concentration of cyclotene in the 16 barrel–stored model wines was strongly correlated with other products of toasting, regardless of their accumulation profiles (guaiacol, 4–methylguaiacol, vanillin, maltol, 'estimated extracted furfural' and 5–methylfurfural; $p<0.01$, 0.01, 0.01, 0.001, 0.001 and 0.001; Appx. Tab. C.11). Furthermore, the barrel with the lowest apparent toast level, as judged by the final concentration of these compounds, also had the slowest rate of accumulation of cyclotene.

The accumulation profile for vanillin (Fig. 8.3) indicates that this compound is probably formed by two mechanisms. Lignin pyrolysis during coopering could yield vanillin, extracted relatively quickly, while acid hydrolysis and/or oxidation of lignin might also generate vanillin, but more slowly. Unlike cyclotene, initial coopering heat appears to have had little or no influence on the rate of accumulation of vanillin between weeks 32 and 93. With the exception of the least strongly toasted barrel, all barrels showed similar rates of accumulation of vanillin, in µg/week, during this latter period of storage, regardless of the concentration of vanillin at week 32 (data not shown).

8.3 Summary and conclusion

The accumulation curves for *cis*– and *trans*–oak lactone and eugenol were asymptotic and virtually identical in shape. Guaiacol was extracted more rapidly in the first six weeks, and then continued to accumulate in a near–linear fashion. 4–Methylguaiacol and maltol did not increase significantly in concentration beyond the first year of storage. The accumulation curve for vanillin was asymptotic during the first 32 weeks and linear thereafter, indicating more than one mechanism for generating this compound in oak. The extraction curve for cyclotene was linear throughout the maturation period and was consistent with a generation by slow acid hydrolysis of precursors formed during coopering.

Oak wood–derived volatiles are of diverse origin, are formed in different ways and accumulate at different rates. Not only will the absolute concentration of these volatiles in wines be determined by barrel maturation times, but the relative proportions, and hence their impact on wine aroma, will also depend on this factor.

Chapter 9

'Preference,' and recommendations for oak wood use efficiency and wine quality optimisation

Chapter outline

9.1 Shifting the focus from description to 'preference'

The panels described the aroma variability among each set of wines, without consideration of their like or dislike of each aroma. Later, each set of wines was ranked according to their general liking of the overall aroma, without specific consideration of individual aromas. Thus, the 'preference' differentiations achieved by the panels required a process of integrating the various aromas under the banner of personal 'preference' and then, of analytically, integrating the various personal 'preferences' into a group 'preference.'

Consensus, according to wine aroma 'preference,' can be difficult to reach because different individuals may put different emphases on their personal impression of the quality–impact of particular aromas. Descriptive profiles are probably less subject to disagreement.

The differentiation achieved among the Chardonnay wines, according to 'preference' is illustrated in Table 3.1, and full 'preference' rank and Fisher–Yates rank transformation details are in Appendix E. Similar results for the Cabernet Sauvignon wines are in Table 3.3 and Appendix E.

These results have defined the 'preference' of each panel for the wines; they are not meant to be indicative of any absolute quality or as an estimation of 'preference' among a given population. Nevertheless, the results may be useful as case studies. Each of the panels were comprised of 30 predominantly experienced and regular wine appreciators, some of which were familiar with the wines in this study, having participated in the 'descriptive' panels (panel demographics shown in Appx. D.2).

9.2 Treatment effects according to 'preference'

Significant oak origin treatment effects were found for 'preference' among the combined Chardonnay and Cabernet Sauvignon wine data. Figure 9.1a shows that, among the French oak woods, the Vosges and Tronçais barrel wines were preferred over the wines from the Limousin barrels ($p<0.05$), and Figure 9.1b shows that, for the Australia seasoned and coopered oak wood, the Vosges and Tronçais barrel wines were preferred over the wines from the American barrels ($p<0.05$). ANOVA details are in Appendix Tables K.1 and K.8.

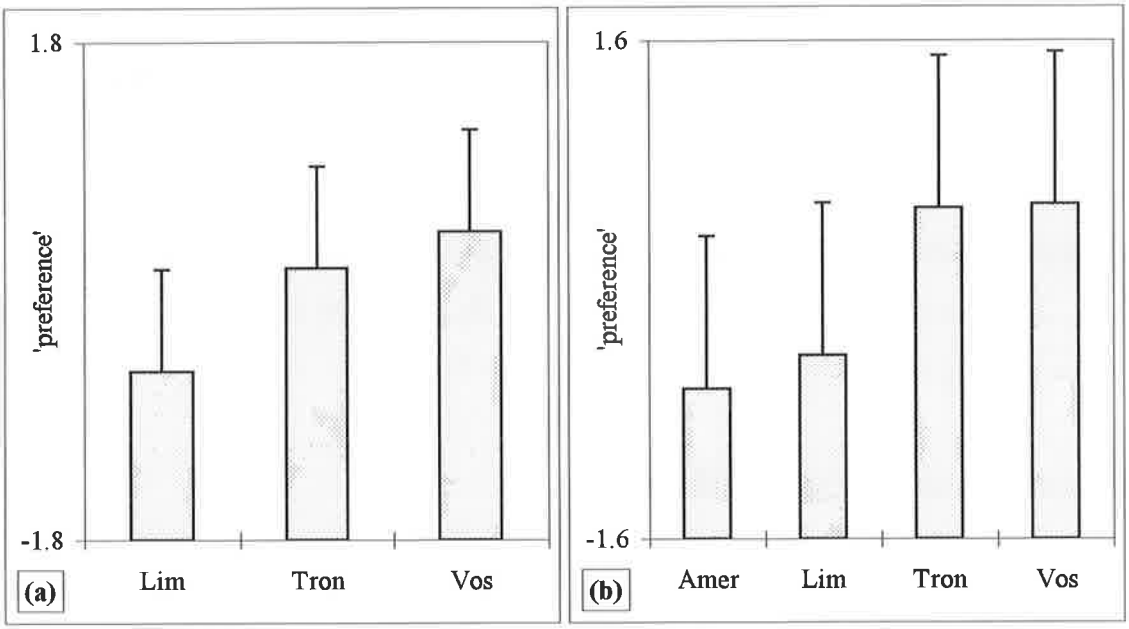


Figure 9.1. Oak origin effects, according to 'preference' among the Chardonnay and the Cabernet Sauvignon wines.

(a): French oak (n=12). Limousin < Troncais & Vosges, p<0.05. No interaction (Appx. Tab. K.1).
(b): American oak, relative to the French oaks (all Australia seasoned and coopered) (n=6).
American < Troncais & Vosges, p<0.05. No interaction (Appx. Tab. K.8).
LSD (5%) bars are shown.
The scale in each figure represents the approximate range of the individual Fisher-Yates rank transformation values.

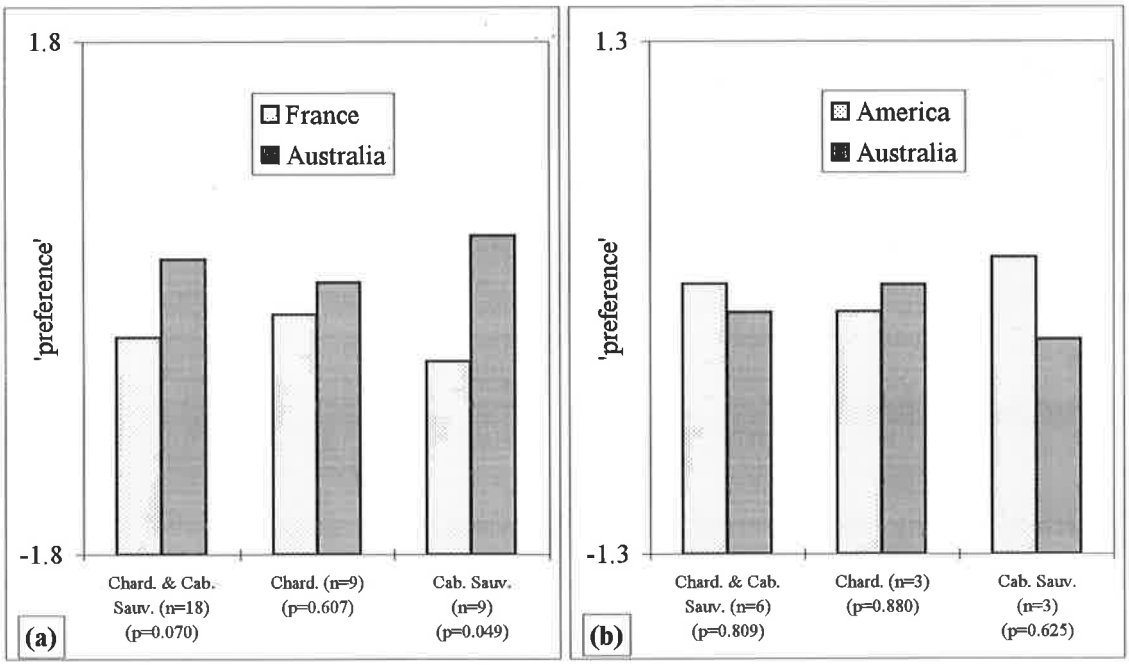


Figure 9.2. Seasoning location and/or cooper effects, according to 'preference' among the Chardonnay and the Cabernet Sauvignon wines.

(a): French oak seasoning and cooper location. No interaction (Appx. Tabs. K.1, K.2 & K.3).
(b): American oak seasoning location. No interaction (Appx. Tab. K.14).
The scale in each figure represents the approximate range of the individual Fisher-Yates rank transformation values.

There was little evidence of a seasoning location and/or cooper treatment effect on 'preference.' The nine Australia seasoned and coopered French oak barrel Cabernet Sauvignon wines were preferred over the corresponding wines from the France seasoned and coopered barrels (p=0.049) (Fig. 9.2a) but no effect was found among the Chardonnay wines. There was no seasoning location effect for the American oak (Fig. 9.2b). ANOVA details are in Appendix Tables K.1, K.2, K.3 and K.14.

9.3 Aromas associated with 'preference'

Correlation analysis between 'preference' and the aroma principal components can indicate which of the aroma groups (PCs) are likely to have contributed to the 'preference' expressed by the panels. Individual aromas were similarly tested for association with 'preference' but, since the combined 'preference' judgement is likely to involve a combination of aromas, the associations with the PCs have been of primary interest.

Chardonnay wine

'Preference' among the Chardonnay wines was associated with aroma–PC2, involving an emphasis on 'vanilla,' 'allspice' & 'cinnamon' (p<0.01); it was not associated with either PC1 or PC3 (Appx. Tab. F.5). Thus, it seems likely that 'preference' has been affected more by the vanilla– and spice–like aromas than by variations among the other Chardonnay wine aromas.

Cabernet Sauvignon wine

'Preference' among the Cabernet Sauvignon wines was associated with aroma–PC1, involving an emphasis on 'vanilla,' 'berry,' 'coconut,' 'caramel,' 'dark chocolate,' 'coffee' and 'allspice' ('rich aromas') versus 'earthy' (p<0.01); it was not associated with either PC2 or PC3 (Appx. Tab. F.10). Thus, it seems likely that 'preference' has been affected more by the 'rich aromas' than by variations among the other Cabernet Sauvignon wine aromas.

9.4 Possible compositional causes or indicators of these 'preference' effects and variations

Single compositional causes of 'preference' are unlikely. Consequently, individual compound correlations (and the specific aroma 'impact–pattern conformity' test for the Cabernet Sauvignon wines; Chapter 4) which indicate the possible impact of an individual compound, are of secondary interest to the composition–PCs in this consideration.

Chardonnay wine

None of the Chardonnay wine composition–PCs were associated with 'preference.' The differentiation of 'preference' in this wine is, therefore, not likely to have resulted from any one of the main compositional variation 'directions' (PCs, Fig. 2.1). There was also no association between malate consumption and 'preference' (Appx. Tab. M.4 & Appx. Fig. M.1a). Any compositional cause or indicator of 'preference' among these wines is, therefore, likely to involve either more or less complexity than the variations summarised by the PC analysis, or to involve compounds not quantified. The *cis*–oak lactone was the only individual compound (apart from 4–vinylphenol, which was present in insubstantial quantities) to be associated with 'preference' (Fig. 9.3a) (Appx. Tab. G.2).

Cabernet Sauvignon wine

The *cis*–oak lactone was also positively associated with 'preference' in the Cabernet Sauvignon wines (Fig. 9.3b) (Appx. Tab. H.2) but there were many other associations, including those for the composition–PCs. Nevertheless, the *cis*–oak lactone was the only compound associated with 'preference' in both of the wines.

The many correlations found between 'preference' and the individual compounds in the Cabernet Sauvignon wines are adequately summarised by the correlations found between 'preference' and the composition–PCs (Appx. Tab. H.2). 'Preference' among these Cabernet Sauvignon wines was positively associated with the 'natural oak products' and the 'coopering heat products,' and was negatively correlated with 'some microbial products' (Fig. 9.4). The most 'preferred' wines in the Cabernet Sauvignon study came from barrels

made from oak wood containing relatively high quantities of the oak lactones and eugenol, from barrels which had been subjected to relatively high 'medium toast' levels, and from barrels in which relatively low levels of microbial activity (especially from *Brettanomyces/ Dekkera* species) had occurred during barrel maturation.

9.5 Recommendations

Each wine processing choice should be made based on reliable estimations of the aroma consequences. However, current oak selection processes and coopering treatments provide poor estimations, and unintended microbial activity can provide further interference. Three recommendations are made, below, with the aim of improving the predictability of oak wood and wine processing choices.

The term 'quality' is used, below, to mean the general acceptability of wine from the perspective of winemakers, wine judges or other wine 'experts.' It is acknowledged that many of the panelists may not necessarily fall into one of these groups (although some did) and that the 'preference' rankings do not necessarily equate to this definition of 'quality.'

Oak selection based on cis–oak lactone quantification

Oak wood selection based on an estimation of the mean and variance of the *cis*–oak lactone concentration in each oak wood lot prior to usage in winemaking (and preferably prior to coopering) may constitute an improved strategy over the current reliance on oak origin for wood selection. If oak selection processes were to be based on *cis*–oak lactone estimations, the probable aroma potential of an oak lot could be estimated, possibly manipulated or, if beyond remedy, the wood could be diverted to another use. This could generally raise oak wood–affected wine quality. Further quality improvements could follow when the aroma consequence of each lot of wood could be relied upon and appropriately matched to a known batch of grapes or wine. The commercial trading of lots could also be facilitated.

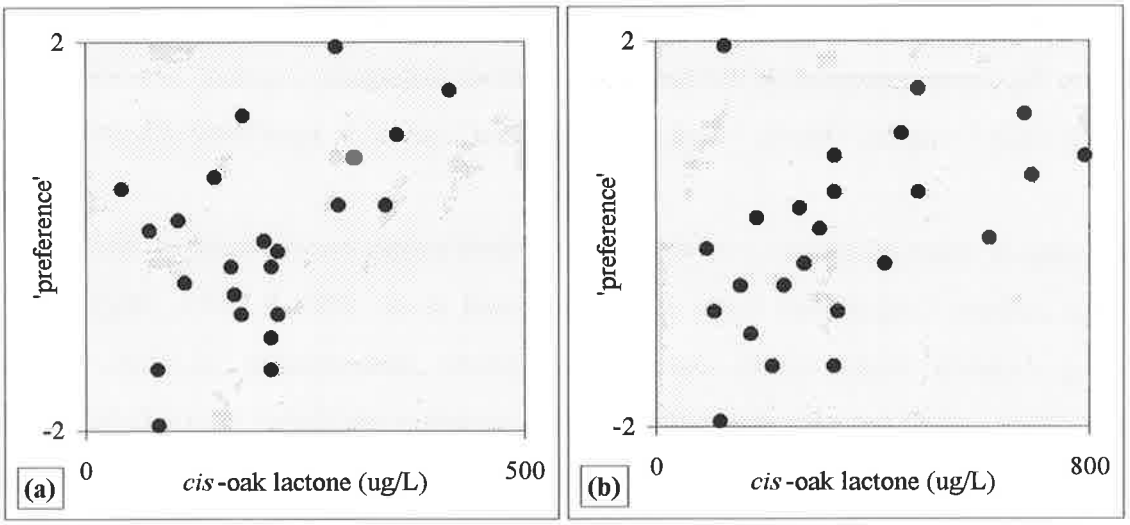


Figure 9.3. 'Preference' associations with the *cis*-oak lactone.
(a): Chardonnay wine (positive correlation, $p<0.01$).
(b): Cabernet Sauvignon wine (positive correlation, $p<0.05$).
The 'preference' scale is in Fisher-Yates rank transformation values.

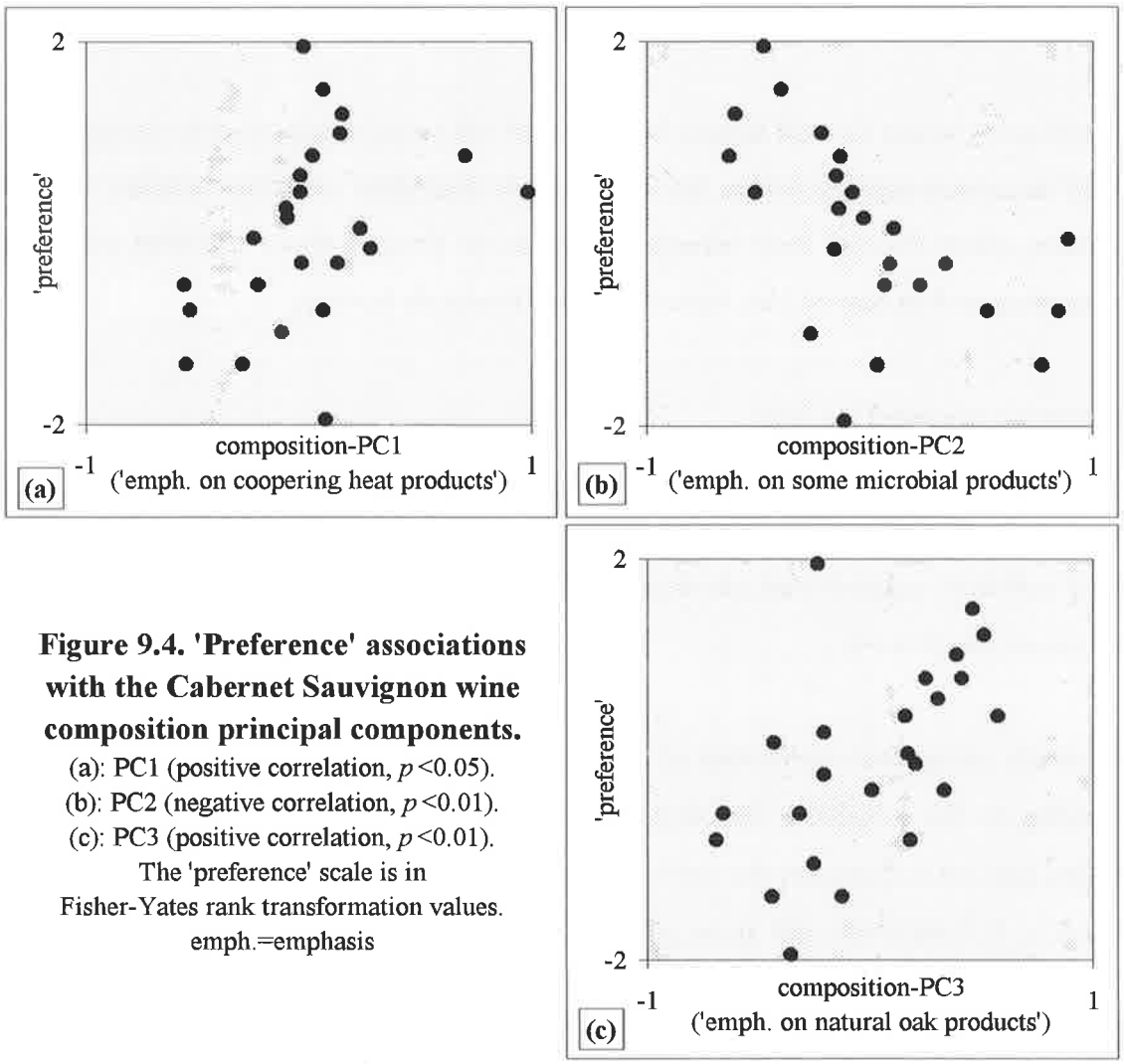


Figure 9.4. 'Preference' associations with the Cabernet Sauvignon wine composition principal components.
(a): PC1 (positive correlation, $p<0.05$).
(b): PC2 (negative correlation, $p<0.01$).
(c): PC3 (positive correlation, $p<0.01$).
The 'preference' scale is in Fisher-Yates rank transformation values.
emph.=emphasis

Based on association, the quantification of this compound in a batch of oak wood may indicate the aroma potential of the batch to positively influence 'coconut' in a white wine, and 'coconut,' 'vanilla,' 'berry,' 'dark chocolate' and 'coffee' in a red wine (Chapter 5).

Coopering or other treatments may also be customised to suit various batches. According to various authors (Marsal and Sarre 1987, Chatonnet *et al.* 1989 & 1991, Maga 1989a), heating variation might modify the *cis*–oak lactone concentration (although no such modification was observed in this study). Heating variation could also substantially augment any aroma provided by this compound, or it could partially compensate for a lack of any such aroma in the case of a batch of low concentration, by the addition of other important aroma compounds. Alternatively, there may be other techniques available to modify oak wood potential, relative to *cis*–oak lactone richness. Towey and Waterhouse (1996), for example, have suggested that SO_2 gas can increase the yield of oak lactones from oak wood. If this is true, poor batches may be subjected to remedial action prior to deployment in winemaking, unless the pool of precursors is also poor in these oak woods.

Quantification of the *cis*–oak lactone in batches of oak wood using gas chromatography is a quality assurance activity within the reach of all Australian wineries, coopers and other suppliers, either through their internal resources or through those provided by external laboratories such as that of The Australian Wine Research Institute.

Consistency of coopering heat

The identification of heating variation during coopering as a substantial contributor to oak aroma variation, suggests that advances in quality assurance can be found by tightening the controls on this process.

The clearly antagonistic behaviour of 'smoky' and 'green apple' in the Chardonnay wine, according to the 'emphasis on coopering heat products'–PC (Fig. 6.6) and the strong positive association between the corresponding PC and 'coffee' in the Cabernet Sauvignon wine (Fig. 6.7) represent just some of the aroma variability that can arise from unintended variation around 'medium toast' coopering. Improvements in the thermal control of this process are, therefore, likely to be useful.

Control of microbial activity during and after the contact period

Chapter 7 illustrated how some oak wood–derived compounds could be readily transformed during alcoholic– and malolactic–fermentations or simply during the microbial activity that can occur in a sugar– and malate–depleted and sanitised wine. While the aroma consequences of these transformations have not been confirmed, it may be that the use of sterilisation in combination with the timing of the oak wood contact period could provide opportunities for creating different aroma effects in wine, perhaps similar to those found in brandy, a sterile beverage stored in oak wood barrels.

If one were to make use of oak wood that contained low quantities of *cis*–oak lactone, and then compensate by ensuring that it were subject to relatively high 'medium toast' levels, special attention should be paid to any potential for microbial activity during wine storage. This is important because some of the 'coopering heat products' (furfural, 5–methylfurfural and vanillin) are susceptible to biochemical reduction, while the 'natural oak products,' *cis*– and *trans*–oak lactone and eugenol, appear to be stable.

Microbial affects on oak wood–derived aromas in the Cabernet Sauvignon wine, considered to be deleterious by the 'preference' panel in this study (Fig. 9.4b), were identified and are of practical importance. With increases in 4–ethylphenol, a product of microbial activity (particularly from *Brettanomyces/Dekkera* species), 'allspice' and 'coffee' decreased (Appx. Tab. H.2). Thus, sanitation processes may be important to the maintenance of oak wood–derived aromas in red wine.

The yeast species implicated in 4–ethylphenol production are resistant to sulfite (Romano and Suzzi 1993), and the maintenance of total SO_2 concentrations around 100 mg/L (Sponholz 1993) or free SO_2 concentrations of at least 30 mg/L (Chatonnet *et al.* 1992a) have been recommended for red wines exposed to these microorganisms. Current Australian industry practice of red wine maturation at concentrations below this level (Anon. 1995) and its perceived benefit should be viewed in relation to any possible deleterious effect on oak wood–derived aroma. Alternatively, other sanitation practices may be employed to avoid the possibly deleterious consequences of this microbial activity.

9.6 Summary and conclusion

The Vosges and Tronçais oak–stored wines were preferred by the experienced panel over those stored in the Limousin and American oak. This trend was consistent with the positive association between *cis*–oak lactone concentrations and the 'preference' indications for both the Chardonnay and the Cabernet Sauvignon wines. The Cabernet Sauvignon wine panel in this study also showed 'preference' for wines from barrels which had been subjected to relatively high 'medium toast' levels and from barrels in which relatively low levels of microbial activity had occurred during barrel maturation.

The results of this study have shown that barrel–effects on wine aroma can be unpredictable. This is reflected in the practices of some Australian winemakers who, despite attempting to pair appropriate wines with appropriate barrels, wait until the end of the barrel maturation period before finally deciding which particular barrel wines are to be used for each of the commercial wines to be produced.

It should be noted that only two wines, both from Coonawarra (South Australia), were involved in the study, and that the applicability of the conclusions to other wines is unknown. Nevertheless, the illustration of patterns among the aromas and volatile compounds in this Chardonnay and this Cabernet Sauvignon wine has suggested the possible nature of some of the aroma variations. These illustrations may, therefore, allow the development of processes designed to improve the predictability of the aroma–outcomes following oak wood selection, processing and deployment choices, as suggested.

Once winemakers are able to select and deploy oak wood, confidently aware of the aroma consequences of their choices, the blend of aroma–effects may be more substantially created at the beginning of the barrel maturation process. This may be preferable to the practice of responding to the sometimes unexpected aroma–effects, by re–assigning individual barrel wines to different products, at the end of the process.

Appendix A

Treatment details

Appendix outline

An overview of the experimental design is given in Chapter 1; further details are given, below.

A.1 Oak origin (from Sefton *et al.* 1993a)

The American wood was harvested from a 20 hectare mixed species stand (species were not determined), halfway between Columbus and Cincinnati, Ohio. Approximately two hundred 100 – 120 year old trees, grown on flat to rolling terrain, were logged for the sample. The wood from the Vosges region was sourced from the Darney forest, approximately 40 km south east of Epinal in the Department of Marne. These trees were approximately 180 – 220 years old. The wood from the Limousin region was taken from an area approximately 10 km south of Guéret, in the department of Haute–Vienne. The trees were approximately 100 – 150 years old. The fourth lot of wood was from the Tronçais forest of central France.

A.2 Seasoning (from Sefton *et al.* 1993a)

Wood was stacked to allow free circulation of air. The American wood seasoned in its country of origin was kept at Waverly, Ohio. The climate at this location is temperate, with a mean July (summer) temperature of 21 °C and a mean January (winter) temperature between 0 and 5 °C. The annual precipitation mean is close to 1000 mm, and is evenly spread throughout the year (Weil, personal communication). The wood seasoned in Australia was kept on the premises of C.A. Schahinger Pty. Ltd., Adelaide, where the mean January (summer) temperature is 22.6 °C and the mean July (winter) temperature, 11.1 °C. The average annual rainfall at this location is 530 mm, with an average of 67 mm in July and 20 mm in January (Adelaide Bureau of Meteorology). The French oak seasoned in France was kept on the premises of Tonnellerie Ludonnaise, Ludon, Médoc. The mean July (summer) temperature at this location is 20.5 °C, while the mean winter temperature (November to March) is 7.4 °C. The mean annual rainfall is 833 mm and is evenly distributed throughout the year, with a mean July rainfall of 52 mm (Gladstones 1992 p. 200).

Appendix Table A.1. Some conventional wine analysis results for the 1991 Chardonnay vinified at Rouge Homme winery, Coonawarra, South Australia.

	Malic acid (g/L)*		Final sampling (55 weeks)					After bottling**		
Barrel code	11 wks	55 wks	alcohol (% v/v)	total SO_2 (mg/L)	free SO_2 (mg/L)	pH	TA (g/L)#	free SO_2 (mg/L)	pH	TA (g/L)#
control		1.95	12.9	80	2	3.29	5.8	27	as before	as before
AU4	1.95	0.25	13.3	98	6	3.43	5.2	20	3.26	6.1
AU6	2.02	0.12	13.3	99	2	3.48	4.9	26	3.34	6.1
AU70	1.95	0.09	13.4	101	4	3.44	5.1	22	3.36	6.5
AA10	1.99	1.38	13.4	100	7	3.31	6.0	24	3.24	6.1
AA11	1.98	1.78	13.4	93	6	3.31	6.2	23	as before	as before
AA22	1.98	0.40	13.4	88	4	3.38	5.4	24	3.28	6.1
FL3	1.96	1.81	13.4	98	7	3.34	6.0	23	3.30	6.1
FL4	1.98	1.80	13.5	96	7	3.32	5.9	24	3.24	6.2
FL5		1.98	13.4	101	11	3.28	6.4	21	as before	as before
LA27		1.94	13.3	85	6	3.27	6.3	24	as before	as before
LA34		1.97	13.3	100	13	3.28	6.3	29	as before	as before
LA41	1.98	1.39	13.4	84	5	3.33	5.9	22	3.28	6.1
FT3		1.93	13.3	99	15	3.29	6.2	30	as before	as before
FT4		1.95	13.5	99	18	3.28	6.2	20	as before	as before
FT5	1.95	0.64	13.4	82	8	3.37	5.4	25	3.27	6.1
TA23		1.95	13.3	92	8	3.26	6.3	28	as before	as before
TA31	1.98	1.88	13.4	91	11	3.29	6.3	26	as before	as before
TA46		1.98	13.1	94	7	3.28	6.3	28	as before	as before
FV3	1.94	1.63	13.5	99	7	3.31	6.0	24	3.26	6.1
FV4		1.97	13.4	106	20	3.28	6.3	22	as before	as before
FV5		1.95	13.5	101	13	3.28	6.2	34	as before	as before
VA32	1.96	1.85	13.4	94	8	3.29	6.2	31	as before	as before
VA38		1.95	13.5	98	9	3.28	6.3	32	as before	as before
VA39		1.99	13.3	94	4	3.28	6.2	23	as before	as before
barrel mean			13.4	96	9	3.32	6.0	25	3.28	6.2

For meaning of barrel code, see Appendix Table A.2.

Blank cells indicate missing values.

* It is reasonable to conclude, using these malic acid values, that no Chardonnay wine had undergone appreciable MLF at the time of racking, *i.e.* all MLFs occurred between racking and the final sampling (between 11 & 55 weeks).

** Added at bottling: SO_2 (to estimated 25 mg/L free) & dimethyldicarbonate (DMDC) (0.15 mL/L). For 10 L of wine, 1.5 mL DMDC was mixed in 10 mL of ethanol, then stirred into wine, and bottled and sealed within 15 minutes. Also, additions of malic acid were made to counter the deacidifying effect of MLF. A 400 g/L aqueous solution of DL-malic acid (Unilab 2361, 99% pure) was used.

#: Titratable acidity in g/L, as tartaric acid equivalents.

A.3 Chardonnay vinification, conventional wine measurements and sampling

A 1991 Coonawarra (South Australia) Chardonnay was fermented to 6 °Baumé in stainless steel tank and then transferred, at approximately 8 °C, to three of each of the eight treatment barrels (24 barrels) and a 200 L stainless steel drum ('control'). 1.0 g/L of bentonite was added during transfer. The wines reached fermentation 'dryness' five weeks after being transferred to barrel. After 11 weeks, each of the barrels and the control was separately racked to remove bentonite and yeast lees, rinsed, and refilled with the same wine. The wines were stored in a temperature–controlled room at approximately 15 °C. Malolactic fermentation was discouraged — no inoculation was performed and free SO_2 was maintained at around 30 mg/L — but it occurred to varying degrees in some of the barrels during the following 44 weeks maturation. After 55 weeks in barrel or drum, the wines were sampled prior to commercial blending and bottling.

Alcohol concentration, malic acid concentration, pH, titratable acidity, and free and total SO_2 were determined for all of the barrel wines and the stainless steel drum–stored control wine at the end of the maturation period, as shown in Appendix Table A.1. Malic acid determinations were made on some of the 11 week samples to identify the period in which malolactic fermentation (MLF) proceeded. Apart from the malic acid determinations, no indicators of post–fermentation microbial activity were recorded.

Samples for volatile compound analysis were taken at 11 and 55 weeks. All eight treatments were sampled at 55 weeks but only three of them — the Australia seasoned and coopered American oak, and the France seasoned and coopered Limousin and Tronçais oaks — were taken at 11 weeks. The control wine was also sampled at 11 and 55 weeks. These samples were stored under a CO_2 atmosphere in crown–sealed 750 mL bottles at -10 °C until analysis. Appendix Table A.2 indicates the barrel codes applicable to the treatments, and the pattern of sampling for the Chardonnay wine.

Appendix Table A.2. Oak source, seasoning and coopering locations, barrel codes and sampling times for the Chardonnay wines.

4 defined lots of oak wood	Location of seasoning[1]	Location of coopering[2]	Barrel code	Sampling times 11 weeks	Sampling times 55 weeks
			control[3]	✓	✓
American	USA	Australia	*AU4, AU6, AU70*		✓✓✓
	Australia	Australia	*AA10, AA11, AA22*	✓✓✓	✓✓✓
Limousin	France	France	*FL3, FL4, FL5*	✓✓✓	✓✓✓
	Australia	Australia	*LA27, LA34, LA41*		✓✓✓
Tronçais	France	France	*FT3, FT4, FT5*	✓✓✓	✓✓✓
	Australia	Australia	*TA23, TA31, TA46*		✓✓✓
Vosges	France	France	*FV3, FV4, FV5*		✓✓✓
	Australia	Australia	*VA32, VA38, VA39*		✓✓✓

[1] Open–air seasoning for three years.
[2] Coopering: Three 300 L barrels, fired to 'medium toast,' for each of the eight treatments.
[3] Control wine stored in 200 L stainless steel drum.

Separate samples were taken for sensory analysis. Ten litres were taken from each barrel, and 60 L were taken from the control. All were stored in five litre glass 'Winchester' bottles with screw–caps, under a CO_2 atmosphere at approx. 2 °C for 30 weeks, before sterilisation and bottling to crown–sealed 375 mL glass bottles.

Since some of the barrel–wines had undergone MLF, the pH and titratable acidity (TA) values varied between 3.26 and 3.48, and between 4.9 and 6.4 g/L, respectively (Appx. Tab. A.1). Consequently, additions of DL–malic acid (Unilab 2361, 99.0% pure, as a 400 g/L aqueous solution) were made in varying amounts to bring these values to between 3.24 and 3.36, and between 5.8 and 6.5 g/L. Those samples in Appendix Table A.1 with different pH and TA values after bottling, were subject to these acid additions (0.2 to 1.2 g/L). Dimethyldicarbonate (DMDC), at 0.15 mL in 1 mL of ethanol / L of wine, was added, along with varying amounts of a 15 % aqueous SO_2 solution (as sodium metabisulfite) bringing the free SO_2 concentration to between 20 and 34 mg/L (Appx. Tab. A.1), to sterilise each of the wines. The bottling of each 10 L batch, under an atmosphere of CO_2, was complete within 15 minutes of the DMDC addition. The wines were then stored for 110 weeks at approximately 2 °C followed by 12 weeks at approximately 20 °C before sensory analysis.

A.4 Cabernet Sauvignon vinification, conventional wine measurements and sampling

A 1991 Coonawarra (South Australia) Cabernet Sauvignon underwent alcoholic and malolactic fermentation in a stainless steel tank before being transferred to three of each of the eight treatment barrels (24 barrels) and a 200 L stainless steel drum ('control'). The wines were stored in a temperature–controlled room at approximately 15 °C. After 93 weeks in barrel or drum, the wines were sampled prior to commercial blending and bottling.

Alcohol concentration, pH, titratable acidity, and free and total SO_2 were determined for all of the barrel wines and the stainless steel drum–stored control wine at 93 weeks, as shown in Appendix Table A.3. No indicators of microbial activity during barrel storage were recorded.

All eight treatments and the control wine were sampled for volatile compound analysis at 93 weeks. These samples were stored under a CO_2 atmosphere in crown–sealed 750 mL bottles at -10 °C until analysis. Appendix Table A.4 indicates the barrel codes applicable to the treatments, and the pattern of sampling for the Cabernet Sauvignon wine.

Separate samples were taken for sensory analysis. Ten litres were taken from each barrel, and 30 L were taken from the control. All were stored in five litre glass 'Winchester' bottles with screw–caps, under a CO_2 atmosphere at approx. 20 °C for 41 weeks. The wines were then sterilised with DMDC, they received SO_2 additions and were bottled in the same manner as the Chardonnay wines, except that no CO_2 cover was used. The resultant total SO_2 concentrations ranged from 63 to 84 mg/L (Appx. Tab. A.3). The wines were then stored for 59 weeks at approximately 20 °C before sensory analysis.

Appendix Table A.3. Some conventional wine analysis results for the 1991 Cabernet Sauvignon vinified at Rouge Homme winery, Coonawarra, South Australia.

	Final sampling (93 weeks)					Before bottling*		After bottling**	
Barrel code	alcohol (% v/v)	pH	TA (g/L)#	total SO_2 (mg/L)	free SO_2 (mg/L)	total SO_2 (mg/L)	free SO_2 (mg/L)	total SO_2 (mg/L)	free SO_2 (mg/L)
control	13.7	3.53	5.8	49	28	14	5	76	56
AU7	13.5	3.52	6.2	27	4	14	1	75	49
AU8	13.5	3.53	6.2	18	3	6	0	73	52
AU9	13.4	3.52	6.2	20	5	21	4	79	50
AA36	13.4	3.48	6.2	13	3	20	2	78	50
AA40	13.5	3.52	6.2	12	3	9	1	74	51
AA48	13.6	3.51	6.3	17	3	20	4	75	48
NL6	13.8	3.48	6.5	19	2	27	4	80	47
NL7	13.7	3.51	6.2	21	3	31	6	84	49
NL8	13.6	3.52	6.2	17	2	42	10	82	43
LA23	13.6	3.51	6.2	17	2	13	2	71	49
LA30	13.2	3.51	6.1	19	4	14	2	73	48
LA38	13.6	3.52	6.2	15	2	23	4	78	44
NT6	13.7	3.52	6.1	20	4	32	8	76	43
NT7	13.6	3.52	6.1	17	3	11	1	74	44
NT8	13.7	3.53	6.1	10	2	10	1	74	52
TA8	13.6	3.51	6.2	27	6	14	3	77	51
TA25	13.6	3.51	6.2	20	4	4	1	77	56
TA39	13.6	3.51	6.2	28	7	16	2	79	50
NV6	13.7	3.51	6.2	23	5	37	7	79	39
NV7	13.4	3.52	6.1	15	3	10	0	63	44
NV8	13.7	3.52	6.3	18	3	3	0	66	39
VA12	13.6	3.52	6.2	19	3	24	5	78	48
VA21	13.6	3.52	6.2	18	3	4	0	67	48
VA27	13.6	3.55	6.1	20	5	34	8	78	42
barrel mean	13.6	3.52	6.2	19	4	18	3	75	47

For meaning of barrel code, see Appendix Table A.4.

* SO_2 determined, after 41 weeks storage in 5 L glass bottles, prior to final adjustment preceding bottling. 50 mg/L SO_2 was added 41 weeks earlier, at barrel sampling.

** Approximately 40 mg/L SO_2 was added at bottling (to reach around 75 mg/L total SO_2 & probably around 20 mg/L free SO_2, after a few days of equilibration). Dimethyldicarbonate (DMDC) (0.15 mL/L) was also added. For 10 L of wine, 1.5 mL DMDC was mixed in 10 mL of ethanol, then stirred into the wine, and bottled and sealed within 15 minutes.

#: Titratable acidity in g/L, as tartaric acid equivalents.

Appendix Table A.4. Oak source, seasoning and coopering locations, barrel codes and sampling times for the Cabernet Sauvignon wines.

4 defined lots of oak wood	Location of seasoning[1]	Location of coopering[2]	Barrel code	Sampling time: 93 weeks
			control[3]	✓
American	USA	Australia	*AU7, AU8, AU9*	✓✓✓
	Australia	Australia	*AA36, AA40, AA48*	✓✓✓
Limousin	France	France	*NL6, NL7, NL8*	✓✓✓
	Australia	Australia	*LA23, LA30, LA38*	✓✓✓
Tronçais	France	France	*NT6, NT7, NT8*	✓✓✓
	Australia	Australia	*TA8, TA25, TA39*	✓✓✓
Vosges	France	France	*NV6, NV7, NV8*	✓✓✓
	Australia	Australia	*VA12, VA21, VA27*	✓✓✓

[1] Open–air seasoning for three years.
[2] Coopering: Three 300 L barrels, fired to 'medium toast,' for each of the eight treatments.
[3] Control wine stored in 200 L stainless steel drum.

A.5 Model wine concoction, conventional wine measurements and sampling

A 12 % aqueous ethanol solution, saturated with potassium hydrogen tartrate, pH adjusted to 3.45 by addition of tartaric acid and containing 28 mg/L SO_2 was concocted as a model of a real wine, and stored in two of each of the eight treatment barrels (16 barrels) and a 200 L stainless steel drum ('control') for 93 weeks. The model wines were stored in the same temperature–controlled room as the Chardonnay and the Cabernet Sauvignon wines.

Alcohol concentration, pH, titratable acidity, and free and total SO_2 were determined for some or all of the barrel wines at various times throughout the maturation period, as shown in Appendix Tables A.5 to A.8. Additionally, the model wines were screened on seven occasions during the 93 week storage period for the presence of yeast and bacteria. Fifty mL samples were filtered through sterile 0.45 micron membranes (Gelman Sciences Inc. 47 mm GN–6 Grid S–Pack) which were then plated on WL Nutrient media ('Oxoid') for yeast, and MRS Broth ('Oxoid'), supplemented with 20 % clarified apple juice and 10 mg/L cycloheximide, for bacteria. The plates were incubated at 25 °C for two weeks. When 'significant' numbers of yeast or bacteria colony forming units (cfu) were detected, the contents of the barrel were sterilised by the addition of 0.15 mL DMDC in 1 mL of ethanol / L of model wine, together with maintenance of free SO_2 concentrations around 30 mg/L. One barrel at one sampling showed 36 yeast cfu/mL but all others showed less than 7 (usually zero) yeast or bacteria cfu/mL. These results are shown in Appendix Table A.9.

Appendix Table A.5. Total SO_2 (mg/L) of the model wines barrel–aged, from 1991, at Rouge Homme winery, Coonawarra, South Australia.

Barrel	0 wks	3 wks	11 wks	16 wks	22 wks	32 wks	48 wks	55 wks	93 wks	Mean
control									15	
AU2	28	24	12	57	68	56	40	54	27	41
AU3	28	23	12	52	62	45	36	49	27	37
AA34	28	23	12	49	60	45	29	38	21	34
AA47	28	23	10	51	64	32	55	68	43	42
NL1	28	20	9	60	99	84	70	78	47	55
NL2	28	22	11	51	65	58	84	93	61	53
LA33	28	20	8	47	56	46	33	45	39	36
LA42	28	21	6	41	49	45	54	66	27	37
NT1	28	22	8	70	76	62	48	55	30	44
NT2	28	22	7	52	60	44	48	58	25	38
TA9	28	19	7	49	49	41	39	52	27	35
TA10	28	18	8	39	52	38	52	61	32	36
NV1	28	25	11	55	63	47	37	47	22	37
NV2	28	23	10	54	65	48	51	57	25	40
VA2	28	21	9	46	58	42	42	52	26	36
VA28	28	20	8	53	63	49	50	61	34	41
Grand mean										40

For meaning of barrel code, see Appendix Table A.10.

Appendix Table A.6. Free SO_2 (mg/L) of model wines barrel–aged, from 1991, at Rouge Homme winery, Coonawarra, South Australia.

Barrel	0 wks	3 wks	11 wks	16 wks	22 wks	32 wks	48 wks	55 wks	93 wks	Mean
control									9	
AU2	28	18	6	42	52	41	24	30	9	28
AU3	28	19	6	37	50	34	20	28	8	26
AA34	28	19	6	41	51	37	20	28	1	26
AA47	28	19	4	42	52	13	38	36	20	28
NL1	28	18	3	21	64	46	28	28	1	26
NL2	28	17	2	14	24	15	32	25	1	18
LA33	28	15	3	22	31	18	6	8	1	15
LA42	28	16	2	22	29	33	29	36	1	22
NT1	28	19	2	53	60	43	29	27	1	29
NT2	28	18	2	37	44	26	30	37	5	25
TA9	28	15	0	36	32	24	15	24	2	20
TA10	28	13	0	21	33	18	25	34	4	20
NV1	28	20	5	41	47	33	22	27	3	25
NV2	28	19	3	41	53	31	28	35	2	27
VA2	28	17	3	28	37	22	19	20	2	20
VA28	28	14	2	35	43	27	23	22	1	22
Grand mean										23

For meaning of barrel code, see Appendix Table A.10.

Appendix Table A.7. Alcohol (% v/v) of the model wines barrel–aged, from 1991, at Rouge Homme winery, Coonawarra, South Australia.

Barrel	0 wks	3 wks	11 wks	16 wks	22 wks	32 wks	48 wks	55 wks	93 wks	Mean
control									12.2	
AU2	12.0	12.7	12.7	12.4	12.3	12.6	12.3	12.3	12.5	12.5
AU3	12.0	12.7	12.5	12.3	12.3	12.5	12.2	12.2	12.6	12.4
AA34	12.0	12.7	12.5	12.4	12.4	12.7	12.3	12.3	12.6	12.5
AA47	12.0	12.8	12.6	12.3	12.6	12.6	12.3	12.3	12.5	12.5
*NL1**	12.0	12.1	11.9	11.5	11.5	11.9	11.6	11.6	12.0	11.8
*NL2**	12.0	12.1	11.9	11.7	11.7	12.1	11.7	11.8	12.2	11.9
*LA33***	12.0	12.2	12.4	12.2	12.2	12.5	12.2	12.2	12.5	12.3
LA42	12.0	13.4	12.4	12.4	12.4	12.6	12.3	12.4	12.8	12.6
NT1	12.0	12.6	12.6	12.6	12.3	12.9	12.3	12.3	12.7	12.5
NT2	12.0	12.6	12.5	12.3	12.3	12.9	12.4	12.4	12.9	12.5
TA9	12.0	12.8	12.6	12.3	12.3	12.8	12.4	12.4	12.7	12.5
TA10	12.0	12.8	12.7	12.4	12.4	12.6	12.4	12.4	12.6	12.5
NV1	12.0	12.8	12.5	12.4	12.4	12.9	12.4	12.4	12.7	12.6
NV2	12.0	12.6	12.4	12.5	12.3	13.5	12.4	12.4	12.7	12.6
VA2	12.0	12.7	12.5	12.4	12.4	13.2	12.3	12.4	12.6	12.6
VA28	12.0	12.7	12.6	12.4	12.3	12.7	12.4	12.4	12.8	12.5
Grand mean										12.4

For meaning of barrel code, see Appendix Table A.10.

* *NL1 & NL2* were not completely filled on the first day; they were 80 % filled then topped the following day with a different concoction (but the same recipe) of model wine. A slightly different alcohol concentration in the second batch may account for the low values.

** *LA33* was the first barrel filled. The low values may, therefore, be a result of dilution of the model wine by water in the lines, *etc.*

Appendix Table A.8. Dissolved oxygen and acidity of the model wines barrel–aged, from 1991, at Rouge Homme winery, Coonawarra, South Australia.

Barrel code	Oxygen (mg/L) 55 wks	pH 0 wks	pH 55 wks	pH 93 wks	Titratable acidity (g/L)# 55 wks	Titratable acidity (g/L)# 93 wks	Notes
control				3.40		1.8	For meaning of barrel code,
AU2	5.7	3.45	3.15	3.25	2.1	2.4	see Appendix Table A.10.
AU3	2.5	3.45	3.14	3.27	1.9	2.3	
AA34	3.4	3.45		3.26		2.3	Blank cells indicate missing values.
AA47	1.8	3.45	3.13	3.24	2.1	2.5	
*NL1**	1.6		3.13	3.20	1.9	2.6	* *NL1 & NL2* were not completely
*NL2**	1.2		3.09	3.17	2.2	2.7	filled on the first day; they were
LA33	0.8	3.45	3.14	3.25	2.0	2.4	80 % filled then topped the following
LA42	0.8	3.45	3.09	3.18	2.1	2.5	day with a different concoction
NT1	1.5	3.45		3.20		2.4	(but the same recipe) of model wine.
NT2	0.8	3.45	3.11	3.20	1.9	2.3	A slightly different tartaric acid
TA9	0.8	3.45	3.12	3.23	2.0	2.4	concentration in the second batch
TA10	0.8	3.45		3.21		2.4	may account for the slightly low
NV1	2.7	3.45	3.11	3.23	1.9	2.3	final pH values and the slightly
NV2	1.5	3.45		3.22		2.4	high final titratable acidity values.
VA2	1.4	3.45	3.14	3.23	2.0	2.4	
VA28	0.8	3.45	3.08	3.22	2.0	2.4	#: Titratable acidity in g/L,
mean	1.8	3.45	3.12	3.22	2.0	2.4	as tartaric acid equivalents.

Appendix Table A.9. Yeast and bacteria counts (cfu/mL) made of the model wines barrel–aged, from 1991, at Rouge Homme winery, Coonawarra, South Australia.

Barrel code	3 wks yeast or bacteria*	11 wks yeast	11 wks bacteria	22 wks yeast or bacteria*	32 wks yeast**	48 wks yeast**	55 wks yeast**	93 wks yeast	93 wks bacteria	Maximum cell count (cfu/mL)
AU2	0.02	0.01					0.02			0.02
AU3	0.15	0.01						0.10		0.15
AA34	0.01						0.02	36.00		36.00
AA47	0.02		0.01							0.02
NL1	0.03		5.00					4.00		5.00
NL2	0.01	0.01			6.00			1.40		6.00
LA33	0.17	0.01			0.04	0.08		0.50	0.10	0.50
LA42	0.06				4.00		0.02	0.40		4.00
NT1	0.01							1.50		1.50
NT2	0.02	0.01								0.02
TA9	0.03	0.02						1.30		1.30
TA10	0.02							0.90		0.90
NV1	0.01		0.01					0.40		0.40
NV2								3.00		3.00
VA2	0.01							0.10		0.10
VA28	0.03							3.20		3.20

For barrel code meaning, see Appendix Table A.10.
Blank cells indicate that no cfu were detected.
* Not specified.
** Plated for bacteria but bacteria cfu were not detected at weeks 32, 48 & 55.
: DMDC treatments: *NL1, NL2 & LA42* received 0.15 – 0.20 mL/L, 2 – 3 weeks following the 11 or 32 week sampling. Additionally, all barrels received 0.12 mL DMDC/L model wine approximately one week prior to the 22 week sampling. DMDC additions always made with addition of 30 – 50 mg/L SO_2.

Samples for volatile compound analysis were taken at 6, 11, 32, 55 and 93 weeks. All eight treatments were sampled at 55 and 93 weeks but only four of them — the two American oak and the two Limousin oak treatments (Fig. 1.2) — were taken at the earlier three samplings. The control wine was also sampled at week 11. These samples were stored under a CO_2 atmosphere in crown sealed 750 mL bottles at -10 °C until analysis. Appendix Table A.10 indicates the barrel codes applicable to the treatments, and the pattern of sampling for the model wine.

Appendix Table A.10. Oak source, seasoning and coopering locations, barrel codes and sampling times for the model wines.

4 defined lots of oak wood	Location of seasoning[1]	Location of coopering[2]	Barrel code	Sampling times 6 wks	11 wks	32 wks	55 wks	93 wks
			control[3]		✓			
American	USA	Australia	*AU2, AU3*	✓✓	✓✓	✓✓	✓✓	✓✓
	Australia	Australia	*AA34, AA47*	✓✓	✓✓	✓✓	✓✓	✓✓
Limousin	France	France	*NL1, NL2*	✓✓	✓✓	✓✓	✓✓	✓✓
	Australia	Australia	*LA33, LA42*	✓✓	✓✓	✓✓	✓✓	✓✓
Tronçais	France	France	*NT1, NT2*		✓✓		✓✓	✓✓
	Australia	Australia	*TA9, TA10*				✓✓	✓✓
Vosges	France	France	*NV1, NV2*				✓✓	✓✓
	Australia	Australia	*VA2, VA28*				✓✓	✓✓

[1] Open–air seasoning for three years.
[2] Coopering: Two 300 L barrels, fired to 'medium toast,' for each of the eight treatments.
[3] Control model wine stored in 200 L stainless steel drum.

Appendix B

Volatile compound quantification materials and methods

Appendix outline

B.1. Preparation of samples for chromatography

All solvents were analytical grade and distilled prior to use. Methylene chloride was used as the solvent for all standards subject to direct injection to the gas chromatograph (GC), and 96 % ethanol was used for all standards to be added to the wine samples prior to liquid/liquid extraction. Whenever water was used as a solvent, *e.g.* for the saturated aqueous solution of sodium bicarbonate, it was distilled and membrane (MilliQ) filtered.

Vanillin was extracted from the wines and quantified by stable isotope dilution analysis, as described in Spillman *et al.* (1997). The 19 other target–compounds were extracted and analysed in the following manner. An internal standard (2,6–di–*tert*–butyl–4–methylphenol, *i.e.* butylated hydroxytoluene, abbreviated to BHT) was added (0.30 mL of 200 mg/L 96 % ethanol solution) to each 200 mL aliquot of Chardonnay or model wine sample (yielding approx. 300 µg of internal standard per litre of sample) prior to continuous liquid/liquid extraction with Freon F11 for three days (waterbath at approx. 38 °C and condenser at approx. 2 °C) as described by Wilson *et al.* (1984).

The Cabernet Sauvignon wines were extracted in the same manner but a second internal standard (2,5–dimethylphenol, abbreviated to DMP), added just prior to GC injection (0.20 mL of 305 mg/L methylene chloride solution to ~ 1 mL sample, corresponding to an initial concentration of 305 µg/L in the 200 mL wine sample), was used because it allowed greater precision (determined from standard recovery experiments). This methodological improvement was made after the Chardonnay and model wine analyses had been completed. DMP is recommended for future research. No endogenous BHT or DMP was found in the wines.

The Freon extracts (approx. 400 mL) were evaporated, through a Vigreux column packed with Fenske's helices, over a water bath at approximately 38 °C. The solvent was replaced with methylene chloride (approx. 10 mL) and reduced to approximately 1 mL (waterbath at approx. 70 °C). Methylene chloride (approx. 50 mL) was again added, the sample dried over $MgSO_4$, and then further reduced to approximately 1 mL. The samples were stored at -20 °C prior to analysis by gas chromatography – mass spectrometry (GC–MS).

For extracts of the Chardonnay and the Cabernet Sauvignon wines, methylene chloride solutions (at approx. 90 mL) were subject to 'washing' with 2 x 10 mL of saturated aqueous bicarbonate solution to remove chromatography–interfering fatty acids, derived from primary fermentation. This was followed by a 'washing' with 1 x 10 mL of saturated aqueous sodium chloride to aid phase separation. The samples were then dried and concentrated, as described above.

B.2. Standards

On each day of analyses, a 'standards mix' (weighed quantities of 15 of the 20 target compounds, along with the internal standard, in methylene chloride) was subject to GC–MS analysis in the same manner as the experimental samples. The standards and the approximate concentrations used are listed at Appendix Table B.1. The standards and the standards mixes were stored in a freezer (-20 °C), and the standards mixes were renewed within one to two months in most cases. The stability of the compounds in these mixes was determined, occasionally, and found to be adequate over this time frame.

B.3. Gas chromatography – mass spectrometry conditions

Extracts were analysed with a Varian 3400 gas chromatograph coupled with a Finnigan MAT TSQ70 mass spectrometer. The gas chromatograph was fitted with a 30 m x 0.25 mm J&W fused silica capillary column DB–1701, 0.25 μm film thickness. This column had proved adequate for the separation of a variety of compounds in past research conducted in our laboratory. The oven temperature was started at 60 °C, held at this temperature for 1 minute then increased to 200 °C at 4 °C/min, then to 250 °C at 50 °C/min, and held at this temperature for 20 minutes. The injector was held at 220 °C, the transfer line at 250 °C and the detector at 200 °C. The sample volume injected was 3 μL. The splitter, at 1:12, was opened after 18 seconds. Positive ion electron impact spectra at 70 eV were recorded in the range *m/z* 35 – 350 for scan runs. Mass fragments were scanned every 0.5 seconds.

B.4. Compound identification (mass spectrometry and coinjection)

Sixteen of the 20 compound identifications were initially based on comparisons of GC retention time and mass spectra between purified compounds and barrel samples (Appx. Tab. B.2). Fifteen of these identifications were later confirmed by coinjection (4–ethylguaiacol was not confirmed in this manner due to depletion of the purified sample). Three of the compound identities were later confirmed by comparison to synthetic samples (the three ethyl ethers, work of Sefton, in Spillman *et al.* 1998). Two compound identities (5–methylfurfuryl alcohol and 4–vinylphenol) were based only on comparisons of the mass spectra with published spectra in the MS computer data base.

Appendix Table B.1. Purified compounds used as standards.

Name / synonym	Approximate concentration in standards mix (µg/L)†	Source / description
furfural / furaldehyde	1000	Fluka, Switzerland, 100 mL, > 99 % pure
furfuryl alcohol	500	Fluka, Switzerland, 100 mL
5–methylfurfural / 5–methyl–2–furaldehyde	100	Fluka, Switzerland, 25 mL, > 97 % pure
cyclotene	5	Aldrich Chem Co, Milwaukee, Wis. 53233 USA
guaiacol	10	Department of Chemistry, University of Adelaide
maltol / 3–hydroxy–2–methyl–4–pyrone	10	Aldrich Chem Co, Milwaukee, Wis. 53233 USA
4–methylguaiacol	5	Oxford Organic Chemicals Ltd, Brackley, Northamptonshire, UK
2,5–dimethylphenol	100	Department of Chemistry, University of Adelaide
4–ethylphenol	200	Fluka, Switzerland, 250 g, > 97 % pure
4–ethylguaiacol	5	Oxford Organic Chemicals Ltd
4–vinylguaiacol	50	Oxford Organic Chemicals Ltd, Brackley, Northamptonshire, UK, 10 g
cis– & *trans*–oak lactone	100	Allied Flavours, 100 g (whiskey lactone) (isomeric mix of β–methyl–γ–octalactone)
Eugenol BPC Clove Oil Trpnless	10	Bush Boake Allen Aust Ltd, 60–8969, 2/84 sample
2,6–di–*tert*–butyl–4–methylphenol (butylated hydroxy toluene)	100	Department of Chemistry, University of Adelaide
vanillyl ethyl ether	100	synthesised in our laboratory
vanillin	200	Oxford Organic Chemicals Ltd, Brackley, Northamptonshire, UK
vanillyl alcohol	100	Department of Chemistry, University of Adelaide

†: 'Standards mixes' for direct injection to GC were made–up in methylene chloride; 'standards mixes' for standard recovery experiments were made–up in 96 % ethanol; each in an A grade 250 mL volumetric flask. Masses less than 5 mg were usually made–up, in a separate volumetric flask, to ten times the required quantity, then one tenth was volumetrically transferred.

B.5. Effects of sulfite variation on standard recovery

Since sulfite can bind easily with aldehyde functional groups (Peynaud 1981 pp. 269–270), some of which are present in the target–compounds, the small sulfite concentration variability among the samples (Appx. Tabs. A.1, A.3, A.5 & A.6) was identified as a potential interference to measurement accuracy and precision.

800 mL model wine, 0.8 mL BHT (200 mg/L 96 % ethanol solution), and 4.0 mL of a 'standards mix' (96 % ethanol solution) were mixed thoroughly and split into two portions. The sulfur dioxide in one of the portions was adjusted to 50 mg/L using an aqueous solution of sodium metabisulfite. Each of the two portions was split into two, then extracted, and the compounds quantified as normal. Approximately 1 hour lapsed between adding the sodium metabisulfite solution and initiating the extraction which proceeded for 3 days. Any effect for a reaction which had not proceeded within this time frame would not have been detected.

Sulfite effect on furfuryl ethyl ether, 5–methylfurfuryl ethyl ether, 5–methylfurfuryl alcohol, 4–vinylphenol and vanillyl alcohol were not determined. Within the kinetic limitations of the experiment, the 15 other compounds varied within +/- 10 % with addition of sulfite, and were within the 95 % confidence interval deviations from the no SO_2 treatment.

This experiment indicates that, unless a reaction was too slow to have been detected, none of the 15 added compounds, including all of those with aldehyde functional groups, were significantly affected by a variation in SO_2 concentration of 50 mg/L.

B.6. Data analysis

The concentration data were analysed by various parametric data analysis methods. They were also ranked and used in Spearman's rank correlation analysis with the ranked sensory data.

Treatment differences were investigated by analysis of variance (ANOVA). Single factor ANOVAs (Microsoft Excel V5.0) were performed occasionally but most of the analyses were factorial, using fixed factors. When more than one of the wines was included, the analysis was one of three factors (oak origin x seasoning location x wine). Otherwise they were of two factors, and factorial ANOVAs, with interaction, were performed using Excel or SYSTAT V5.0 statistical software. When missing cells were encountered, due to the omission of an outlier or due to the analysis of the model wines (replicated twice) with the Chardonnay and/or the Cabernet Sauvignon wines (replicated three times), an unweighted means model (Kirby 1993 pp. 318–323) was used.

The repeated–measures aspect of some of the analyses, *e.g.* the compound accumulation analyses discussed in Chapter 8, was accommodated by two factor, repeated–measures, ANOVAs, without replication (Microsoft Excel V5.0).

When a multiple comparison was required, following any of the ANOVA designs, a two–tailed Fisher's least significant difference (LSD) ($p<0.05$) was performed to separate the means.

Appendix Table B.2. Compound identification data.

Compound in order eluting from GC column	Retent. time relat. to I.S.	Molec. ion	Major mass ions from mass spectrum (base peak, secondary peak & its proportion relative to base peak)	Did the purified compound and the barrel wine sample share the same mass spec.?	Was compound identity confirmed by coinjection?
furfural	0.337	96	96, 95 (95%)	yes	yes
furfuryl ethyl ether	0.341	126	81, 126 (25%)	yes	yes
furfuryl alcohol	0.407	98	98, 81 (50%)	yes	yes
5–methylfurf.e.e.*	0.491	140	95, 140 (25%)	yes	yes
5–methylfurfural	0.547	110	110, 109 (95%)	yes	yes
5–methylfurf.alc.*	0.669	112	95, 112 (40%)	‡	
cyclotene	0.678	112	112	yes	yes
guaiacol	0.767	124	124, 109 (90%)	yes	yes
maltol	0.874	126	126	yes	yes
4–methylguaiacol	0.967	138	138, 123 (80%)	yes	yes
2,5–dimethylphenol[#]	1.000	123	122, 107 (70%)	not applicable	not applicable
4–ethylphenol	1.084	122	107, 122 (35%)	yes	yes
4–ethylguaiacol	1.131	152	137, 152 (40%)	yes	
4–vinylguaiacol	1.234	150	150, 135 (70%)	yes	yes
4–vinylphenol	1.252	120	120, 91 (60%)		
trans–oak lactone[†]	1.266	99	99	yes	yes
eugenol	1.276	164	164, 149 (45%)	yes	yes
cis–oak lactone[†]	1.332	99	99	yes	yes
BHT[##]	1.440	220	205, 220 (30%)	not applicable	not applicable
vanillyl ethyl ether	1.496	182	137, 182 (45%)	yes	yes
vanillin	1.505	152	151, 152 (85%)	yes	yes
vanillyl alcohol	1.626	154	154, 137 (50%)	yes	yes

* 5–methylfurf.e.e. = 5–methylfurfuryl ethyl ether; and 5–methylfurf.alc. = 5–methylfurfuryl alcohol.
[#] Internal standard (I.S.) used for the Cabernet Sauvignon wines.
[†] Oak lactones = *cis*– and *trans*–β–methyl–γ–octalactone.
[##] Internal standard (butylated hydroxytoluene, *i.e.* 2,6–di–*tert*–butyl–4–methylphenol) used for the model and Chardonnay wines.
‡ A space indicates that the purified compound was not available for mass spectrum comparison or coinjection.

Appendix C

Composition principal components analysis results

Appendix outline

PC analysis was performed using SYSTAT, V5.0 (SYSTAT, Inc.) statistical software. The analysis was based on a Pearson's product-moment correlation matrix, three PCs were retained, and a varimax rotation was performed.

C.1 Chardonnay wine

Raw data were the Chardonnay wine composition data listed in Table 2.2.

Appendix Table C.1. Pearson's product-moment correlation coefficient matrix.

	cis	*trans*	*eug*	*guaiac*	*4mg*	*van*	*malt*	*furf*	*eef*	*5mf*	*falc*	*5mfalc*	*fee*	*5mfee*	*vee*	*4vg*	*4eg*	*4vp*
cis	1																	
trans	0.581	1																
eug	0.684	0.540	1															
guaiac	-0.214	-0.210	-0.137	1														
4mg	-0.153	0.078	0.220	0.722	1													
van	-0.292	-0.316	-0.224	0.354	0.395	1												
malt	-0.472	-0.671	-0.448	0.575	0.176	0.547	1											
furf	-0.053	-0.019	-0.081	0.841	0.735	0.538	0.470	1										
eef	-0.422	-0.430	-0.377	0.801	0.573	0.622	0.709	0.778	1									
5mf	-0.072	-0.015	-0.020	0.653	0.737	0.597	0.376	0.910	0.701	1								
falc	-0.591	-0.654	-0.489	0.128	-0.096	0.245	0.485	-0.125	0.525	-0.124	1							
5mfalc	0.310	0.303	0.065	-0.171	-0.120	0.157	-0.102	0.025	-0.105	-0.033	-0.213	1						
fee	-0.166	-0.018	-0.270	0.029	0.087	0.463	0.207	0.184	0.320	0.195	0.249	0.652	1					
5mfee	-0.459	-0.402	-0.389	-0.107	-0.269	0.253	0.322	-0.278	0.219	-0.265	0.719	0.029	0.142	1				
vee	0.328	0.324	0.267	-0.289	0.093	0.286	-0.288	0.097	-0.131	0.212	-0.350	0.700	0.525	-0.217	1			
4vg	0.531	0.460	0.484	-0.406	-0.010	-0.218	-0.554	-0.116	-0.386	0.019	-0.460	0.508	0.213	-0.352	0.799	1		
4eg	-0.163	0.011	0.126	0.850	0.807	0.163	0.282	0.675	0.571	0.556	-0.011	-0.282	-0.111	-0.148	-0.296	-0.231	1	
4vp	0.455	0.398	0.525	-0.323	0.109	-0.165	-0.482	-0.061	-0.291	0.087	-0.383	0.405	0.198	-0.290	0.730	0.967	-0.089	1

: significant correlation, p <0.05 or stronger.

Critical values for 2-tailed test of correlation, n = 24, *d.f.* = n - 2 = 24 - 2 = 22.

If r is greater than or equal to 0.404, significant correlation, p <0.05.*

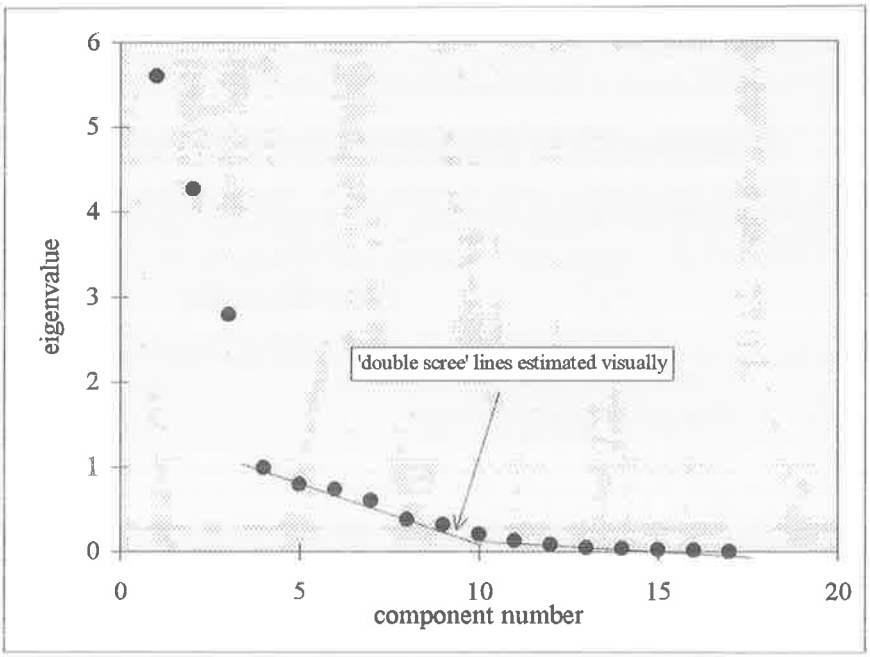
If r is greater than or equal to 0.515, significant correlation, p <0.01.*

If r is greater than or equal to 0.6524, significant correlation, p <0.001.**

*: from Snedecor and Cochran (1967), 22 *d.f.* **: from O'Mahony (1986), 20 d.f.

Compound abbreviations: cis=*cis* -oak lactone, trans=*trans* -oak lactone, eug=eugenol, guaiac=guaiacol, 4mg=4-methylguaiacol, van=vanillin, malt=maltol, furf=furfural, eef='estimated extracted furfural' (furfural + furfuryl alcohol), 5mf=5-methylfurfural, falc=furfuryl alcohol, 5mfalc=5-methylfurfuryl alcohol, fee=furfuryl ethyl ether, 5mfee=5-methylfurfuryl ethyl ether, vee=vanillyl ethyl ether, 4vg=4-vinylguaiacol, 4eg=4-ethylguaiacol, 4vp=4-vinylphenol.

Scree plot	
number	eigenvalue
1	5.598
2	4.261
3	2.792
4	0.986
5	0.792
6	0.734
7	0.604
8	0.375
9	0.316
10	0.201
11	0.126
12	0.083
13	0.048
14	0.040
15	0.026
16	0.016
17	0.003



Appendix Figure C.1. Scree plot of Chardonnay composition-PCA.

Conclusion: The scree test suggests retaining the first three PCs.

Appendix Table C.2. PC characteristics.

PCs description:	**PC1:**	'emphasis on natural oak products and oak origin associations with some microbial products'		
	PC2:	'emphasis on coopering heat products'		
	PC3:	'emphasis on some microbial products'		
Proportion of variance explained by each PC		**PC1**	**PC2**	**PC3**
variance explained:		28.34%	26.91%	19.17%
cumulative variance explained:		28.34%	55.25%	74.41%

Appendix Table C.3. Rotated component loadings.

compounds included	PC load 1	PC load 2	PC load 3
furfuryl alcohol	-0.820	-0.093	-0.043
eugenol	0.779	0.036	-0.008
trans -oak lactone	0.769	-0.040	0.128
cis -oak lactone	0.765	-0.125	0.128
maltol	-0.718	0.452	-0.025
5-methylfurfuryl ethyl ether	-0.704	-0.283	0.067
4-vinylguaiacol	0.663	-0.159	0.620
4-vinylphenol	0.625	-0.062	0.573
furfural	-0.026	0.948	0.113
5-methylfurfural	0.014	0.889	0.207
guaiacol	-0.200	0.887	-0.221
4-methylguaiacol	0.141	0.883	0.009
4-ethylguaiacol	0.061	0.824	-0.317
vanillin	-0.471	0.525	0.440
vanillyl ethyl ether	0.353	0.011	0.888
5-methylfurfuryl alcohol	0.123	-0.104	0.819
furfuryl ethyl ether	-0.311	0.118	0.805

compounds excluded due to imprecision of measurement, *etc.*
cyclotene
vanillyl alcohol
4-ethylphenol

('estimated extracted furfural' was also excluded)

: Loadings with absolute values > 0.5 are highlighted. They contribute most to the corresponding PC.

Component loadings were converted to eigenvectors to determine sample locations in PC space [eigenvector = component loading / sqrt(eigenvalue)]. See following page.

Appendix Table C.4. Rotated component loadings and corresponding eigenvectors.

	PC1		PC2		PC3	
	compnt load	eigenvector	compnt load	eigenvector	compnt load	eigenvector
furfuryl alcohol	-0.820	-0.347	-0.093	-0.045	-0.043	-0.026
eugenol	0.779	0.329	0.036	0.017	-0.008	-0.005
trans -oak lactone	0.769	0.325	-0.040	-0.019	0.128	0.077
cis -oak lactone	0.765	0.323	-0.125	-0.061	0.128	0.077
maltol	-0.718	-0.303	0.452	0.219	-0.025	-0.015
5-methylfurfuryl ethyl ether	-0.704	-0.298	-0.283	-0.137	0.067	0.040
4-vinylguaiacol	0.663	0.280	-0.159	-0.077	0.620	0.371
4-vinylphenol	0.625	0.264	-0.062	-0.030	0.573	0.343
furfural	-0.026	-0.011	0.948	0.459	0.113	0.068
5-methylfurfural	0.014	0.006	0.889	0.431	0.207	0.124
guaiacol	-0.200	-0.085	0.887	0.430	-0.221	-0.132
4-methylguaiacol	0.141	0.060	0.883	0.428	0.009	0.005
4-ethylguaiacol	0.061	0.026	0.824	0.399	-0.317	-0.190
vanillin	-0.471	-0.199	0.525	0.254	0.440	0.263
vanillyl ethyl ether	0.353	0.149	0.011	0.005	0.888	0.531
5-methylfurfuryl alcohol	0.123	0.052	-0.104	-0.050	0.819	0.490
furfuryl ethyl ether	-0.311	-0.131	0.118	0.057	0.805	0.482
eigenvalue:	5.598		4.261		2.792	

Appendix Table C.5. Chardonnay wine samples in rotated PCA space.

Sample locations in PC space were calculated as follows. The raw data for each compound were converted to *z*-scores. The PC1 eigenvector for the first compound was multiplied, separately, by each of the *z*-scores of that compound. The PC1 axis value for each sample was, then, the sum of these 17 products (one for each compound). The other PC axis values for each sample were obtained in a similar manner. Finally, the co-ordinates (axis values) were arbitrarily divided by 6.8 to restrict the range to -1 to 1.

Barrel code	PC1	PC2	PC3	PC1/6.8	PC2/6.8	PC3/6.8
AU4	-4.437	-1.620	-2.507	-0.652	-0.238	-0.369
AU6	-2.735	-0.880	-1.985	-0.402	-0.129	-0.292
AU70	-2.506	-1.323	-2.604	-0.368	-0.195	-0.383
AA10	-3.280	-0.036	0.186	-0.482	-0.005	0.027
AA11	-3.511	4.201	0.119	-0.516	0.618	0.017
AA22	-1.405	-2.039	-0.514	-0.207	-0.300	-0.076
FL3	1.170	-0.728	-2.483	0.172	-0.107	-0.365
FL4	0.069	2.039	1.812	0.010	0.300	0.266
FL5	0.308	1.339	1.297	0.045	0.197	0.191
LA27	0.986	-2.303	1.860	0.145	-0.339	0.274
LA34	-0.279	1.149	1.326	-0.041	0.169	0.195
LA41	0.416	-1.850	3.626	0.061	-0.272	0.533
FT3	0.303	0.664	-2.311	0.045	0.098	-0.340
FT4	1.444	0.668	-0.093	0.212	0.098	-0.014
FT5	0.577	-1.763	-1.882	0.085	-0.259	-0.277
TA23	-1.654	6.725	-2.254	-0.243	0.989	-0.331
TA31	0.745	-2.020	-1.322	0.110	-0.297	-0.194
TA46	0.160	1.822	-0.611	0.024	0.268	-0.090
FV3	0.983	-0.347	-0.114	0.145	-0.051	-0.017
FV4	2.433	-0.091	0.979	0.358	-0.013	0.144
FV5	2.033	1.151	0.212	0.299	0.169	0.031
VA32	2.437	-1.090	5.023	0.358	-0.160	0.739
VA38	2.637	-0.180	2.398	0.388	-0.027	0.353
VA39	3.105	-3.489	-0.159	0.457	-0.513	-0.023

C.2 Cabernet Sauvignon wine

Raw data were the Cabernet Sauvignon wine composition data listed in Table 2.5.

Appendix Table C.6. Pearson's product-moment correlation coefficient matrix.

	cis	*trans*	*eug*	*guaiac*	*4mg*	*van*	*cyc*	*malt*	*furf*	*5mf*	*falc*	*5mfalc*	*fee*	*4vg*	*4eg*	*4vp*	*4ep*
cis	1																
trans	0.604	1															
eug	0.727	0.778	1														
guaiac	0.100	0.406	0.324	1													
4mg	0.122	0.593	0.391	0.828	1												
van	0.317	0.354	0.171	0.409	0.568	1											
cyc	-0.033	0.197	0.093	0.760	0.625	0.254	1										
malt	0.207	0.403	0.322	0.741	0.646	0.265	0.782	1									
furf	0.355	0.272	0.193	0.655	0.552	0.574	0.451	0.459	1								
5mf	0.436	0.323	0.434	0.449	0.344	0.444	0.158	0.132	0.650	1							
falc	0.269	0.263	0.150	0.728	0.621	0.737	0.664	0.620	0.820	0.505	1						
5mfalc	0.007	0.077	-0.091	-0.208	-0.180	-0.414	-0.163	-0.107	-0.301	-0.312	-0.388	1					
fee	0.256	0.386	0.075	0.570	0.577	0.553	0.517	0.623	0.618	0.298	0.795	0.072	1				
4vg	0.175	-0.004	0.045	-0.370	-0.367	-0.292	-0.288	-0.298	-0.423	-0.213	-0.354	0.484	-0.133	1			
4eg	0.019	0.179	-0.018	-0.212	-0.116	-0.419	-0.139	-0.126	-0.389	-0.389	-0.431	0.887	0.033	0.551	1		
4vp	-0.051	-0.083	-0.004	-0.292	-0.366	-0.647	-0.145	-0.128	-0.426	-0.395	-0.442	0.696	-0.112	0.568	0.797	1	
4ep	-0.188	0.016	0.067	0.051	0.027	-0.542	0.142	-0.113	-0.289	-0.222	-0.406	0.448	-0.309	0.278	0.569	0.611	1

: significant correlation, p <0.05 or stronger.

Critical values for 2-tailed test of correlation, $n = 24$, $d.f. = n - 2 = 24 - 2 = 22$.

If r is greater than or equal to 0.404, significant correlation, p <0.05.*

If r is greater than or equal to 0.515, significant correlation, p <0.01.*

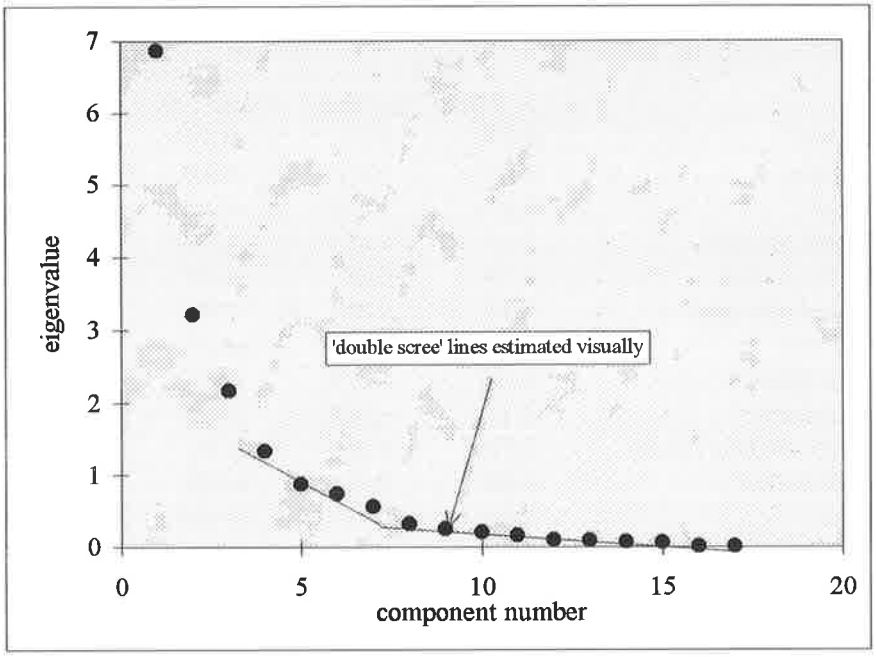
If r is greater than or equal to 0.6524, significant correlation, p <0.001.**

*: from Snedecor and Cochran (1967), 22 *d.f.*

**: from O'Mahony (1986), 20 *d.f.*

Compound abbreviations: cis=*cis* -oak lactone, trans=*trans* -oak lactone, eug=eugenol, guaiac=guaiacol, 4mg=4-methylguaiacol, van=vanillin, cyc=cyclotene, malt=maltol, furf=furfural, 5mf=5-methylfurfural, falc=furfuryl alcohol, 5mfalc=5-methylfurfuryl alcohol, fee=furfuryl ethyl ether, 4vg=4-vinylguaiacol, 4eg=4-ethylguaiacol, 4vp=4-vinylphenol, 4-ethylphenol.

Scree plot	
number	eigenvalue
1	6.863
2	3.211
3	2.161
4	1.330
5	0.867
6	0.737
7	0.563
8	0.319
9	0.249
10	0.201
11	0.158
12	0.098
13	0.088
14	0.075
15	0.061
16	0.012
17	0.008



Appendix Figure C.2. Scree plot of Cab. Sauv. composition-PCA.

Conclusion: The scree test suggests retaining the first three PCs.

Appendix Table C.7. PC characteristics.

PCs description:	**PC1:**	'emphasis on coopering heat products'		
	PC2:	'emphasis on some microbial products'		
	PC3:	'emphasis on natural oak products'		
Proportion of variance explained by each PC		**PC1**	**PC2**	**PC3**
variance explained:		29.58%	25.09%	17.29%
cumulative variance explained:		29.58%	54.68%	71.97%

PC2, describing microbial activity products, was altered after the Systat PCA: the signs were changed on all of the rotated component loadings to make the effect positive (*i.e.* to make a positive value indicative of more microbial activity products). This change was carried through all of the calculations.

Appendix Table C.8. Rotated component loadings.

compounds included	PC load 1	PC load 2	PC load 3	compounds excluded due to
guaiacol	0.895	-0.138	0.145	imprecision of measurement, *etc.*
cyclotene	0.887	-0.003	-0.125	vanillyl alcohol
maltol	0.839	-0.010	0.126	5-methylfurfuryl ethyl ether
4-methylguaiacol	0.819	-0.128	0.257	vanillyl ethyl ether
furfuryl alcohol	0.768	-0.467	0.191	('estimated extracted furfural'
furfuryl ethyl ether	0.748	-0.066	0.229	was also excluded)
furfural	0.625	-0.454	0.288	
4-ethylguaiacol	-0.055	0.922	0.090	:
4-vinylphenol	-0.156	0.883	-0.038	Loadings with
5-methylfurfuryl alcohol	-0.057	0.838	0.048	absolute values > 0.5 are
4-ethylphenol	0.062	0.738	-0.125	highlighted. They contribute
vanillin	0.401	-0.606	0.344	most to the corresponding PC.
4-vinylguaiacol	-0.358	0.578	0.227	
cis -oak lactone	0.015	-0.055	0.900	Component loadings were converted to
eugenol	0.141	0.046	0.855	eigenvectors to determine sample
trans -oak lactone	0.355	0.121	0.791	locations in PC space [eigenvector =
5-methylfurfural	0.243	-0.451	0.534	component loading / sqrt(eigenvalue)].

Appendix Table C.9. Rotated component loadings and corresponding eigenvectors.

	PC1		PC2		PC3	
	compnt load	eigenvector	compnt load	eigenvector	compnt load	eigenvector
guaiacol	0.895	0.342	-0.138	-0.077	0.145	0.099
cyclotene	0.887	0.339	-0.003	-0.002	-0.125	-0.085
maltol	0.839	0.320	-0.010	-0.006	0.126	0.086
4-methylguaiacol	0.819	0.313	-0.128	-0.071	0.257	0.175
furfuryl alcohol	0.768	0.293	-0.467	-0.261	0.191	0.130
furfuryl ethyl ether	0.748	0.286	-0.066	-0.037	0.229	0.156
furfural	0.625	0.239	-0.454	-0.253	0.288	0.196
4-ethylguaiacol	-0.055	-0.021	0.922	0.515	0.090	0.061
4-vinylphenol	-0.156	-0.060	0.883	0.493	-0.038	-0.026
5-methylfurfuryl alcohol	-0.057	-0.022	0.838	0.468	0.048	0.033
4-ethylphenol	0.062	0.024	0.738	0.412	-0.125	-0.085
vanillin	0.401	0.153	-0.606	-0.338	0.344	0.234
4-vinylguaiacol	-0.358	-0.137	0.578	0.323	0.227	0.154
cis -oak lactone	0.015	0.006	-0.055	-0.031	0.900	0.612
eugenol	0.141	0.054	0.046	0.026	0.855	0.582
trans -oak lactone	0.355	0.136	0.121	0.068	0.791	0.538
5-methylfurfural	0.243	0.093	-0.451	-0.252	0.534	0.363
eigenvalue:	6.863		3.211		2.161	

Appendix Table C.10. Cab. Sauv. wine samples in rotated PCA space.

Sample locations in PC space were calculated as follows. The raw data for each compound were converted to *z* -scores. The PC1 eigenvector for the first compound was multiplied, separately, by each of the *z* -scores of that compound. The PC1 axis value for each sample was, then, the sum of these 17 products (one for each compound). The other PC axis values for each sample were obtained in a similar manner. Finally, the co-ordinates (axis values) were arbitrarily divided by 6.0 to restrict the range to -1 to 1.

Barrel code	PC1	PC2	PC3	PC1/6.0	PC2/6.0	PC3/6.0
AU7	-0.607	-0.824	-1.240	-0.101	-0.137	-0.207
AU8	-3.175	5.089	-4.134	-0.529	0.848	-0.689
AU9	-3.340	0.403	-3.935	-0.557	0.067	-0.656
AA36	-3.278	4.628	-2.651	-0.546	0.771	-0.442
AA40	-1.348	1.348	-1.903	-0.225	0.225	-0.317
AA48	-0.576	-0.176	-2.590	-0.096	-0.029	-0.432
NL6	-0.709	-1.584	-1.529	-0.118	-0.264	-0.255
NL7	0.450	-0.688	-2.143	0.075	-0.115	-0.357
NL8	1.655	-0.956	-1.248	0.276	-0.159	-0.208
LA23	-1.772	0.189	-0.755	-0.295	0.032	-0.126
LA30	-0.190	0.543	0.030	-0.032	0.091	0.005
LA38	-0.136	-2.845	-1.410	-0.023	-0.474	-0.235
NT6	0.772	2.047	1.991	0.129	0.341	0.332
NT7	-0.228	-0.471	0.927	-0.038	-0.079	0.154
NT8	1.386	0.648	1.001	0.231	0.108	0.167
TA8	0.399	-2.384	2.745	0.066	-0.397	0.457
TA25	0.900	-3.619	3.059	0.150	-0.603	0.510
TA39	4.224	-3.772	1.489	0.704	-0.629	0.248
NV6	-1.465	5.346	1.206	-0.244	0.891	0.201
NV7	0.852	-1.300	2.313	0.142	-0.217	0.386
NV8	0.404	3.162	1.074	0.067	0.527	0.179
VA12	5.910	-3.083	3.433	0.985	-0.514	0.572
VA21	0.102	-0.801	2.457	0.017	-0.134	0.409
VA27	-0.231	-0.901	1.813	-0.038	-0.150	0.302

C.3 Model wine

Raw data were the 93 week model wine composition data listed in Table 2.7.

Appendix Table C.11. Pearson's product-moment correlation coefficient matrix.

	cis	*trans*	*eug*	*guaiac*	*4mg*	*van*	*cyc*	*malt*	*furf*	*eef*	*5mf*	*falc*	*fee*	*4eg*
cis	1													
trans	0.608	1												
eug	0.692	0.535	1											
guaiac	0.405	0.161	0.482	1										
4mg	0.683	0.480	0.698	0.859	1									
van	0.409	0.366	0.184	0.538	0.573	1								
cyc	0.323	0.127	0.338	0.653	0.637	0.737	1							
malt	0.110	-0.165	0.074	0.760	0.483	0.663	0.745	1						
furf	0.219	0.082	0.279	0.788	0.564	0.645	0.789	0.868	1					
eef	0.299	0.116	0.240	0.837	0.648	0.746	0.833	0.879	0.942	1				
5mf	0.371	0.181	0.422	0.878	0.732	0.756	0.850	0.890	0.923	0.943	1			
falc	0.195	0.076	-0.153	0.049	0.162	0.218	0.035	-0.064	-0.275	0.064	-0.051	1		
fee	-0.083	0.028	-0.337	-0.191	-0.017	0.117	-0.074	-0.202	-0.460	-0.170	-0.256	0.874	1	
4eg	0.343	0.564	0.577	0.584	0.612	0.368	0.358	0.396	0.467	0.400	0.581	-0.262	-0.257	1

: significant correlation, p <0.05 or stronger.

Critical values for 2-tailed test of correlation, n = 16, $d.f.$ = n - 2 = 16 - 2 = 14, from O'Mahony (1986).

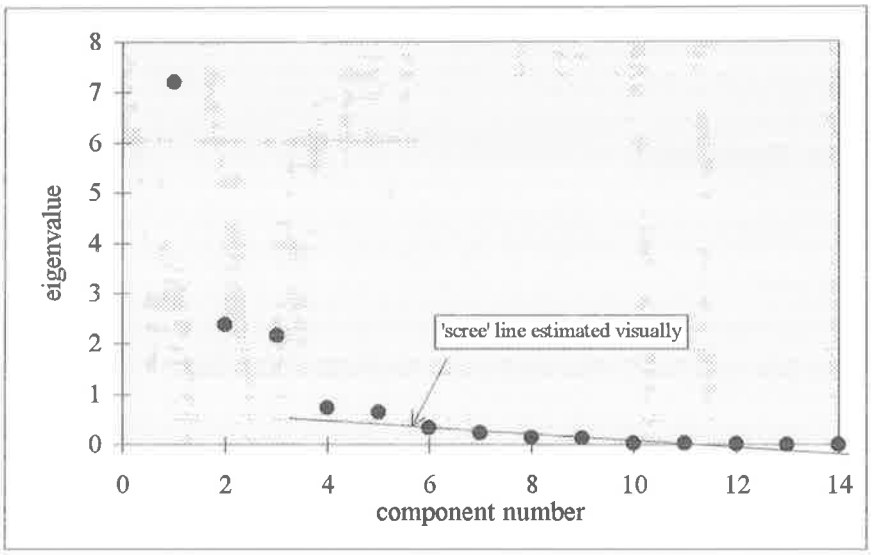
If r is greater than or equal to 0.4973, significant correlation, p <0.05.

If r is greater than or equal to 0.6226, significant correlation, p <0.01.

If r is greater than or equal to 0.7420, significant correlation, p <0.001.

Compound abbreviations: cis=*cis* -oak lactone, trans=*trans* -oak lactone, eug=eugenol, guaiac=guaiacol, 4mg=4-methylguaiacol, van=vanillin, cyc=cyclotene, malt=maltol, furf=furfural, eef='estimated extracted furfural' (furfural + furfuryl alcohol), 5mf=5-methylfurfural, falc=furfuryl alcohol, fee=furfuryl ethyl ether, 4eg=4-ethylguaiacol.

Scree plot	
number	eigenvalue
1	7.211
2	2.372
3	2.159
4	0.725
5	0.632
6	0.329
7	0.231
8	0.146
9	0.124
10	0.030
11	0.025
12	0.012
13	0.004
14	0.000



Appendix Figure C.3. Scree plot of 93 week model wine composition-PCA.

Conclusion: The scree test suggests retaining the first three PCs.

Appendix Table C.12. Rotated component loadings and PC characteristics.

compounds included	PC load 1	PC load 2	PC load 3	compounds excluded due to
'estimated extracted furfural'	0.965	0.129	-0.005	imprecision of measurement, *etc.*
maltol	0.953	-0.099	-0.108	5-methyl furfuryl alcohol
5-methyl furfural	0.943	0.274	-0.119	vanillyl alcohol
furfural	0.916	0.102	-0.329	5-methylfurfuryl ethyl ether
cyclotene	0.861	0.186	0.044	vanillyl ethyl ether
guaiacol	0.818	0.366	-0.070	4-vinylguaiacol
vanillin	0.761	0.266	0.279	4-vinylphenol
4-methylguaiacol	0.603	0.704	0.105	4-ethylphenol
eugenol	0.150	0.850	-0.257	
trans-oak lactone	-0.040	0.849	0.099	:
cis-oak lactone	0.186	0.821	0.134	Loadings with
4-ethylguaiacol	0.378	0.630	-0.303	absolute values > 0.5 are
furfuryl alcohol	0.039	0.048	0.962	highlighted. They contribute
furfuryl ethyl ether	-0.128	-0.090	0.945	most to the corresponding PC.
PCs description:	**PC1:**	'emphasis on coopering heat products'		
	PC2:	'emphasis on natural oak products'		
	PC3:	'emphasis on microbial products'		

Proportion of variance explained by each PC	PC1	PC2	PC3
variance explained:	43.85%	24.08%	15.94%
cumulative variance explained:	43.85%	67.93%	83.87%

Component loadings were converted to eigenvectors to determine sample locations in PC space [eigenvector = component loading / sqrt(eigenvalue)].

Appendix Table C.13. Rotated component loadings and corresponding eigenvectors.

	PC1		PC2		PC3	
	compnt load	eigenvector	compnt load	eigenvector	compnt load	eigenvector
'estimated extracted furfural'	0.965	0.359	0.129	0.084	-0.005	-0.003
maltol	0.953	0.355	-0.099	-0.064	-0.108	-0.074
5-methyl furfural	0.943	0.351	0.274	0.178	-0.119	-0.081
furfural	0.916	0.341	0.102	0.066	-0.329	-0.224
cyclotene	0.861	0.321	0.186	0.121	0.044	0.030
guaiacol	0.818	0.305	0.366	0.238	-0.070	-0.048
vanillin	0.761	0.283	0.266	0.173	0.279	0.190
4-methylguaiacol	0.603	0.225	0.704	0.457	0.105	0.071
eugenol	0.150	0.056	0.850	0.552	-0.257	-0.175
trans-oak lactone	-0.040	-0.015	0.849	0.551	0.099	0.067
cis-oak lactone	0.186	0.069	0.821	0.533	0.134	0.091
4-ethylguaiacol	0.378	0.141	0.630	0.409	-0.303	-0.206
furfuryl alcohol	0.039	0.015	0.048	0.031	0.962	0.655
furfuryl ethyl ether	-0.128	-0.048	-0.090	-0.058	0.945	0.643
eigenvalue:	7.211		2.372		2.159	

Appendix Table C.14. 93 week model wine samples in rotated PCA space.

Sample locations in PC space were calculated as follows. The raw data for each compound were converted to z-scores. The PC1 eigenvector for the first compound was multiplied, separately, by each of the z-scores of that compound. The PC1 axis value for each sample was, then, the sum of these 14 products (one for each compound). The other PC axis values for each sample were obtained in a similar manner. Finally, the co-ordinates (axis values) were arbitrarily divided by 5.4 to restrict the range to -1 to 1.

Barrel code	PC1	PC2	PC3	PC1/5.4	PC2/5.4	PC3/5.4
AU2	-0.802	-3.012	-0.853	-0.149	-0.558	-0.158
AU3	1.826	-1.165	-1.444	0.338	-0.216	-0.267
AA34	-5.357	-5.253	-0.199	-0.992	-0.973	-0.037
AA47	1.591	-1.254	-1.405	0.295	-0.232	-0.260
NL1	-1.849	-1.886	1.449	-0.342	-0.349	0.268
NL2	-2.797	-2.473	1.261	-0.518	-0.458	0.234
LA33	-0.536	-0.672	4.777	-0.099	-0.124	0.885
LA42	-1.394	-1.179	0.152	-0.258	-0.218	0.028
NT1	0.961	1.959	-0.151	0.178	0.363	-0.028
NT2	-1.691	1.805	-0.919	-0.313	0.334	-0.170
TA9	1.567	0.402	-0.129	0.290	0.074	-0.024
TA10	0.979	2.957	-0.773	0.181	0.548	-0.143
NV1	0.151	1.155	-0.565	0.028	0.214	-0.105
NV2	1.136	2.993	-1.363	0.210	0.554	-0.252
VA2	5.391	4.099	-0.766	0.998	0.759	-0.142
VA28	0.825	1.524	0.929	0.153	0.282	0.172

Appendix D

Sensory (aroma) analysis materials and methods

Appendix outline

D.1 Sample size effects on consecutive concentration interval statistics

As discussed in Chapter 3, an experiment was conducted to test the effect of increasing the size of a sample of barrel wines on the size and variance of the volatile compound concentration intervals between each consecutive unit of the sample. Compound concentration data were employed but the results were used to make inferences about the nature of the sensory (aroma) measurements. If the mean size of the consecutive concentration intervals were to decrease, then it could be inferred that increasing the sample size would result in increasing the difficulty of the sensory measurement of the intervals. If the variance of the consecutive concentration intervals were to decrease, then the data would become more rank–like (the variance of the consecutive intervals in rank data equals zero). These two results could indicate the suitability of a sensory ranking procedure, relative to a rating procedure, when relatively large sample sizes are involved.

A selection of the volatile compound concentration data for the 93 week barrel–stored Cabernet Sauvignon wines was used. The 24 concentration values for the oak lactones, eugenol, guaiacol, 4–methylguaiacol, and 4–ethylphenol were subjected to random sampling, as follows. The order in which the compounds were to be tested was randomly determined. For the first compound, a random sample of 6, 12 and then 18 concentration values was taken. The sampling of 24 for this compound consisted of the whole population. This process was repeated for each of the other five compounds.

The units within each sampling for each compound were arranged in order of ascending concentration, and the mean and variance for the intervals between each consecutive unit were determined. Each of these statistics was expressed, in percentage terms, relative to the corresponding 'n=6' sample which was designated a value of 100 % (Appx. Fig. D.1).

There were three sample–size increases for each of the six compounds. Thus, there were 18 sample–size increase in the experiment. The mean of the consecutive concentration intervals decreased over 17 of these 18 sample–size increases; and the variance decreased over 14 of them. The implications of these results are discussed in Section 3.2.

D.2 Preparations

Materials and environmental conditions

The wine samples to be used at each stage of each experiment were stored in the sensory analysis room for convenience and to ensure that the samples, on any one occasion, were of the same temperature. Throughout the sensory analyses, wines and standards were served in the standard, tulip–shaped, international wine tasting glass (XL5). Approximately 20 mL of each sample was poured into each glass as close as possible, in time, to the beginning of the session but commonly around one hour prior to it. A plastic petri dish was used to cover the glass to minimise the contamination of the sensory analysis environment with wine aromas.

Clean glasses were individually smelled by the experimenter to isolate any aroma–contaminated (*e.g.* 'dusty') glass. Each of the glasses used was marked with a random three–digit number on its base.

The sensory analysis environment was made as free as possible of interferences such as noise, odours, colours and large temperature variations, and the room was ventilated to allow the removal of sample aromas. Group sessions and discussions were held in a large, quiet meeting room, free of aromas. All of the individual rankings (and triangle difference tests) were performed in individual, white coloured, relatively noise–free sensory analysis booths, under a combination of red artificial lighting and diffuse natural lighting. Temperatures were usually close to 20 °C (all aroma ranking sessions were between 18 and 23 °C) but were occasionally up to 29 °C (for some triangle difference tests). A positive atmospheric pressure was established in the booths by fan, and a vent in each booth allowed for adequate air flow.

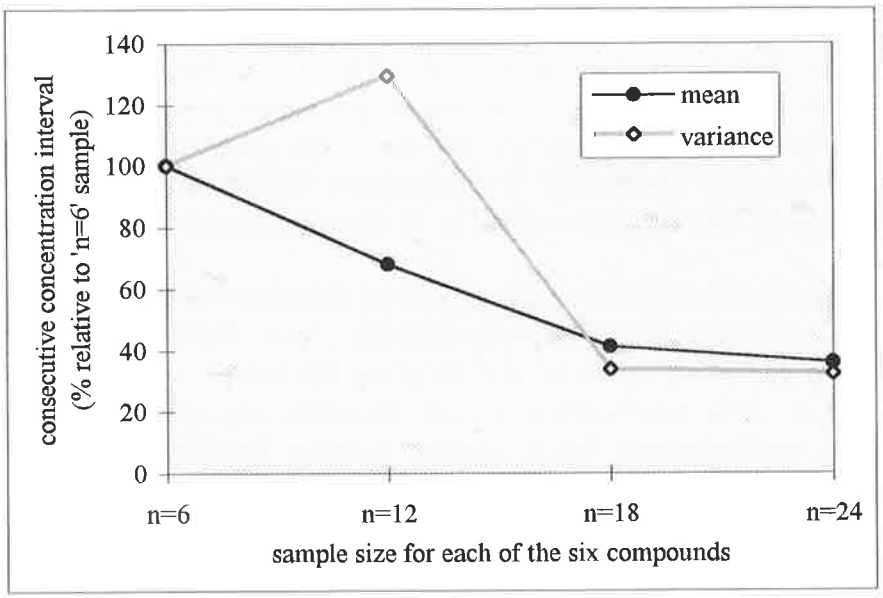
All of the ranking sessions and most of the training sessions were performed between 10 am and 1 pm, but usually before 11 am. Beyond breakfast each day, panelists were asked to refrain from the consumption of food, beverages and tobacco until after their session.

Similarly, they were asked to refrain from wearing any personal odorant beyond a simple antiperspirant.

Could glasses of the samples be shared by different panelists?

As preliminaries to the sensory descriptive analysis, this and the two questions on the following page were considered.

If it were possible to share glasses, it would minimise the preparation time and sample volume requirements. Two of the Chardonnay wine samples — *LA41* and *TA46* — were selected. Approximately 20 mL of each was poured to a separate batch of glasses and covered with lids around an hour prior to the difference testing. Each of the two batches was split into two portions, one to be 'used' and the other to be 'non–used.' The 'non–used' treatment of each batch was left to stand while the 'used' treatment for each was subjected to simulated use. This involved six of the following applications over 30 minutes for each glass. The wine was caused to vortex three times in the glass, over three seconds, by three quick, circular and horizontal hand movements, the lid was lifted and the air in the glass was drawn through the nose with moderate force. Each of the two pairs of treatments was presented twice to 15 panelists for triangle difference testing (Meilgaard *et al.* 1991 pp. 60–62) during the among–replicate difference test sessions (Appx. Tab. D.1). The simulated use (sniffing) imposed on sample *TA46* resulted in a significant aroma difference from the same but 'non–used' sample ($p<0.001$). Thus, it was apparent that glasses of at least one of the samples could not be shared. Consequently, each panelist was served separate samples throughout all of the sensory analyses.



Appendix Figure D.1. Sample size effects on consecutive concentration interval statistics.

Did the replacement of malic acid in the malolactic fermentation–affected Chardonnay wines alter their aromas?

Ten of the 24 Chardonnay barrel wines, having experienced malic acid degradation of more than 10 %, received DL–malic acid additions (AR grade, 40 % aqueous solution) (Appx. Tab. A.1) at bottling, to titratable acidity and pH values approximately equal to the initial values. This was a precaution against any possible variability in pH–mediated aroma transformations during bottle storage. Some non–acidified samples were bottled at the same time, and kept for later comparison. Two of them — *AU6* and *AA22* — were subjected to triangle difference testing, by smelling only, against their acidified counterparts during the among–replicate difference test sessions (Appx. Tab. D.1). No significant aroma difference was found to result from the practice of acidifying some of the samples ($p>0.10$). Thus, the possibility of variability in pH–mediated aroma transformations during bottle storage needed no further consideration.

For the replicate samples within each treatment, were the aromas significantly different, requiring the inclusion of each replicate in the sensory descriptive analysis?

If the replicate samples were not significantly different, it may have been appropriate to reduce the number of samples requiring description.

Each of the three replicate barrel wines within each treatment were compared. Thus, three triangle difference tests for aroma only were performed among the replicates of each treatment. This meant 24 tests for the Chardonnay wine (Appx. Tab. D.1) and 24 tests for the Cabernet Sauvignon wine (Appx. Tab. D.2).

The order of the sample–comparisons was determined randomly. The panels were similar to those described below. All were instructed in the triangle difference test procedure, and a practice day preceded the 'real' sessions. Each of the 15 participating panelist was served four triangle difference tests in an isolation booth, one comparison in tray positions one and three, and another in tray positions two and four. The panelists were not aware of this pattern. The allocation of comparison to tray position and the order of glasses within a set were made randomly. This method resulted in 30 triangle tests for each comparison.

Thirteen and fourteen of the 24 Chardonnay and 24 Cabernet Sauvignon wine comparisons, respectively, were found to be significantly different (Appx. Tabs. D.1 & D.2), resulting in the possibility of excluding seven of the 24 wines from each of the sensory descriptive analyses. However, since there were numerous differences among replicates which required description, and since retaining equal replicate numbers was desirable, no samples were excluded from the analyses.

Appendix Table D.1. Chardonnay wine triangle difference test results with corresponding 'preference' and aroma differences determined by ranking.

Date	Temp (°C)	Set	Comparison	Correct (of 30†)	Sig diff by diff test?	Descriptor significantly different by Friedman analysis? If so, which barrel was higher?	
8/11/94	?	1st & 3rd	practice wines	25/40	$p<0.001$		
		2nd & 4th	practice wines	14/40	n.s.		
9/11/94	21	1st & 3rd	*AA10-AA11*	15	$p<0.05$		
		2nd & 4th	*TA31-TA46*	14	n.s.	allspice, $p<0.05$, *TA46*	cinnamon, $p<0.001$, *TA46*
10/11/94	23	1st & 3rd	*FT4-FT5*	11	n.s.		
		2nd & 4th	*LA27-LA41*	13	n.s.	vanilla, $p<0.05$, *LA41*	butter, $p<0.05$, *LA41*
11/11/94	24	1st & 3rd	*FT3-FT4*	13	n.s.	smoky, $p<0.05$, *FT3*	
		2nd & 4th	*AA22-AA22**	10	n.s.		
14/11/94	19	1st & 3rd	*AU6-AU6**	13	n.s.		
		2nd & 4th	*FV4-FV5*	12	n.s.		
15/11/94	20	1st & 3rd	*FV3-FV4*	7	n.s.		
		2nd & 4th	*AA11-AA22*	22	$p<0.001$	coconut, $p<0.05$, *AA22*	pencil shavings, $p<0.05$, *AA11*
						allspice, $p<0.01$, *AA11*	smoky, $p<0.05$, *AA11*
16/11/94	19	1st & 3rd	*LA34-LA41*	14	n.s.	pencil shavings, $p<0.05$, *LA34*	smoky, $p<0.01$, *LA34*
		2nd & 4th	*AU6-AU70*	22	$p<0.001$		
17/11/94	21	1st & 3rd	*FL4-FL5*	22	$p<0.001$	preference, $p<0.01$, *FL4*	coconut, $p<0.01$, *FL4*
						caramel, $p<0.05$, *FL4*	vanilla, $p<0.01$, *FL4*
						allspice, $p<0.05$, *FL4*	
		2nd & 4th	*AU4-AU70*	14	n.s.		
18/11/94	23	1st & 3rd	*AA10-AA22*	14	n.s.	coconut, $p<0.05$, *AA22*	
		2nd & 4th	*FV3-FV5*	17	$p<0.01$	butter, $p<0.05$, *FV3*	
21/11/94	21	1st & 3rd	*VA32-VA38*	9	n.s.		
		2nd & 4th	*FT3-FT5*	17	$p<0.01$	smoky, $p<0.05$, *FT3*	
22/11/94	20	1st & 3rd	*FL3-FL4*	16	$p<0.05$	preference, $p<0.05$, *FL4*	allspice, $p<0.05$, *FL4*
						smoky, $p<0.05$, *FL4*	green apple, $p<0.01$, *FL3*
		2nd & 4th	*TA23-TA31*	22	$p<0.001$	butter, $p<0.05$, *TA31*	smoky, $p<0.001$, *TA23*
						green apple, $p<0.05$, *TA31*	cinnamon, $p<0.01$, *TA23*
23/11/94	?	1st & 3rd	*AU4-AU6*	21	$p<0.001$	preference, $p<0.05$, *AU4*	green apple, $p<0.05$, *AU6*
		2nd & 4th	*LA27-LA34*	20	$p<0.001$	preference, $p<0.05$, *LA34*	vanilla, $p<0.05$, *LA34*
						butter, $p<0.05$, *LA34*	smoky, $p<0.001$, *LA34*
24/11/94	24	1st & 3rd	*TA23-TA46*	14	n.s.	vanilla, $p<0.01$, *TA46*	smoky, $p<0.05$, *TA23*
						green apple, $p<0.05$, *TA46*	
		2nd & 4th	*VA38-VA39*	19	$p<0.001$	cashew nut, $p<0.05$, *VA38*	
25/11/94	24	1st & 3rd	*VA32-VA39*	21	$p<0.001$		
		2nd & 4th	*FL3-FL5*	15	$p<0.05$	coconut, $p<0.001$, *FL3*	pencil shavings, $p<0.05$, *FL5*
28/11/94	20	1st & 3rd	*LA41-LA41***	13	n.s.		
		2nd & 4th	*TA46**-TA46*	20	$p<0.001$		

* Sample had acidity adjusted to pre-MLF level with DL-malic acid.
** Sample was subject to simulated use, *i.e.* swirling and sniffing.
† Number correct was out of 30 except for the first session which was out of 40.
n.s. = not significant.

The 'descriptive' panels — selection, training and demographics

Panels may be made up of untrained or inexperienced personnel — these are often used in consumer preference tests — but in the present case, training and experience were considered advantages since discrimination based on sometimes very subtle differences was required.

Fifteen panelists were used for the descriptive tests but a pool of 20 was required to account for absenteeism. These panelists were staff or postgraduate students of The Australian Wine Research Institute or of the Department of Horticulture, Viticulture and Oenology, The University of Adelaide. The candidates had participated in the triangle difference tests so they were familiar with the wines, the experimenter's general methods and the sensory analysis facilities and environment of the laboratory. Most of the 20 panelists comprising the Chardonnay panel (Appx. Tab. D.3) and the Cabernet Sauvignon panel (Appx. Tab. D.4) considered themselves to be moderately to very well accustomed to paying more than very brief attention to wine aroma during consumption, most of them consumed wine more frequently than weekly, and the largest proportion were aged 31 to 40 years. There was an equal proportion of males and females in the Chardonnay wine aroma panel, and 13 of 20 were males in the Cabernet Sauvignon wine aroma panel. Health and medication consumption responses were acceptable, and each panelist considered their sense of smell to be at least 'average.'

Each candidate was invited to participate in aptitude tests after ascertaining that they were available, that they possessed sufficient interest and an amenable and co–operative nature. Further to the aptitude testing, day one involved a general verbal introduction to the scope, aims and methods of the experiment. Each panelist completed a questionnaire requesting some personal information, an indication of availability and commitment, and a self–assessment of general wine experience and state of health relevant to the task (Appx. Fig. D.2).

Also on day one, each panelist completed aroma matching and identification tests as described in Meilgaard *et al.* (1991 pp. 138–140), and on days two and three, aroma ranking tests were performed as per Meilgaard *et al.* (1991 pp. 138–140). The results of the four tests for the Chardonnay wine panel are summarised in Appendix Table D.5, while those for the Cabernet Sauvignon wine panel are summarised in Appendix Table D.6. Meilgaard *et al.* (1991 pp. 140–141) recommend rejecting those candidates who score less than 75 % correct matches, less than 60 % correct identifications, or those who rank any more than one adjacent pair incorrectly. As a result of the generally adequate results of these tests, of the questionnaire responses, of an informal estimation of candidate suitability, and of a consideration of the available resources, only one candidate (number 9) was excluded from the Chardonnay wine aroma panel, and no candidate was excluded from the Cabernet Sauvignon wine aroma panel. Thus, the tests are referred to as aptitude — rather than screening — tests.

Panelists are often selected on the basis of their sensitivity using these or similar tests. Interestingly, however, recent work by Lesschaeve and Issanchou (1996) has shown that "an initial olfactive culture" and a good memory for odours were important criteria for good descriptive analysis performance, while sensitivity and "verbal creativity" were not.

A pool of 20 panelists was available to supply the 15 required at each session. The first 15 to attend were used.

Training sessions two to six–or–seven involved aroma ranking practice and a process of descriptor– and standard–generation (discussed below). The focus of each of the training sessions is listed in Appendix Tables D.7 and D.8.

The final training occasion, immediately prior to the commencement of the formal ranking occasions for each experiment, involved an isolation booth practice session. The intention was to initiate the process as a routine for the panelists and the experimenter.

Appendix Table D.2. Cabernet Sauvignon wine triangle difference test results with corresponding 'preference' and aroma differences determined by ranking.

Date	Temp (°C)	Sets	Comparison	Correct (of 30)	Sig diff by diff test?	Descriptor significantly different by Friedman analysis? If so, which barrel was higher?	
6/12/94	29	1st & 3rd	*LA30-LA38*	14	n.s.	preference, $p<0.05$, *LA38*	Band-aid, $p<0.05$, *LA38*
						smoky, $p<0.05$, *LA38*	vanilla, $p<0.001$, *LA30*
		2nd & 4th	*TA25-TA39*	18	$p<0.01$	berry, $p<0.01$, *TA25*	smoky, $p<0.05$, *TA39*
7/12/94	27	1st & 3rd	*NV6-NV8*	22	$p<0.001$	berry, $p<0.05$, *NV8*	coffee, $p<0.05$, *NV8*
		2nd & 4th	*AU7-AU8*	11	n.s.	berry, $p<0.05$, *AU7*	
8/12/94	22	1st & 3rd	*NL6-NL8*	16	$p<0.05$	caramel, $p<0.05$, *NL8*	
		2nd & 4th	*AU7-AU9*	15	$p<0.05$	berry, $p<0.05$, *AU7*	vanilla, $p<0.01$, *AU7*
9/12/94	21	1st & 3rd	*NL7-NL8*	12	n.s.		
		2nd & 4th	*NV6-NV7*	15	$p<0.05$		
12/12/94	27	1st & 3rd	*NT6-NT8*	10	n.s.	dark chocolate, $p<0.01$, *NT6*	coffee, $p<0.05$, *NT6*
		2nd & 4th	*LA23-LA38*	19	$p<0.001$	preference, $p<0.01$, *LA38*	
13/12/94	26	1st & 3rd	*VA21-VA27*	19	$p<0.001$	dark chocolate, $p<0.05$, *VA21*	pencil shavings, $p<0.01$, *VA27*
		2nd & 4th	*VA12-VA21*	21	$p<0.001$	allspice, $p<0.05$, *VA21*	caramel, $p<0.05$, *VA12*
						berry, $p<0.05$, *VA21*	
14/12/94	22	1st & 3rd	*AU8-AU9*	16	$p<0.05$		
		2nd & 4th	*TA8-TA25*	11	n.s.	berry, $p<0.05$, *TA25*	
15/12/94	22	1st & 3rd	*AA40-AA48*	7	n.s.		
		2nd & 4th	*AA36-AA48*	19	$p<0.001$	caramel, $p<0.05$, *AA48*	
16/12/94	24	1st & 3rd	*NT7-NT8*	19	$p<0.001$	Band-aid, $p<0.01$, *NT8*	caramel, $p<0.05$, *NT7*
						berry, $p<0.01$, *NT7*	pencil shavings, $p<0.01$, *NT8*
		2nd & 4th	*VA12-VA27*	17	$p<0.01$	caramel, $p<0.05$, *VA12*	pencil shavings, $p<0.01$, *VA27*
19/12/94	29	1st & 3rd	*TA8-TA39*	7	n.s.	allspice, $p<0.05$, *TA39*	smoky, $p<0.05$, *TA39*
		2nd & 4th	*NL6-NL7*	11	n.s.		
20/12/94	27	1st & 3rd	*NV7-NV8*	14	n.s.	preference, $p<0.05$, *NV7*	
		2nd & 4th	*LA23-LA30*	23	$p<0.001$	Band-aid, $p<0.01$, *LA23*	smoky, $p<0.001$, *LA23*
						vanilla, $p<0.01$, *LA30*	coffee, $p<0.01$, *LA23*
21/12/94	27	1st & 3rd	*NT6-NT7*	21	$p<0.001$	coconut, $p<0.05$, *NT7*	allspice, $p<0.05$, *NT7*
						caramel, $p<0.05$, *NT7*	berry, $p<0.05$, *NT7*
						pencil shavings, $p<0.01$, *NT6*	
		2nd & 4th	*AA36-AA40*	14	n.s.	caramel, $p<0.05$, *AA40*	

n.s. = not significant.

Appendix Table D.3. Wine evaluation experience, consumption frequency and age of the 20 panelists used for the Chardonnay wine aroma description.

Accustomisation to paying more than very brief attention to wine aroma during consumption			
unaccustomed	*moderately accustomed*	*well accustomed*	*very well accustomed*
0	7	8	5
Wine consumption frequency			
more frequently than weekly	*more frequently than monthly but less frequently than weekly*	*more frequently than yearly but less frequently than monthly*	*less frequently than yearly*
15	5	0	0
Age			
20 to 25 years	*26 to 30 years*	*31 to 40 years*	*41 to 60 years*
4	5	9	2

Appendix Table D.4. Wine evaluation experience, consumption frequency and age of the 20 panelists used for the Cabernet Sauvignon wine aroma description.

Accustomisation to paying more than very brief attention to wine aroma during consumption			
unaccustomed	*moderately accustomed*	*well accustomed*	*very well accustomed*
1	8	4	7
Wine consumption frequency			
more frequently than weekly	*more frequently than monthly but less frequently than weekly*	*more frequently than yearly but less frequently than monthly*	*less frequently than yearly*
16	4	0	0
Age			
20 to 25 years	*26 to 30 years*	*31 to 40 years*	*41 to 60 years*
4	3	9	4

The 'preference' panels — selection and demographics

Each 'descriptive' panel consisted of 15 persons, each of which ranked two trays of three sets of five wines. The 'preference' panels, selected from the same population, consisted of twice the number of persons, each of which ranked only one tray of three sets of five wines. The panelist number was increased in this manner for the two 'preference' occasions because the subjectivity of the 'preference' ranking procedure recommended the canvassing of wider opinion. The panels were not constituted of random samples of persons so no estimation of population parameters is possible. Nevertheless, the panels' demographics are provided in Appendix Tables D.9 and D.10.

Panelist questionnaire

name: .....

age (tick one):

<20yrs	20–25yrs	26–30yrs	31–40yrs	41–60yrs

wine experience:

(a): I consider myself (tick one) ...

(i): ☐ Unaccustomed to paying anything more than very brief attention to wine aroma during consumption.

(ii): ☐ Moderately accustomed (*e.g.* 1–2 years of paying this attention).

(iii): ☐ Well accustomed (*e.g.* 2–5 years of paying this attention).

(iv): ☐ Very well accustomed (*e.g.* >5 years of paying this attention).

(b): I consume wine (tick one) ...

(i): ☐ Weekly or more frequently.

(ii): ☐ Less than weekly but more than monthly.

(iii): ☐ Less than monthly but more than yearly.

(iv): ☐ Less frequently than yearly.

availability:

Are there any weekdays (M–F) that you will not be available on a regular basis over the next month?

.....

health:

(a): Do you have any of the following? (tick those which apply)

(i): ☐ Oral, gum or nasal disease.

(ii): ☐ Frequent nasal congestion, hay fever or colds. If so, how often?

(b): Do you take any medications which affect your senses, especially smell?

.....

general:

(a): Is your sense of smell (tick one) ...

(i): ☐ Worse than average?

(ii): ☐ Average?

(iii): ☐ Better than average?

(b): Members of the trained panel should not use heavy perfumes/colognes on evaluation days. They should not smoke an hour before the panel meets. Nor should they consume coffee, tea or chocolate drinks or food (especially strongly flavoured items such as chocolate or cakes) an hour before the panel meets. If food or drink is inadvertently consumed, the panelist should rinse his/her mouth with water and wait at least 15 minutes before attending the session. Are you willing to follow these rules?

(i): ☐ Yes

(ii): ☐ No

Appendix Figure D.2. Panelist questionnaire.

Appendix Table D.5. Candidate aptitude test results summary — Chardonnay aroma 'description' panel.

Cand-idate N°.	Matching test		Identification test		'Coconut' ranking test		'Smoky' ranking test	
	% correct	recom. action[1]	% correct	recom. action[1]	result	recom. action[1]	result	recom. action[1]
1	100	accept	100	accept	all correct	accept	1adj.pr.wrong	accept
2					all correct	accept	all correct	accept
3	80	accept	80	accept				
4	100	accept	100	accept	all correct	accept	1adj.pr.wrong	accept
5					1adj.pr.wrong[2]	accept	all correct	accept
6	80	accept	80	accept				
7	80	accept	60	accept	1adj.pr.wrong	accept	all correct	accept
8	100	accept	100	accept	all correct	accept	>1adj.pr.wrong	reject
9	60	reject	0	reject	>1adj.pr.wrong	reject	all correct	accept
10	80	accept	20	reject	>1adj.pr.wrong	reject	>1adj.pr.wrong	reject
11	100	accept	100	accept	1adj.pr.wrong	accept		
12	80	accept	60	accept	1adj.pr.wrong	accept	all correct	accept
13	80	accept	80	accept	all correct	accept	all correct	accept
14	100	accept	100	accept	all correct	accept	1adj.pr.wrong	accept
15	100	accept	100	accept	>1adj.pr.wrong	reject		
16								
17	100	accept	60	accept	1adj.pr.wrong	accept	all correct	accept
18	100	accept	100	accept	1adj.pr.wrong	accept	all correct	accept
19	40	reject	40	reject	>1adj.pr.wrong	reject		
20					all correct	accept	all correct	accept
21	100	accept	100	accept	all correct	accept	all correct	accept

[1] Recommended according to Meilgaard *et al.* (1991 pp. 140–141).
[2] One adjacent pair wrong.

Appendix Table D.6. Candidate aptitude test results summary — Cabernet Sauvignon aroma 'description' panel.

Cand-idate N°.	Matching test		Identification test		'Vanilla' ranking test		'Clove' ranking test	
	% correct	recom. action[1]	% correct	recom. action[1]	result	recom. action[1]	result	recom. action[1]
1	100	accept	80	accept	all correct	accept	1adj.pr.wrong	accept
2	100	accept	80	accept	all correct	accept	all correct	accept
3	100	accept	40	reject	all correct	accept	all correct	accept
4	100	accept	40	reject	all correct	accept	all correct	accept
5	80	accept	20	reject	1adj.pr.wrong[2]	accept	all correct	accept
6	100	accept	40	reject	all correct	accept	all correct	accept
7	100	accept	40	reject	all correct	accept	1adj.pr.wrong	accept
8	100	accept	60	accept				
9	100	accept	40	reject	1adj.pr.wrong	accept	all correct	accept
10	20	reject	20	reject	1adj.pr.wrong	accept	>1adj.pr.wrong	reject
11	100	accept	40	reject	all correct	accept	1adj.pr.wrong	accept
12	100	accept	20	reject	all correct	accept	all correct	accept
13	100	accept	20	reject	all correct	accept	all correct	accept
14	60	reject	40	reject	all correct	accept	all correct	accept
15	100	accept	60	accept	all correct	accept	all correct	accept
16	40	reject	20	reject	1adj.pr.wrong	accept	>1adj.pr.wrong	reject
17	60	reject	20	reject	all correct	accept	all correct	accept
18	60	reject	20	reject	>1adj.pr.wrong	reject	>1adj.pr.wrong	reject
19	100	accept	20	reject	>1adj.pr.wrong	reject	1adj.pr.wrong	accept
20	100	accept	100	accept	1adj.pr.wrong	accept	>1adj.pr.wrong	reject

[1] Recommended action, according to Meilgaard *et al.* (1991 pp. 140–141).
[2] One adjacent pair wrong.

Appendix Table D.7. Chardonnay wine aroma 'descriptive' panel training summary.

Training session	Focus of training session
1	Introduction, questionnaire and aroma matching and identification tests
2	Aroma descriptor generation and aroma ranking test
3	Aroma descriptor refinement, aroma ranking test and aroma ranking practice
4	Aroma standard generation
5	Aroma standard refinement
6	Aroma ranking practice and final instruction
7	Aroma ranking practice in isolation booths

Appendix Table D.8. Cabernet Sauvignon wine aroma 'descriptive' panel training summary.

Training session	Focus of training session
1	Introduction, questionnaire and aroma matching test
2	Aroma descriptor generation, aroma ranking practice and aroma ranking test
3	Aroma descriptor refinement and aroma ranking practice
4	Aroma descriptor refinement and aroma ranking practice
5	Aroma standard generation
6	Aroma standard refinement
7	Confirmation of adequacy of aroma standards
8	Aroma ranking practice in isolation booths

Appendix Table D.9. Wine evaluation experience, consumption frequency and age of the 30 panelists used for the Chardonnay wine aroma 'preference' ranking.

Accustomisation to paying attention to wine aroma during consumption			
unaccustomed	*moderately accustomed*	*well accustomed*	*very well accustomed*
1	14	9	6
Wine consumption frequency			
more frequently than weekly	*more frequently than monthly but less frequently than weekly*	*more frequently than yearly but less frequently than monthly*	*less frequently than yearly*
20	10	0	0
Age			
20 to 25 years	*26 to 30 years*	*31 to 40 years*	*41 to 60 years*
5	6	13	6

Appendix Table D.10. Wine evaluation experience, consumption frequency and age of the 30 panelists used for the Cabernet Sauvignon wine aroma 'preference' ranking.

Accustomisation to paying attention to wine aroma during consumption			
unaccustomed	*moderately accustomed*	*well accustomed*	*very well accustomed*
0	14	9	7
Wine consumption frequency			
more frequently than weekly	*more frequently than monthly but less frequently than weekly*	*more frequently than yearly but less frequently than monthly*	*less frequently than yearly*
23	7	0	0
Age			
20 to 25 years	*26 to 30 years*	*31 to 40 years*	*41 to 60 years*
4	7	13	6

The questionnaire results for the Chardonnay and the Cabernet Sauvignon wine 'preference' panels indicate that most of the panelists considered themselves to be at least moderately accustomed to paying more than very brief attention to wine aroma during consumption, that most of them consumed wine more frequently than weekly, and that the largest proportion were aged 31 to 40 years. Health and medication consumption responses were acceptable.

The Chardonnay and the Cabernet Sauvignon wine 'preference' panels contained 16 and 17 males (of 30 panelists), respectively. All but one panelist, who was on the Cabernet Sauvignon wine 'preference' panel, considered their sense of smell to be at least 'average.'

Aroma descriptor– and standard–generation

The initial generation of a list of descriptive terms to be considered and refined comes from introspection and depends on the experiences of the panelists. Little can be done to optimise this process except, perhaps, to incorporate an adequate number of individuals for the variety of experience they provide. The use of 15 to 20 panelists for these experiments is adequate in this regard (King *et al.* 1995).

The aroma descriptor refinement and standard generation and refinement processes should allow a combination of individual introspection and group discussion. In this way, the importance of the individual sensory response and the translation of the sensory responses by the group, for sharing with a wider audience, is respected.

Following an initial occasion of descriptor generation using a representative selection of the experimental samples, the descriptors were compiled into a list together with the frequency of their use by the panelists. At the following one or two occasions, panelists were able to consider the suitability of the descriptors on the list with reference to other experimental samples. This process allowed and encouraged consideration of the popularity and groupings of the descriptors. For example, a number of descriptors representing the same sensory stimulus may have been individually unpopular but then adequately popular when combined under a single descriptor which was more suitable.

The first batch of standards was prepared following the first couple of descriptor generation/refinement sessions. They were presented to the panelists, along with a selection of experimental samples, for consideration. A process of standard modification, reconsideration and discussion followed, leading to agreements among the panelists and the experimenter for ten and twelve standards for the Chardonnay (Appx. Tab. D.11) and the Cabernet Sauvignon wines (Appx. Tab. D.12), respectively.

Where possible, natural or commonly available products were preferred for use as standards because the names of these products are more likely to invoke the aroma, in the memories of a large proportion of the audience, than are the names of purified compounds or obscure natural products.

Appendix Table D.11. Chardonnay wine aroma descriptive analysis standards.

Descriptor	Standard (representative of the aroma)
'preference'	not applicable
'coconut'	¼ teaspoon desiccated coconut (Anchor Foods Pty. Ltd., Australia)
'pencil shavings'	15 turns of A.W.FABER–CASTEL Goldfaber 1221 HB in pencil shaver
'caramel'	¼ teaspoon Golden Syrup (CSR Ltd., Pyrmont, Australia)
'vanilla'	20 mg/L vanillin (BDH Laboratory Supplies AnalaR) in water (20 mL)
'butter'	approx. ½ cm^3 piece of butter in a glass placed in hot tap water to melt
'allspice'	¼ teaspoon allspice powder (Master Foods of Australia)
'smoky'	1 freshly half–burnt match (Redhead matches, Australia)
'cashew nut'	1 roasted cashew nut, cut to small pieces and placed in approx. 20 mL water
'green apple'	approx. 1 cm^3 freshly cut Granny Smith apple
'cinnamon'	½ cm cinnamon stick (Ward McKenzie Pty. Ltd., Australia), cut to small pieces

All standards were presented in an XL5 wine tasting glass and covered with a lid.

Appendix Table D.12. Cabernet Sauvignon wine aroma descriptive analysis standards.

Descriptor	Standard (representative of the aroma)
'preference'	not applicable
'coconut'	¼ teaspoon desiccated coconut (Anchor Foods Pty. Ltd., Australia)
'pencil shavings'	15 turns of A.W.FABER–CASTEL Goldfaber 1221 HB in pencil shaver
'allspice'	¼ teaspoon allspice powder (Master Foods of Australia)
'berry'	½ teaspoon Cottee's Fruit of the forest Conserve jam (Cottee's Foods, Liverpool, Australia)*
'smoky'	1 freshly half–burnt match (Redhead matches, Australia)
'caramel'	¼ teaspoon Golden Syrup (CSR Ltd., Pyrmont, Australia)
'vanilla'	20 mg/L vanillin (BDH Laboratory Supplies AnalaR) in water (20 mL)
'coffee'	½ teaspoon Moccona freeze dried coffee, wetted with a couple of drops of water
'dark chocolate'	square of Cadbury Old Gold (dark) chocolate (Cadbury Confectionary, Tasmania, Australia)
'Band-aid'	1 Band-aid brand plastic strip (Johnson & Johnson Australia Pty. Ltd.), paper wrapper torn
'earthy'	garden soil with a few drops of water
'mint'	½ teaspoon dried mint (Spencers General Foods, Perth, Western Australia)

All standards were presented in an XL5 wine tasting glass, and covered with a lid.
* (containing boysenberries, strawberries & blueberries).

Appendix Table D.13. BIB design for 25 treatments: 30 blocks of 5 (6 fold)
(Yates 1970 p. 194)

B#[1]	block	B#	block	B#	block	B#	block	B#	block
1	*a b c d e*	7	*b g l q v*	13	*c i o p v*	19	*d f m t v*	25	*e h k s v*
2	*f g h i j*	8	*c h m r w*	14	*d j k q w*	20	*e g n p w*	26	*a j n r v*
3	*k l m n o*	9	*d i n s x*	15	*e f l r x*	21	*a i l t w*	27	*b f o s w*
4	*p q r s t*	10	*e j o t y*	16	*a h o q x*	22	*b j m p x*	28	*c g k t x*
5	*u v w x y*	11	*a g m s y*	17	*b i k r y*	23	*c f n q y*	29	*d h l p y*
6	*a f k p u*	12	*b h n t u*	18	*c j l s u*	24	*d g o r u*	30	*e i m q u*

[1] Block number
For each occasion, each of the 25 wines was randomly assigned a lower–case letter, from *a* to *y*.

D.3. Descriptions

Experimental design — Balanced Incomplete Block (BIB)

Meilgaard *et al.* (1991 p. 107) suggested that a person is unlikely to effectively rank more than around four to six items at a single sitting. For an experiment involving a large number of samples, a balanced incomplete block (BIB) design, which allows the ranking of subsets of the samples in a way that does not bias the outcome, can be used. An advantage of this type of design, according to McDaniel *et al.* (1987), is that it allows for the ranking of a small number of samples over a large number of occasions which can lead to more discriminatory results than if the number of samples was increased to reduce the occasion number.

The design chosen (Yates 1970 p. 194) allowed the samples to be ranked in blocks of five (Appx. Tab. D.13). When the design was triplicated, each sample was ranked with each of the other samples three times, and each was ranked a total of 18 times. Ninety subsets (blocks) of five were taken from the 25 wines (24 barrels plus 1 stainless steel drum) for each aroma ranking. Each of 15 panelist received two sets of five wines from each of the three repetitions, at random. The order of presentation within each set (120 permutations) was also determined randomly.

One 375 mL crown sealed bottle of each of the 25 wines was used. Eighteen glasses were required for each wine. Thus, 25 groups of 18 (450) glasses were assembled on a bench. The trays were made–up from these batches, with reference to a worksheet, and the identifying glass numbers were entered on the worksheet.

The ranking procedure

A standard was prepared freshly each day. Before entering an isolation booth, each panelist smelled the standard, and committed it to memory. The booth could be left at any time to re–smell the standard. Each panelist ranked six sets of five wines, according to a single aroma attribute, at each sitting. It is important that the ranking process not be overly demanding of the panelists' time, senses or concentration capacity. Limiting each session to the consideration of only one aroma helped to minimise these demands.

The panelists were provided with a tray containing three sets of five wines, and a glass of odourless water for refreshment. They were instructed to smell (not taste) the first set of five wines, and to rank them from lowest to highest, according to the standard smelled outside of the booth. They were, further, advised that the sniffing and ranking procedure may require numerous repetitions before each could be confident in their response. On completion of the first set, the second and then the third set were similarly ranked. On completion of the first tray, it was removed by the experimenter and a second tray of three sets of five wines was presented for ranking in the same manner. When samples were difficult to separate, the panelists were required to make a best guess; ties were not allowed.

Determining what significant differentiation was achieved

To test whether significant differentiation among the wines was achieved for each aroma, a Friedman–type statistic (Meilgaard *et al.* 1991 p. 264) was calculated using the rank sums.

When significant differentiation was achieved, the samples were separated according to a multiple comparison procedure. The non–parametric analogue to Fisher's least significant difference (LSD) for rank sums from a BIB design, as described in Meilgaard *et al.* (1991 p. 264), was performed. For these experiments, the parameters appropriate to the analyses were as follows.

t = number of samples in the experiment = 25
k = number of samples in each block = 5
r = number of times each sample was evaluated in a single repetition of the design = 6
λ = number of times each pair was evaluated in a single repetition of the design = 1
b = number of blocks required to complete a single repetition of the design = 30
p = number of repetitions of the design = 3

The ranking repeatability

No absolute reference–anchored scale is used in ranking; perceived intensity is only measured relative to the other samples. For confidence to be assigned to the data, one must test for the repeatability of the ranking for each sample relative to the other samples. This sort of test is intrinsic to a method generating rank sums. Each wine is ranked numerous times so, for differentiation to be significant, some of the samples must be repeatedly ranked lowly or highly. To further test the repeatability of some of the rankings, however, some full repetitions may be performed, and each pair of ranks compared by Spearman's rank correlation calculation.

Although not a requirement of the method, six of the ten Chardonnay wine ranking occasions and five of the twelve Cabernet Sauvignon wine ranking occasions were repeated so that an estimation of ranking repeatability could be made via inter–occasion correlation. It was appropriate to use a one–tailed test since the direction of the correlation (positive) was predicted. Some of the Chardonnay wine sample stocks had become depleted so smaller sets (21 or 16 of the 25) were ranked for these wines. See Appendix D.5 for details.

The repeat occasion Cabernet Sauvignon wine rankings were of full sets of the samples, so they were incorporated into the Friedman–type statistic calculations. The rank sums for the two occasions were simply summed, and p, the number of repeats of the fundamental design, was changed from 3 to 6 for the calculation. The ranks and, therefore, the rank transformations were also based on the combined set of data for the five repeated descriptors. The repeat occasion Chardonnay rankings, however, being subsets of the samples, were not incorporated into these calculations as doing so would leave the design unbalanced.

D.4. Data analysis

When using non–parametric data such as ranks, some forms of data analysis either do not exist or are not commonly available in the form of statistical software packages. A transformation of the data, using the Fisher–Yates rank transformation, may help to overcome this problem by making them amenable to parametric analysis. The transformation assumes that the rank sums can be considered percentage points along a standard normal distribution (Fisher and Yates 1963 p. 94).

The rank sums were unsuitable for analysis beyond that used to generate the data in Tables 3.1 and 3.3. Consequently, they were transformed to ranks and to Fisher–Yates rank transformations. Separate transformations were required for each subset of samples to be analysed. Thus, they were obtained for the data for (1) the set of 6 American oak barrel wines, (2) the set of 12 Australia seasoned/coopered barrel wines, (3) the set of 18 French oak barrel wines, and (4) the full set of 24 barrel wines (Appx. E). The ranks were used in Spearman's rank correlation calculations, and the Fisher–Yates rank transformations were used in a variety of parametric data analyses, particularly analysis of variance and principal components analysis (PCA).

The single– and two–factor analyses of variance (ANOVAs) were performed using Microsoft Excel V5.0 spreadsheet software. The three–factor ANOVAs were performed using SYSTAT V5.0 (SYSTAT, Inc.) statistical software. The variables were treated as 'fixed' and factorial ANOVAs were performed (Kirby 1993 pp. 279–323). 95 % Confidence intervals were calculated using individual cell variances (Kirby 1993 pp. 269–270). The PCAs were also performed using SYSTAT V5.0 software, using the Pearson's product–moment correlation matrix and varimax rotation. The scree test, described by Cattell (1966), was used to determine the number of factors to retain in each PCA. Then, the PCA output was adapted according to Broschat (1979) and Federer *et al.* (1987).

D.5. Oddities

Overcoming the shortfall in stocks for 'cinnamon' and 'green apple' in the Chardonnay wine

AA10 and *FV3* samples were not available for testing the 'green apple' and 'cinnamon' descriptors due to depletion of stocks. *AA22* and *FV4* were of the same treatment as *AA10* and *FV3*, respectively, and neither pair was significantly different, according to triangle difference test ($p<0.05$) (Appx. Tab. D.1). Consequently, two *AA22* samples and two *FV4* samples were used. The mean rank sum obtained for the two *AA22* samples was applied to both *AA22* and *AA10*. Similarly, the mean rank sum obtained for the two *FV4* samples was applied to both *FV4* and *FV3*. These schemes are detailed in Appendix Table D.14.

BIB designs and procedures for the Chardonnay wine repeat rankings

While the BIB design had to be changed to accommodate 21 or 16 wines (Appx. Tabs. D.15 & D.16), the sensory protocol remained virtually unchanged. Appendix Table D.17 details the samples omitted from the repeat occasion rankings due to depletion of the Chardonnay wine stocks. The matching wines from the first occasion were assigned new ranks, using only those samples in the new subset and then tested for Spearman's correlation with the repeat set rankings. 'Coconut' was repeated a second time.

For the 21 sample design (Appx. Tab. D.15), 14 panelists ranked 6 sets of 5 wines each, giving a total of 84 sets of 5 wines. Thus, each of the 21 wines (4 omitted) was ranked 20 times. For the 16 sample design (Appx. Tab. D.16), 16 panelists ranked 5 sets of 4 wines each, giving a total of 80 sets of 4 wines. Thus, each of the 16 wines (9 omitted) was ranked 20 times.

It is possible to calculate the significance of any differences for these new designs but this was not done since only the rankings were of interest. The full designs (all 25 wines) were used to calculate any significance of difference; the new design results were used only to check the repeatability of ranking (by correlation between occasions). Appendix Table D.18 details these correlation analysis results.

Appendix Table D.14. 'Green apple' and 'cinnamon' rank sums.

See text (Appx. D.5) for an explanation.

green apple			
rank sums obtained		rank sums used	
control	73	*control*	73
LA27	70	*LA27*	70
VA39	69	*VA39*	69
FL3	67	*FL3*	67
LA41	62	*LA41*	62
LA34	59	*LA34*	59
AU6	59	*AU6*	59
FT5	58	*FT5*	58
FV4	58	*VA32*	57
VA32	57	*FL5*	55
FL5	55	*TA31*	55
TA31	55	*TA46*	55
TA46	55	*FV3*	55
AA22	55	*FV4*	55
VA38	54	*VA38*	54
FV4	52	*AA10*	51.5
AU70	51	*AA22*	51.5
AA22	48	*AU70*	51
FT4	46	*FT4*	46
FV5	45	*FV5*	45
FT3	44	*FT3*	44
AU4	41	*AU4*	41
AA11	41	*AA11*	41
FL4	39	*FL4*	39
TA23	37	*TA23*	37

cinnamon			
rank sums obtained		rank sums used	
TA46	65	*TA46*	65
AU6	61	*AU6*	61
LA34	60	*LA34*	60
LA41	60	*LA41*	60
AA11	60	*AA11*	60
FL4	59	*FL4*	59
FT4	59	*FT4*	59
VA32	59	*VA32*	59
FL5	58	*FL5*	58
FT5	58	*FT5*	58
FV4	58	*FL3*	57
AA22	58	*TA23*	57
FL3	57	*AU4*	57
TA23	57	*FV3*	56
AU4	57	*FV4*	56
VA38	55	*VA38*	55
FV4	54	*VA39*	53
VA39	53	*AA10*	52.5
AU70	52	*AA22*	52.5
FV5	51	*AU70*	52
FT3	50	*FV5*	51
AA22	47	*FT3*	50
LA27	44	*LA27*	44
TA31	33	*TA31*	33
control	25	*control*	25

Appendix Table D.15. BIB design for 21 treatments: 21 blocks of 5 (5 fold)

(Yates 1970 p. 193)

B#[1]	block	B#	block	B#	block	B#	block	B#	block
1	*a b c d e*	5	*a r s t u*	9	*b i m q u*	13	*c i j p s*	17	*d i l o r*
2	*a f g h i*	6	*b f j n r*	10	*c f m o t*	14	*d f k p u*	18	*e f l q s*
3	*a j k l m*	7	*b g k o s*	11	*c g l n u*	15	*d g j q t*	19	*e g m p r*
4	*a n o p q*	8	*b h l p t*	12	*c h k q r*	16	*d h m n s*	20	*e h j o u*
								21	*e i k n t*

[1] Block number

Appendix Table D.16. BIB design for 16 treatments: 20 blocks of 4 (5 fold)

(Yates 1970 p. 194)

B#[1]	block	B#	block	B#	block	B#	block	B#	block
1	*a b c d*	5	*a e i m*	9	*a f k p*	13	*a h j o*	17	*a g l n*
2	*e f g h*	6	*b f j n*	10	*b g l m*	14	*b e k p*	18	*b h i o*
3	*i j k l*	7	*c g k o*	11	*c h i n*	15	*c f l m*	19	*c e j p*
4	*m n o p*	8	*d h l p*	12	*d e j o*	16	*d g i n*	20	*d f k m*

[1] Block number

Appendix Table D.17. Samples omitted (X) from the Chardonnay wine repeat occasion rankings due to depletion of stocks.

Barrel code	coconut #2	coconut #3	vanilla #2	butter #2	pencil shavings #2	green apple #2	smoky #2
control							
AU4		X	X		X		
AU6			X				X
AU70		X	X				
AA10	X	X	X	X	X	X	X
AA11	X	X	X				
AA22			X				
FL3							
FL4							
FL5							
LA27							
LA34					X		
LA41	X	X	X	X	X	X	X
FT3							
FT4				X			
FT5		X		X			
TA23		X		X	X		
TA31					X		
TA46							
FV3	X	X	X	X	X	X	X
FV4				X	X	X	
FV5			X				
VA32							
VA38				X			
VA39		X		X	X		
n	21	16	16	16	16	21	21

Appendix Table D.18. Chardonnay wine aroma ranking inter–occasion correlations.

Comparison		*n*	rho	Significance[†]
coconut #1	coconut #2	21	0.346	
coconut #1	coconut #3	16	0.510	$p<0.05^{/}$
coconut #2	coconut #3	16	0.575	
vanilla #1	vanilla #2	16	0.499	$p<0.05$
penc shavs #1	penc shavs #2	16	0.099	n.s. ($p>0.10$)
butter #1	butter #2	16	0.742	$p<0.001$
grn apple #1	grn apple #2	21	0.458	$p<0.05$
smoky #1	smoky #2	21	0.791	$p<0.001$

†: Significance determined with reference to a Table in O'Mahony (1986 p. 458).
/: Mean of rho (0.477) used.

Appendix E

Wine aroma ranks and Fisher-Yates rank transformations

For both the Chardonnay and the Cabernet Sauvignon wines, analyses were performed separately on ...
(1): the set of 6 American oak barrel wines
(2): the set of 12 Australia seasoned/coopered barrel wines
(3): the set of 18 French oak barrel wines
(4): the full set of 24 barrel wines
... so ranks and Fisher-Yates rank transformations for these subsets were prepared separately.

Rank sums were converted to ranks and ties were given mean ranks.
These ranks were used in Spearman's rank correlation calculations.

The Fisher-Yates rank transformations were used in Pearson's correlation calculations, ANOVAs, and PC analyses.

Higher rank number or transformation indicates higher intensity.

The Fisher-Yates rank transformations used for these subsets are listed in Appendix Table E.1.
Ties were allocated means.

Appendix Table E.1. Fisher-Yates rank transformation values.

All barrels	All French oak	All Australia seasoning	All American oak
-1.95	-1.82	-1.63	-1.27
-1.5	-1.35	-1.12	-0.64
-1.24	-1.07	-0.79	-0.2
-1.04	-0.85	-0.54	0.2
-0.88	-0.67	-0.31	0.64
-0.73	-0.5	-0.1	1.27
-0.6	-0.35	0.1	
-0.48	-0.21	0.31	
-0.37	-0.07	0.54	
-0.26	0.07	0.79	
-0.16	0.21	1.12	
-0.05	0.35	1.63	
0.05	0.5		
0.16	0.67		
0.26	0.85		
0.37	1.07		
0.48	1.35		
0.6	1.82		
0.73			
0.88			
1.04			
1.24			
1.5			
1.95			

Reference:
Fisher R.A., Yates F. 1963 Statistical Tables for Biological, Agricultural and Medical Research. Longman, Edinburgh, U.K. p. 94.

Appendix Table E.2. American oak-barrel Chardonnay wine aroma ranks.

barrel	preference	coconut	pene shavs	caramel	vanilla	butter	allspice	smoky	cashew nut	green apple	cinnamon
AU4	6	4	2.5	3.5	5.5	4	4	5	5	1.5	4
AU6	1	1.5	5	3.5	1	6	3	2.5	3	6	6
AU70	3	5	4	5	2	5	2	2.5	1	3	1
AA10	5	3	2.5	2	3.5	2	5	4	6	4.5	2.5
AA11	4	1.5	6	1	5.5	1	6	6	2	1.5	5
AA22	2	6	1	6	3.5	3	1	1	4	4.5	2.5

Appendix Table E.3. American oak-barrel Chardonnay aroma rank transformations.

barrel	preference	coconut	pene shavs	caramel	vanilla	butter	allspice	smoky	cashew nut	green apple	cinnamon
AU4	1.27	0.2	-0.42	0	0.955	0.2	0.2	0.64	0.64	-0.955	0.2
AU6	-1.27	-0.955	0.64	0	-1.27	1.27	-0.2	-0.42	-0.2	1.27	1.27
AU70	-0.2	0.64	0.2	0.64	-0.64	0.64	-0.64	-0.42	-1.27	-0.2	-1.27
AA10	0.64	-0.2	-0.42	-0.64	0	-0.64	0.64	0.2	1.27	0.42	-0.42
AA11	0.2	-0.955	1.27	-1.27	0.955	-1.27	1.27	1.27	-0.64	-0.955	0.64
AA22	-0.64	1.27	-1.27	1.27	0	-0.2	-1.27	-1.27	0.2	0.42	-0.42

Appendix Table E.4. Australia seasoned and coopered barrel Chardonnay wine aroma ranks.

barrel	preference	coconut	pene shavs	caramel	vanilla	butter	allspice	smoky	cashew nut	green apple	cinnamon
AA10	6	2	2	11	4.5	5	7	6	8	3.5	3.5
AA11	5	1	4	10	9	4	11	9.5	1	2	10
AA22	2.5	9	1	12	4.5	10	1	3	4	3.5	3.5
LA27	1	3	6.5	8.5	1	1	7	1	7	12	2
LA34	8	6	10	8.5	9	9	3.5	11	10	9	10
LA41	2.5	4.5	3	4	11	11.5	3.5	4.5	5	10	10
TA23	4	4.5	9	5	2	2	10	12	9	1	7
TA31	9.5	8	5	7	9	11.5	5	4.5	2.5	6.5	1
TA46	9.5	7	11	6	12	6.5	12	9.5	11	6.5	12
VA32	7	10	8	2	6.5	6.5	7	8	6	8	8
VA38	12	11	12	3	3	3	2	7	12	5	6
VA39	11	12	6.5	1	6.5	8	9	2	2.5	11	5

Appendix Table E.5. Australia seasoned and coopered barrel Chardonnay aroma rank transformations.

barrel	preference	coconut	pene shavs	caramel	vanilla	butter	allspice	smoky	cashew nut	green apple	cinnamon
AA10	-0.1	-1.12	-1.12	1.12	-0.425	-0.31	0.1033	-0.1	0.31	-0.665	-0.665
AA11	-0.31	-1.63	-0.54	0.79	0.5467	-0.54	1.12	0.665	-1.63	-1.12	0.8167
AA22	-0.955	0.54	-1.63	1.63	-0.425	0.79	-1.63	-0.79	-0.54	-0.665	-0.665
LA27	-1.63	-0.79	0	0.425	-1.63	-1.63	0.1033	-1.63	0.1	1.63	-1.12
LA34	0.31	-0.1	0.79	0.425	0.5467	0.54	-0.665	1.12	0.79	0.54	0.8167
LA41	-0.955	-0.425	-0.79	-0.54	1.12	1.375	-0.665	-0.425	-0.31	0.79	0.8167
TA23	-0.54	-0.425	0.54	-0.31	-1.12	-1.12	0.79	1.63	0.54	-1.63	0.1
TA31	0.665	0.31	-0.31	0.1	0.5467	1.375	-0.31	-0.425	-0.955	0	-1.63
TA46	0.665	0.1	1.12	-0.1	1.63	0	1.63	0.665	1.12	0	1.63
VA32	0.1	0.79	0.31	-1.12	0	0	0.1033	0.31	-0.1	0.31	0.31
VA38	1.63	1.12	1.63	-0.79	-0.79	-0.79	-1.12	0.1	1.63	-0.31	-0.1
VA39	1.12	1.63	0	-1.63	0	0.31	0.54	-1.12	-0.955	1.12	-0.31

Appendix Table E.6. French oak barrel Chardonnay wine aroma ranks.

barrel	preference	coconut	pene shavs	caramel	vanilla	butter	allspice	smoky	cashew nut	green apple	cinnamon
FL3	3	15	3.5	13.5	7.5	4	8.5	3	16	16	9.5
FL4	16	10	12	17	17	2	17.5	15	15	2	14
FL5	1.5	1	18	1	1	13	4.5	12	10.5	9	11.5
LA27	1.5	7	6	17	2	4	10.5	1	9	18	2
LA34	12	11	13.5	17	14.5	16	6.5	17	13	14	16.5
LA41	4	8.5	1	6.5	16	17.5	6.5	4.5	6.5	15	16.5
FT3	5	2	15.5	3	9	4	3	16	1	3	3
FT4	6.5	6	6	11	7.5	9	1	10.5	2	5	14
FT5	8	3	13.5	13.5	3.5	10	4.5	10.5	6.5	13	11.5
TA23	6.5	8.5	10	8.5	3.5	6	16	18	12	1	9.5
TA31	13.5	14	3.5	13.5	14.5	17.5	8.5	4.5	3.5	9	1
TA46	13.5	12	15.5	10	18	11.5	17.5	14	14	9	18
FV3	10.5	13	2	6.5	11	14	13	6	17	9	7.5
FV4	18	4.5	9	13.5	13	7	14	7.5	10.5	9	7.5
FV5	9	4.5	11	8.5	5	1	15	13	5	4	4
VA32	10.5	16	8	4	11	11.5	10.5	9	8	12	14
VA38	17	17	17	5	6	8	2	7.5	18	6	6
VA39	15	18	6	2	11	15	12	2	3.5	17	5

Appendix Table E.7. French oak barrel Chardonnay aroma rank transformations.

barrel	preference	coconut	pene shavs	caramel	vanilla	butter	allspice	smoky	cashew nut	green apple	cinnamon
FL3	-1.07	0.85	-0.96	0.5925	-0.28	-0.863	-0.14	-1.07	1.07	1.07	0
FL4	1.07	0.07	0.35	1.4133	1.35	-1.35	1.585	0.85	0.85	-1.35	0.6733
FL5	-1.585	-1.82	1.82	-1.82	-1.82	0.5	-0.76	0.35	0.14	-0.07	0.28
LA27	-1.585	-0.35	-0.507	1.4133	-1.35	-0.863	0.14	-1.82	-0.07	1.82	-1.35
LA34	0.35	0.21	0.585	1.4133	0.76	1.07	-0.425	1.35	0.5	0.67	1.21
LA41	-0.85	-0.14	-1.82	-0.425	1.07	1.585	-0.425	-0.76	-0.425	0.85	1.21
FT3	-0.67	-1.35	0.96	-1.07	-0.07	-0.863	-1.07	1.07	-1.82	-1.07	-1.07
FT4	-0.425	-0.5	-0.507	0.21	-0.28	-0.07	-1.82	0.14	-1.35	-0.67	0.6733
FT5	-0.21	-1.07	0.585	0.5925	-0.96	0.07	-0.76	0.14	-0.425	0.5	0.28
TA23	-0.425	-0.14	0.07	-0.14	-0.96	-0.5	1.07	1.82	0.35	-1.82	0
TA31	0.585	0.67	-0.96	0.5925	0.76	1.585	-0.14	-0.76	-0.96	-0.07	-1.82
TA46	0.585	0.35	0.96	0.07	1.82	0.28	1.585	0.67	0.67	-0.07	1.82
FV3	0.14	0.5	-1.35	-0.425	0.21	0.67	0.5	-0.5	1.35	-0.07	-0.28
FV4	1.82	-0.76	-0.07	0.5925	0.5	-0.35	0.67	-0.28	0.14	-0.07	-0.28
FV5	-0.07	-0.76	0.21	-0.14	-0.67	-1.82	0.85	0.5	-0.67	-0.85	-0.85
VA32	0.14	1.07	-0.21	-0.85	0.21	0.28	0.14	-0.07	-0.21	0.35	0.6733
VA38	1.35	1.35	1.35	-0.67	-0.5	-0.21	-1.35	-0.28	1.82	-0.5	-0.5
VA39	0.85	1.82	-0.507	-1.35	0.21	0.85	0.35	-1.35	-0.96	1.35	-0.67

Appendix Table E.8. All oak barrel Chardonnay wine aroma ranks.

barrel	preference	coconut	pene shavs	caramel	vanilla	butter	allspice	smoky	cashew nut	green apple	cinnamon
AU4	17	9	2.5	21.5	19.5	21	12	18	13	3.5	13
AU6	1	3.5	6.5	21.5	5	24	5.5	6.5	4.5	19.5	23
AU70	8	10.5	5	23	7.5	23	3	6.5	1	8	5
AA10	14	5.5	2.5	20	12	12	15	9.5	16	9.5	6.5
AA11	13	3.5	8	19	19.5	9.5	22	19.5	3	3.5	21
AA22	5.5	21	1	24	12	19	2	3	8	9.5	6.5
FL3	4	20	9.5	13.5	9.5	4	12	4	22	22	13
FL4	22	15	18	17	23	2	23.5	21	21	2	18
FL5	2.5	1	24	1	1	15	7.5	16	16	14	15.5
LA27	2.5	12	12	17	2	4	15	1	14	24	2
LA34	18	16	19.5	17	19.5	18	9.5	23	19	19.5	21
LA41	5.5	13.5	4	6.5	22	21	9.5	6.5	10.5	21	21
FT3	7	2	21.5	3	12	4	5.5	22	2	5	3
FT4	9.5	10.5	12	11	9.5	9.5	1	14.5	4.5	7	18
FT5	11	5.5	19.5	13.5	3.5	11	7.5	14.5	10.5	18	15.5
TA23	9.5	13.5	16	8.5	3.5	6	21	24	18	1	13
TA31	19.5	19	9.5	13.5	19.5	21	12	6.5	6.5	14	1
TA46	19.5	17	21.5	10	24	13.5	23.5	19.5	20	14	24
FV3	15.5	18	6.5	6.5	15	16	18	9.5	23	14	10.5
FV4	24	7.5	15	13.5	17	7	19	11.5	16	14	10.5
FV5	12	7.5	17	8.5	6	1	20	17	9	6	4
VA32	15.5	22	14	4	15	13.5	15	13	12	17	18
VA38	23	23	23	5	7.5	8	4	11.5	24	11	9
VA39	21	24	12	2	15	17	17	2	6.5	23	8

Appendix Table E.9. All oak barrel Chardonnay aroma rank transformations.

barrel	preference	coconut	pene shavs	caramel	vanilla	butter	allspice	smoky	cashew nut	green apple	cinnamon
AU4	0.48	-0.37	-1.37	1.14	0.8125	1.0533	-0.053	0.6	0.05	-1.14	0.0533
AU6	-1.95	-1.14	-0.665	1.14	-0.88	1.95	-0.805	-0.673	-0.96	0.805	1.5
AU70	-0.48	-0.21	-0.88	1.5	-0.54	1.5	-1.24	-0.673	-1.95	-0.48	-0.88
AA10	0.16	-0.805	-1.37	0.88	-0.053	-0.05	0.2633	-0.315	0.37	-0.315	-0.665
AA11	0.05	-1.14	-0.48	0.73	0.8125	-0.315	1.24	0.805	-1.24	-1.14	1.0533
AA22	-0.805	1.04	-1.95	1.95	-0.053	0.73	-1.5	-1.24	-0.48	-0.315	-0.665
FL3	-1.04	0.88	-0.315	0.105	-0.315	-1.053	-0.053	-1.04	1.24	1.24	0.0533
FL4	1.24	0.26	0.6	0.4833	1.5	-1.5	1.725	1.04	1.04	-1.5	0.6033
FL5	-1.37	-1.95	1.95	-1.95	-1.95	0.26	-0.54	0.37	0.37	0.158	0.315
LA27	-1.37	-0.05	-0.053	0.4833	-1.5	-1.053	0.2633	-1.95	0.16	1.95	-1.5
LA34	0.6	0.37	0.805	0.4833	0.8125	0.6	-0.315	1.5	0.73	0.805	1.0533
LA41	-0.805	0.105	-1.04	-0.665	1.24	1.0533	-0.315	-0.673	-0.21	1.04	1.0533
FT3	-0.6	-1.5	1.14	-1.24	-0.053	-1.053	-0.805	1.24	-1.5	-0.88	-1.24
FT4	-0.315	-0.21	-0.053	-0.16	-0.315	-0.315	-1.95	0.21	-0.96	-0.6	0.6033
FT5	-0.16	-0.805	0.805	0.105	-1.14	-0.16	-0.54	0.21	-0.21	0.6	0.315
TA23	-0.315	0.105	0.37	-0.425	-1.14	-0.73	1.04	1.95	0.6	-1.95	0.0533
TA31	0.805	0.73	-0.315	0.105	0.8125	1.0533	-0.053	-0.673	-0.665	0.158	-1.95
TA46	0.805	0.48	1.14	-0.26	1.95	0.105	1.725	0.805	0.88	0.158	1.95
FV3	0.315	0.6	-0.665	-0.665	0.2633	0.37	0.6	-0.315	1.5	0.158	-0.21
FV4	1.95	-0.54	0.26	0.105	0.48	-0.6	0.73	-0.105	0.37	0.158	-0.21
FV5	-0.05	-0.54	0.48	-0.425	-0.73	-1.95	0.88	0.48	-0.37	-0.73	-1.04
VA32	0.315	1.24	0.16	-1.04	0.2633	0.105	0.2633	0.05	-0.05	0.48	0.6033
VA38	1.5	1.5	1.5	-0.88	-0.54	-0.48	-1.04	-0.105	1.95	-0.16	-0.37
VA39	1.04	1.95	-0.053	-1.5	0.2633	0.48	0.48	-1.5	-0.665	1.5	-0.48

Appendix Table E.10. American oak barrel Cabernet Sauvignon wine aroma ranks.

barrel	preference	coconut	penc shav	allspice	berry	smoky	caramel	vanilla	coffee	dark choc	Band-aid	earthy	mint
AU7	6	6	1.5	2	6	1	4.5	6	4	6	1	1	6
AU8	2	2	5	1	5	6	3	4	3	4	4	2	2
AU9	3.5	1	1.5	6	2	3	2	2	1	1.5	3	5	4
AA36	1	3	3.5	3.5	1	5	1	1	2	1.5	6	6	4
AA40	3.5	5	6	3.5	4	2	4.5	3	5	5	2	4	1
AA48	5	4	3.5	5	3	4	6	5	6	3	5	3	4

Appendix Table E.11. American oak barrel Cabernet Sauvignon wine aroma rank transformations.

barrel	preference	coconut	penc shav	allspice	berry	smoky	caramel	vanilla	coffee	dark choc	Band-aid	earthy	mint
AU7	1.27	1.27	-0.96	-0.64	1.27	-1.27	0.42	1.27	0.2	1.27	-1.27	-1.27	1.27
AU8	-0.64	-0.64	0.64	-1.27	0.64	1.27	-0.2	0.2	-0.2	0.2	0.2	-0.64	-0.64
AU9	0	-1.27	-0.96	1.27	-0.64	-0.2	-0.64	-0.64	-1.27	-0.96	-0.2	0.64	0.213
AA36	-1.27	-0.2	0	0	-1.27	0.64	-1.27	-1.27	-0.64	-0.96	1.27	1.27	0.213
AA40	0	0.64	1.27	0	0.2	-0.64	0.42	-0.2	0.64	0.64	-0.64	0.2	-1.27
AA48	0.64	0.2	0	0.64	-0.2	0.2	1.27	0.64	1.27	-0.2	0.64	-0.2	0.213

Appendix Table E.12. Australia seasoned and coopered barrel Cabernet Sauvignon wine aroma ranks.

barrel	preference	coconut	penc shav	allspice	berry	smoky	caramel	vanilla	coffee	dark choc	Band-aid	earthy	mint
AA36	1.5	1	1.5	1.5	1	5	1	1	1	1	10	12	4.5
AA40	3	4.5	7	1.5	4	1	4.5	2.5	2	4	3	8	1.5
AA48	5	2	1.5	3	2.5	4	6.5	5	3	2.5	8	6	4.5
LA23	1.5	4.5	3.5	7	6.5	12	3	4	12	5	12	11	6
LA30	4	6	10	10	8	6	11	10	4	9	1	5	7.5
LA38	12	3	11	8.5	10	11	8	2.5	8	6.5	11	9	9
TA8	11	9	9	5	6.5	3	4.5	7	9	6.5	9	1	3
TA25	10	12	3.5	8.5	12	2	9.5	11	7	10.5	6	3	12
TA39	8.5	7	8	11	2.5	9	9.5	6	10	8	2	10	1.5
VA12	6	10	5	4	5	10	12	9	11	10.5	6	7	10
VA21	8.5	11	6	12	11	8	2	12	5	12	6	4	7.5
VA27	7	8	12	6	9	7	6.5	8	6	2.5	4	2	11

Appendix Table E.13. Australia seasoned and coopered barrel Cabernet Sauvignon aroma rank transformations.

barrel	preference	coconut	penc shav	allspice	berry	smoky	caramel	vanilla	coffee	dark choc	Band-aid	earthy	mint
AA36	-1.38	-1.63	-1.38	-1.38	-1.63	-0.31	-1.63	-1.63	-1.63	-1.63	0.79	1.63	-0.43
AA40	-0.79	-0.43	0.1	-1.38	-0.54	-1.63	-0.43	-0.96	-1.12	-0.54	-0.79	0.31	-1.38
AA48	-0.31	-1.12	-1.38	-0.79	-0.96	-0.54	0	-0.31	-0.79	-0.96	0.31	-0.1	-0.43
LA23	-1.38	-0.43	-0.67	0.1	0	1.63	-0.79	-0.54	1.63	-0.31	1.63	1.12	-0.1
LA30	-0.54	-0.1	0.79	0.79	0.31	-0.1	1.12	0.79	-0.54	0.54	-1.63	-0.31	0.205
LA38	1.63	-0.79	1.12	0.425	0.79	1.12	0.31	-0.96	0.31	0	1.12	0.54	0.54
TA8	1.12	0.54	0.54	-0.31	0	-0.79	-0.43	0.1	0.54	0	0.54	-1.63	-0.79
TA25	0.79	1.63	-0.67	0.425	1.63	-1.12	0.665	1.12	0.1	0.955	-0.1	-0.79	1.63
TA39	0.425	0.1	0.31	1.12	-0.96	0.54	0.665	-0.1	0.79	0.31	-1.12	0.79	-1.38
VA12	-0.1	0.79	-0.31	-0.54	-0.31	0.79	1.63	0.54	1.12	0.955	-0.1	0.1	0.79
VA21	0.425	1.12	-0.1	1.63	1.12	0.31	-1.12	1.63	-0.31	1.63	-0.1	-0.54	0.205
VA27	0.1	0.31	1.63	-0.1	0.54	0.1	0	0.31	-0.1	-0.96	-0.54	-1.12	1.12

Appendix Table E.14.

French oak barrel Cabernet Sauvignon wine aroma ranks.

barrel	preference	coconut	pene shav	allspice	berry	smoky	caramel	vanilla	coffee	dark choc	Band-aid	earthy	mint
NL6	3	1	16	11	3	3	1	2	2	3	11	15.5	17
NL7	1	2	17	3	1	9.5	3	5	4	2	11	11	9.5
NL8	7	3	6	7.5	2	13	10.5	1	8	6	17	15.5	9.5
LA23	2	5	3.5	11	9	18	6	4	18	7.5	18	18	7.5
LA30	5.5	6	12	16	11	11	14	16	7	13	3	7.5	11.5
LA38	18	4	13.5	13.5	13	17	10.5	3	14	10.5	15	12.5	14
NT6	5.5	7	13.5	1	7	9.5	4	6	11.5	17.5	8	6	5
NT7	10.5	18	1	11	16.5	2	16	8.5	5	5	2	3	4
NT8	9	9	15	2	4	8	2	8.5	3	1	16	14	6
TA8	17	11	10	7.5	9	7	7.5	11	15	10.5	14	1	3
TA25	16	17	3.5	13.5	18	6	12.5	17	13	14.5	11	4	18
TA39	13.5	8	9	17	5	15	12.5	10	16	12	5.5	17	2
NV6	8	13	11	4	9	4	7.5	7	1	7.5	1	12.5	13
NV7	15	14.5	8	15	14	5	15	13	6	16	5.5	10	1
NV8	4	16	2	5.5	16.5	1	17	12	10	9	4	7.5	7.5
VA12	10.5	12	5	5.5	6	16	18	15	17	14.5	11	9	15
VA21	13.5	14.5	7	18	15	14	5	18	9	17.5	11	5	11.5
VA27	12	10	18	9	12	12	9	14	11.5	4	7	2	16

Appendix Table E.15.

French oak barrel Cabernet Sauvignon aroma rank transformations.

barrel	preference	coconut	pene shav	allspice	berry	smoky	caramel	vanilla	coffee	dark choc	Band-aid	earthy	mint
NL6	-1.07	-1.82	1.07	0.21	-1.07	-1.07	-1.82	-1.35	-1.35	-1.07	0.212	0.96	1.35
NL7	-1.82	-1.35	1.35	-1.07	-1.82	0	-1.07	-0.67	-0.85	-1.35	0.212	0.21	0
NL8	-0.35	-1.07	-0.5	-0.28	-1.35	0.5	0.14	-1.82	-0.21	-0.5	1.35	0.96	0
LA23	-1.35	-0.67	-0.96	0.21	-0.07	1.82	-0.5	-0.85	1.82	-0.28	1.82	1.82	-0.28
LA30	-0.59	-0.5	0.35	1.07	0.21	0.21	0.67	1.07	-0.35	0.5	-1.07	-0.28	0.28
LA38	1.82	-0.85	0.585	0.585	0.5	1.35	0.14	-1.07	0.67	0.14	0.85	0.425	0.67
NT6	-0.59	-0.35	0.585	-1.82	-0.35	0	-0.85	-0.5	0.28	1.585	-0.21	-0.5	-0.67
NT7	0.14	1.82	-1.82	0.21	1.21	-1.35	1.07	-0.14	-0.67	-0.67	-1.35	-1.07	-0.85
NT8	-0.07	-0.07	0.85	-1.35	-0.85	-0.21	-1.35	-0.14	-1.07	-1.82	1.07	0.67	-0.5
TA8	1.35	0.21	0.07	-0.28	-0.07	-0.35	-0.28	0.21	0.85	0.14	0.67	-1.82	-1.07
TA25	1.07	1.35	-0.96	0.585	1.82	-0.5	0.425	1.35	0.5	0.76	0.212	-0.85	1.82
TA39	0.585	-0.21	-0.07	1.35	-0.67	0.85	0.425	0.07	1.07	0.35	-0.59	1.35	-1.35
NV6	-0.21	0.5	0.21	-0.85	-0.07	-0.85	-0.28	-0.35	-1.82	-0.28	-1.82	0.425	0.5
NV7	0.85	0.76	-0.21	0.85	0.67	-0.67	0.85	0.5	-0.5	1.07	-0.59	0.07	-1.82
NV8	-0.85	1.07	-1.35	-0.59	1.21	-1.82	1.35	0.35	0.07	-0.07	-0.85	-0.28	-0.28
VA12	0.14	0.35	-0.67	-0.59	-0.5	1.07	1.82	0.85	1.35	0.76	0.212	-0.07	0.85
VA21	0.585	0.76	-0.35	1.82	0.85	0.67	-0.67	1.82	-0.07	1.585	0.212	-0.67	0.28
VA27	0.35	0.07	1.82	-0.07	0.35	0.35	-0.07	0.67	0.28	-0.85	-0.35	-1.35	1.07

Appendix Table E.16. All oak barrel Cabernet Sauvignon wine aroma ranks.

barrel	preference	coconut	pene shav	allspice	berry	smoky	caramel	vanilla	coffee	dark choc	Band-aid	earthy	mint
AU7	15	11.5	3.5	3.5	21	3	11.5	16.5	5	16	4	4.5	12
AU8	5.5	4	10.5	2	11	16	8.5	9	4	8.5	10	10	5
AU9	7.5	3	3.5	13	6	6.5	4.5	2	1	3.5	9	17	7
AA36	2.5	6	5.5	5.5	2	15	2	1	2	3.5	20	24	7
AA40	7.5	9.5	14	5.5	9	4.5	11.5	5.5	7.5	10.5	8	13.5	2.5
AA48	14	7	5.5	8	7.5	11.5	14.5	10	11	6.5	18	11	7
NL6	4	1	22	17	4	4.5	1	4	6	5	15	20.5	23
NL7	1	2	23	7	1	13.5	4.5	8	9	2	15	15	15.5
NL8	11	5	10.5	13	3	19	16.5	3	14	10.5	23	20.5	15.5
LA23	2.5	9.5	7.5	17	14	24	8.5	7	24	12.5	24	23	13.5
LA30	9.5	11.5	18	22	16	17	20	22	13	19	3	8.5	17.5
LA38	24	8	19.5	19.5	18	23	16.5	5.5	20	16	21	17	20
NT6	9.5	13	19.5	1	12	13.5	6	11	17.5	23.5	12	7	10
NT7	16.5	24	1	17	22.5	2	22	13.5	10	8.5	2	3	9
NT8	13	15	21	3.5	5	11.5	3	13.5	7.5	1	22	19	11
TA8	23	17	16	13	14	10	11.5	16.5	21	16	19	1	4
TA25	22	23	7.5	19.5	24	9	18.5	23	19	20.5	15	4.5	24
TA39	19.5	14	15	23	7.5	21	18.5	15	22	18	6.5	22	2.5
NV6	12	19	17	9	14	6.5	11.5	12	3	12.5	1	17	19
NV7	21	20.5	13	21	19	8	21	19	12	22	6.5	13.5	1
NV8	5.5	22	2	10.5	22.5	1	23	18	16	14	5	8.5	13.5
VA12	16.5	18	9	10.5	10	22	24	21	23	20.5	15	12	21
VA21	19.5	20.5	12	24	20	20	7	24	15	23.5	15	6	17.5
VA27	18	16	24	15	17	18	14.5	20	17.5	6.5	11	2	22

Appendix Table E.17.

All oak barrel Cabernet Sauvignon aroma rank transformations.

barrel	preference	coconut	pene shav	allspice	berry	smoky	caramel	vanilla	coffee	dark choc	Band-aid	earthy	mint
AU7	0.26	-0.11	-1.14	-1.14	1.04	-1.24	-0.11	0.425	-0.88	0.37	-1.04	-0.96	-0.05
AU8	-0.81	-1.04	-0.21	-1.5	-0.16	0.37	-0.43	-0.37	-1.04	-0.43	-0.26	-0.26	-0.88
AU9	-0.54	-1.24	-1.14	0.053	-0.73	-0.67	-0.96	-1.5	-1.95	-1.14	-0.37	0.483	-0.6
AA36	-1.37	-0.73	-0.81	-0.81	-1.5	0.26	-1.5	-1.95	-1.5	-1.14	0.88	1.95	-0.6
AA40	-0.54	-0.32	0.16	-0.81	-0.37	-0.96	-0.11	-0.81	-0.54	-0.21	-0.48	0.105	-1.37
AA48	0.16	-0.6	-0.81	-0.48	-0.54	-0.11	0.21	-0.26	-0.16	-0.67	0.6	-0.16	-0.6
NL6	-1.04	-1.95	1.24	0.483	-1.04	-0.96	-1.95	-1.04	-0.73	-0.88	0.264	0.96	1.5
NL7	-1.95	-1.5	1.5	-0.6	-1.95	0.105	-0.96	-0.48	-0.37	-1.5	0.264	0.26	0.315
NL8	-0.16	-0.88	-0.21	0.053	-1.24	0.73	0.425	-1.24	0.16	-0.21	1.5	0.96	0.315
LA23	-1.37	-0.32	-0.54	0.483	0.157	1.95	-0.43	-0.6	1.95	0	1.95	1.5	0.105
LA30	-0.32	-0.11	0.6	1.24	0.37	0.48	0.88	1.24	0.05	0.73	-1.24	-0.43	0.54
LA38	1.95	-0.48	0.805	0.805	0.6	1.5	0.425	-0.81	0.88	0.37	1.04	0.483	0.88
NT6	-0.32	0.05	0.805	-1.95	-0.05	0.105	-0.73	-0.16	0.54	1.725	-0.05	-0.6	-0.26
NT7	0.425	1.95	-1.95	0.483	1.37	-1.5	1.24	0.105	-0.26	-0.43	-1.5	-1.24	-0.37
NT8	0.05	0.26	1.04	-1.14	-0.88	-0.11	-1.24	0.105	-0.54	-1.95	1.24	0.73	-0.16
TA8	1.5	0.48	0.37	0.053	0.157	-0.26	-0.11	0.425	1.04	0.37	0.73	-1.95	-1.04
TA25	1.24	1.5	-0.54	0.805	1.95	-0.37	0.665	1.5	0.73	0.96	0.264	-0.96	1.95
TA39	0.805	0.16	0.26	1.5	-0.54	1.04	0.665	0.26	1.24	0.6	-0.67	1.24	-1.37
NV6	-0.05	0.73	0.48	-0.37	0.157	-0.67	-0.11	-0.05	-1.24	0	-1.95	0.483	0.73
NV7	1.04	0.96	0.05	1.04	0.73	-0.48	1.04	0.73	-0.05	1.24	-0.67	0.105	-1.95
NV8	-0.81	1.24	-1.5	-0.21	1.37	-1.95	1.5	0.6	0.37	0.16	-0.88	-0.43	0.105
VA12	0.425	0.6	-0.37	-0.21	-0.26	1.24	1.95	1.04	1.5	0.96	0.264	-0.05	1.04
VA21	0.805	0.96	-0.05	1.95	0.88	0.88	-0.6	1.95	0.26	1.725	0.264	-0.73	0.54
VA27	0.6	0.37	1.95	0.26	0.48	0.6	0.21	0.88	0.54	-0.67	-0.16	-1.5	1.24

Appendix F

Wine aroma principal components analysis results

Appendix outline

PC analysis was performed using SYSTAT V5.0 (SYSTAT, Inc.) statistical software. The analysis was based on a Pearson's product-moment correlation matrix, three PCs were retained, and a varimax rotation was performed. The Spearman's rank correlation matrix is also shown.

F.1 Chardonnay wine

Raw data were the Fisher-Yates rank transformations of the Chardonnay wine aroma ranks (excluding 'preference') (Appx. Tab. E.9). 'Preference' is included in Appendix Tables F.1 & F.2 only coincidentally

Appendix Table F.1. Spearman's rank correlation matrix.

	preference	*coconut*	*penc shavs*	*caramel*	*vanilla*	*butter*	*allspice*	*smoky*	*cashew nut*	*green apple*	*cinnamon*
coconut	0.402	1									
penc shavs	0.243	-0.066	1								
caramel	-0.128	-0.174	-0.588	1							
vanilla	0.598	0.307	-0.174	0.090	1						
butter	-0.083	0.106	-0.508	0.268	0.213	1					
allspice	0.448	0.079	0.128	-0.114	0.394	-0.400	1				
smoky	0.270	-0.346	0.548	-0.158	0.198	-0.330	0.301	1			
cashew nut	0.347	0.378	0.316	-0.194	0.104	-0.306	0.374	0.161	1		
green apple	-0.178	0.303	-0.015	-0.170	-0.088	0.266	-0.142	-0.621	0.126	1	
cinnamon	0.008	-0.078	0.131	0.005	0.324	0.185	0.135	0.382	0.180	0.095	1

Critical values for 2-tailed test of correlation, $n = 24$, from O'Mahony (1986). : significant correlation, $p < 0.05$ or stronger.

If rho is greater than or equal to 0.407, significant correlation, $p<0.05$. penc shavs = 'pencil shavings'

If rho is greater than or equal to 0.521, significant correlation, $p < 0.01$.

If rho is greater than or equal to 0.608, significant correlation, $p < 0.002$.

Appendix Table F.2. Pearson's product-moment correlation coefficient matrix.

	preference	*coconut*	*penc shavs*	*caramel*	*vanilla*	*butter*	*allspice*	*smoky*	*cashew nut*	*green apple*	*cinnamon*
coconut	0.431	1									
penc shavs	0.207	-0.144	1								
caramel	-0.133	-0.069	-0.672	1							
vanilla	0.575	0.330	-0.197	0.137	1						
butter	-0.187	0.058	-0.454	0.298	0.134	1					
allspice	0.413	0.067	0.164	-0.139	0.423	-0.406	1				
smoky	0.256	-0.346	0.471	-0.171	0.199	-0.282	0.302	1			
cashew nut	0.360	0.374	0.304	-0.234	0.102	-0.315	0.360	0.123	1		
green apple	-0.222	0.287	-0.010	-0.159	-0.127	0.241	-0.161	-0.695	0.099	1	
cinnamon	-0.032	-0.090	0.140	-0.008	0.341	0.214	0.192	0.373	0.185	0.007	1

: correlation coefficients for 'preference' are included in the Table but they were not included in the PCA.

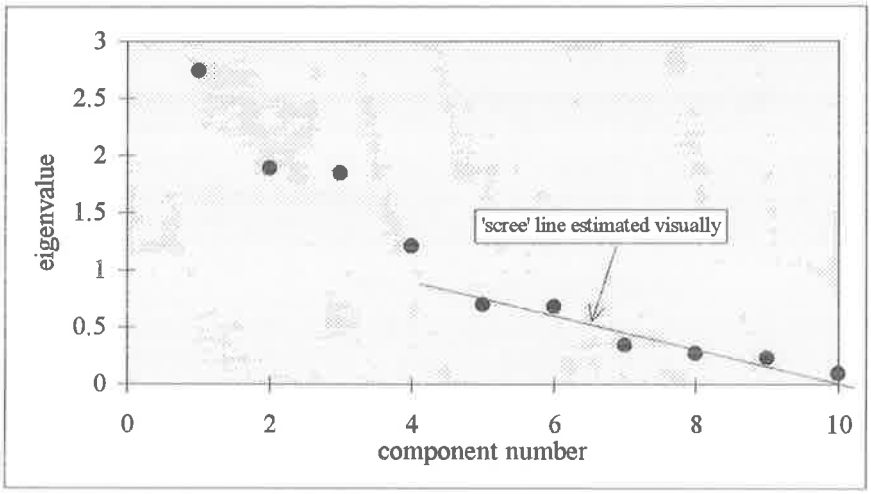
Critical values for 2-tailed test of correlation, $n = 24$, $d.f. = n - 2 = 24 - 2 = 22$. penc shavs = 'pencil shavings'

If r is greater than or equal to 0.404, significant correlation, $p < 0.05$.* : significant correlation, $p < 0.05$ or stronger.

If r is greater than or equal to 0.515, significant correlation, $p < 0.01$.* *: from Snedecor and Cochran (1967), 22 *d.f.*

If r is greater than or equal to 0.6524, significant correlation, $p < 0.001$.** **: from O'Mahony (1986), 20 *d.f.*

Scree plot	
number	eigenvalue
1	2.744
2	1.889
3	1.841
4	1.209
5	0.698
6	0.682
7	0.342
8	0.268
9	0.228
10	0.098



Appendix Figure F.1. Scree plot of Chardonnay aroma PCA.

Conclusion: The scree test suggests retaining the first four PCs but this is not clear. Interpretability suggests retaining the first three so three were retained.

N.B. PCs were re-ordered following rotation because the proportion of variance changed sufficiently to alter their order. The original PCs 1, 2 & 3 were changed to PCs 1, 3 & 2.

Appendix Table F.3. Rotated component loadings and PC characteristics.

	PC load 1	PC load 2	PC load 3
penc shavs	0.863	-0.024	0.174
caramel	-0.784	0.08	0.135
butter	-0.701	-0.05	-0.178
cashew nut	0.523	0.456	-0.358
green apple	0.017	-0.24	-0.795
smoky	0.346	0.417	0.762
coconut	-0.002	0.341	-0.75
vanilla	-0.26	0.829	-0.068
allspice	0.334	0.676	0.055
cinnamon	-0.032	0.569	0.158

: Loadings with absolute values > 0.5 are highlighted. They contribute most to the corresponding PC.

penc shavs = 'pencil shavings'

PCs description:

PC1: emphasis on 'pencil shavings' & 'cashew nut' versus 'caramel & 'butter.'

PC2: emphasis on 'vanilla,' 'allspice' & 'cinnamon.'

PC3: emphasis on 'smoky' versus 'green apple' & 'coconut.'

Proportion of variance explained by each PC	PC1	PC2	PC3
variance explained:	24.24%	20.34%	20.16%
cumulative variance explained:	24.24%	44.58%	64.74%

Component loadings were converted to eigenvectors to determine sample locations in PC space [eigenvector = component loading / sqrt(eigenvalue)]. See following page.

Appendix Table F.4. Rotated component loadings and corresponding eigenvectors.

	PC1		PC2		PC3	
	compnt load	eigenvector	compnt load	eigenvector	compnt load	eigenvector
penc shavs	0.863	0.521	-0.024	-0.018	0.174	0.127
caramel	-0.784	-0.473	0.080	0.059	0.135	0.098
butter	-0.701	-0.423	-0.050	-0.037	-0.178	-0.130
cashew nut	0.523	0.316	0.456	0.336	-0.358	-0.260
green apple	0.017	0.010	-0.240	-0.177	-0.795	-0.578
smoky	0.346	0.209	0.417	0.307	0.762	0.554
coconut	-0.002	-0.001	0.341	0.251	-0.750	-0.546
vanilla	-0.260	-0.157	0.829	0.611	-0.068	-0.049
allspice	0.334	0.202	0.676	0.498	0.055	0.040
cinnamon	-0.032	-0.019	0.569	0.419	0.158	0.115
eigenvalue:	2.744		1.841		1.889	

Appendix Table F.5. Chardonnay wine samples in rotated PCA space.

(Fisher-Yates rank transforms of 'preference' ranks, and correlation analysis also included.)

Sample locations in PC space were calculated as follows. The raw data for each aroma were converted to z-scores. The PC1 eigenvector for the first aroma was multiplied, separately, by each of the z-scores of that aroma. The PC1 axis value for each sample was, then, the sum of these 10 products (one for each aroma). The other PC axis values for each sample were obtained in a similar manner. Finally, the co-ordinates (axis values) were arbitrarily divided by 3.6 to restrict the range to -1 to 1.

Barrel code	PC1	PC2	PC3	PC1/3.6	PC2/3.6	PC3/3.6	preference
AU4	-1.768	0.885	0.980	-0.491	0.246	0.272	0.48
AU6	-2.274	-1.305	-0.009	-0.632	-0.362	-0.003	-1.95
AU70	-2.805	-2.171	0.252	-0.779	-0.603	0.070	-0.48
AA10	-1.020	-0.229	0.213	-0.283	-0.064	0.059	0.16
AA11	-0.613	1.415	2.309	-0.170	0.393	0.641	0.05
AA22	-3.046	-1.203	-1.273	-0.846	-0.334	-0.354	-0.805
FL3	0.471	-0.050	-2.038	0.131	-0.014	-0.566	-1.04
FL4	1.396	3.212	1.464	0.388	0.892	0.407	1.24
FL5	2.294	-1.830	1.255	0.637	-0.508	0.349	-1.37
LA27	0.177	-2.329	-2.209	0.049	-0.647	-0.613	-1.37
LA34	0.286	1.480	0.118	0.079	0.411	0.033	0.6
LA41	-1.188	0.568	-1.308	-0.330	0.158	-0.363	-0.805
FT3	1.319	-1.399	2.474	0.367	-0.388	0.687	-0.6
FT4	-0.454	-1.152	0.883	-0.126	-0.320	0.245	-0.315
FT5	0.503	-1.191	0.482	0.140	-0.331	0.134	-0.16
TA23	1.725	1.046	2.277	0.479	0.291	0.632	-0.315
TA31	-1.148	-0.672	-1.161	-0.319	-0.187	-0.322	0.805
TA46	1.162	3.588	0.172	0.323	0.997	0.048	0.805
FV3	0.314	0.890	-1.235	0.087	0.247	-0.343	0.315
FV4	0.530	0.540	0.156	0.147	0.150	0.043	1.95
FV5	1.620	-0.402	1.348	0.450	-0.112	0.374	-0.05
VA32	0.548	0.727	-0.974	0.152	0.202	-0.271	0.315
VA38	1.937	-0.040	-1.223	0.538	-0.011	-0.340	1.5
VA39	0.035	-0.379	-2.953	0.010	-0.105	-0.820	1.04

Pearson's correlation coefficients for aroma-PC comparisons with 'preference.'

	PC1	*PC2*	*PC3*
preference	0.275	0.582	-0.025

F.2. Cabernet Sauvignon wine

Raw data were the Fisher-Yates rank transformations of the Cab. Sauv. wine aroma ranks (excluding 'preference') (Appx. Tab. E.17). 'Preference' is included in Appx. Tab. F.6 & F.7 only coincidentally.

Appendix Table F.6. Spearman's rank correlation matrix.

	pref	*coconut*	*penc shv*	*allspice*	*berry*	*smoky*	*caramel*	*vanilla*	*coffee*	*dk choc*	*Band-aid*	*earthy*	*mint*
pref	1												
coconut	0.598	1											
penc shv	0.060	-0.196	1										
allspice	0.476	0.317	0.047	1									
berry	0.536	0.753	-0.287	0.380	1								
smoky	0.135	-0.187	0.296	0.278	-0.205	1							
caramel	0.511	0.595	-0.300	0.415	0.570	0.022	1						
vanilla	0.574	0.785	0.020	0.374	0.679	0.027	0.545	1					
coffee	0.472	0.398	0.124	0.466	0.334	0.553	0.506	0.447	1				
dk choc	0.544	0.561	-0.052	0.408	0.606	0.217	0.514	0.629	0.589	1			
Band-aid	-0.039	-0.363	0.161	-0.058	-0.427	0.536	-0.374	-0.351	0.301	-0.221	1		
earthy	-0.451	-0.539	0.094	-0.011	-0.677	0.289	-0.351	-0.667	-0.189	-0.372	0.370	1	
mint	0.056	0.085	0.278	0.253	0.194	0.207	0.063	0.241	0.219	0.080	0.151	-0.089	1

: significant correlation, p <0.05 or stronger.

Critical values for 2-tailed test of correlation, n = 24, from O'Mahony (1986).

If rho is greater than or equal to 0.407, significant correlation, p <0.05.

If rho is greater than or equal to 0.521, significant correlation, p <0.01.

If rho is greater than or equal to 0.608, significant correlation, p <0.002.

pref = 'preference'

penc shv = 'pencil shavings'

dk choc = 'dark chocolate'

Appendix Table F.7. Pearson's product-moment correlation coefficient matrix.

	pref	*coconut*	*penc shv*	*allspice*	*berry*	*smoky*	*caramel*	*vanilla*	*coffee*	*dk choc*	*Band-aid*	*earthy*	*mint*
pref	1												
coconut	0.553	1											
penc shv	0.027	-0.328	1										
allspice	0.440	0.293	0.011	1									
berry	0.566	0.784	-0.361	0.336	1								
smoky	0.124	-0.212	0.352	0.274	-0.243	1							
caramel	0.474	0.646	-0.352	0.327	0.573	-0.003	1						
vanilla	0.510	0.707	0.054	0.417	0.674	0.016	0.530	1					
coffee	0.394	0.360	0.135	0.388	0.312	0.550	0.500	0.451	1				
dk choc	0.510	0.505	-0.099	0.364	0.594	0.193	0.492	0.609	0.523	1			
Band-aid	-0.062	-0.371	0.181	-0.043	-0.394	0.587	-0.343	-0.317	0.357	-0.210	1		
earthy	-0.473	-0.517	0.032	-0.008	-0.625	0.333	-0.367	-0.645	-0.163	-0.353	0.366	1	
mint	0.038	0.025	0.259	0.186	0.191	0.157	0.002	0.250	0.186	0.031	0.126	-0.107	1

: correlation coefficients for 'preference' are included in the Table but they were not included in the PCA.

: significant correlation, p <0.05 or stronger.

Critical values for 2-tailed test of correlation, n = 24, $d.f.$ = n - 2 = 24 - 2 = 22.

If r is greater than or equal to 0.404, significant correlation, p <0.05.*

If r is greater than or equal to 0.515, significant correlation, p <0.01.*

If r is greater than or equal to 0.6524, significant correlation, p <0.001.**

pref = 'preference'

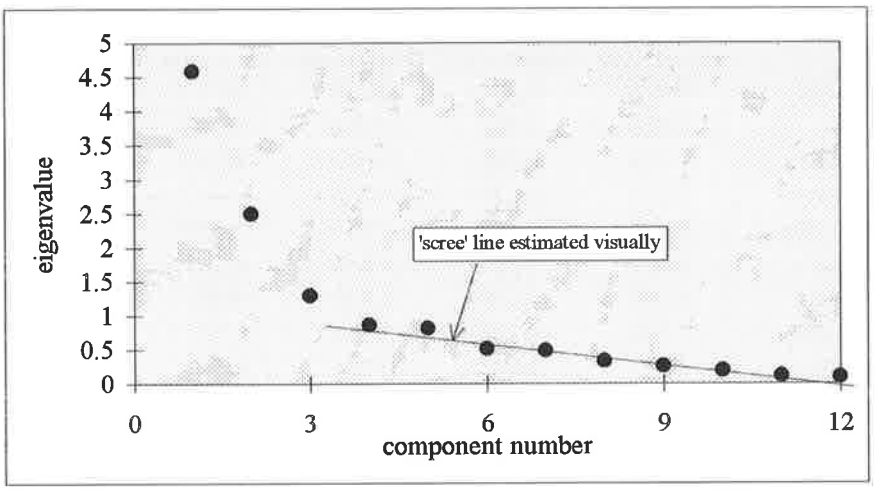
penc shv = 'pencil shavings'

dk choc = 'dark chocolate'

*: from Snedecor and Cochran (1967), 22 $d.f.$

**: from O'Mahony (1986), 20 $d.f.$

Scree plot	
number	eigenvalue
1	4.586
2	2.498
3	1.295
4	0.854
5	0.807
6	0.505
7	0.481
8	0.326
9	0.256
10	0.184
11	0.111
12	0.097



Appendix Figure F.2. Scree plot of Cabernet Sauvignon aroma PCA.

Conclusion: Retain first three PCs.

Appendix Table F.8. Rotated component loadings and PC characteristics.

	PC load 1	PC load 2	PC load 3
vanilla	0.829	-0.175	0.343
berry	0.829	-0.349	-0.006
coconut	0.825	-0.283	-0.121
caramel	0.792	-0.027	-0.269
dark choc	0.777	0.113	-0.004
coffee	0.652	0.606	0.147
earthy	-0.555	0.539	-0.298
allspice	0.541	0.319	0.079
smoky	0.087	0.886	0.206
Band-aid	-0.270	0.754	0.112
penc shavs	-0.232	0.231	0.781
mint	0.146	0.049	0.716

: Loadings with absolute values > 0.5 are highlighted. They contribute most to the corresponding PC.

dark choc = 'dark chocolate'
penc shavs = 'pencil shavings'

Principal components description

PC1: emphasis on 'vanilla,' 'berry,' 'coconut,' 'caramel,' 'dark chocolate,' 'coffee' & 'allspice' ('rich aromas') versus 'earthy.'

PC2: emphasis on 'smoky,' 'Band-aid' (medicinal), 'coffee' & 'earthy.'

PC3: emphasis on 'pencil shavings' & 'mint.'

Proportion of variance explained by each PC	PC1	PC2	PC3
variance explained:	37.22%	20.12%	12.49%
cumulative variance explained:	37.22%	57.34%	69.82%

Component loadings were converted to eigenvectors to determine sample locations in PC space [eigenvector = component loading / sqrt(eigenvalue)]. See following page.

Appendix Table F.9. Rotated component loadings and corresponding eigenvectors.

	PC1		PC2		PC3	
	compnt load	eigenvector	compnt load	eigenvector	compnt load	eigenvector
vanilla	0.829	0.387	-0.175	-0.111	0.343	0.301
berry	0.829	0.387	-0.349	-0.221	-0.006	-0.005
coconut	0.825	0.385	-0.283	-0.179	-0.121	-0.106
caramel	0.792	0.370	-0.027	-0.017	-0.269	-0.236
dark choc	0.777	0.363	0.113	0.071	-0.004	-0.004
coffee	0.652	0.304	0.606	0.383	0.147	0.129
earthy	-0.555	-0.259	0.539	0.341	-0.298	-0.262
allspice	0.541	0.253	0.319	0.202	0.079	0.069
smoky	0.087	0.041	0.886	0.561	0.206	0.181
Band-aid	-0.270	-0.126	0.754	0.477	0.112	0.098
penc shavs	-0.232	-0.108	0.231	0.146	0.781	0.686
mint	0.146	0.068	0.049	0.031	0.716	0.629
eigenvalue:	4.586		2.498		1.295	

Appendix Table F.10. Cabernet Sauvignon wine samples in rotated PCA space.

(Fisher-Yates rank transforms of 'preference' ranks, and correlation analysis also included.)

Sample locations in PC space were calculated as follows. The raw data for each aroma were converted to *z*-scores. The PC1 eigenvector for the first aroma was multiplied, separately, by each of the *z*-scores of that aroma. The PC1 axis value for each sample was, then, the sum of these 12 products (one for each aroma). The other PC axis values for each sample were obtained in a similar manner. Finally, the co-ordinates (axis values) were arbitrarily divided by 4 to restrict the range to -1 to 1.

Barrel code	PC1	PC2	PC3	PC1/4	PC2/4	PC3/4	preference
AU7	0.534	-2.567	-0.955	0.133	-0.642	-0.239	0.26
AU8	-1.586	-0.543	-0.749	-0.396	-0.136	-0.187	-0.805
AU9	-2.803	-0.850	-1.838	-0.701	-0.212	-0.459	-0.54
AA36	-3.932	1.012	-1.758	-0.983	0.253	-0.440	-1.37
AA40	-1.214	-0.936	-1.352	-0.304	-0.234	-0.338	-0.54
AA48	-0.896	0.086	-0.995	-0.224	0.021	-0.249	0.16
NL6	-3.152	0.646	1.756	-0.788	0.162	0.439	-1.04
NL7	-3.018	0.931	1.418	-0.754	0.233	0.355	-1.95
NL8	-1.570	2.118	-0.275	-0.393	0.530	-0.069	-0.16
LA23	-0.232	3.513	0.088	-0.058	0.878	0.022	-1.37
LA30	1.819	-0.266	1.128	0.455	-0.066	0.282	-0.315
LA38	0.286	2.282	1.268	0.071	0.570	0.317	1.95
NT6	0.029	-0.094	0.628	0.007	-0.024	0.157	-0.315
NT7	2.382	-3.092	-2.211	0.595	-0.773	-0.553	0.425
NT8	-2.371	0.527	0.708	-0.593	0.132	0.177	0.05
TA8	1.164	-0.176	0.385	0.291	-0.044	0.096	1.5
TA25	3.436	-0.819	1.384	0.859	-0.205	0.346	1.24
TA39	0.886	1.606	-0.741	0.221	0.402	-0.185	0.805
NV6	-0.099	-1.812	0.099	-0.025	-0.453	0.025	-0.05
NV7	1.981	-0.781	-1.492	0.495	-0.195	-0.373	1.04
NV8	2.298	-2.459	-1.624	0.575	-0.615	-0.406	-0.805
VA12	2.219	1.224	0.650	0.555	0.306	0.163	0.425
VA21	2.765	0.439	1.516	0.691	0.110	0.379	0.805
VA27	1.076	0.010	2.963	0.269	0.003	0.741	0.6

Pearson's correlation coefficients for aroma-PC comparisons with 'preference.'

	PC1	*PC2*	*PC3*
preference	0.646	-0.081	0.176

Appendix G

Compound and composition-PC correlations with aromas - Chardonnay barrel wine

Three compounds (cyclotene, vanillyl alcohol and 4-ethylphenol) have been omitted due to imprecision of measurement, *etc.*, as discussed in Section 2.4.

Appendix Table G.1. Spearman's rank-order correlation matrix - 24 Chardonnay barrel wines at 55 weeks - sensory & composition.

	cis#	trans	eug	guaiac	4mg	van	malt	furf	eef	5mf	falc	5mfalc	fee	5mfee	vee	4vg	4eg	4vp
preference	0.459	0.027	0.329	0.067	0.101	0.093	-0.028	0.263	0.117	0.256	-0.029	0.125	-0.047	-0.223	0.258	0.342	0.082	0.362
coconut	0.744	0.324	0.381	-0.432	-0.327	0.021	-0.320	-0.095	-0.356	-0.158	-0.235	0.280	-0.101	-0.104	0.242	0.349	-0.355	0.232
pencil shavings	0.271	0.359	0.455	0.303	0.612	-0.032	-0.149	0.599	0.158	0.549	-0.417	0.009	-0.034	-0.593	0.289	0.383	0.283	0.325
caramel	-0.446	-0.544	-0.433	0.105	-0.345	0.095	0.553	-0.332	0.210	-0.309	0.571	-0.190	0.078	0.507	-0.330	-0.556	-0.046	-0.507
vanilla	0.094	0.137	-0.059	0.057	-0.047	0.365	-0.009	0.179	0.200	0.190	0.165	0.023	0.141	0.176	0.237	0.065	-0.085	0.066
butter	-0.161	-0.423	-0.374	-0.349	-0.536	0.099	0.092	-0.508	0.048	-0.522	0.434	-0.170	0.064	0.235	-0.164	-0.233	-0.618	-0.304
allspice	0.196	0.029	-0.075	0.305	0.354	0.234	-0.058	0.436	0.179	0.481	-0.122	0.144	0.120	0.079	0.187	0.084	0.342	0.078
smoky	-0.286	-0.058	0.013	0.762	0.801	0.438	0.344	0.699	0.717	0.704	0.162	-0.078	0.072	-0.100	0.020	-0.174	0.607	-0.092
cashew nut	0.329	0.140	0.306	0.036	0.335	0.348	-0.125	0.280	0.070	0.282	-0.091	0.311	0.203	0.032	0.329	0.217	0.206	0.171
green apple	0.340	0.300	0.114	-0.664	-0.486	-0.277	-0.535	-0.398	-0.627	-0.459	-0.422	0.158	0.055	-0.181	0.291	0.349	-0.609	0.152
cinnamon	-0.224	0.017	0.032	0.300	0.340	0.561	0.084	0.303	0.494	0.296	0.374	0.010	0.368	0.199	0.273	-0.054	0.108	-0.130

Critical values for 2-tailed test of correlation, $n = 24$, from O'Mahony (1986). : significant correlation, $p < 0.05$ or stronger.

If rho is greater than or equal to 0.407, significant correlation, $p < 0.05$.

If rho is greater than or equal to 0.521, significant correlation, $p < 0.01$.

If rho is greater than or equal to 0.608, significant correlation, $p < 0.002$.

#: Compound abbreviations: cis=*cis* -oak lactone, trans=*trans* -oak lactone, eug=eugenol, guaiac=guaiacol, 4mg=4-methylguaiacol, van=vanillin, malt=maltol, furf=furfural, eef='estimated extracted furfural' (furfural + furfuryl alcohol), 5mf=5-methylfurfural, falc=furfuryl alcohol, 5mfalc=5-methylfurfuryl alcohol, fee=furfuryl ethyl ether, 5mfee=5-methylfurfuryl ethyl ether, vee=vanillyl ethyl ether, 4vg=4-vinylguaiacol, 4eg=4-ethylguaiacol, 4vp=4-vinylphenol.

Appendix Table G.2. Pearson's product-moment correlation coefficient matrix - 24 Chardonnay barrel wines at 55 weeks - sensory & composition.

	cis#	trans	eug	guaiac	4mg	van	malt	furf	eef	5mf	falc	5mfalc	fee	5mfee	vee	4vg	4eg	4vp	PC1	PC2	PC3
pref#	0.536	0.181	0.390	-0.019	0.054	0.044	-0.088	0.166	0.088	0.200	-0.077	0.024	-0.069	-0.005	0.247	0.351	0.086	0.412	0.319	0.061	0.232
cocon	0.752	0.321	0.391	-0.254	-0.394	-0.086	-0.377	-0.157	-0.339	-0.226	-0.322	0.296	-0.121	-0.084	0.253	0.311	-0.264	0.234	0.456	-0.325	0.260
pncshv	0.293	0.411	0.434	0.184	0.581	-0.055	-0.255	0.405	0.069	0.496	-0.439	-0.048	-0.051	-0.644	0.270	0.361	0.253	0.367	0.512	0.362	0.197
caraml	-0.444	-0.598	-0.390	0.017	-0.347	0.131	0.473	-0.209	0.201	-0.243	0.606	-0.206	0.085	0.543	-0.370	-0.543	-0.076	-0.523	-0.669	-0.105	-0.352
vanilla	0.150	0.182	-0.037	-0.097	-0.129	0.350	0.009	0.091	0.166	0.174	0.149	0.003	0.189	0.294	0.163	-0.029	-0.119	-0.006	-0.061	0.008	0.174
butter	-0.200	-0.452	-0.373	-0.299	-0.498	0.058	0.058	-0.407	-0.033	-0.448	0.497	-0.129	0.095	0.282	-0.154	-0.299	-0.567	-0.346	-0.409	-0.413	-0.149
allspc	0.153	0.109	-0.108	0.351	0.328	0.246	0.116	0.487	0.334	0.513	-0.129	0.102	0.147	-0.030	0.138	0.059	0.374	0.060	0.027	0.432	0.149
smoky	-0.231	-0.046	0.027	0.713	0.802	0.461	0.361	0.721	0.731	0.694	0.179	-0.166	0.088	-0.033	-0.046	-0.227	0.658	-0.104	-0.216	0.798	-0.086
cashew	0.406	0.229	0.389	0.053	0.301	0.294	-0.215	0.169	-0.002	0.180	-0.238	0.232	0.153	-0.050	0.313	0.232	0.189	0.242	0.298	0.169	0.318
grnap	0.296	0.286	0.080	-0.689	-0.564	-0.359	-0.560	-0.561	-0.761	-0.519	-0.449	0.267	0.002	-0.303	0.324	0.329	-0.678	0.146	0.433	-0.673	0.270
cinn	-0.192	-0.086	0.035	0.217	0.318	0.550	0.145	0.285	0.392	0.377	0.224	0.008	0.346	0.053	0.238	-0.069	0.087	-0.067	-0.177	0.337	0.191

Critical values for 2-tailed test of correlation, $n = 24$, $d.f. = n - 2 = 24 - 2 = 22$. : significant correlation, $p < 0.05$ or stronger.

If r is greater than or equal to 0.404, significant correlation, $p < 0.05$.*

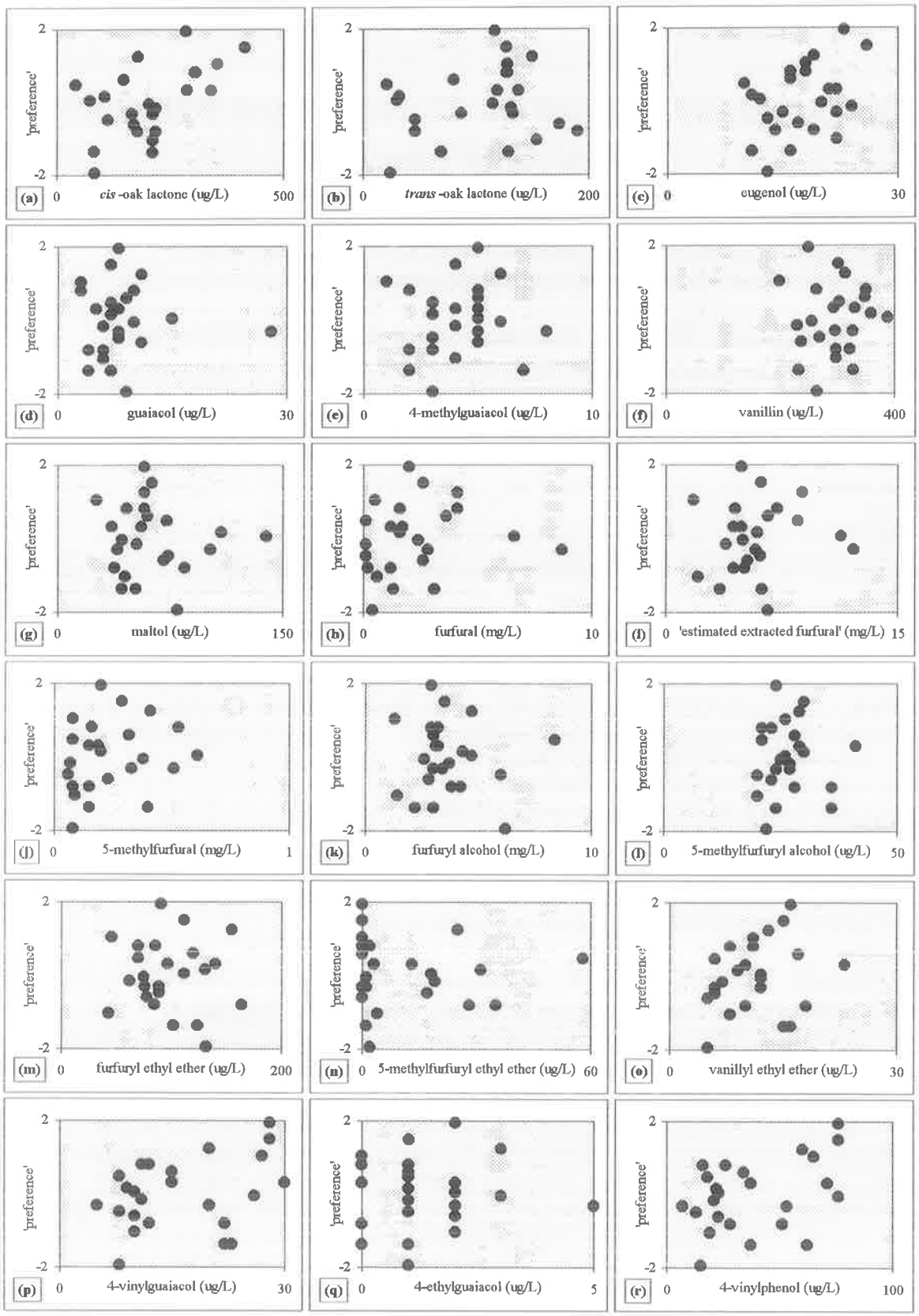
If r is greater than or equal to 0.515, significant correlation, $p < 0.01$.*

If r is greater than or equal to 0.6524, significant correlation, $p < 0.001$.**

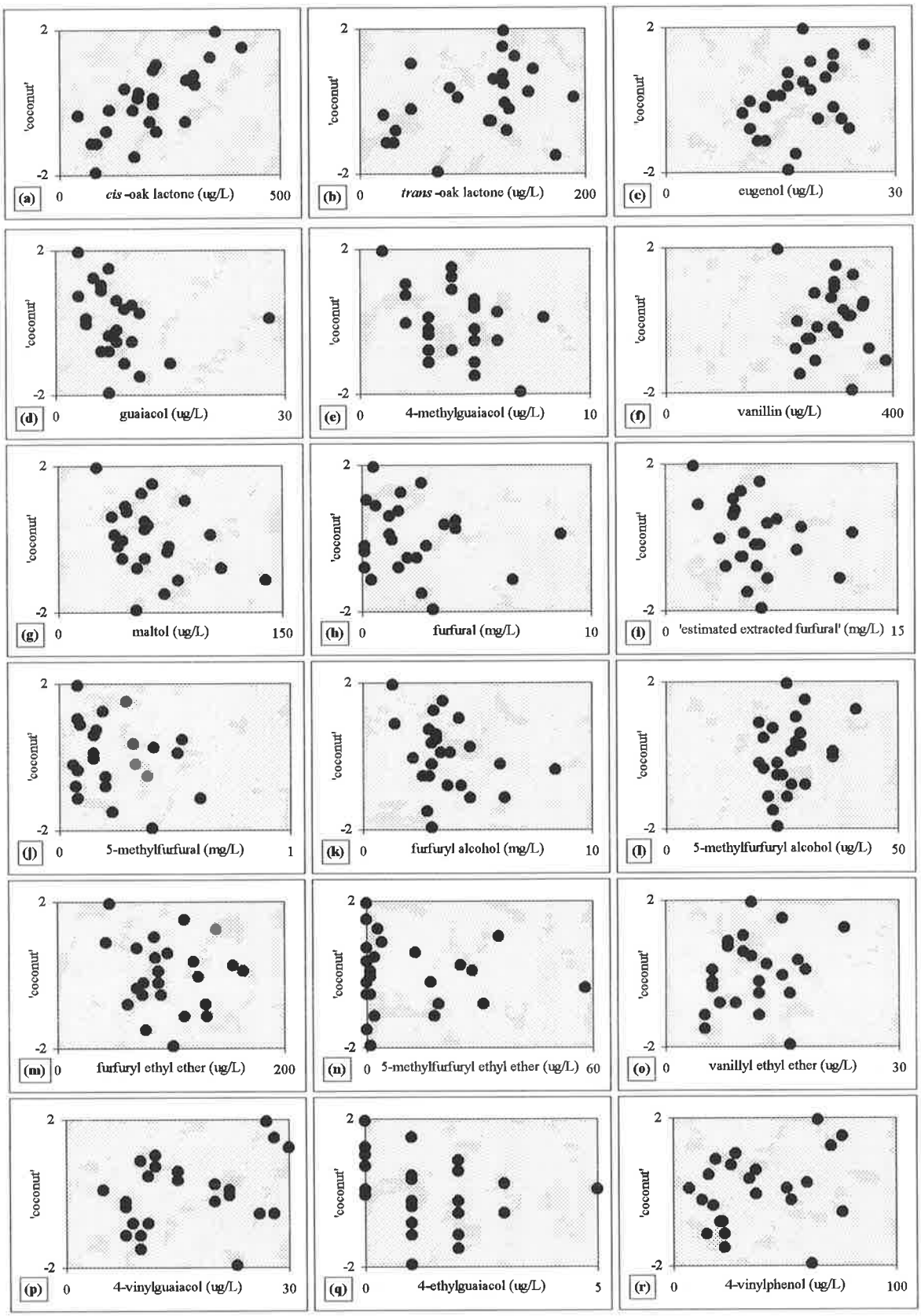
*: from Snedecor and Cochran (1967), 22 *d.f.*

**: from O'Mahony (1986), 20 *d.f.*

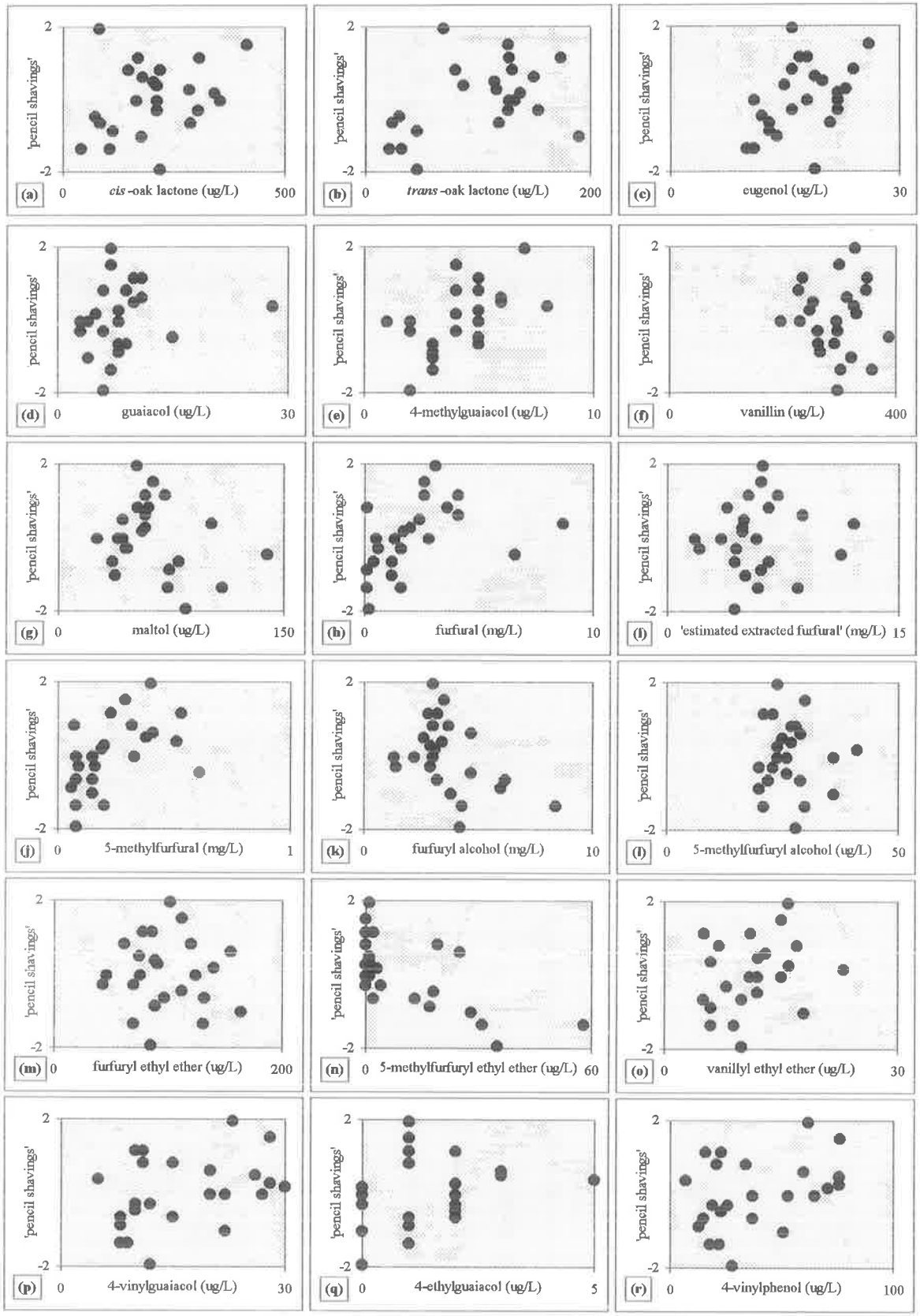
#: For abbreviations, see Appendix Table G.1.



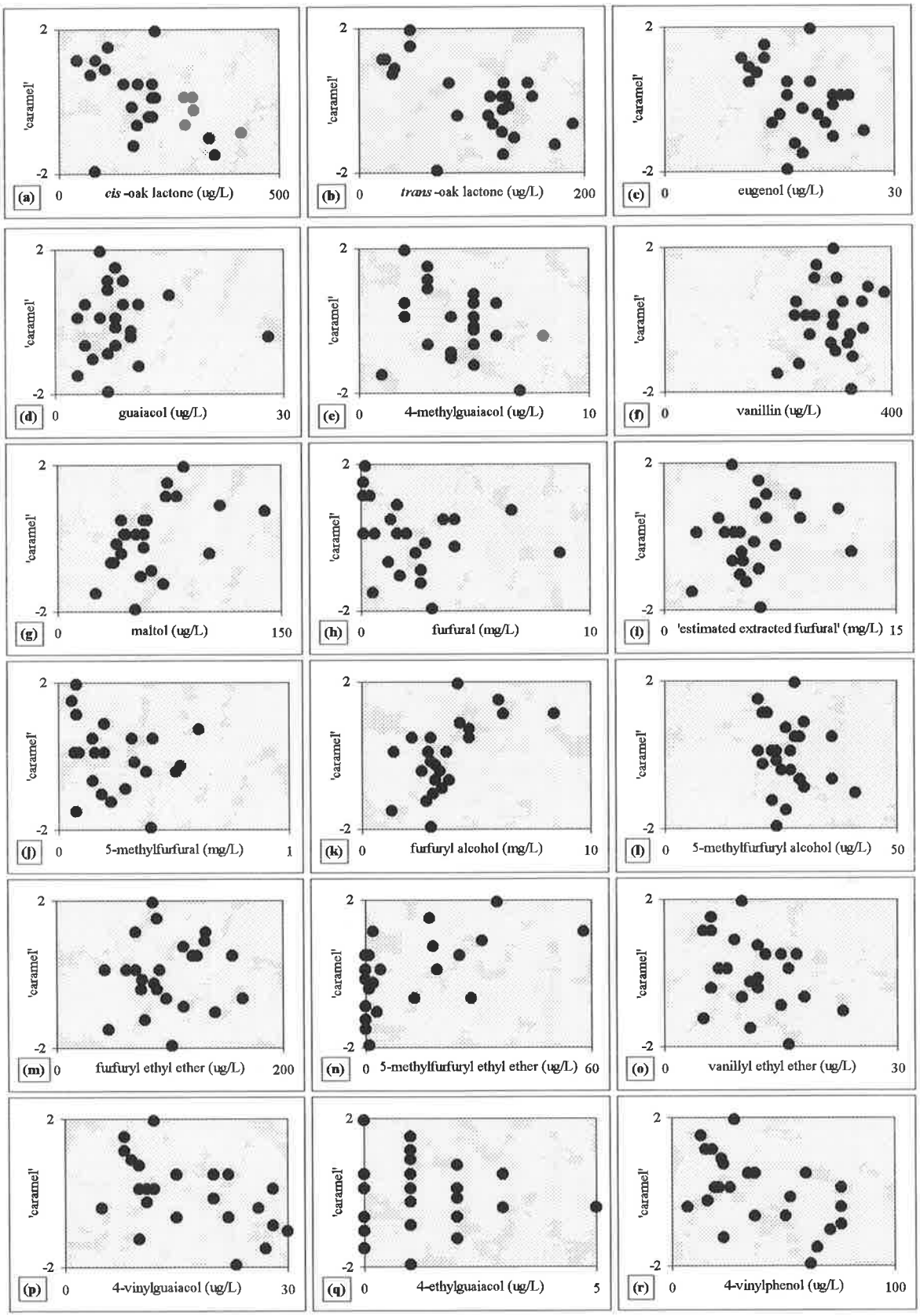
Appendix Figure G.1. Scatter plots of the Chardonnay wine Fisher-Yates rank transformation of 'preference' versus volatile compound concentrations.



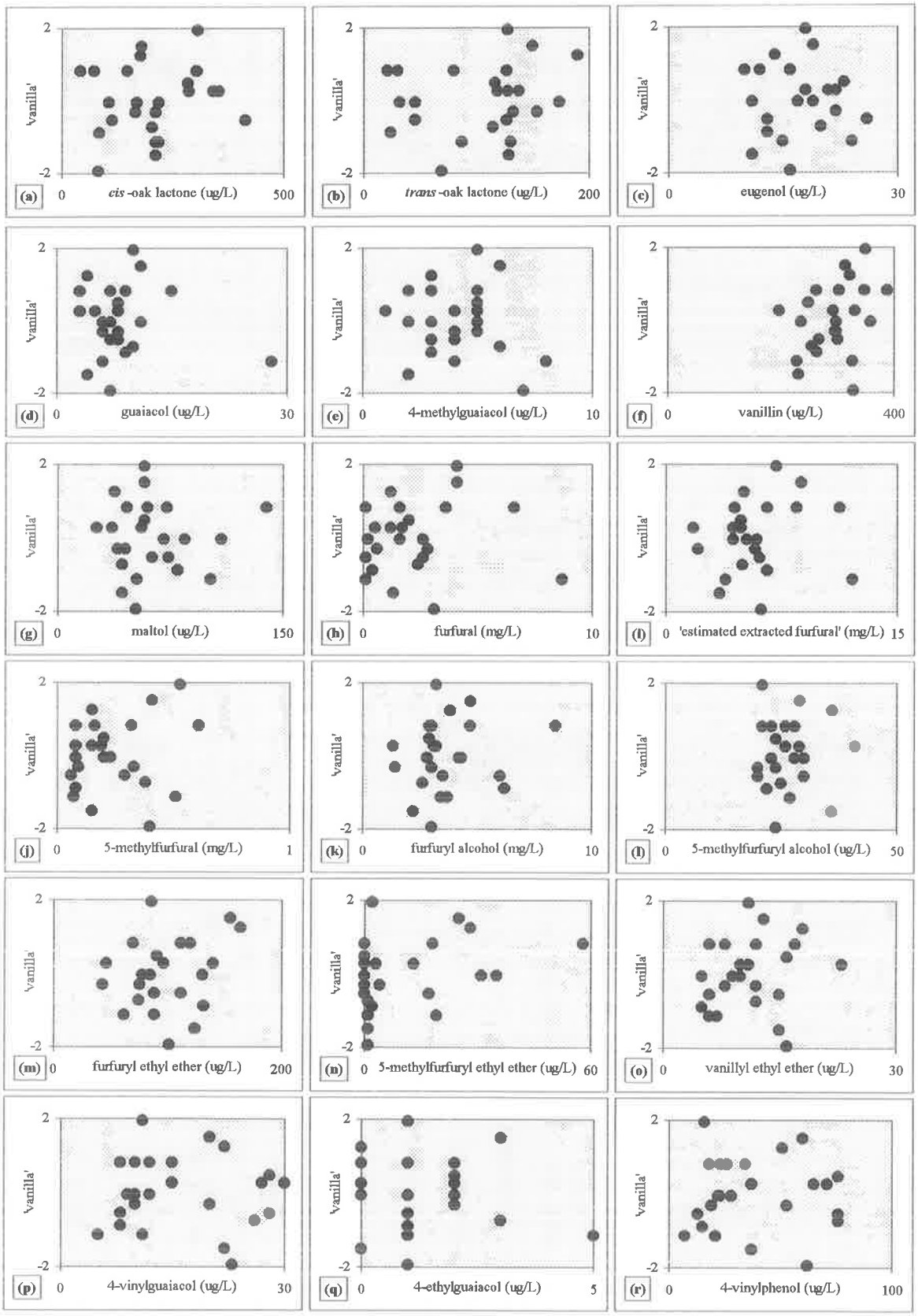
Appendix Figure G.2. Scatter plots of the Chardonnay wine Fisher-Yates rank transformation of 'coconut' versus volatile compound concentrations.



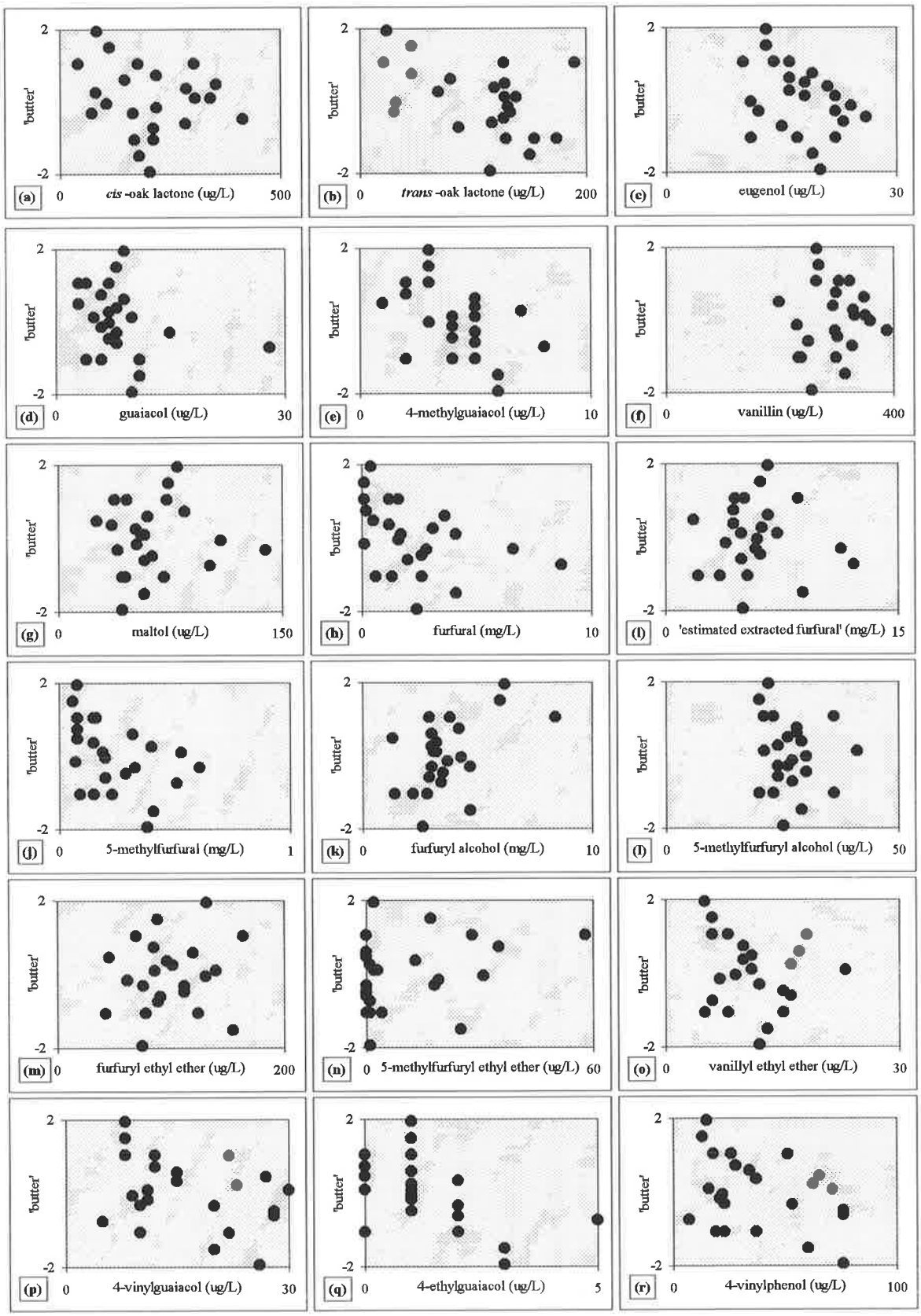
Appendix Figure G.3. Scatter plots of the Chardonnay wine Fisher-Yates rank transformation of 'pencil shavings' versus volatile compound concentrations.



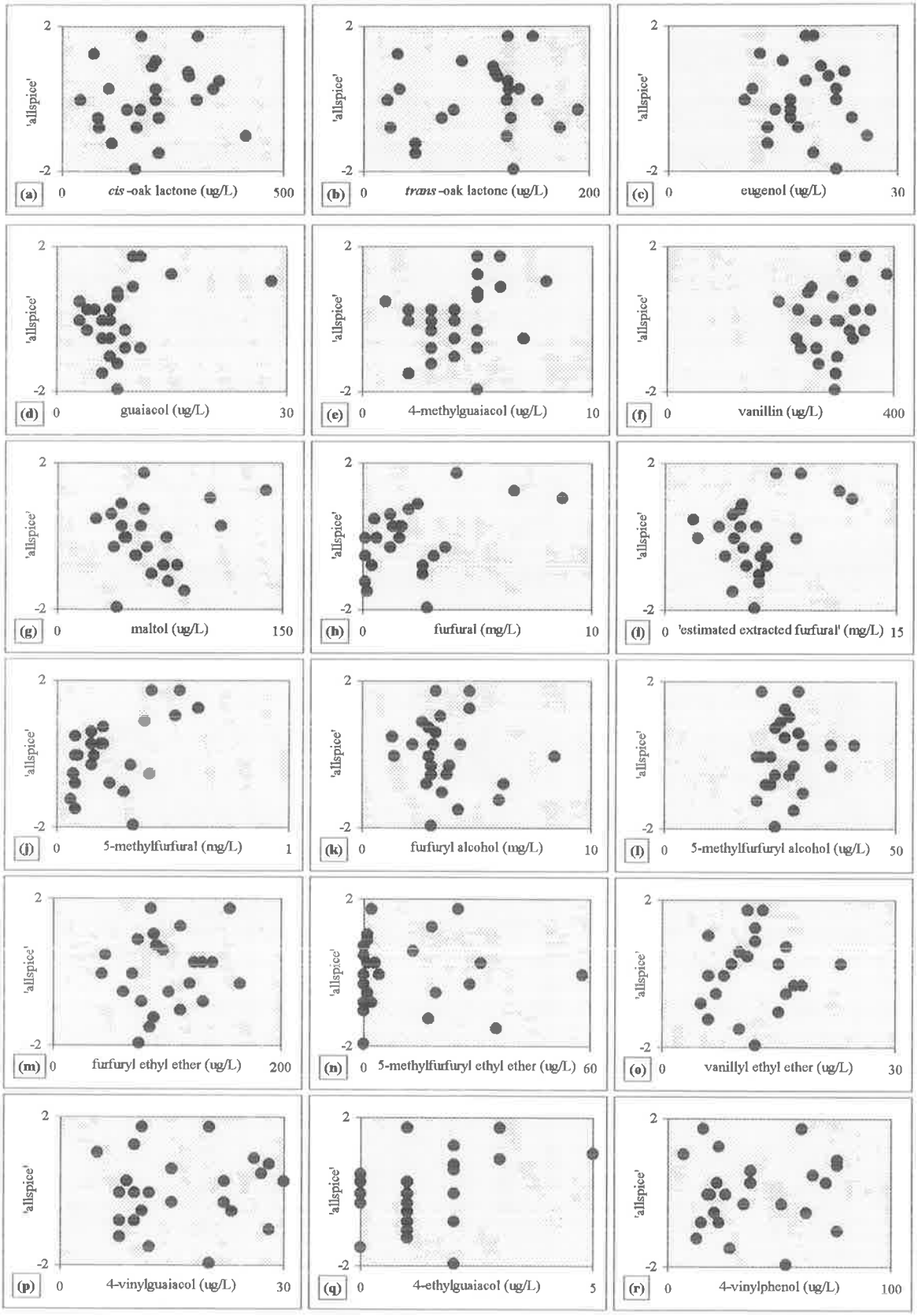
Appendix Figure G.4. Scatter plots of the Chardonnay wine Fisher-Yates rank transformation of 'caramel' versus volatile compound concentrations.



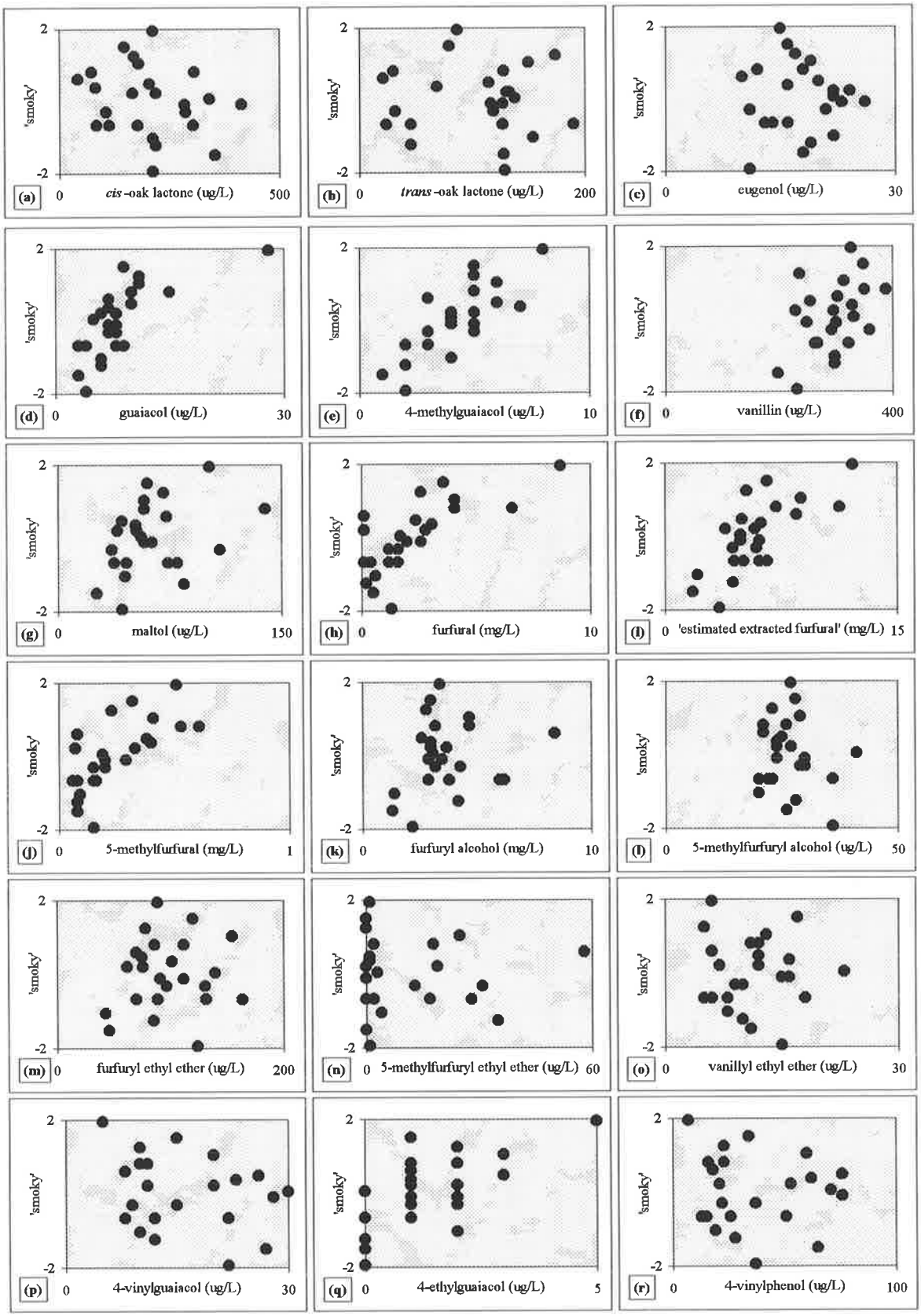
Appendix Figure G.5. Scatter plots of the Chardonnay wine Fisher-Yates rank transformation of 'vanilla' versus volatile compound concentrations.



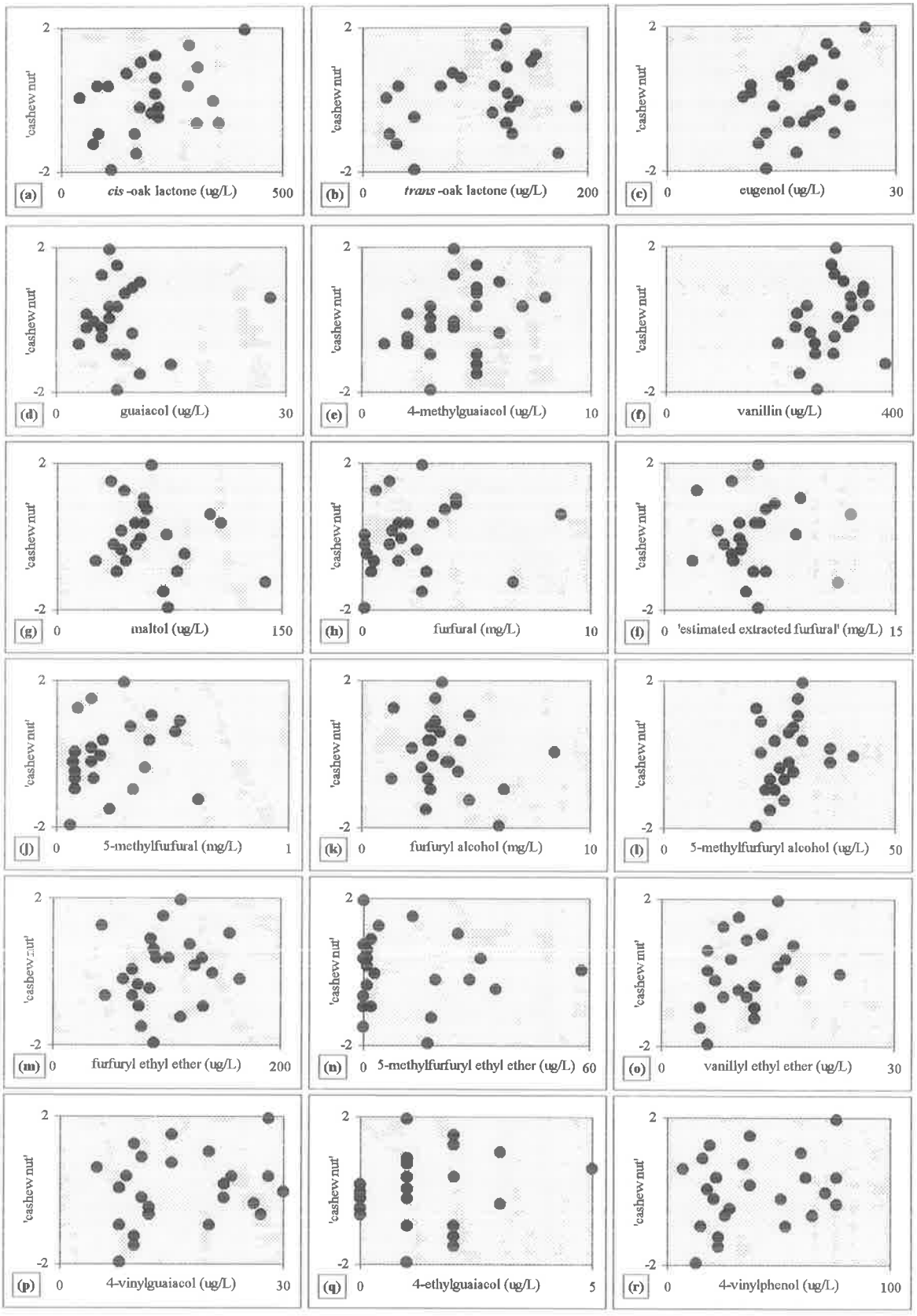
Appendix Figure G.6. Scatter plots of the Chardonnay wine Fisher-Yates rank transformation of 'butter' versus volatile compound concentrations.



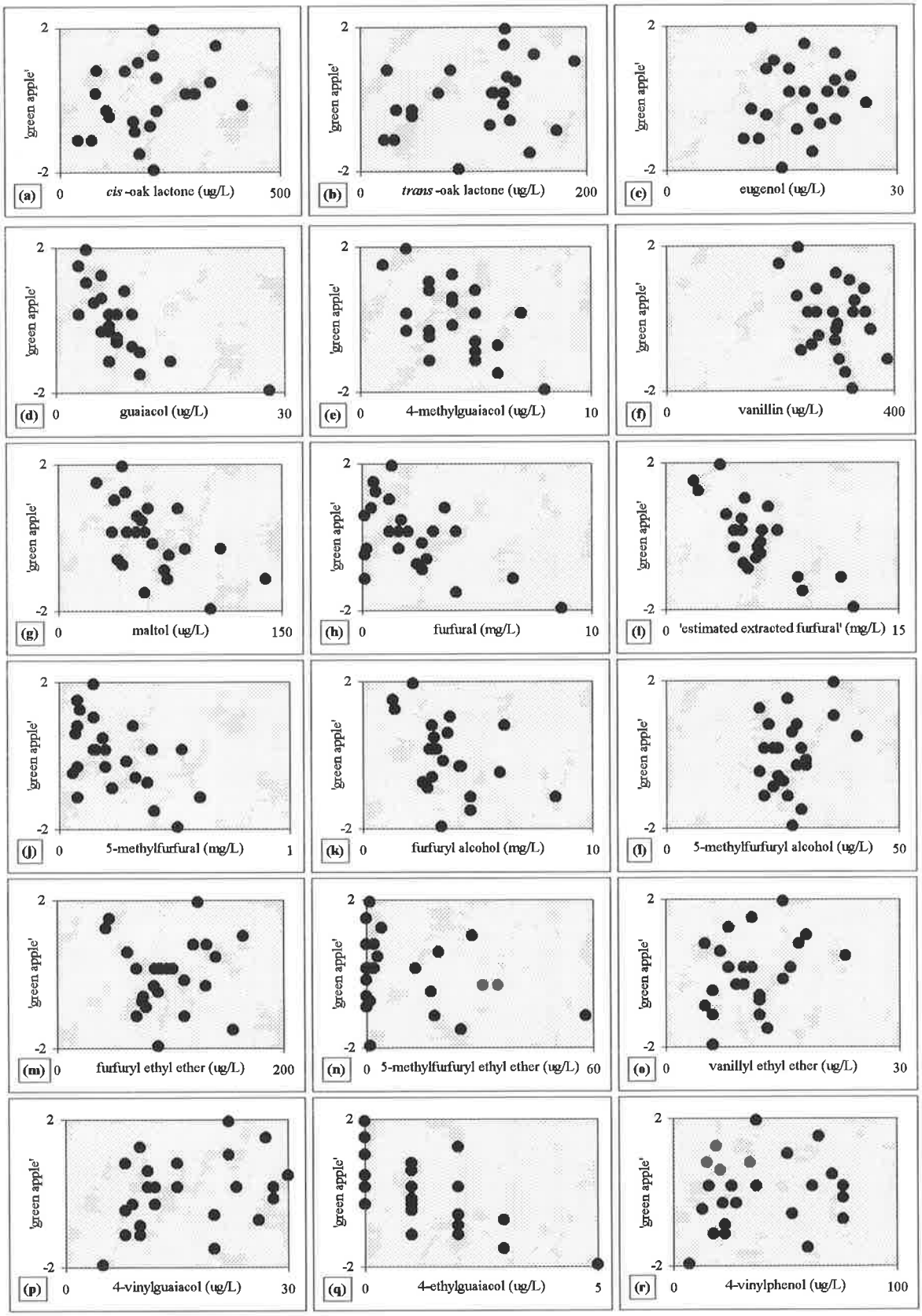
Appendix Figure G.7. Scatter plots of the Chardonnay wine Fisher-Yates rank transformation of 'allspice' versus volatile compound concentrations.



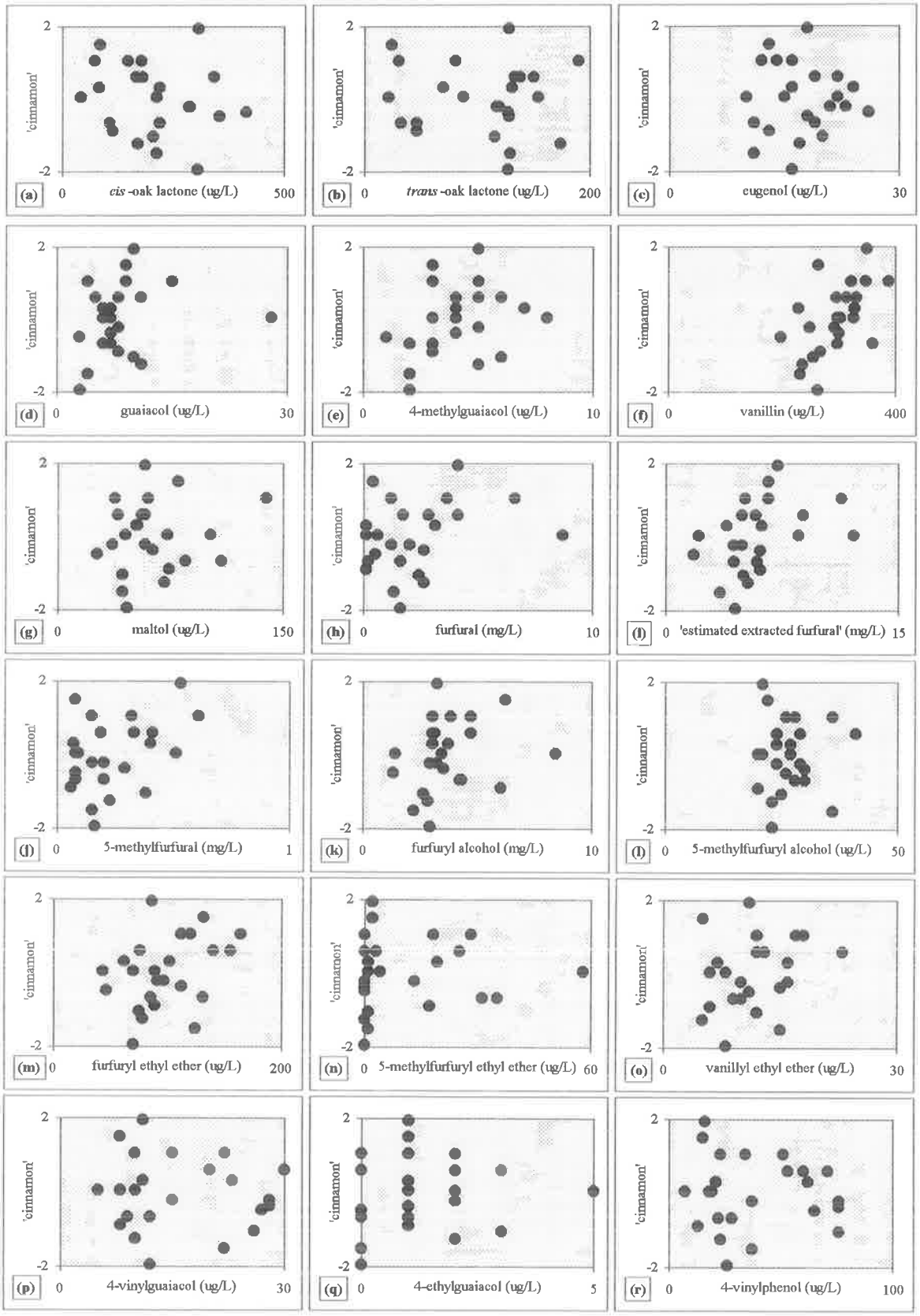
Appendix Figure G.8. Scatter plots of the Chardonnay wine Fisher-Yates rank transformation of 'smoky' versus volatile compound concentrations.



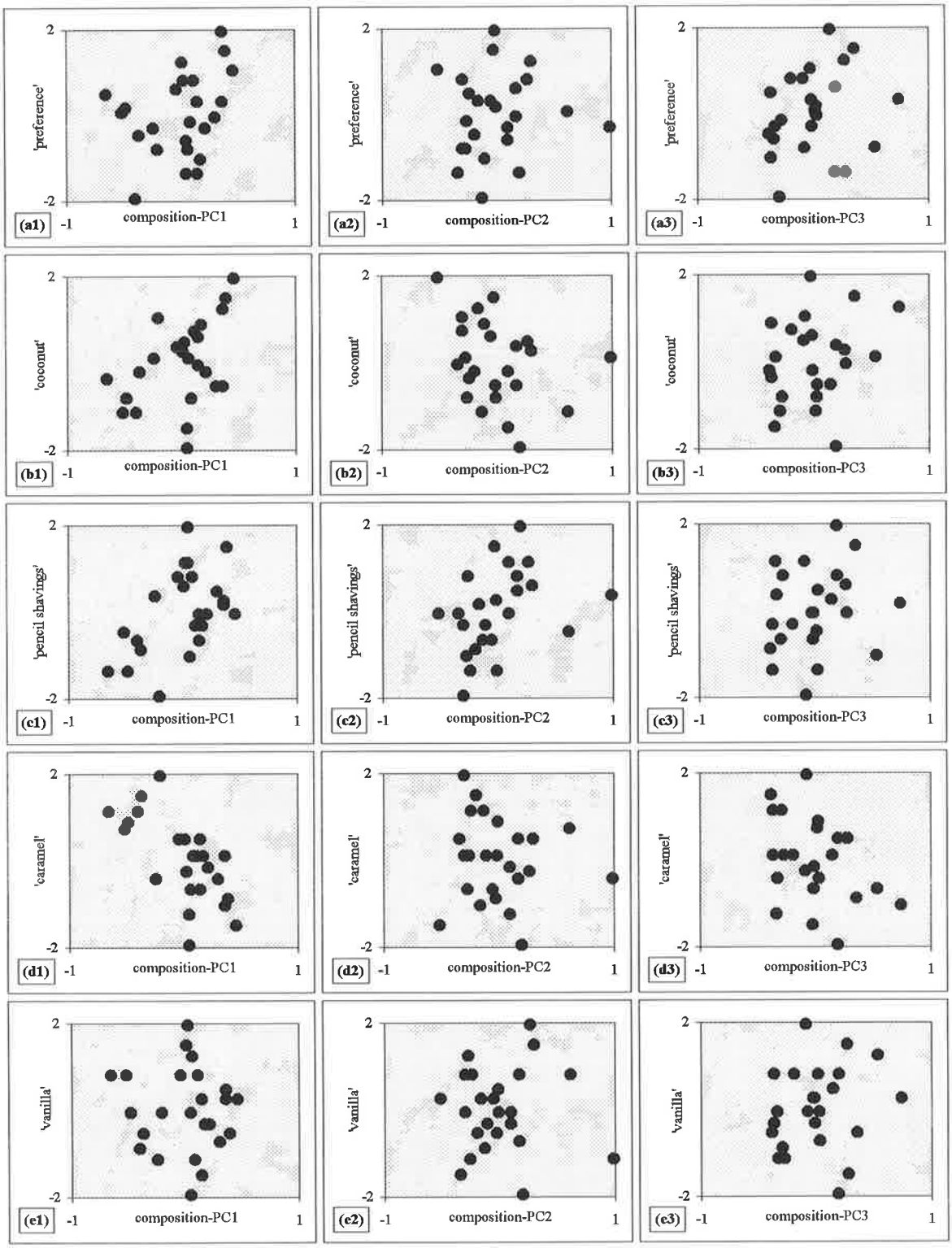
Appendix Figure G.9. Scatter plots of the Chardonnay wine Fisher-Yates rank transformation of 'cashew nut' versus volatile compound concentrations.



Appendix Figure G.10. Scatter plots of the Chardonnay wine Fisher-Yates rank transformation of 'green apple' versus volatile compound concentrations.

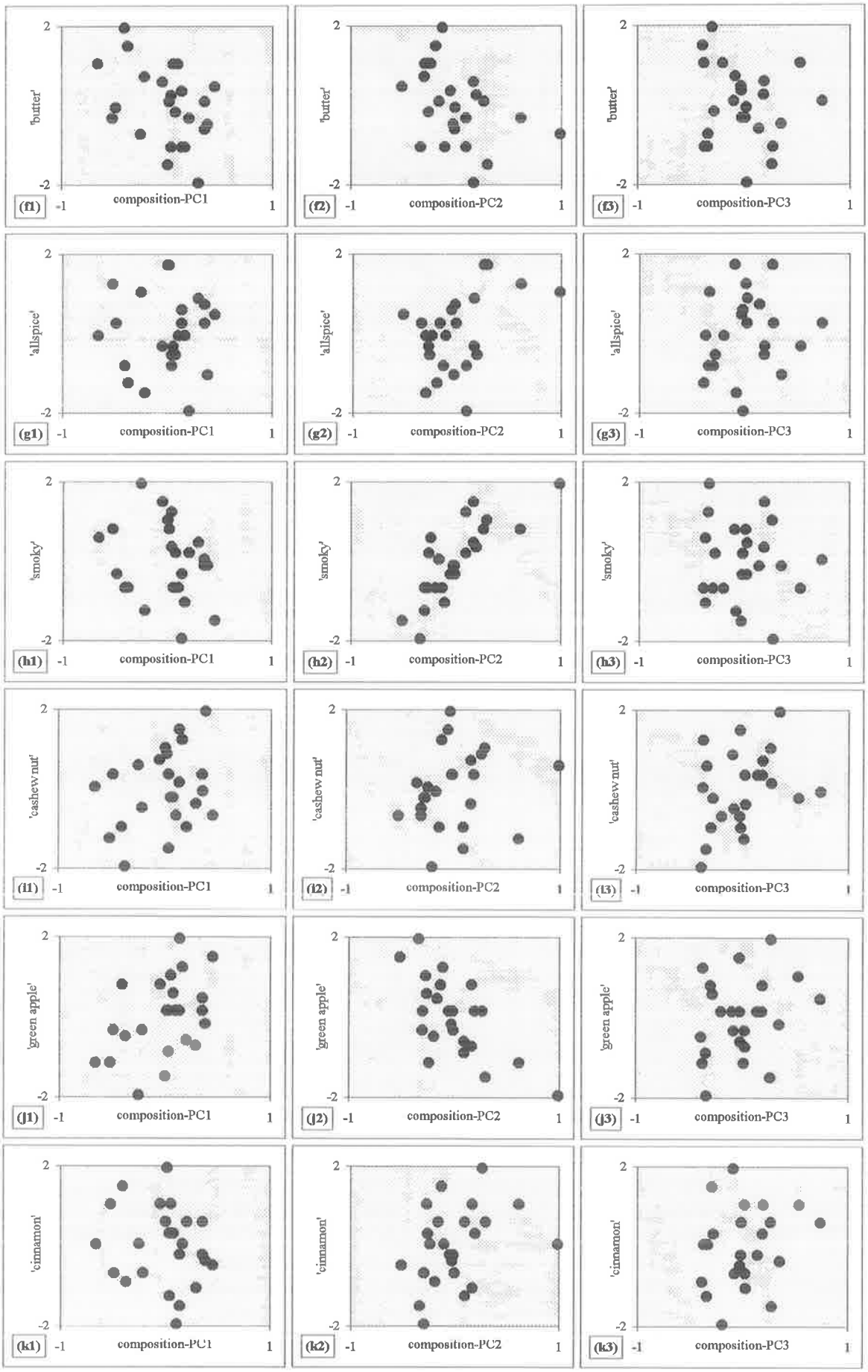


Appendix Figure G.11. Scatter plots of the Chardonnay wine Fisher-Yates rank transformation of 'cinnamon' versus volatile compound concentrations.



Appendix Figure G.12. Scatter plots of the Chardonnay wine Fisher-Yates rank transformations versus composition-PCs.

'Preference,' 'coconut,' 'pencil shavings,' 'caramel' and 'vanilla' on this page;
'butter,' 'allspice,' 'smoky,' 'cashew nut,' 'green apple' and 'cinnamon' on the following page.
For details of principal components analysis, see Appendix C.
PC1 (28% of the variance): 'emphasis on natural oak products and oak origin associations with some microbial products.'
PC2 (27% of the variance): 'emphasis on coopering heat products.'
PC3 (19% of the variance): 'emphasis on some microbial products.'


(f1) 'butter' composition-PC1
(f2) 'butter' composition-PC2
(f3) 'butter' composition-PC3
(g1) 'allspice' composition-PC1
(g2) 'allspice' composition-PC2
(g3) 'allspice' composition-PC3
(h1) 'smoky' composition-PC1
(h2) 'smoky' composition-PC2
(h3) 'smoky' composition-PC3
(i1) 'cashew nut' composition-PC1
(i2) 'cashew nut' composition-PC2
(i3) 'cashew nut' composition-PC3
(j1) 'green apple' composition-PC1
(j2) 'green apple' composition-PC2
(j3) 'green apple' composition-PC3
(k1) 'cinnamon' composition-PC1
(k2) 'cinnamon' composition-PC2
(k3) 'cinnamon' composition-PC3
2
-2
-1
1

Appendix H

Compound and composition-PC correlations with aromas - Cabernet Sauvignon barrel wine

Three compounds (vanillyl alcohol, 5-methylfurfuryl ethyl ether and vanillyl ethyl ether) have been omitted due to imprecision of measurement, *etc.*, as discussed in Section 2.4. 'Estimated extracted furfural' has been omitted since it is very similar to furfuryl alcohol.

Appendix Table H.1. Spearman's rank-order correlation matrix - 24 Cabernet Sauvignon barrel wines at 93 weeks - sensory & composition.

	cis^	*trans*	*eug*	*guaiac*	*4mg*	*van-f*#	*van-c*#	*cyc*	*malt*	*furf*	*eef*	*5mf*	*falc*	*5mfalc*	*fee*	*4vg*	*4eg*	*4vp*	*4ep*
preference	0.524	0.315	0.541	0.352	0.275	0.502	0.340	0.175	0.061	0.504	0.672	0.537	0.674	-0.610	0.386	-0.185	-0.555	-0.321	-0.410
coconut	0.861	0.768	0.865	0.468	0.337	0.217	0.427	0.101	0.252	0.351	0.290	0.545	0.288	-0.041	0.306	0.119	-0.020	0.057	-0.031
pencil shavings	0.045	0.113	0.123	0.057	0.340	0.204	-0.082	0.146	0.169	0.183	0.216	0.180	0.211	0.035	0.172	-0.035	0.012	-0.245	-0.101
allspice	0.317	0.231	0.291	0.097	0.301	0.381	0.427	-0.212	0.038	0.392	0.357	0.273	0.351	-0.378	0.209	-0.365	-0.513	-0.630	-0.534
berry	0.597	0.434	0.633	0.087	0.038	0.249	0.493	-0.170	-0.106	0.164	0.138	0.218	0.134	-0.166	0.083	0.112	-0.059	-0.031	-0.236
smoky	-0.026	-0.027	-0.293	-0.007	0.196	0.569	0.214	0.187	-0.113	0.353	0.456	0.031	0.453	0.120	0.348	-0.074	-0.005	-0.261	-0.262
caramel	0.319	0.407	0.390	0.478	0.440	0.390	0.546	0.319	0.171	0.350	0.484	0.380	0.481	-0.151	0.441	-0.236	-0.128	-0.103	-0.196
vanilla	0.776	0.551	0.713	0.417	0.330	0.320	0.482	0.264	0.273	0.571	0.502	0.545	0.497	-0.074	0.350	0.116	-0.107	-0.091	-0.197
coffee	0.443	0.440	0.305	0.438	0.522	0.878	0.581	0.199	0.141	0.565	0.739	0.506	0.738	-0.136	0.566	-0.212	-0.257	-0.429	-0.480
dark chocolate	0.564	0.514	0.446	0.356	0.348	0.627	0.482	0.124	0.149	0.349	0.477	0.362	0.471	-0.023	0.684	0.025	-0.014	-0.120	-0.359
Band-aid	-0.247	-0.181	-0.414	-0.054	0.024	0.400	-0.053	-0.056	-0.229	0.230	0.292	0.068	0.298	-0.073	0.055	-0.048	-0.156	-0.283	-0.096
earthy	-0.504	-0.226	-0.579	-0.095	0.091	-0.163	-0.377	-0.046	-0.079	-0.172	-0.238	-0.231	-0.236	0.183	-0.006	-0.133	0.174	-0.187	0.083
mint	0.196	0.227	0.135	-0.039	0.178	0.314	0.299	-0.086	0.066	0.385	0.235	0.129	0.231	0.111	0.020	-0.331	0.026	-0.236	-0.287

^: cis=*cis*-oak lactone, trans=*trans*-oak lactone, eug=eugenol, guaiac=guaiacol, 4mg=4-methylguaiacol, van=vanillin, cyc=cyclotene, malt=maltol, furf=furfural, eef='estimated extracted furfural' (furfural + furfuryl alcohol), 5mf=5-methylfurfural, falc=furfuryl alcohol, 5mfalc=5-methylfurfuryl alcohol, fee=furfuryl ethyl ether, 4vg=4-vinylguaiacol, 4eg=4-ethylguaiacol, 4vp=4-vinylphenol, 4ep=4-ethylphenol.

#: vanillin sample - freezer or cellar-stored. See footnote to Appx.Tab.H.2.

: significant correlation, $p<0.05$ or stronger.

Critical values for 2-tailed test of correlation, $n = 24$, from O'Mahony (1986).

If rho is greater than or equal to 0.407, significant correlation, $p<0.05$.

If rho is greater than or equal to 0.521, significant correlation, $p<0.01$.

If rho is greater than or equal to 0.608, significant correlation, $p<0.002$.

Appendix Table H.2. Pearson's product-moment correlation coefficient matrix - 24 Cab. Sauv. barrel wines at 93 weeks - sensory & composition.

																				composition		
	cis^	*trans*	*eug*	*guaiac*	*4mg*	*van-f*#	*van-c*#	*cyc*	*malt*	*furf*	*eef*	*5mf*	*falc*	*5mfalc*	*fee*	*4vg*	*4eg*	*4vp*	*4ep*	*PC1*	*PC2*	*PC3*
pref^	0.469	0.294	0.486	0.285	0.251	0.511	0.366	0.142	0.107	0.441	0.557	0.556	0.559	-0.531	0.253	-0.201	-0.462	-0.334	-0.405	0.410	-0.579	0.570
cocon	0.717	0.704	0.826	0.345	0.298	0.265	0.444	0.065	0.239	0.324	0.268	0.443	0.268	-0.044	0.229	0.088	0.028	0.029	-0.011	0.355	-0.129	0.786
pncsh	0.120	0.060	0.023	0.052	0.256	0.240	-0.073	0.145	0.104	0.087	0.144	0.092	0.145	-0.013	0.086	-0.032	-0.024	-0.242	-0.087	0.163	-0.145	0.138
allspc	0.355	0.151	0.234	0.165	0.202	0.400	0.459	-0.106	0.099	0.357	0.306	0.316	0.305	-0.337	0.087	-0.333	-0.504	-0.579	-0.547	0.251	-0.563	0.357
berry	0.567	0.437	0.603	-0.021	0.031	0.249	0.474	-0.224	-0.078	0.172	0.091	0.286	0.091	-0.203	-0.007	0.027	-0.054	-0.030	-0.244	0.060	-0.165	0.520
smoky	0.047	-0.029	-0.349	0.082	0.143	0.447	0.169	0.218	0.024	0.323	0.414	-0.013	0.413	0.019	0.387	-0.068	-0.083	-0.288	-0.294	0.253	-0.273	0.054
caram	0.243	0.383	0.365	0.466	0.460	0.392	0.522	0.383	0.382	0.404	0.535	0.296	0.535	-0.141	0.507	-0.239	-0.069	-0.071	-0.150	0.550	-0.306	0.484
vanilla	0.752	0.547	0.669	0.345	0.335	0.369	0.534	0.249	0.386	0.508	0.480	0.442	0.481	-0.206	0.355	0.017	-0.168	-0.176	-0.209	0.486	-0.341	0.764
coffee	0.340	0.447	0.238	0.456	0.543	0.821	0.603	0.349	0.329	0.567	0.683	0.454	0.681	-0.254	0.542	-0.215	-0.252	-0.457	-0.427	0.641	-0.586	0.603
dkchc	0.534	0.498	0.391	0.216	0.311	0.533	0.502	0.106	0.258	0.301	0.417	0.340	0.416	-0.067	0.462	0.010	0.013	-0.100	-0.346	0.391	-0.270	0.612
B-aid	-0.208	-0.221	-0.432	-0.037	0.055	0.368	0.012	0.015	-0.231	0.240	0.193	0.104	0.191	-0.171	0.081	-0.014	-0.225	-0.305	-0.055	0.053	-0.256	-0.139
earthy	-0.482	-0.277	-0.598	0.048	0.030	-0.175	-0.337	0.013	-0.054	-0.143	-0.173	-0.339	-0.176	0.336	0.000	-0.015	0.240	-0.034	0.122	-0.100	0.199	-0.439
mint	0.302	0.150	0.065	-0.110	0.107	0.301	0.247	-0.077	0.043	0.380	0.179	-0.007	0.178	0.066	0.172	-0.339	-0.035	-0.208	-0.246	0.143	-0.213	0.217

^: For abbreviations, see Appendix Table H.1.

#: *van-f*=vanillin from freezer-stored samples (-10 degC for 3 years since barrel sampling); *van-c*=vanillin from cellar-stored samples (~20 degC for 1 year from barrel sampling, then sterilised with DMDC and stored for a further 2 years at ~20 degC). The sensory analyses were performed on the cellar-stored samples approximately 1 year after sterilisation.

: significant correlation, $p<0.05$ or stronger.

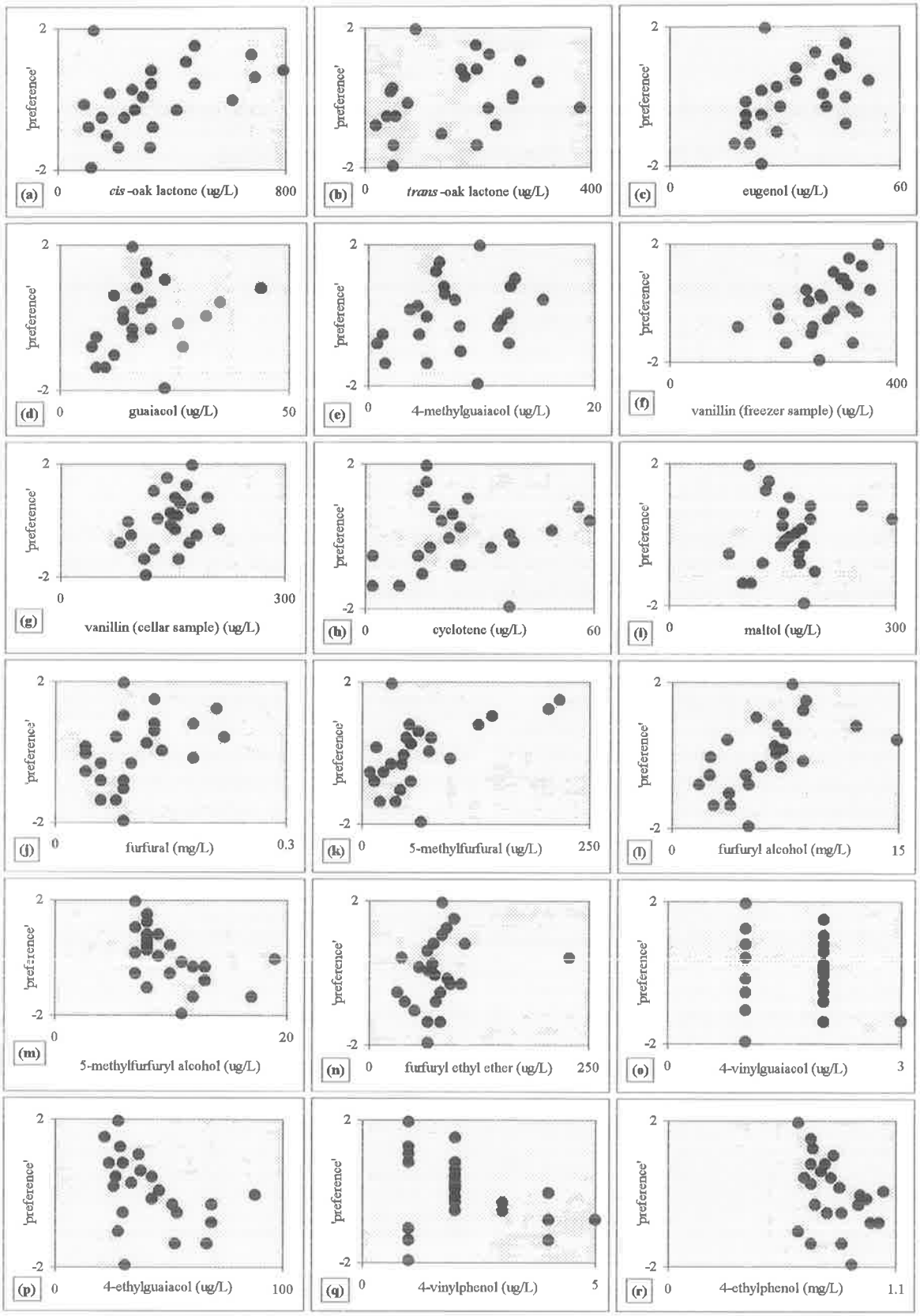
Critical values for 2-tailed test of correlation, $n = 24$, $d.f. = n - 2 = 24 - 2 = 22$.

If r is greater than or equal to 0.404, significant correlation, $p<0.05$.*

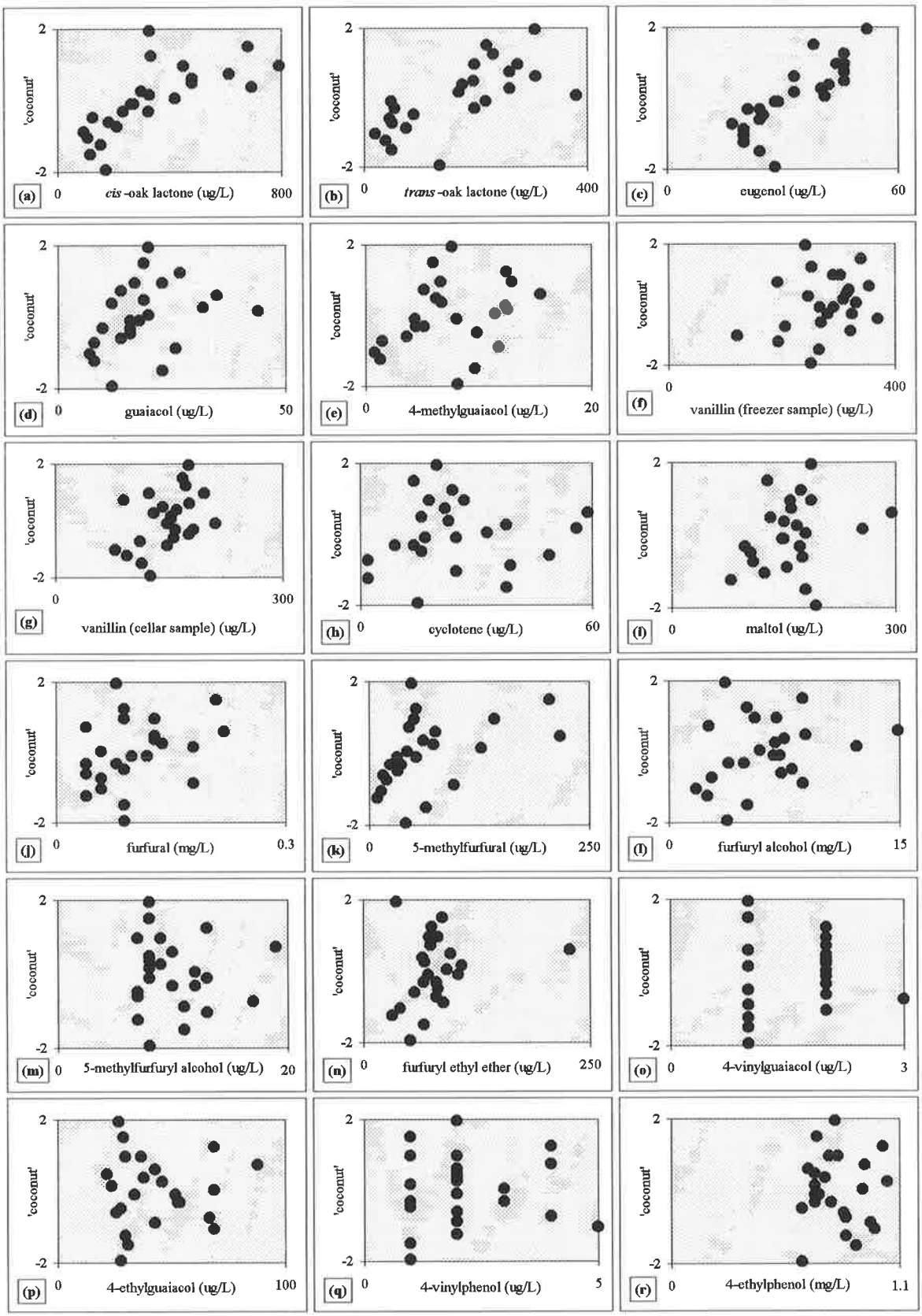
If r is greater than or equal to 0.515, significant correlation, $p<0.01$.*

If r is greater than or equal to 0.6524, significant correlation, $p<0.001$.**

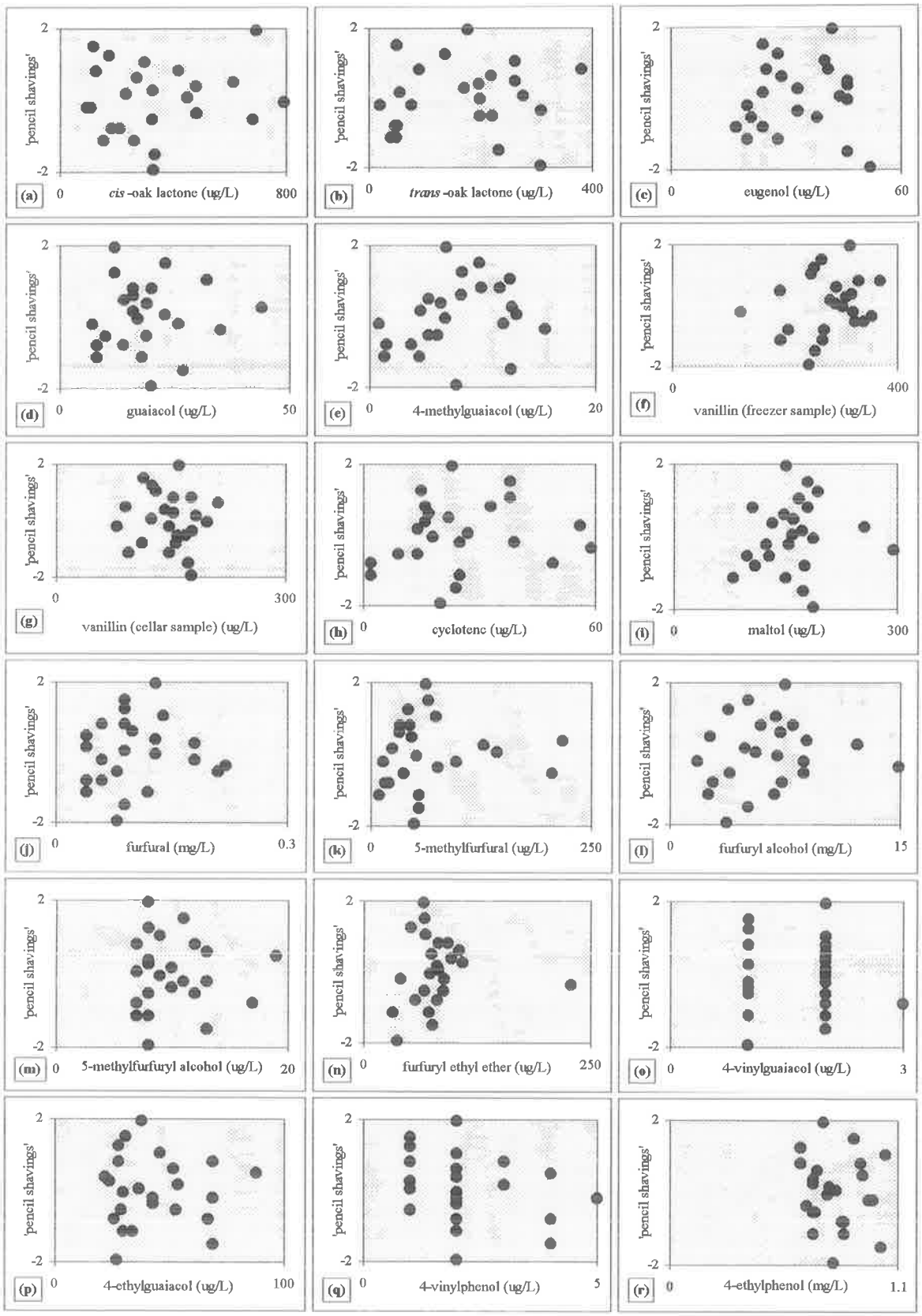
*: from Snedecor and Cochran (1967), 22 *d.f.* **: from O'Mahony (1986), 20 *d.f*



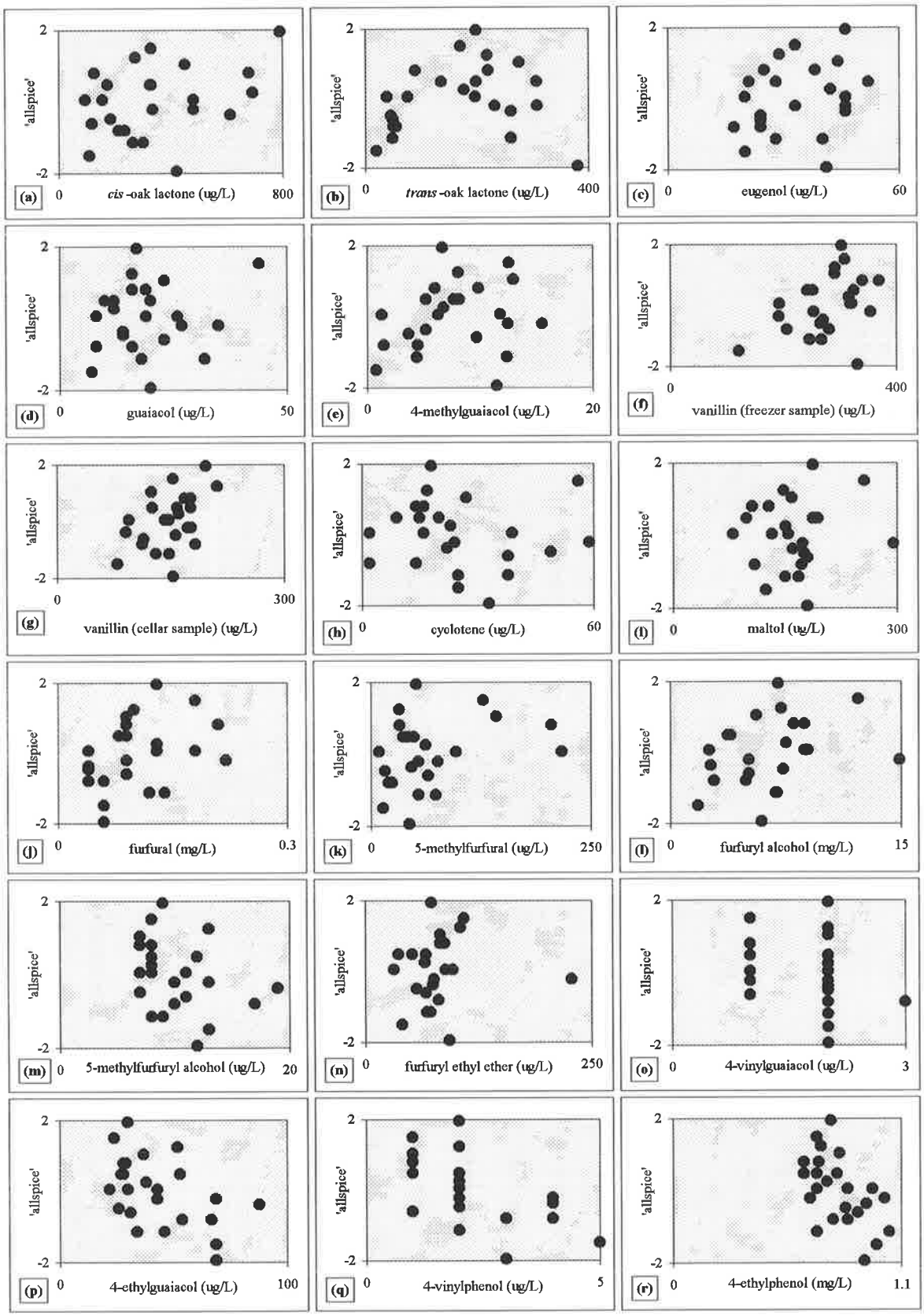
Appendix Figure H.1. Scatter plots of the Cabernet Sauvignon wine Fisher-Yates rank transformation of 'preference' versus volatile compound concentrations.



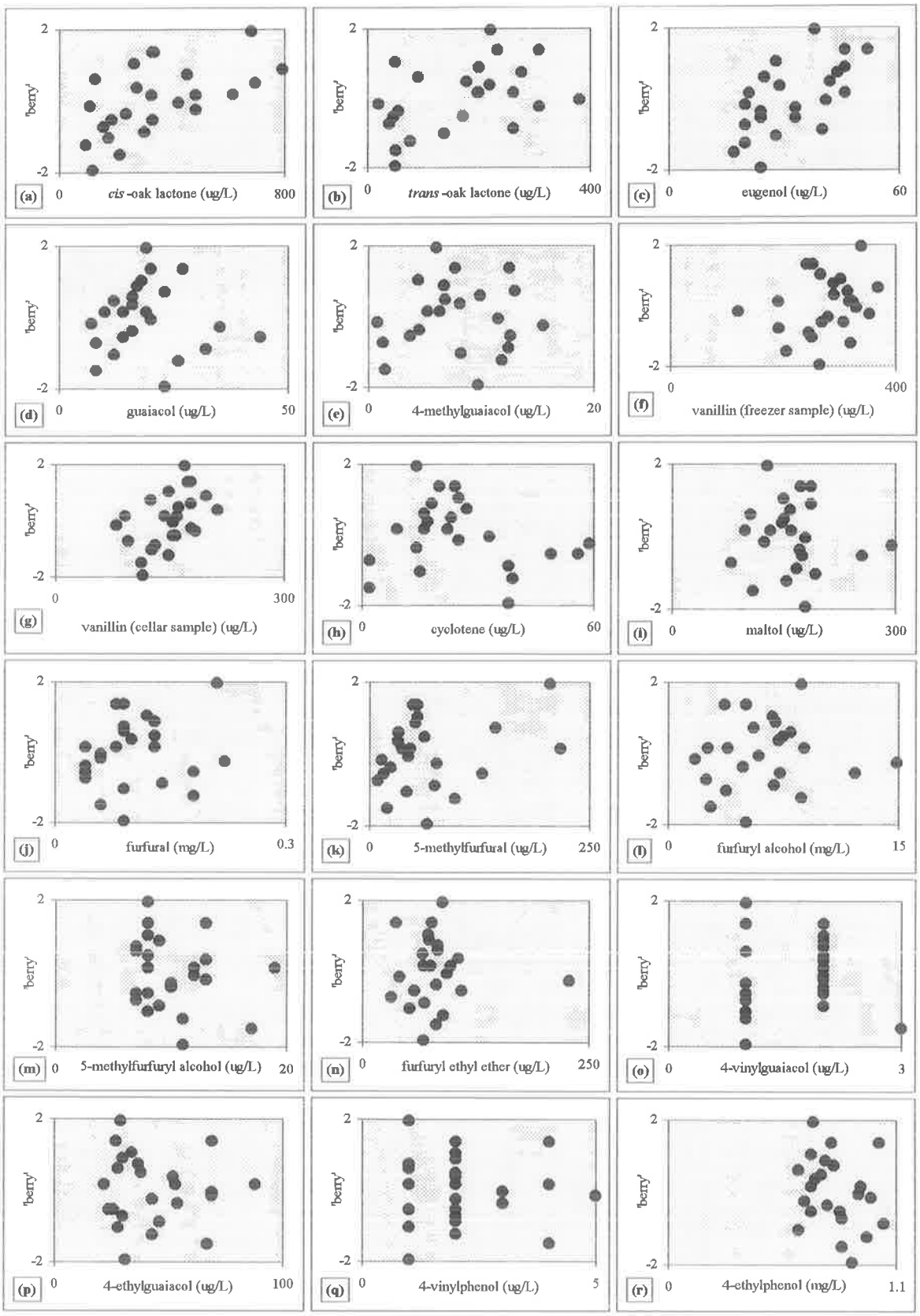
Appendix Figure H.2. Scatter plots of the Cabernet Sauvignon wine Fisher-Yates rank transformation of 'coconut' versus volatile compound concentrations.



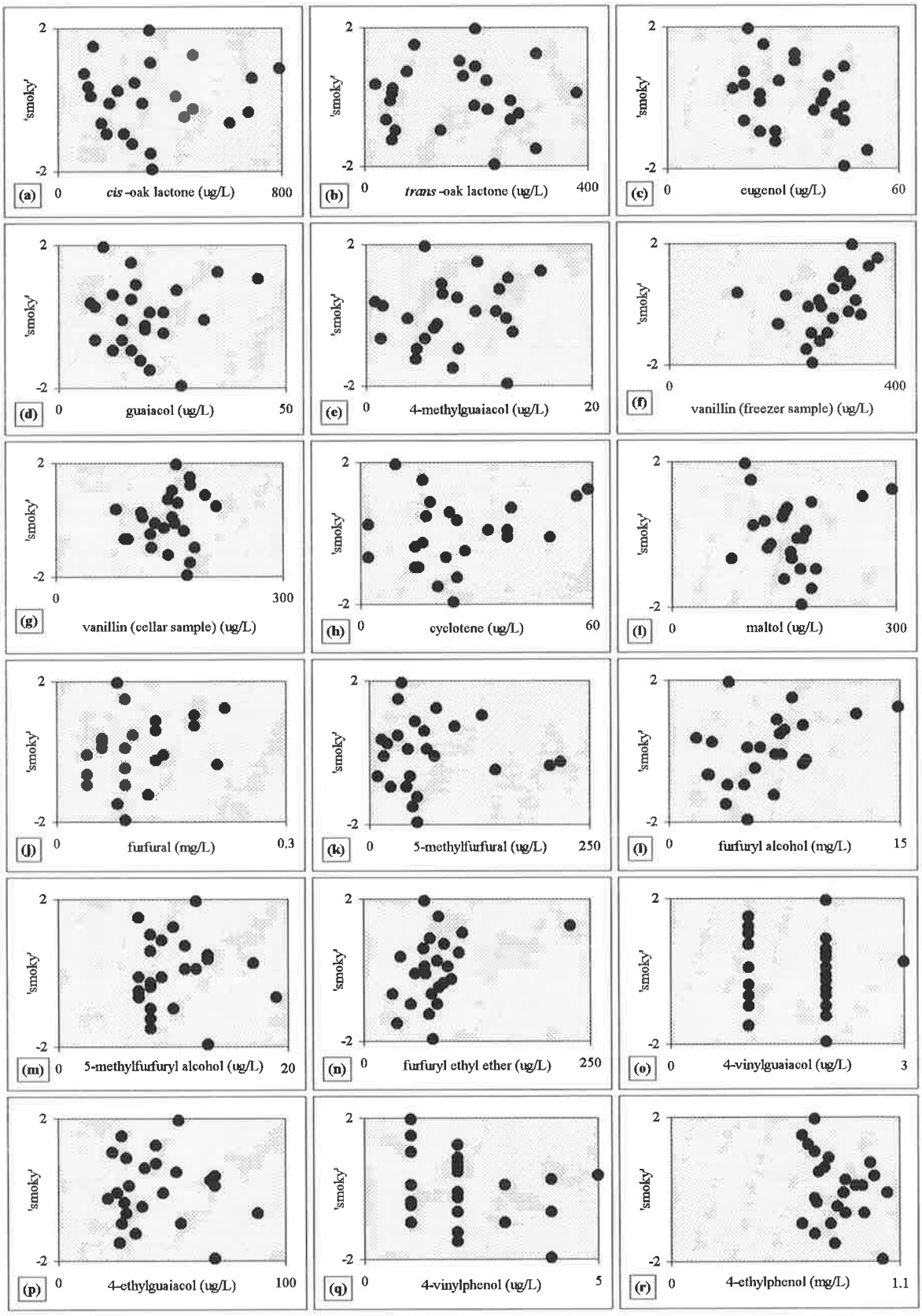
Appendix Figure H.3. Scatter plots of the Cabernet Sauvignon wine Fisher-Yates rank transformation of 'pencil shavings' versus volatile compound concentrations.



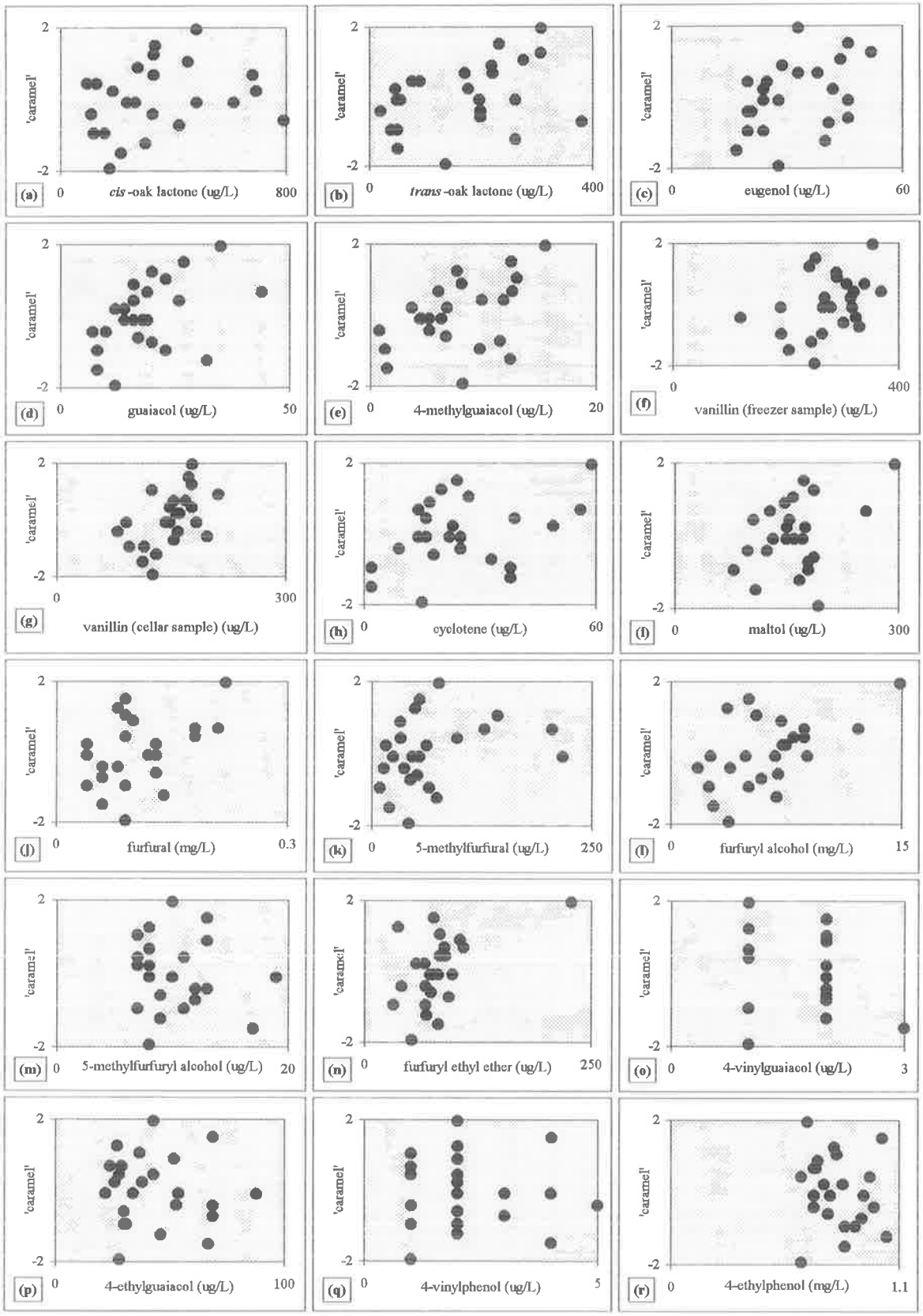
Appendix Figure H.4. Scatter plots of the Cabernet Sauvignon wine Fisher-Yates rank transformation of 'allspice' versus volatile compound concentrations.



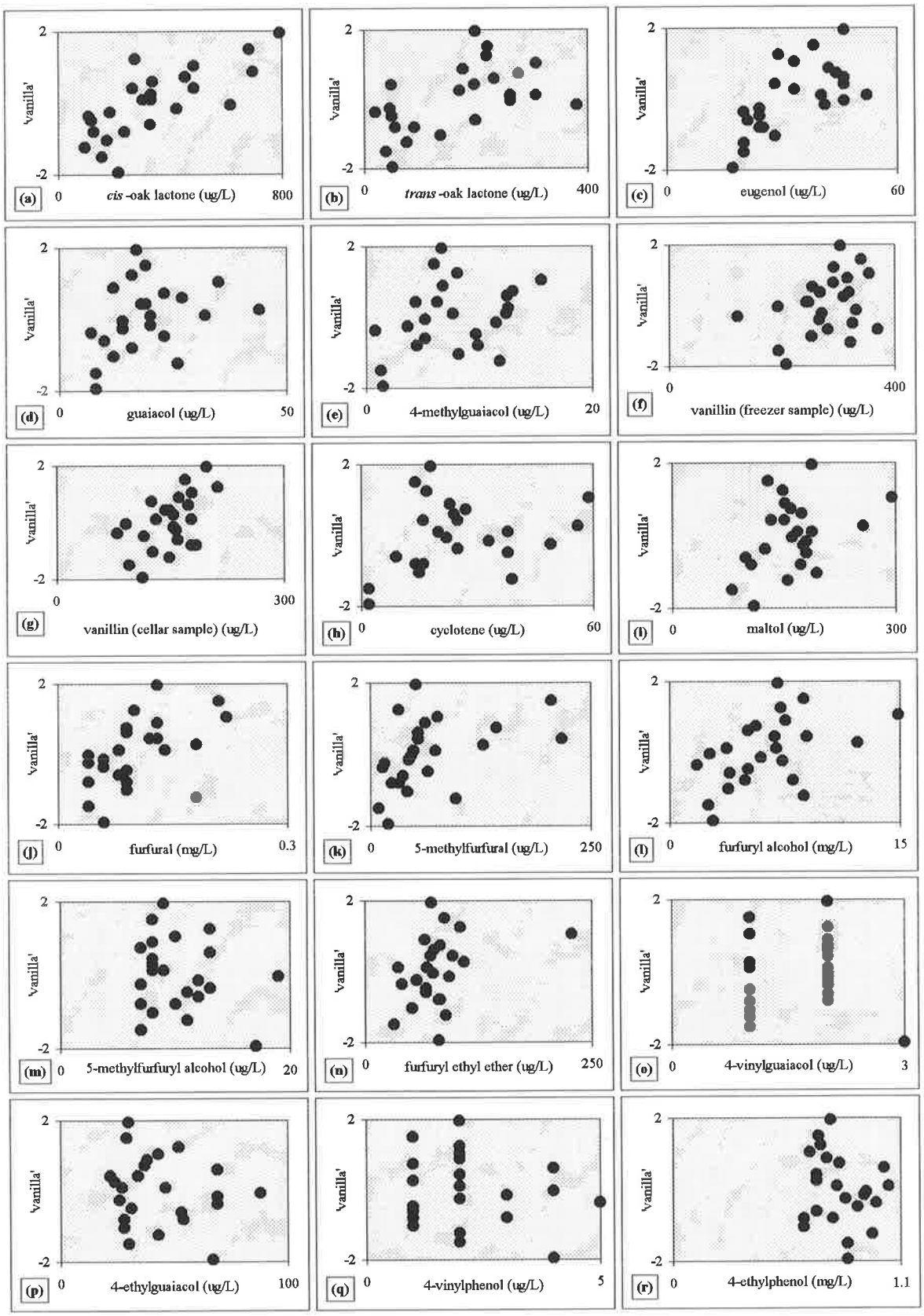
Appendix Figure H.5. Scatter plots of the Cabernet Sauvignon wine Fisher-Yates rank transformation of 'berry' versus volatile compound concentrations.



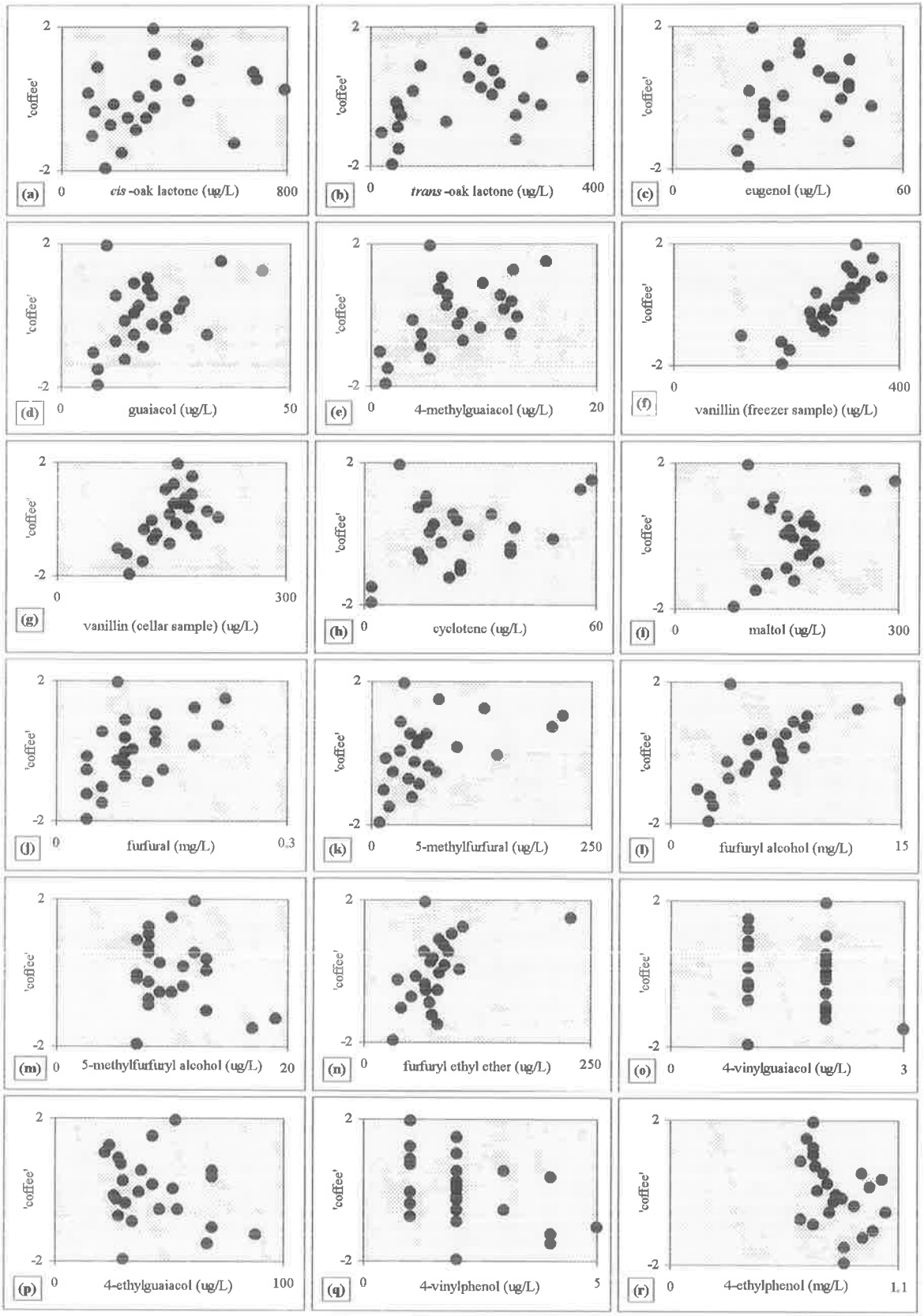
Appendix Figure H.6. Scatter plots of the Cabernet Sauvignon wine Fisher-Yates rank transformation of 'smoky' versus volatile compound concentrations.



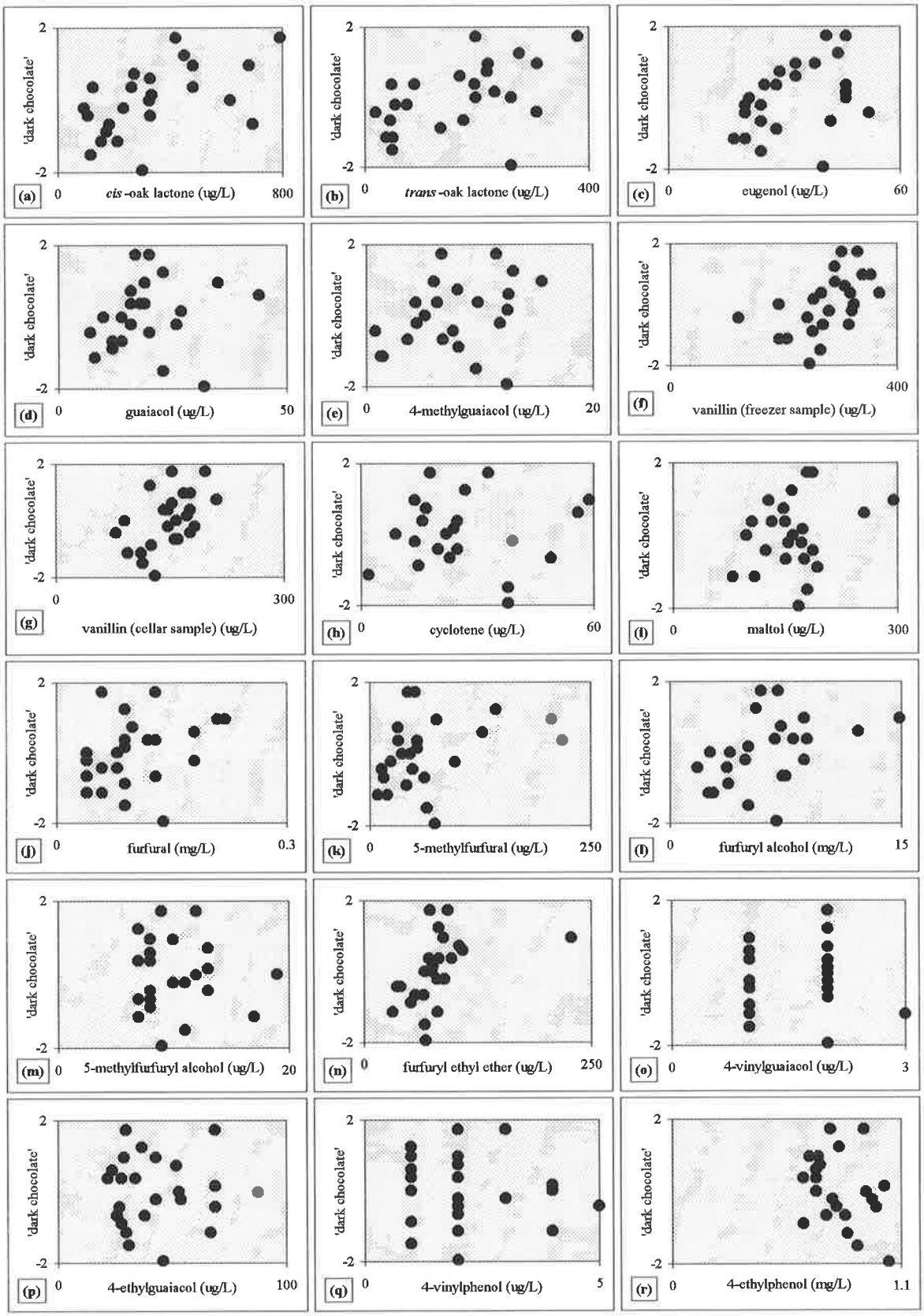
Appendix Figure H.7. Scatter plots of the Cabernet Sauvignon wine Fisher-Yates rank transformation of 'caramel' versus volatile compound concentrations.



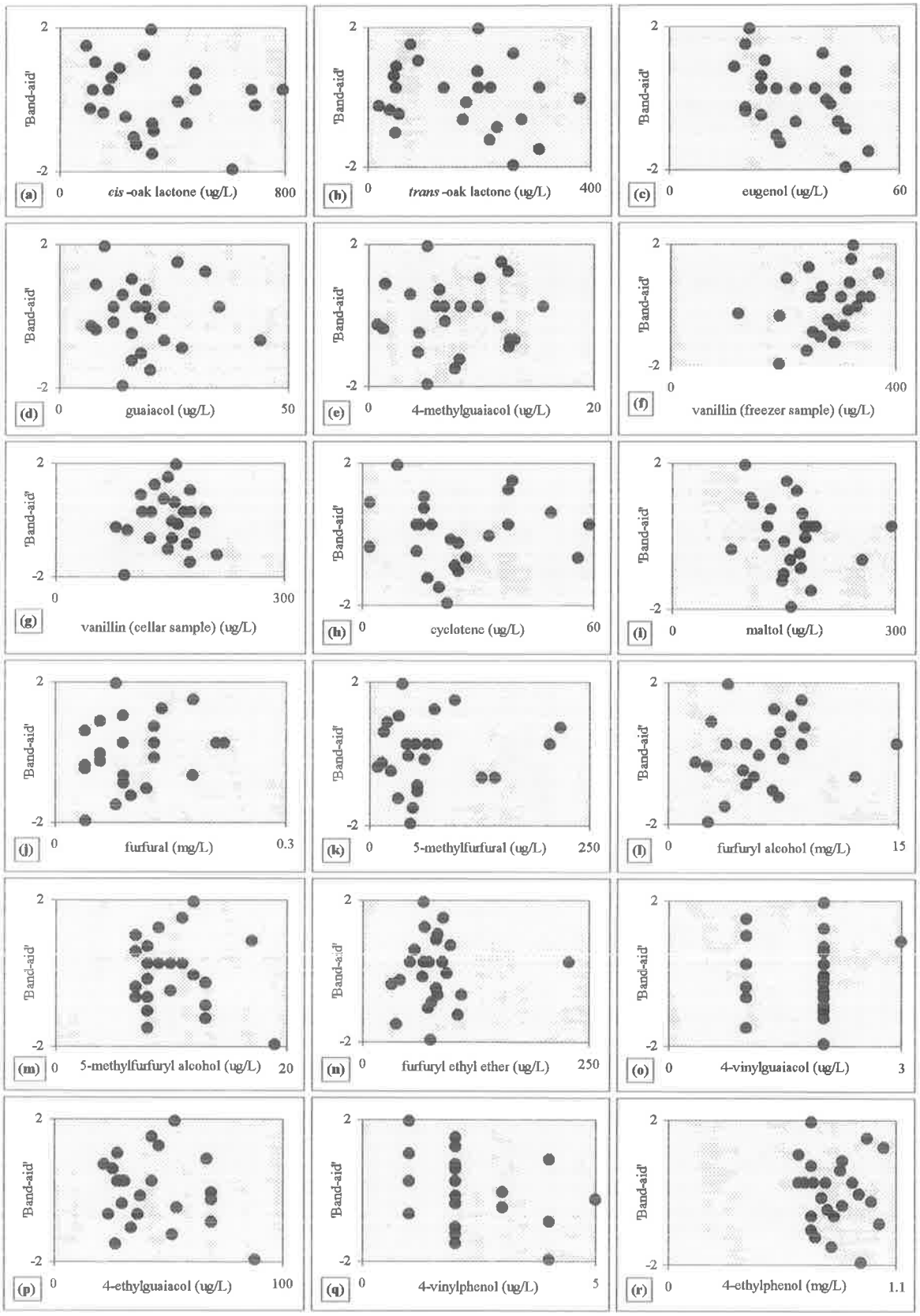
Appendix Figure H.8. Scatter plots of the Cabernet Sauvignon wine Fisher-Yates rank transformation of 'vanilla' versus volatile compound concentrations.



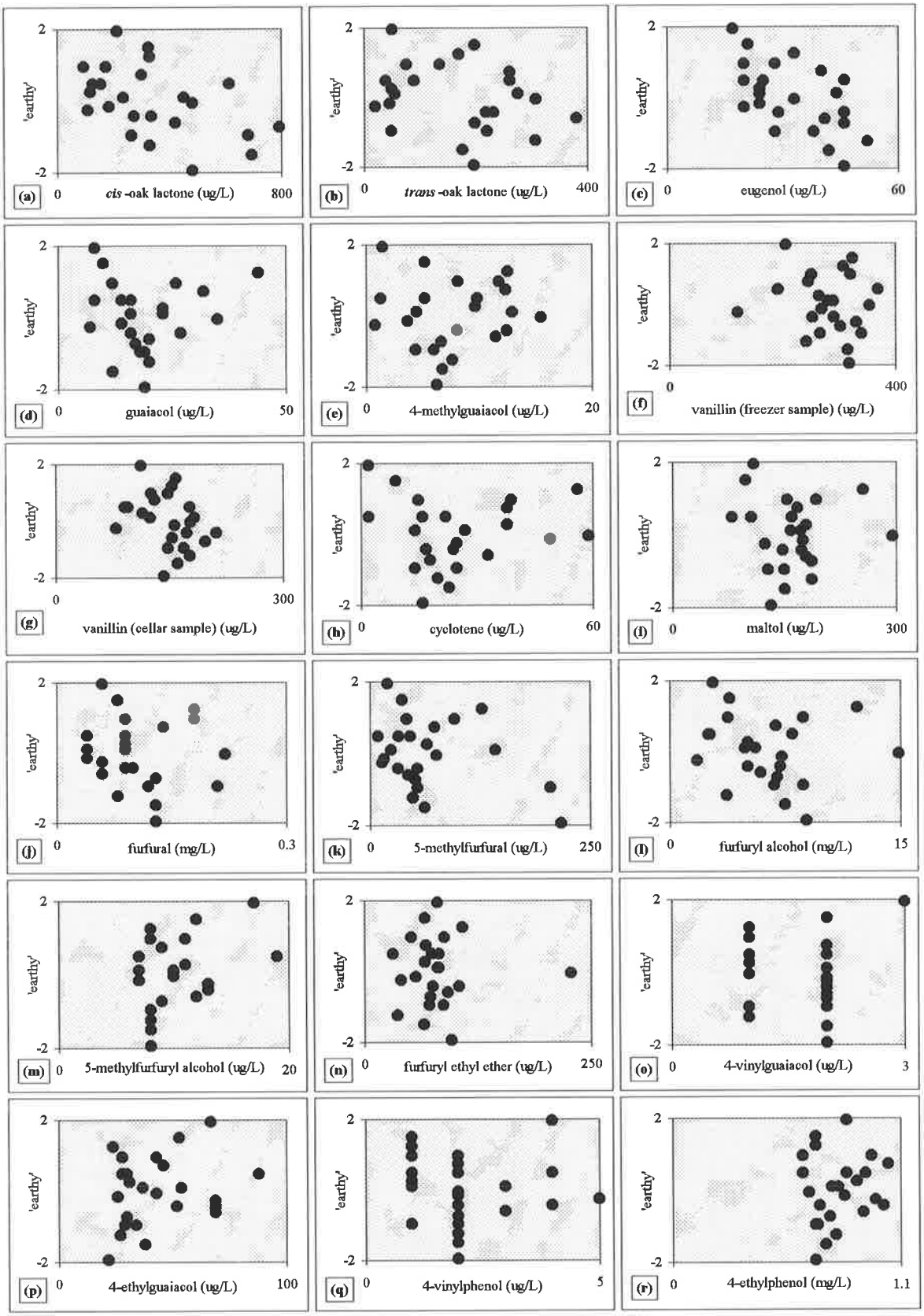
Appendix Figure H.9. Scatter plots of the Cabernet Sauvignon wine Fisher-Yates rank transformation of 'coffee' versus volatile compound concentrations.



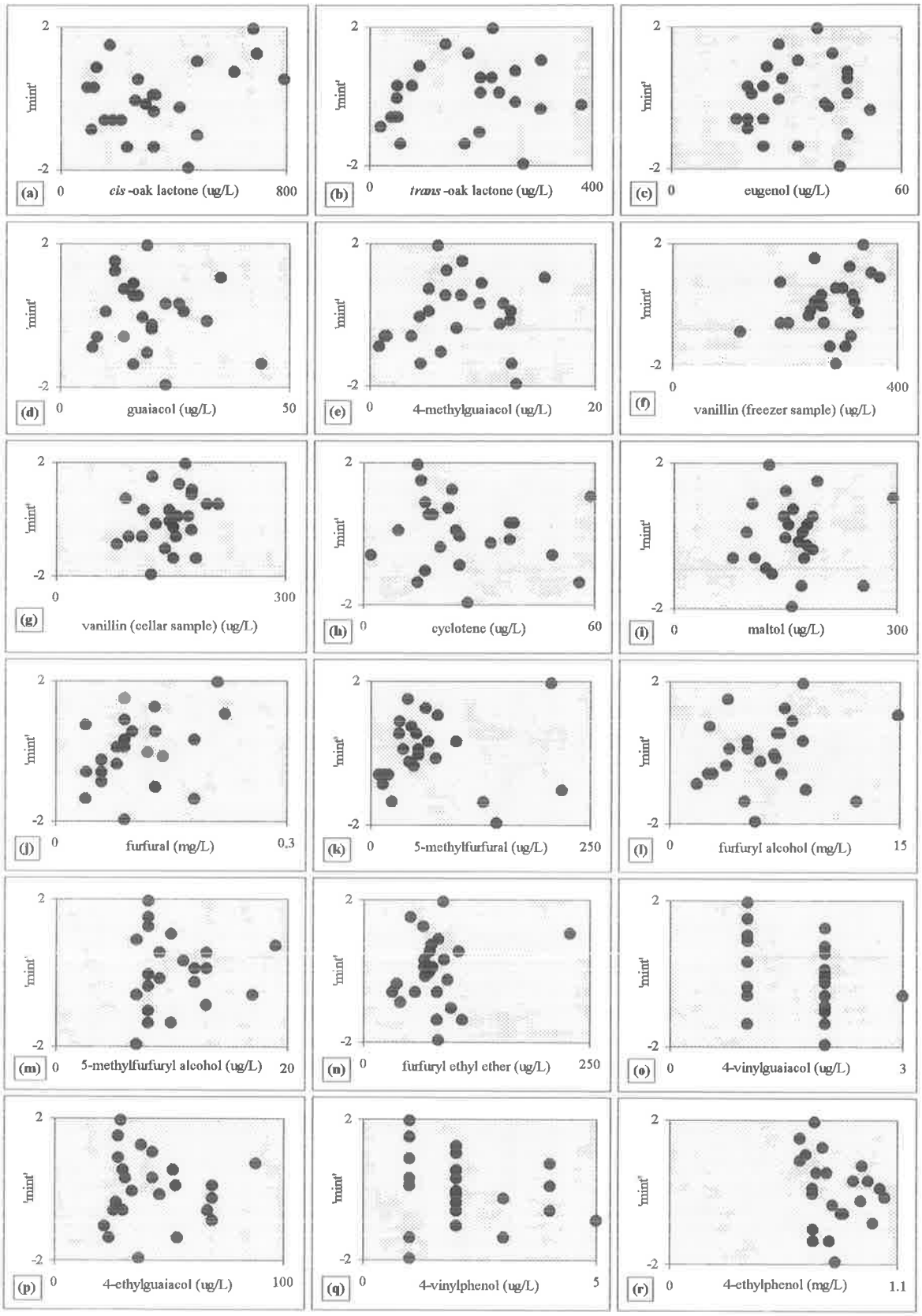
Appendix Figure H.10. Scatter plots of the Cabernet Sauvignon wine Fisher-Yates rank transformation of 'dark chocolate' versus volatile compound concentrations.



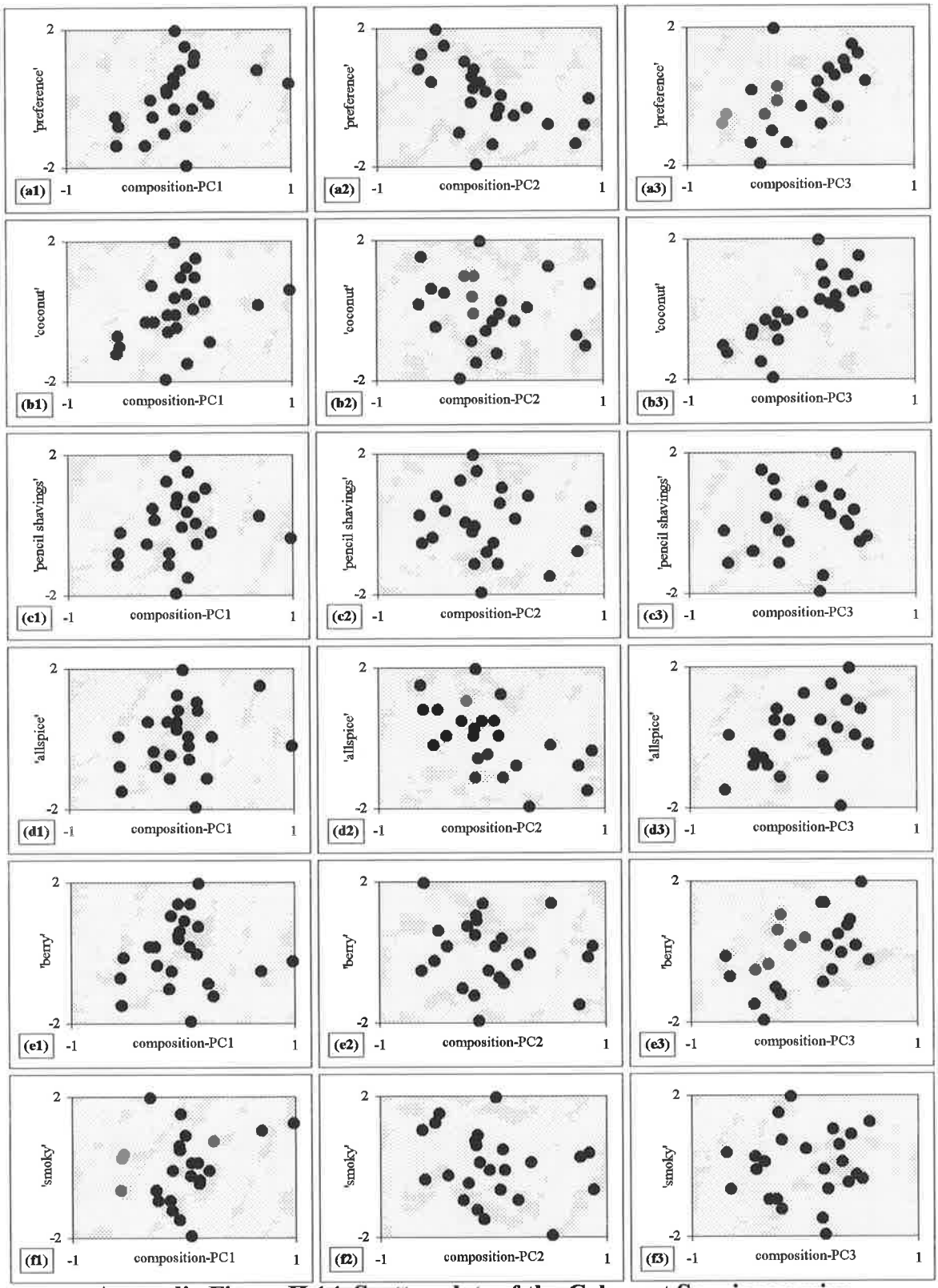
Appendix Figure H.11. Scatter plots of the Cabernet Sauvignon wine Fisher-Yates rank transformation of 'Band-aid' versus volatile compound concentrations.



Appendix Figure H.12. Scatter plots of the Cabernet Sauvignon wine Fisher-Yates rank transformation of 'earthy' versus volatile compound concentrations.

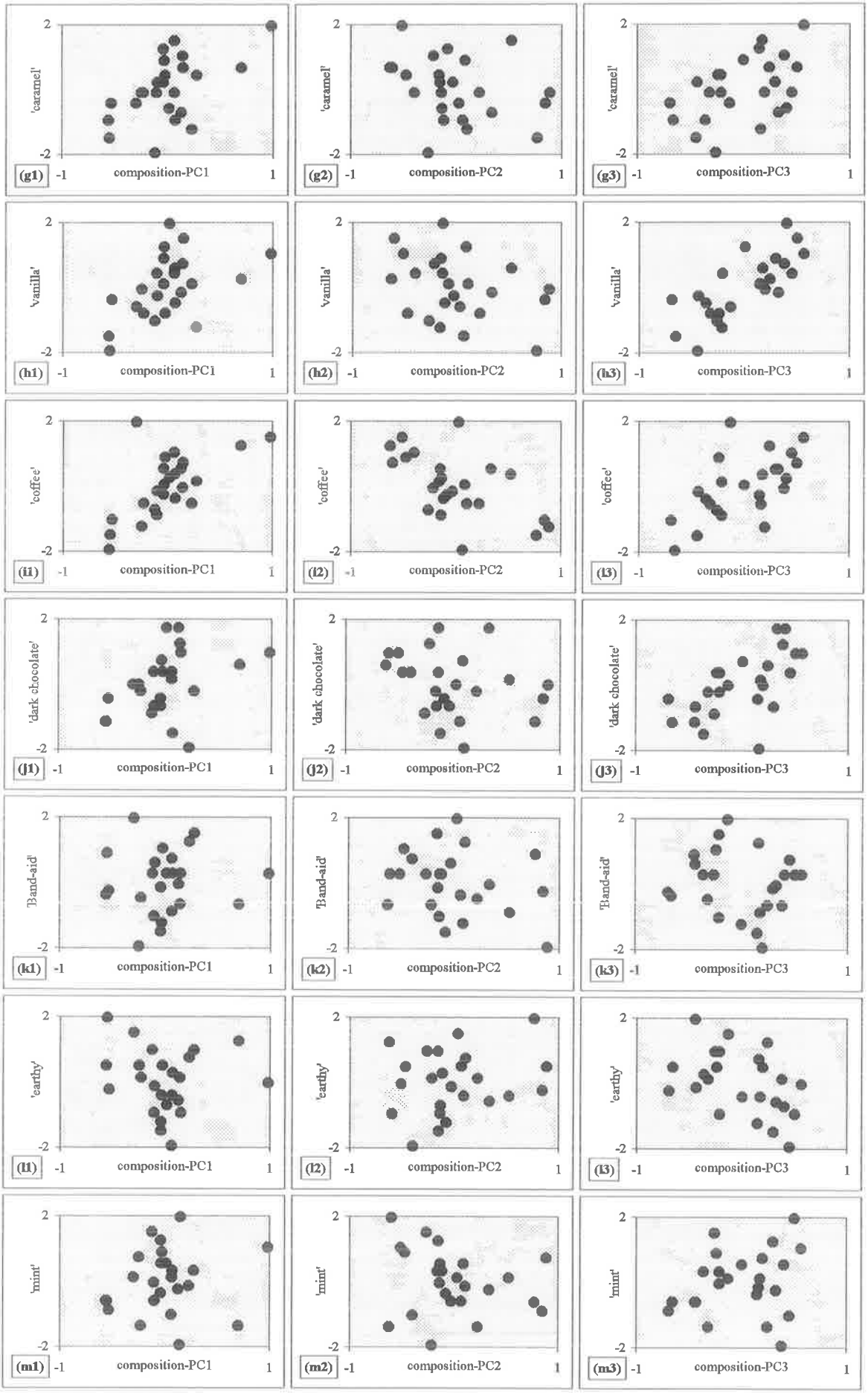


Appendix Figure H.13. Scatter plots of the Cabernet Sauvignon wine Fisher-Yates rank transformation of 'mint' versus volatile compound concentrations.



Appendix Figure H.14. Scatter plots of the Cabernet Sauvignon wine Fisher-Yates rank transformations versus composition-PCs.

'Preference,' 'coconut,' 'pencil shavings,' 'allspice,' 'berry' and 'smoky' on this page;
'caramel,' 'vanilla,' 'coffee,' 'dark chocolate,' 'Band-aid,' 'earthy' and 'mint' on the following page.
For details of principal components analysis, see Appendix C.
PC1 (30% of the variance): 'emphasis on coopering heat products.'
PC2 (25% of the variance): 'emphasis on some microbial products.'
PC3 (17% of the variance): 'emphasis on natural oak products.'


'caramel'
'vanilla'
'coffee'
'dark chocolate'
'Band-aid'
'earthy'
'mint'
composition-PC1
composition-PC2
composition-PC3
(g1)
(g2)
(g3)
(h1)
(h2)
(h3)
(i1)
(i2)
(i3)
(j1)
(j2)
(j3)
(k1)
(k2)
(k3)
(l1)
(l2)
(l3)
(m1)
(m2)
(m3)
2
-2
-1
1

Appendix I

Potency of the overall, oak wood–derived, aroma–effect of selected individual barrel wines — materials, methods and results

Appendix outline

An introduction and a discussion of the results of this experiment may be found in Section 4.4. Further details are shown below.

I.1. Materials and methods

The relative compositions of the three selected barrel–stored wines were shown in Figure 4.1.

The ASTM (American Society of Testing and Materials) rapid method (E679), as described in Meilgaard *et al.* (1991 pp. 124–128), was used. This is based on the Ascending Method of Limits and on a method of sample presentation known as the 3–Alternative Forced Choice (3–AFC) in which 3 samples are presented; 2 are controls and one contains the test substance. So–called Best Estimate Thresholds (BETs) were determined using this method. This gives a very approximate "best estimate" determination of each panelist's threshold — but more panelists can be tested, using a given amount of effort, leading to a more reliable group threshold and distribution.

Twenty panelists (16 male; other demographics in Appx. Tab. I.1), familiar with the barrel–stored Chardonnay wines and triangle difference testing from the difference testing described in Appendix D (15 days of tests within the month preceding this experiment), were used. Each received six 3–AFC tests containing ascending concentrations of a barrel–stored Chardonnay wine mixed with the stainless steel–stored Chardonnay (control) wine. They were not told that the sets were presented in an ascending order.

The proportion of barrel–aged wine presented was from 50 % to 1.56 %, spaced by a factor of 2 in 5 steps. Those panelists correct at the lowest level and those who failed at the highest level were retested, once. If failing at the 50 % level, they were retested at the 50 % and 100 % levels; if correct at the 1.56 % level, they were retested at the 1.56 %, the 0.78 % and the 0.39 % levels. On one occasion, a panelist was correct at the 0.39 % level so was retested at the 0.39 %, the 0.20 % and the 0.10 % levels.

The position of the different wine in each set was determined randomly. Bottles of control wine were first homogenised in a 10 L conical flask. Then the greatest dilution to the smallest dilution was prepared in a 2 L conical flask, and poured (approx. 18 mL) into each glass.

I.2. Results

A summary of the results, in relation to published thresholds, is presented in Table 4.1. More detail is shown in Appendix Table I.2 and Appendix Figure I.1 which show the concentration ranges corresponding to the individual BETs, and the distribution of the individual BETs, respectively. Details of the results for wines *VA39*, *AA11* and *AU4* are shown in Appendix Tables I.3, I.4 and I.5, respectively.

The BET for each subject is the geometric mean of the highest concentration missed and the next higher concentration. The group BET is the geometric mean of the individual BETs. When retesting extreme results, if there was no discreet space between the highest concentration incorrectly identified and the next higher (*i.e.* there was an overlap), the concentration served which was correctly identified once and incorrectly identified once was quoted as the BET.

Meilgaard *et al.* (1991 p. 127) suggest repeating the test series with the same subjects on at least one other day, and to continue repeating the test until the group BET decreases by less than 20 %. The threshold will often decrease as the subjects become familiar with the procedure and the substance. Given limited wine stocks and that the panelists had just experienced weeks of difference testing with the same wines, only one of the three tests (*VA39*) was repeated (Appx. Tab. I.6). This showed a decrease in the group BET from 6.01 % to 4.84 %, an improvement of 19 %, below the 20 % level recommended as the minimum for discontinuation of retesting. Thus, no more retesting was performed. The final result for *VA39* was obtained by averaging the performances of the 16 panelists who participated in both sessions, and taking the results of the other 4 panelists from whichever session they had attended (Appx. Tab. I.3).

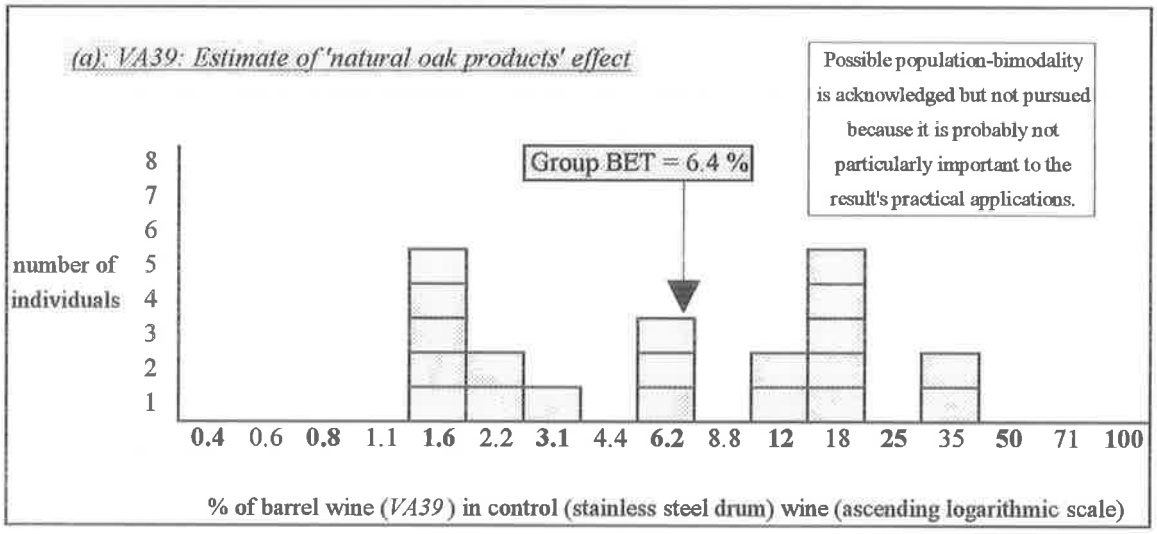
Appendix Table I.1. Wine evaluation experience, consumption frequency and age of the 20 panelists who participated in the 'potency of the overall, oak wood–derived, aroma–effect of selected individual barrel wines' experiment.

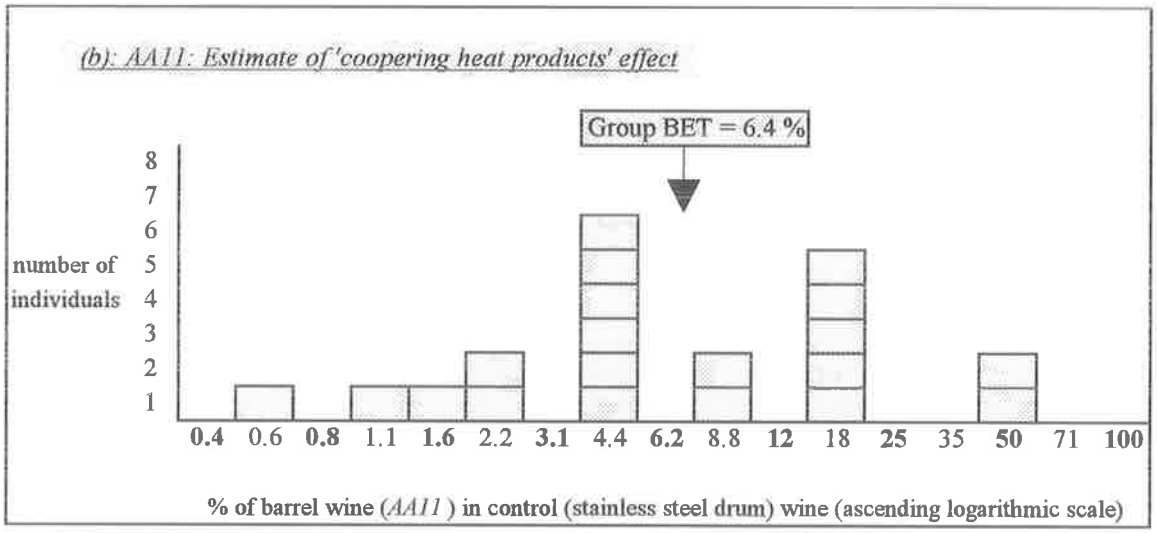
Accustomisation to paying more than very brief attention to wine aroma during consumption			
unaccustomed	*moderately accustomed*	*well accustomed*	*very well accustomed*
1	5	4	10
Wine consumption frequency			
more frequently than weekly	*more frequently than monthly but less frequently than weekly*	*more frequently than yearly but less frequently than monthly*	*less frequently than yearly*
15	5	0	0
Age			
20 to 25 years	*26 to 30 years*	*31 to 40 years*	*41 to 60 years*
4	3	9	4

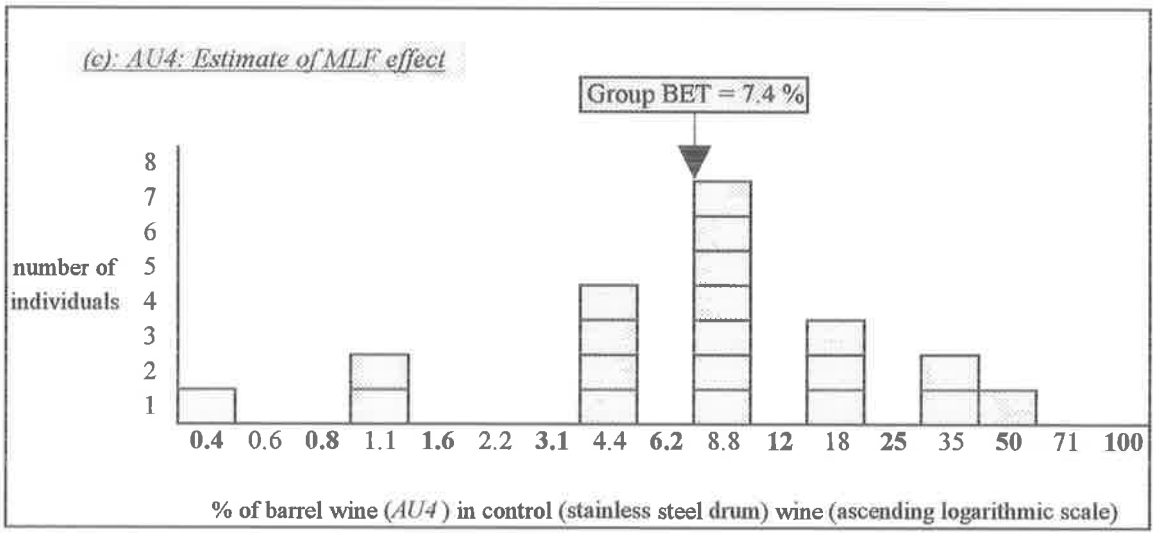
Appendix Table I.2 Compound concentration ranges (20 panelists) corresponding to best estimate thresholds (BETs) for the 'potency of the overall, oak wood–derived, aroma–effect of selected individual barrel wines' experiment.

	Barrel *VA39*			**Barrel *AA11***			**Barrel *AU4***		
Compound (µg/L)*	Group BET	Lowest individual BET	Highest individual BET	Group BET	Lowest individual BET	Highest individual BET	Group BET	Lowest individual BET	Highest individual BET
cis–oak lactone	23	6	124						
trans–oak lactone	8	2	45						
eugenol	1	0.3	6						
4–vinylguaiacol	2	0.4	9						
4–vinylphenol	4	1	23						
guaiacol				1	0.1	8			
4–methylguaiacol				0.3	0.03	3			
4–ethylguaiacol				0.1	0.01	1.0			
vanillin				25	2	194			
maltol				9	1	70			
furfural*				0.42*	0.04*	3.3*			
5–methylfurfural*				0.04*	0.004*	0.31*			
furfuryl alcohol*							0.62*	0.03*	4.2*
5–methylfurfuryl ethyl ether							4	0.2	29

*: Concentration values in mg/L for furfural, 5–methylfurfural and furfuryl alcohol.







Appendix Figure I.1. Histograms of individual Best Estimate Thresholds.

Appendix Table I.3. Potency of *VA39* (featuring 'natural oak products').

Procedure: ASTM E679 Ascending Concentration Series Method of Limits (Meilgaard *et al.* 1991 pp. 125-128).

Equipment: approx. 18 mL wine in each XL5 wine tasting glass, with petri dish lid.

Sample: Chardonnay wine from barrel *VA39* in same, but stainless steel drum-stored, wine.

2 occasions (& 3rd for some) presented.	Number of subjects: 20	Concentration factor per step: 2
Temperature (degrees C): 22, 23 & 29	Number of scale steps: 6	"High" & "low" results confirmed: Yes

Panelist number	Dilutions presented (% barrel wine in stainless steel drum wine)											BET (%)*
	0.1	0.2	0.4	0.8	1.6	3.1	6.2	12.5	25	50	100	
1					+	+	+	+	+	+		
					+	+	+	+	+	+		
			+	+	0							1.6
2					+	0	+	0	0	+		
					-	-	-	-	-	-		35.4
3					+	0	0	0	+	+		
					0	+	0	0	+	+		17.7
4					0	+	+	+	+	+		
					+	+	+	+	+	+		1.6
5					+	0	+	+	+	0		
					+	0	+	+	+	+		17.7
6					0	0	0	+	+	+		
					+	0	+	+	+	+		6.2
7					0	0	0	+	+	+		
					0	0	0	+	0	+		17.7
8					0	0	+	0	+	+		
					0	+	0	+	+	+		12.5
9					0	+	+	+	+	+		
					+	0	+	+	+	+		3.1
10					0	0	+	+	+	+		
					0	0	0	+	+	+		6.2
11					+	+	0	0	+	+		
					-	-	-	-	-	-		17.7
12					+	+	+	+	+	+		
					+	0	+	+	+	+		2.2
13					0	+	+	+	+	+		
					+	+	+	0	+	+		6.2
14					+	+	+	+	+	+		
					0	+	+	+	+	+		1.6
15					+	0	0	0	+	0		
					0	+	+	+	+	+		12.5
16					+	0	+	+	+	+		
					+	+	+	+	+	+		2.2
17					+	+	+	0	0	+		
					+	0	0	+	+	+		17.7
18					+	+	+	+	+	+		
					+	+	+	+	+	+		
			0	+	0							1.6
19					-	-	-	-	-	-		
					0	+	0	0	0	+		35.4
20					-	-	-	-	-	-		
					+	+	+	+	+	+		
			+	0	0							1.6
											Group BET, geometric mean, % of barrel wine in drum wine:	6.39

+: correct; 0: incorrect; -: not done. *: mean of two occasions.

Appendix Table I.4. Potency of *AA11* (featuring 'coopering heat products').

Procedure: ASTM E679 Ascending Concentration Series Method of Limits.
(Ref. Meilgaard *et al.* 1991 pp. 125-128).
Equipment: approx. 18 mL wine in each XL5 wine tasting glass, with petri dish lid.
Sample: Chardonnay wine from barrel *AA11* in same, but stainless steel drum-stored, wine.

1 occasion (& 2nd for some) presented.
Temperature (degrees C): 22 & 29
Number of subjects: 20

Number of scale steps: 6
Concentration factor per step: 2
"High" & "low" results confirmed: Yes

Panelist	Dilutions presented (% barrel wine in stainless steel drum wine)											BET
number	0.1	0.2	0.4	0.8	1.6	3.1	6.2	12.5	25	50	100	(%)
1					+	+	+	+	+	+		
			0	+	+							0.6
2					0	+	0	0	0	0		
										+	+	50
3					+	+	+	+	+	+		
			0	+	0							1.6
4					+	+	+	+	+	+		
			+	0	+							1.1
5					+	0	+	+	+	+		4.4
6					0	+	+	+	+	+		2.2
7					+	+	0	0	0	0		
										+	+	50
8					0	0	+	+	+	+		4.4
9					0	+	+	+	+	+		2.2
10					+	+	+	0	+	+		17.7
11					0	+	0	0	+	+		17.7
12					+	0	+	+	+	+		4.4
13					0	0	+	+	+	+		4.4
14					+	0	+	+	+	+		4.4
15					0	0	0	0	+	+		17.7
16					0	+	0	+	+	+		8.8
17					+	0	+	0	+	+		17.7
18					0	0	+	+	+	+		4.4
19					+	+	0	+	+	+		8.8
20					+	+	+	0	+	+		17.7
												Group BET, geometric mean, % of barrel wine in drum wine: 6.38

+: correct; 0: incorrect.

Appendix Table I.5. Potency of *AU4* (featuring MLF-associated products).

Procedure: ASTM E679 Ascending Concentration Series Method of Limits.
(Ref. Meilgaard *et al.* 1991 pp. 125-128).
Equipment: approx. 18 mL wine in each XL5 wine tasting glass, with petri dish lid.
Sample: Chardonnay wine from barrel *AU4* in same, but stainless steel drum-stored wine.

1 occasion (& 2nd for some) presented.
Temperature (degrees C): 20 & 29
Number of subjects: 20

Number of scale steps: 6
Concentration factor per step: 2
"High" & "low" results confirmed: Yes

Panelist	Dilutions presented (% barrel wine in stainless steel drum wine)											BET
number	0.1	0.2	0.4	0.8	1.6	3.1	6.2	12.5	25	50	100	(%)
1					+	+	0	+	+	+		8.8
2					+	+	0	+	0	+		35.4
3					+	0	+	+	+	+		4.4
4					0	+	0	+	+	+		8.8
5					0	0	+	+	+	+		4.4
6					+	+	0	+	+	+		8.8
7					0	+	0	0	0	+		35.4
8					+	+	+	+	+	+		
			+	0	+							1.1
9					0	0	0	+	+	+		8.8
10					+	0	0	+	+	+		8.8
11					0	+	0	+	+	+		8.8
12					+	+	+	0	+	+		17.7
13					0	0	0	0	+	0		
										+	+	50
14					+	+	+	+	+	+		
			+	+	+							
	+	0	0									0.4
15					0	0	+	0	+	+		17.7
16					+	0	+	+	+	+		4.4
17					0	+	0	+	+	+		8.8
18					+	+	+	+	+	+		
			0	0	+							1.1
19					0	0	+	+	+	+		4.4
20					+	+	0	0	+	+		17.7
					Group BET, geometric mean, % of barrel wine in drum wine:							7.42

+: correct; 0: incorrect.

Appendix Table I.6. Repeatability of the potency determinations (*e.g. VA39*).

Procedure: ASTM E679 Ascending Concentration Series Method of Limits (Meilgaard *et al.* 1991 pp. 125-128).

Equipment: approx. 18 mL wine in each XL5 wine tasting glass, with petri dish lid.

Sample: Chardonnay wine from barrel *VA39* in same, but stainless steel drum-stored wine.

2 occasions presented.	Number of subjects: 16	Concentration factor per step: 2
Temperature (degrees C): 22 & 23	Number of scale steps: 6	"High" & "low" results not confirmed; 50 & 1.6 used.

Panelist number	Dilutions presented (% barrel wine in stainless steel drum wine)										BET (%)
	0.1	0.2	0.4	0.8	1.6	3.1	6.2	12.5	25	50	
1					+	+	+	+	+	+	<2.2 (say 1.6)
					+	+	+	+	+	+	<2.2 (say 1.6)
3					+	0	0	0	+	+	17.7
					0	+	0	0	+	+	17.7
4					0	+	+	+	+	+	2.2
					+	+	+	+	+	+	<2.2 (say 1.6)
5					+	0	+	+	+	0	>35.4 (say 50)
					+	0	+	+	+	+	4.4
6					0	0	0	+	+	+	8.8
					+	0	+	+	+	+	4.4
7					0	0	0	+	+	+	8.8
					0	0	0	+	0	+	35.4
8					0	0	+	0	+	+	17.7
					0	+	0	+	+	+	8.8
9					0	+	+	+	+	+	2.2
					+	0	+	+	+	+	4.4
10					0	0	+	+	+	+	4.4
					0	0	0	+	+	+	8.8
12					+	+	+	+	+	+	<2.2 (say 1.6)
					+	0	+	+	+	+	4.4
13					0	+	+	+	+	+	2.2
					+	+	+	0	+	+	17.7
14					+	+	+	+	+	+	<2.2 (say 1.6)
					0	+	+	+	+	+	2.2
15					+	0	0	0	+	0	>35.4 (say 50)
					0	+	+	+	+	+	2.2
16					+	0	+	+	+	+	4.4
					+	+	+	+	+	+	<2.2 (say 1.6)
17					+	+	+	0	0	+	35.4
					+	0	0	+	+	+	8.8
18					+	+	+	+	+	+	<2.2 (say 1.6)
					+	+	+	+	+	+	<2.2 (say 1.6)

+: correct; 0: incorrect.

Comparison of occasions was based on only those 16 panelists to participate on both occasions.

Panelist	Occ. #1	Occ. #2
1	1.6	1.6
3	17.7	17.7
4	2.2	1.6
5	50	4.4
6	8.8	4.4
7	8.8	35.4
8	17.7	8.8
9	2.2	4.4
10	4.4	8.8
12	1.6	4.4
13	2.2	17.7
14	1.6	2.2
15	50	2.2
16	4.4	1.6
17	35.4	8.8
18	1.6	1.6
Product:	3E+12	9E+10
Group BET:	6.01	4.84

Group BET (*i.e.* geometric mean of the percentage of the barrel wine in the drum wine): Occasion 1 = 6.01 %; and Occasion 2 = 4.84 %. This represents a 19 % improvement (*i.e.* less than the recommended 20 %) so retesting was discontinued. Further, it is likely that there would have been less improvement had the extreme results been retested, here.

Appendix J

Patterns arising from specific aroma differentiations in relation to compound concentration differences

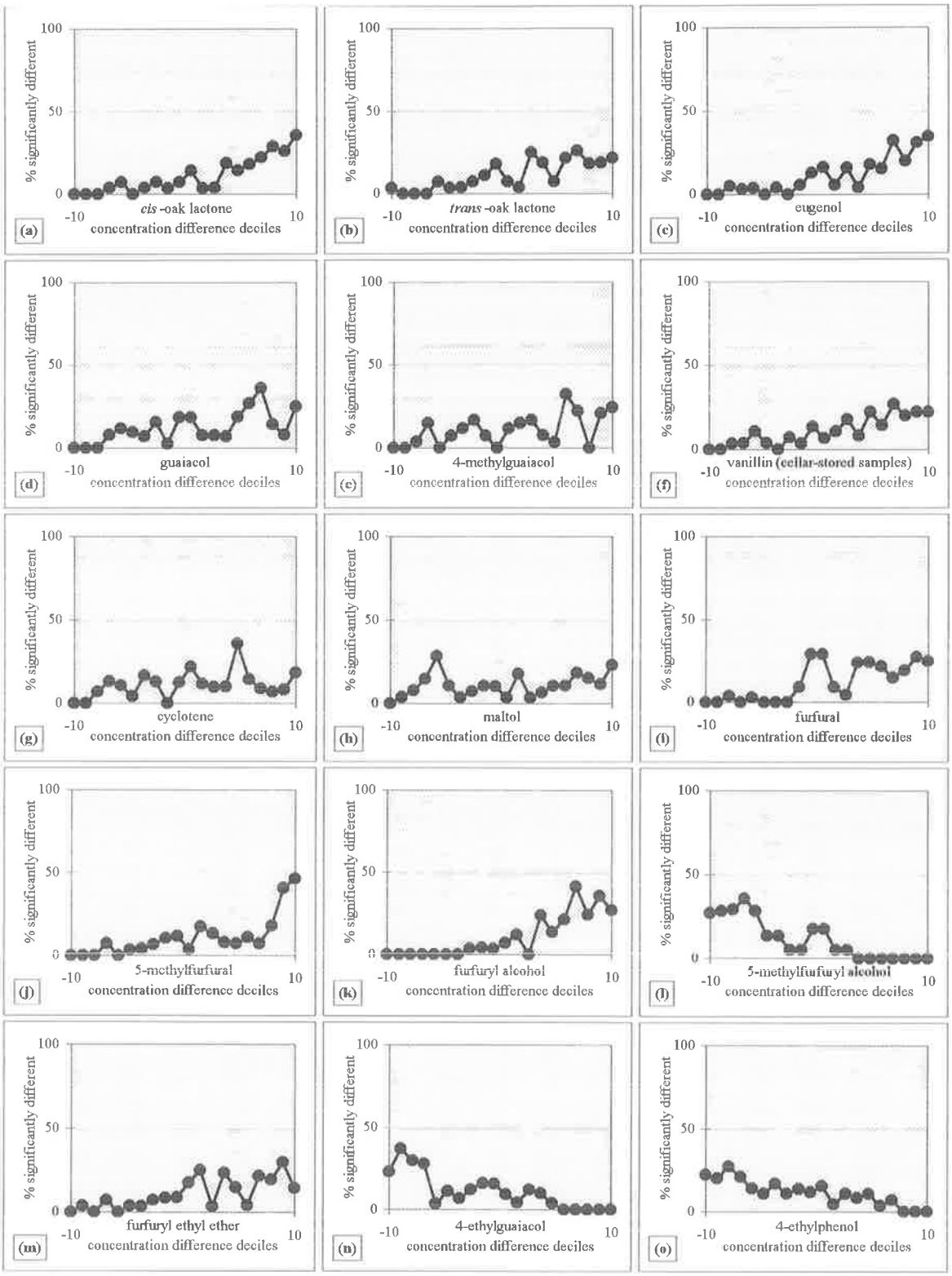
[incorporating the specific aroma 'impact-pattern conformity' (IPC) test]. See Section 4.5 for an explanation of the analysis and Table 4.2 for the naturally occurring 'differentiation potency or accompaniments' (DPAs) and for an explanation of the omissions.

Appendix Table J.1. The Cabernet Sauvignon wine volatile compound concentration ranges and the approximate deciles for the concentration differences (276 comparisons divided, where possible, into 10 roughly equal-sized groups).

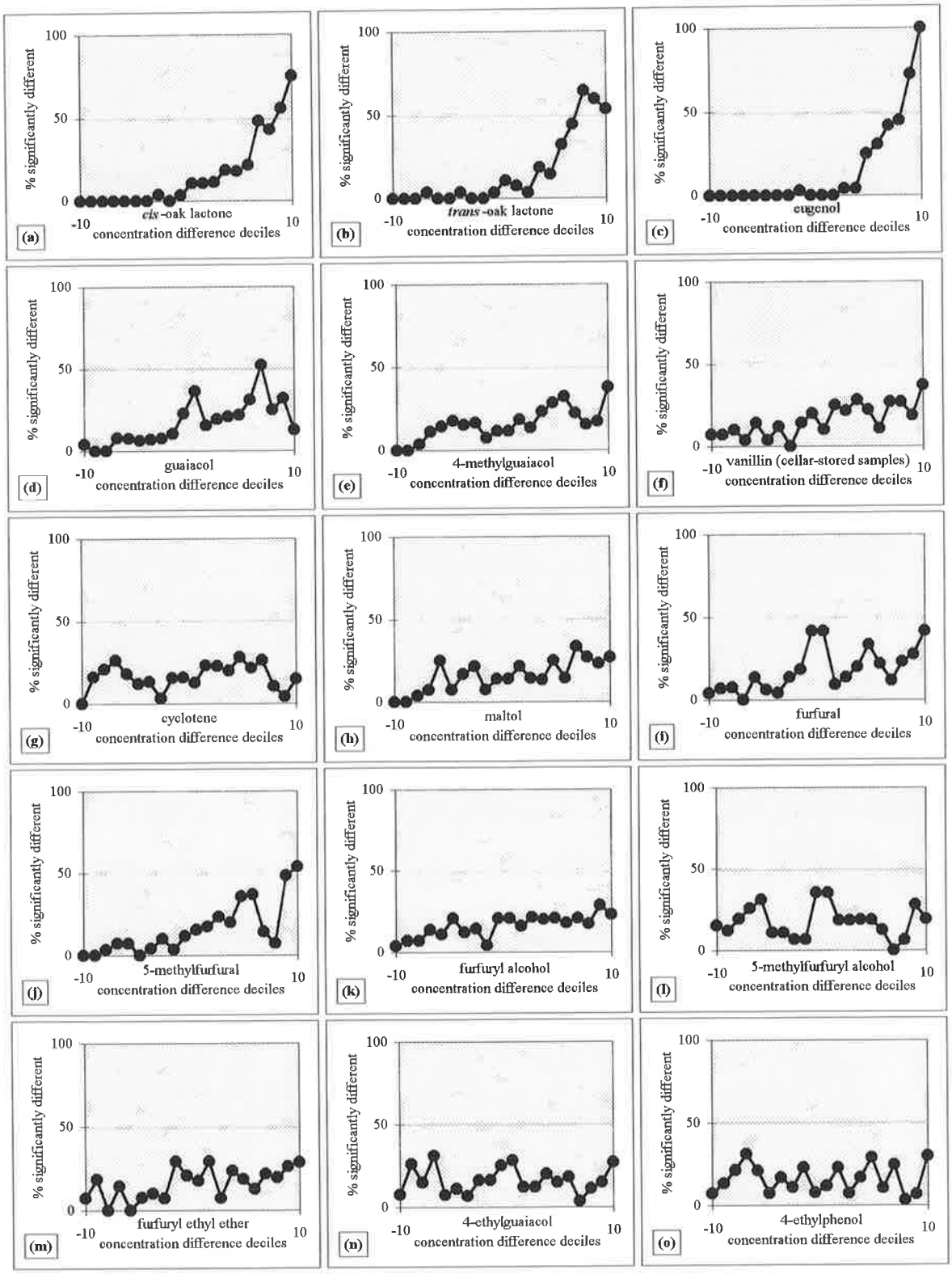
Compounds:	*cis*	*trans*	*eug*	*guaiac*	*4mg*	*van-f*#	*van-c*#	*cyc*	*malt*	*furf*	*5mf*	*falc*	*5mfalc*	*fee*	*4eg*	*4ep*
	(concentrations in ug/L except for furf, falc & 4ep which are in mg/L)															
	Concentration ranges															
from:	94	20	17	7	0.8	121	80	2	80	0.04	9	1.8	7	32	22	0.63
to:	793	381	52	44	15.5	369	212	59	295	0.22	217	14.9	19	228	88	1.04
	Concentration-difference ranges and frequencies within each decile															
1 *from:*	0	0	0	0	0.0	1	0	0	0	0.00	0	0.0	0	0	0	0.00
to:	32	19	2	1	0.7	12	6	2	7	0.00	7	0.5	0	5	2	0.02
frequency:	28	28	31	22	26	29	30	32	29	24	26	29	34	34	32	26
2 *from:*	34	20	3	2	0.8	13	7	3	8	0.01	8	0.6	1	6	3	0.03
to:	66	34	4	3	1.5	21	14	5	14	0.01	12	1.1	1	8	5	0.04
frequency:	28	27	36	39	27	26	28	26	28	22	29	25	60	24	25	22
3 *from:*	67	35	5	4	1.6	22	15	6	15	0.02	13	1.2	as	9	6	0.05
to:	112	56	6	4	2.5	32	20	8	21	0.02	20	1.6	above	13	8	0.06
frequency:	27	28	25	26	30	27	28	31	28	22	30	28		28	25	28
4 *from:*	113	57	7	5	2.6	33	21	9	22	0.03	21	1.7	2	14	9	0.07
to:	147	85	8	6	3.2	44	26	11	28	0.03	27	2.0	2	16	13	0.08
frequency:	27	27	24	29	26	28	25	30	30	25	25	25	37	30	30	24
5 *from:*	148	86	9	7	3.3	45	28	12	29	0.04	28	2.1	as	17	14	0.09
to:	181	116	12	8	4.3	54	33	14	40	0.04	34	2.8	above	22	16	0.11
frequency:	28	28	28	32	28	28	27	25	28	33	28	29		27	27	28
6 *from:*	182	119	13	9	4.4	55	34	15	41	0.05	35	2.9	3	23	17	0.12
to:	221	143	15	10	5.2	66	41	18	51	0.05	45	3.4	3	28	22	0.14
frequency:	28	28	26	26	28	28	28	28	28	37	27	28	32	24	28	29
7 *from:*	227	144	16	11	5.3	67	42	19	52	0.06	46	3.5	4	29	23	0.15
to:	298	168	18	12	6.2	79	51	22	68	0.08	66	4.3	4	35	26	0.18
frequency:	27	27	31	25	27	28	26	23	27	34	28	29	31	28	29	29
8 *from:*	302	169	19	13	6.3	80	52	23	69	0.09	67	4.4	5	36	27	0.19
to:	365	194	21	16	7.3	103	63	29	81	0.09	97	5.2	5	43	37	0.23
frequency:	28	28	20	28	26	27	30	29	26	26	28	29	31	26	27	33
9 *from:*	366	199	22	17	7.4	104	64	30	83	0.10	99	5.3	6	44	38	0.24
to:	491	232	25	23	9.1	132	81	36	113	0.13	153	7.1	8	65	42	0.29
frequency:	27	27	29	25	29	28	27	25	26	29	27	28	25	27	27	30
10 *from:*	495	237	26	24	9.2	134	82	37	116	0.14	155	7.3	9	68	43	0.30
to:	699	361	35	37	14.7	248	132	57	215	0.18	208	13.1	12	196	66	0.41
frequency:	28	28	26	24	29	27	27	27	26	24	28	26	26	28	26	27

Compound abbreviations: cis=*cis* -oak lactone, trans=*trans* -oak lactone, eug=eugenol, guaiac=guaiacol, 4mg=4-methylguaiacol, van=vanillin, cyc=cyclotene, malt=maltol, furf=furfural, 5mf=5-methylfurfural, falc=furfuryl alcohol, 5mfalc=5-methylfurfuryl alcohol, fee=furfuryl ethyl ether, 4eg=4-ethylguaiacol, 4ep=4-ethylphenol.

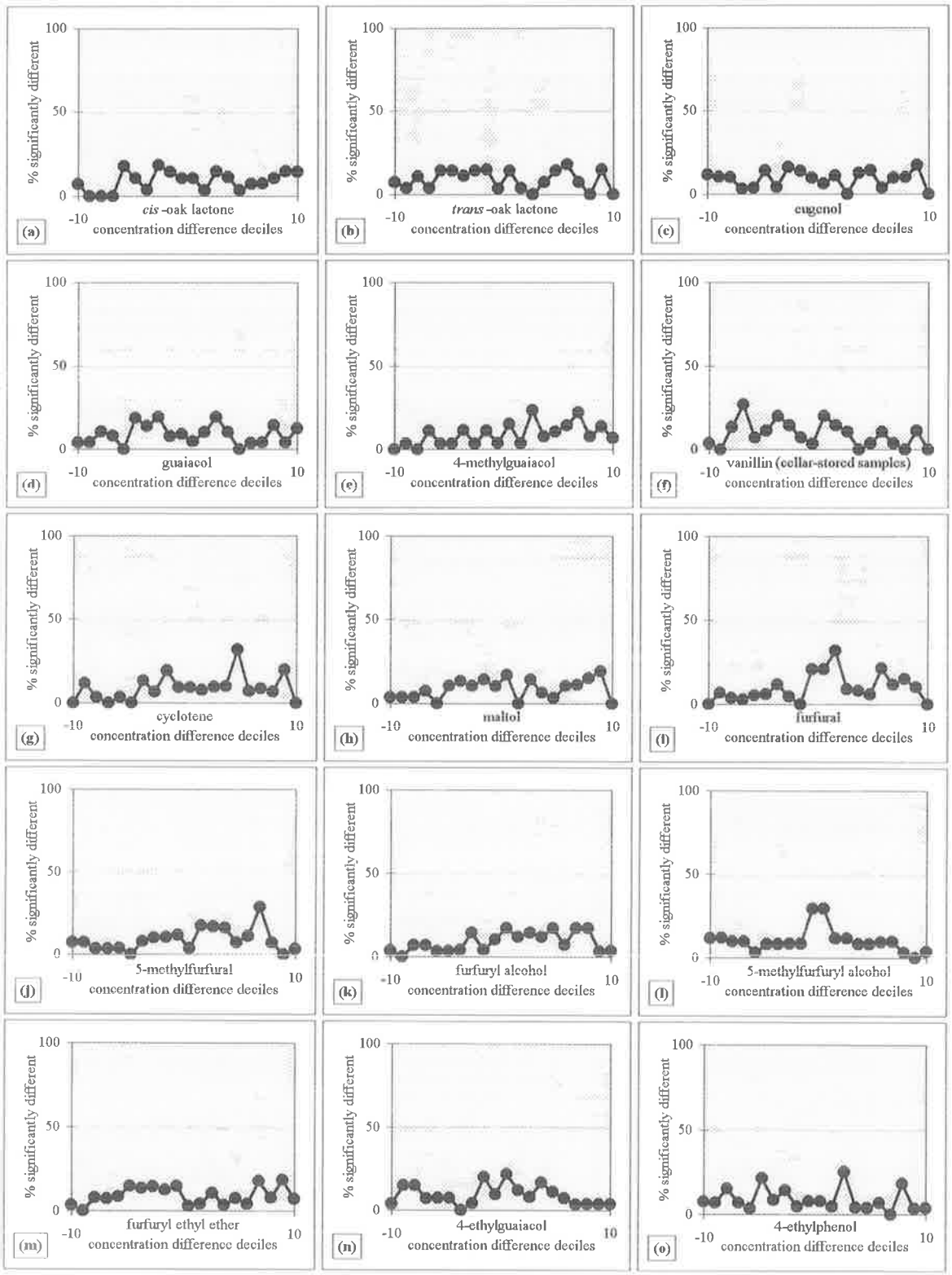
van-f=vanillin from freezer-stored samples (-10 degC for 3 years since barrel sampling); *van-c*=vanillin from cellar-stored samples (~20 degC for 1 year from barrel sampling, then sterilised with DMDC and stored for a further 2 years at ~20 degC). The sensory analysis was performed on the cellar-stored samples approximately 1 year after DMDC sterilisation.



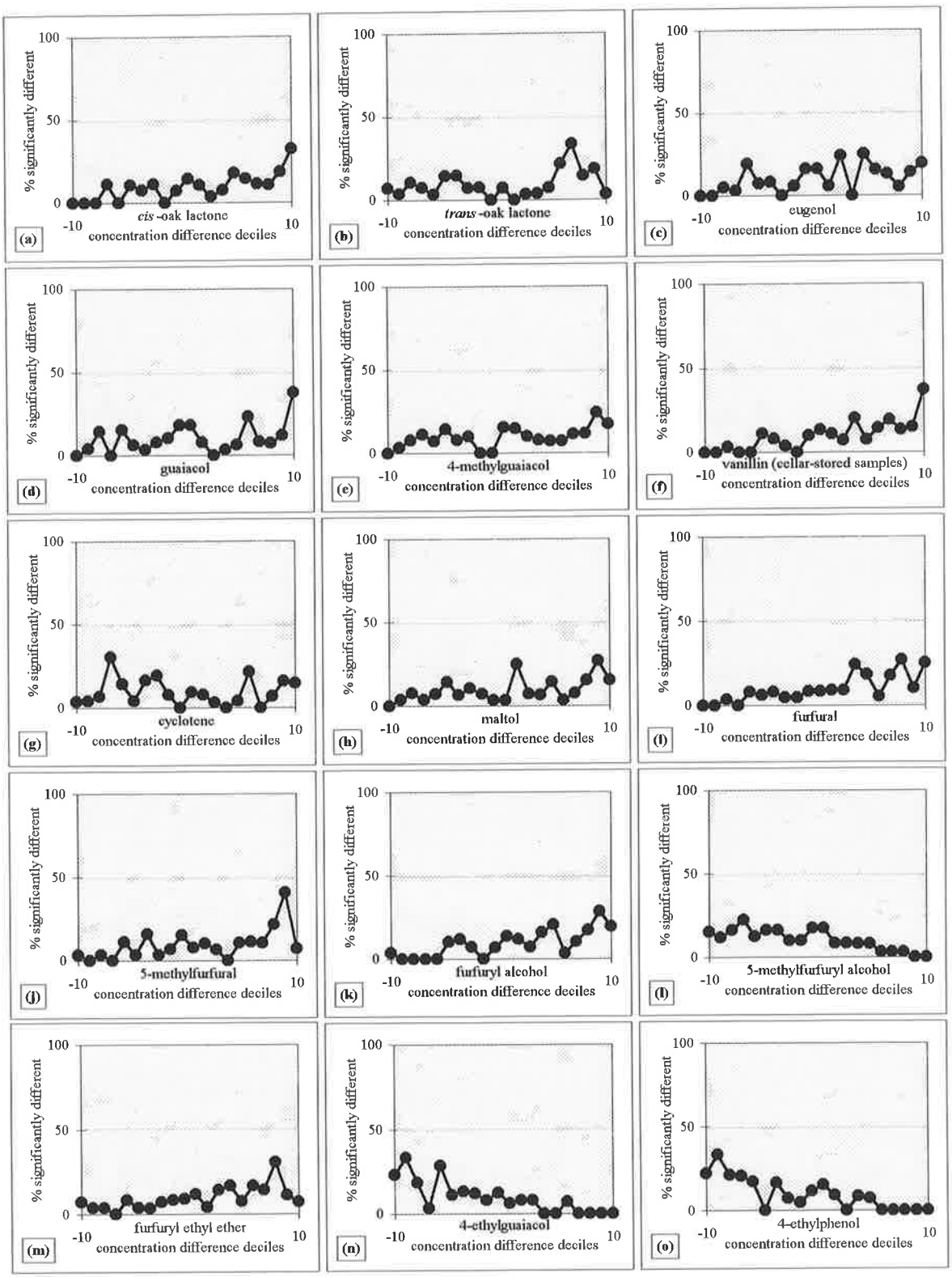
Appendix Figure J.1. 'Preference' aroma 'impact-pattern conformity' (IPC) test in the Cabernet Sauvignon wines.



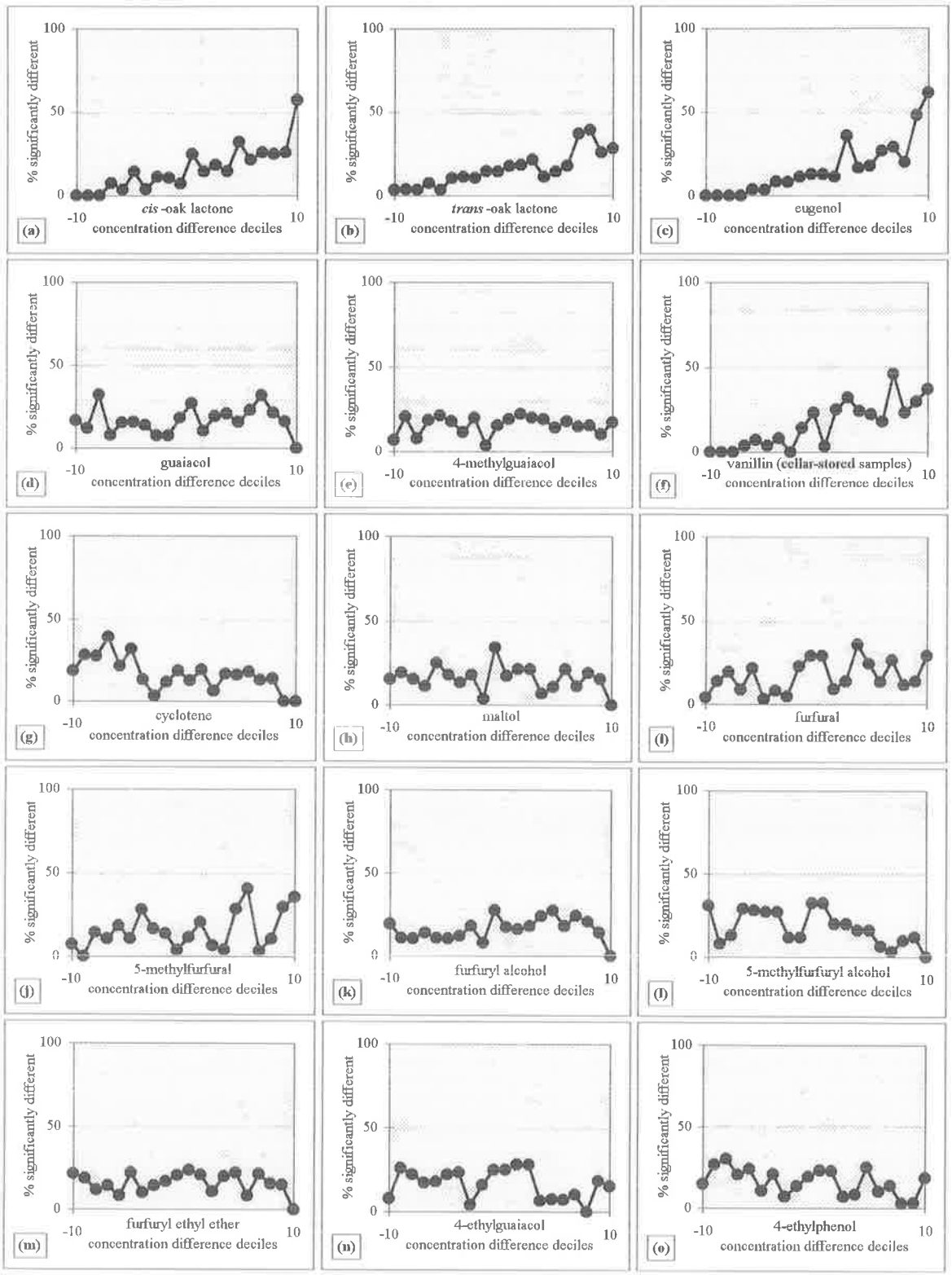
Appendix Figure J.2. 'Coconut' aroma 'impact-pattern conformity' (IPC) test in the Cabernet Sauvignon wines.



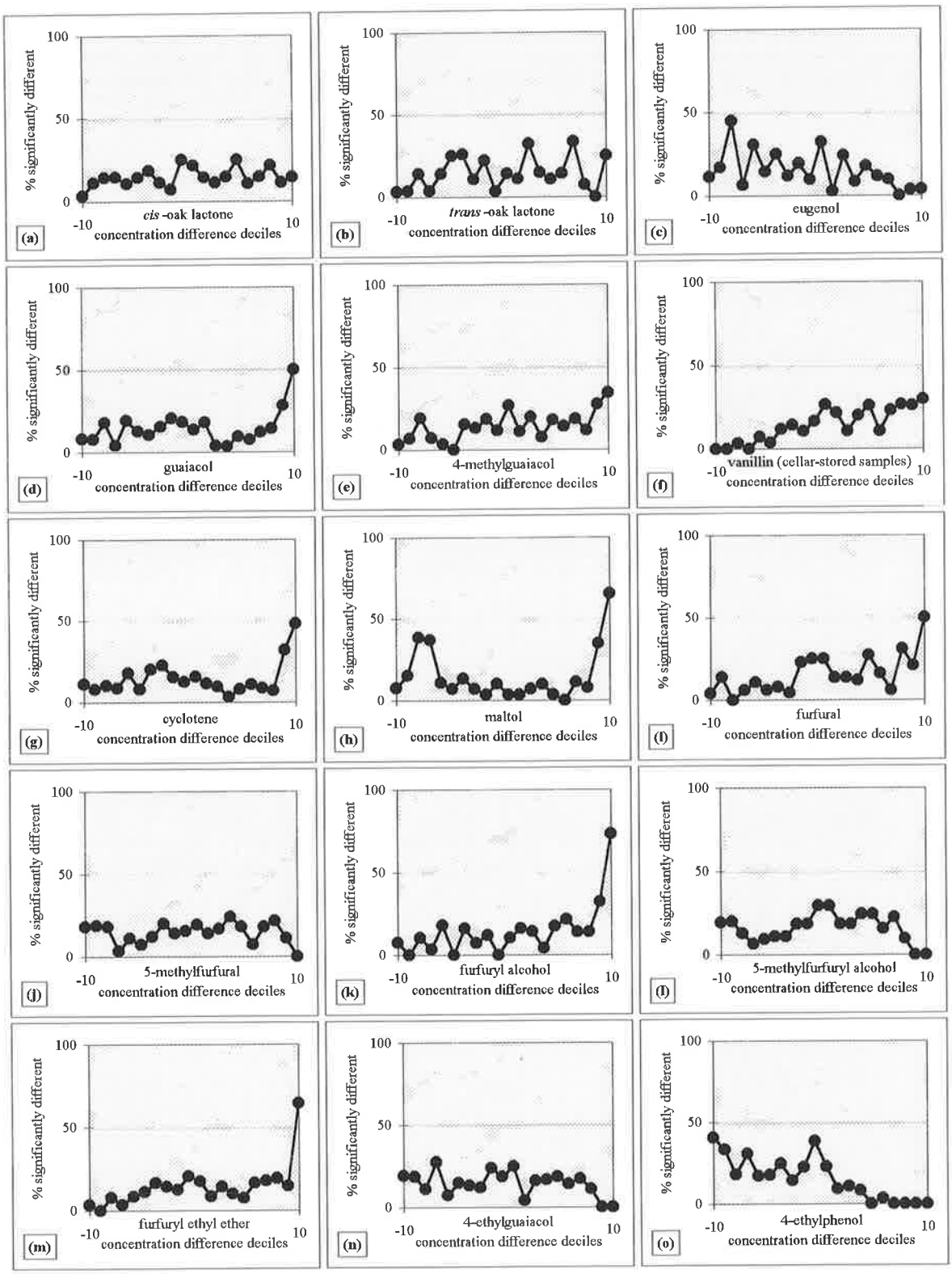
Appendix Figure J.3. 'Pencil shavings' aroma 'impact-pattern conformity' (IPC) test in the Cabernet Sauvignon wines.



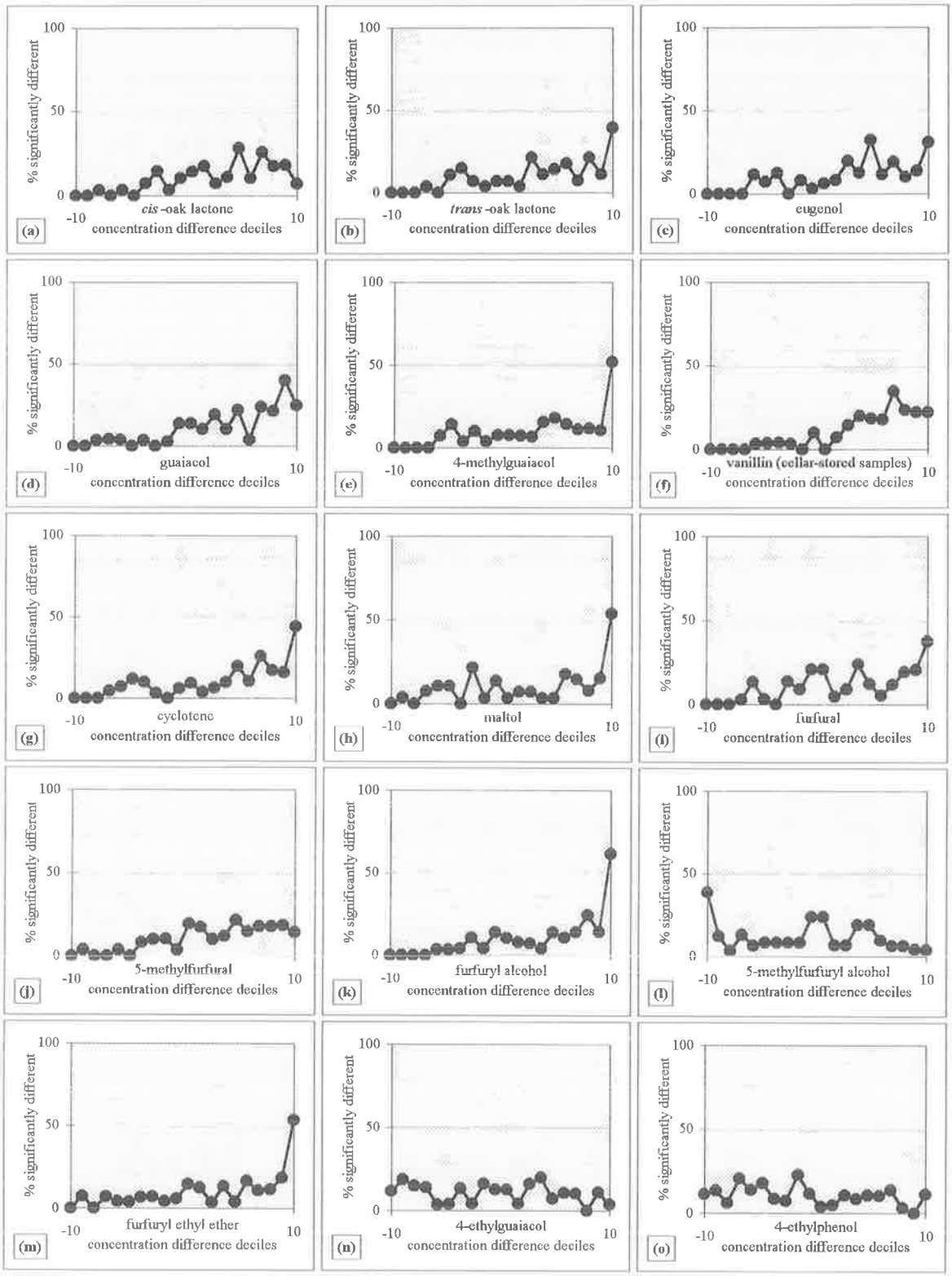
Appendix Figure J.4. 'Allspice' aroma 'impact-pattern conformity' (IPC) test in the Cabernet Sauvignon wines.



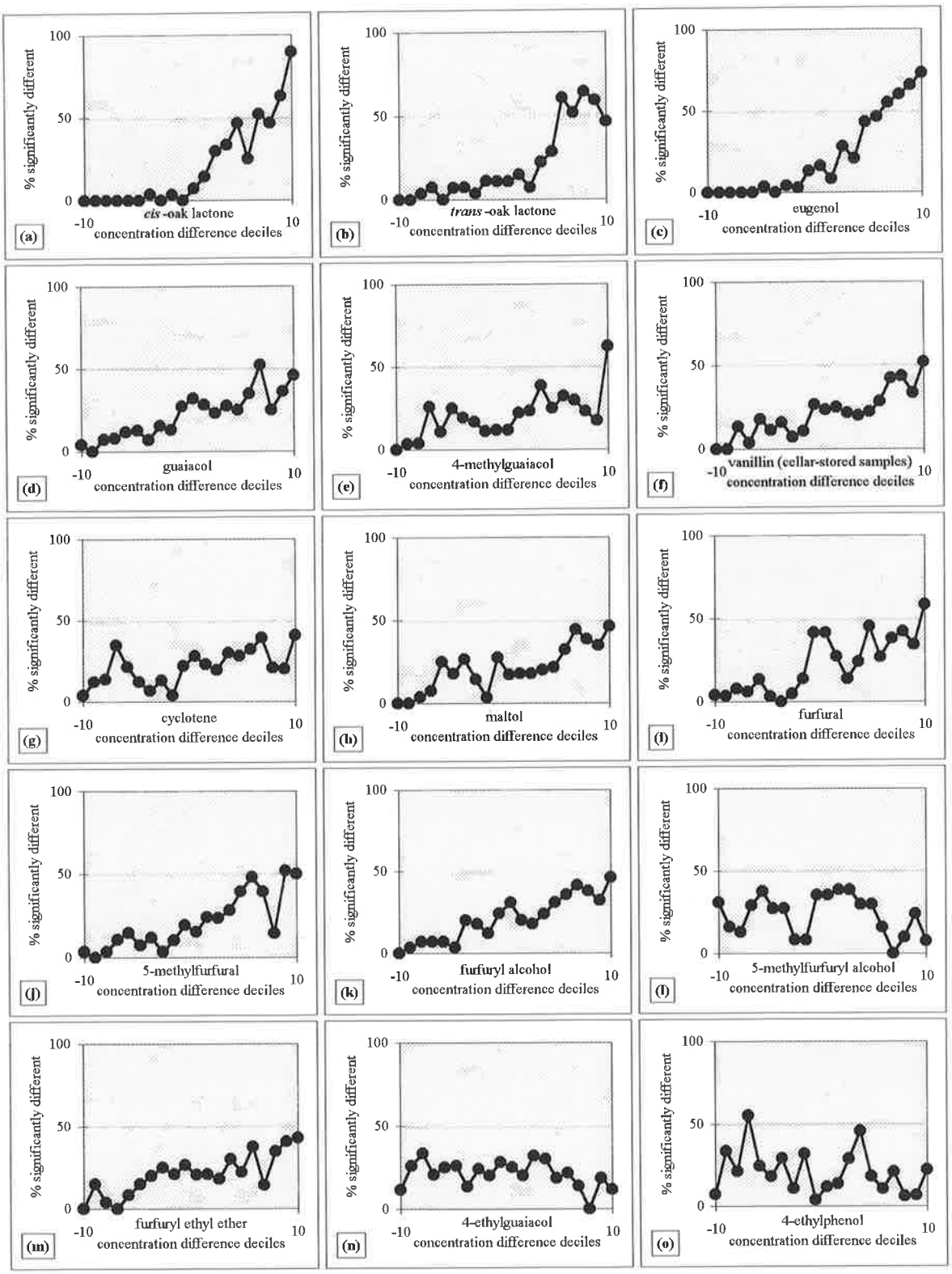
Appendix Figure J.5. 'Berry' aroma 'impact-pattern conformity' (IPC) test in the Cabernet Sauvignon wines.



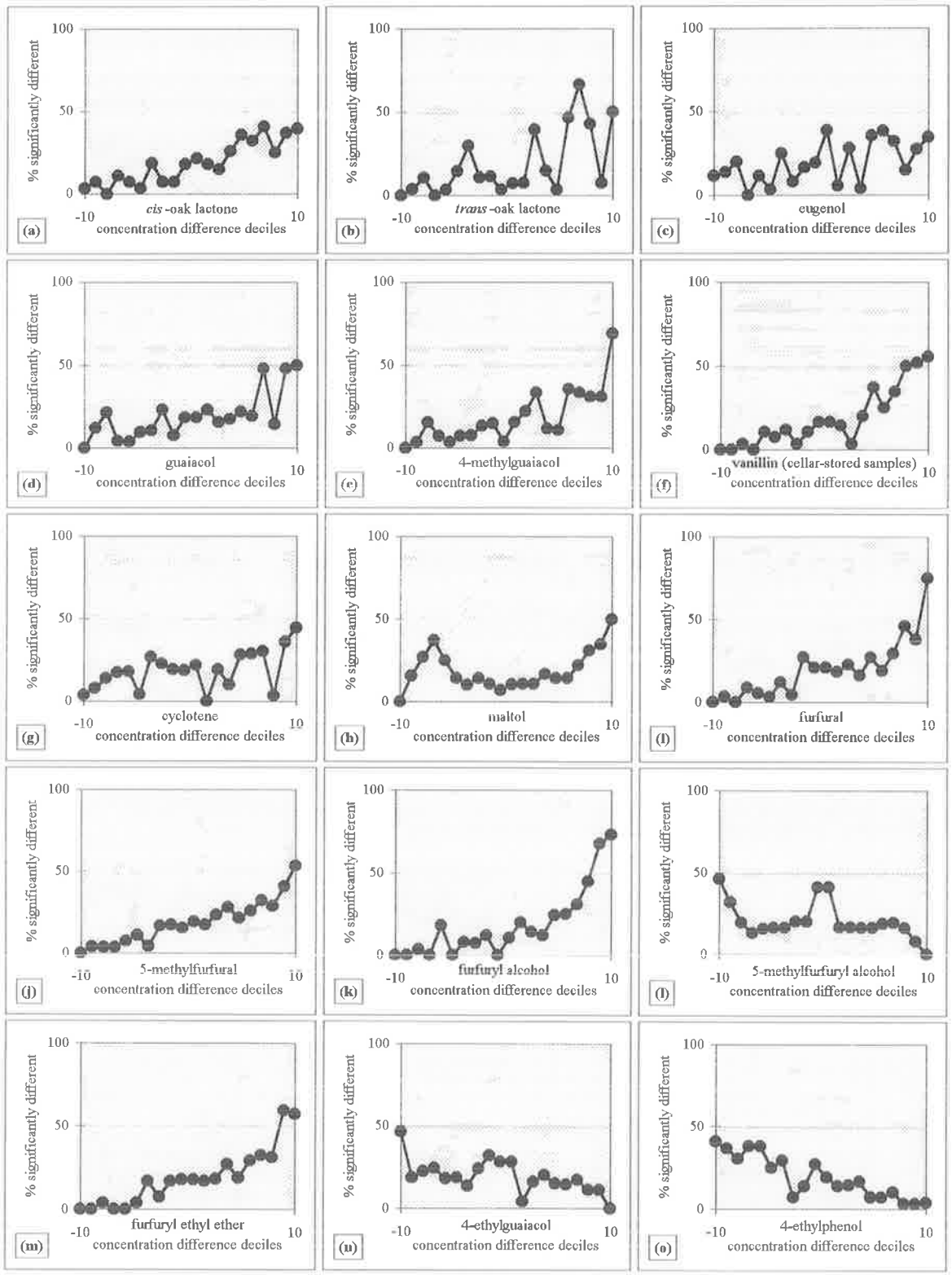
Appendix Figure J.6. 'Smoky' aroma 'impact-pattern conformity' (IPC) test in the Cabernet Sauvignon wines.



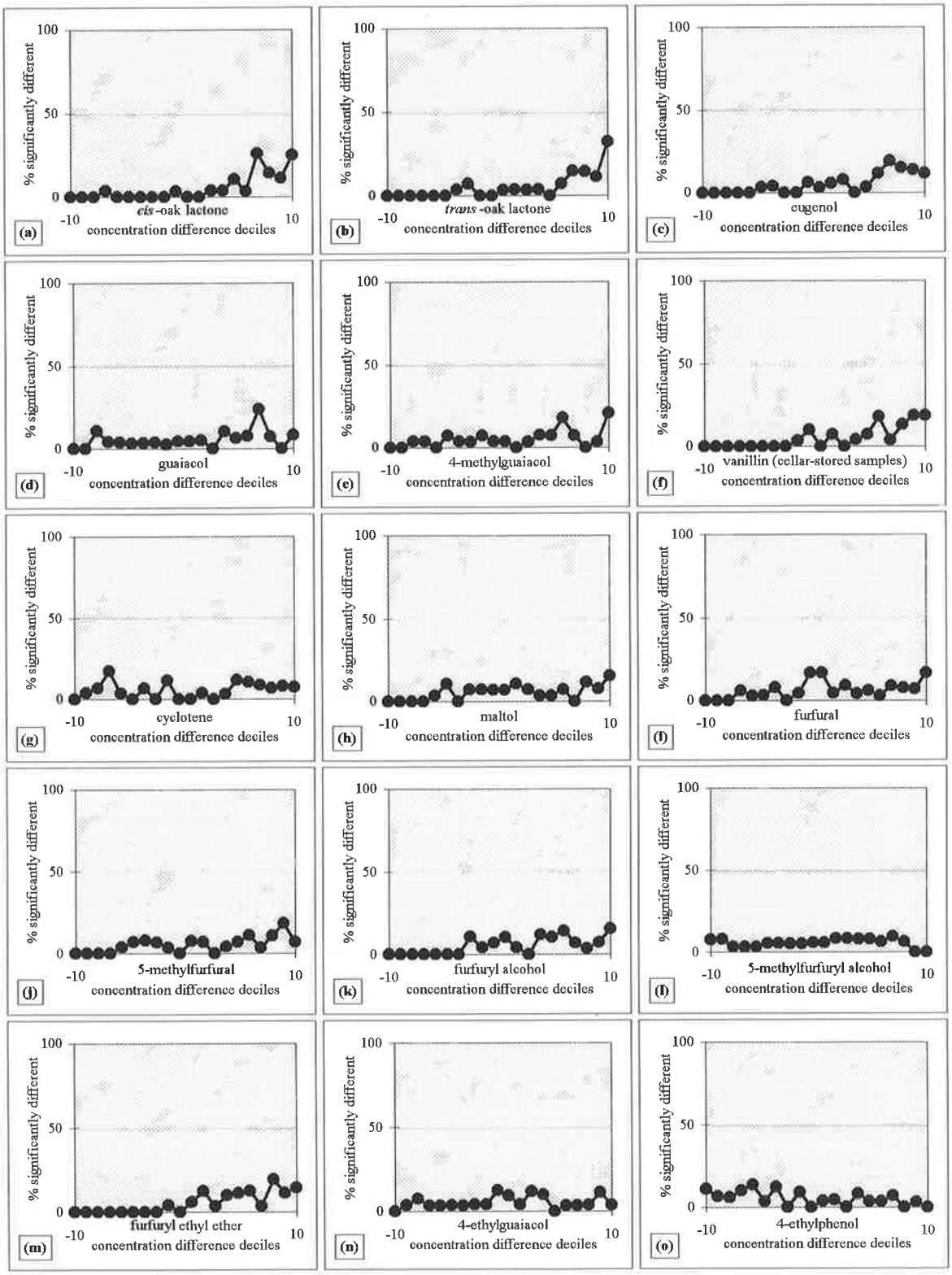
Appendix Figure J.7. 'Caramel' aroma 'impact-pattern conformity' (IPC) test in the Cabernet Sauvignon wines.



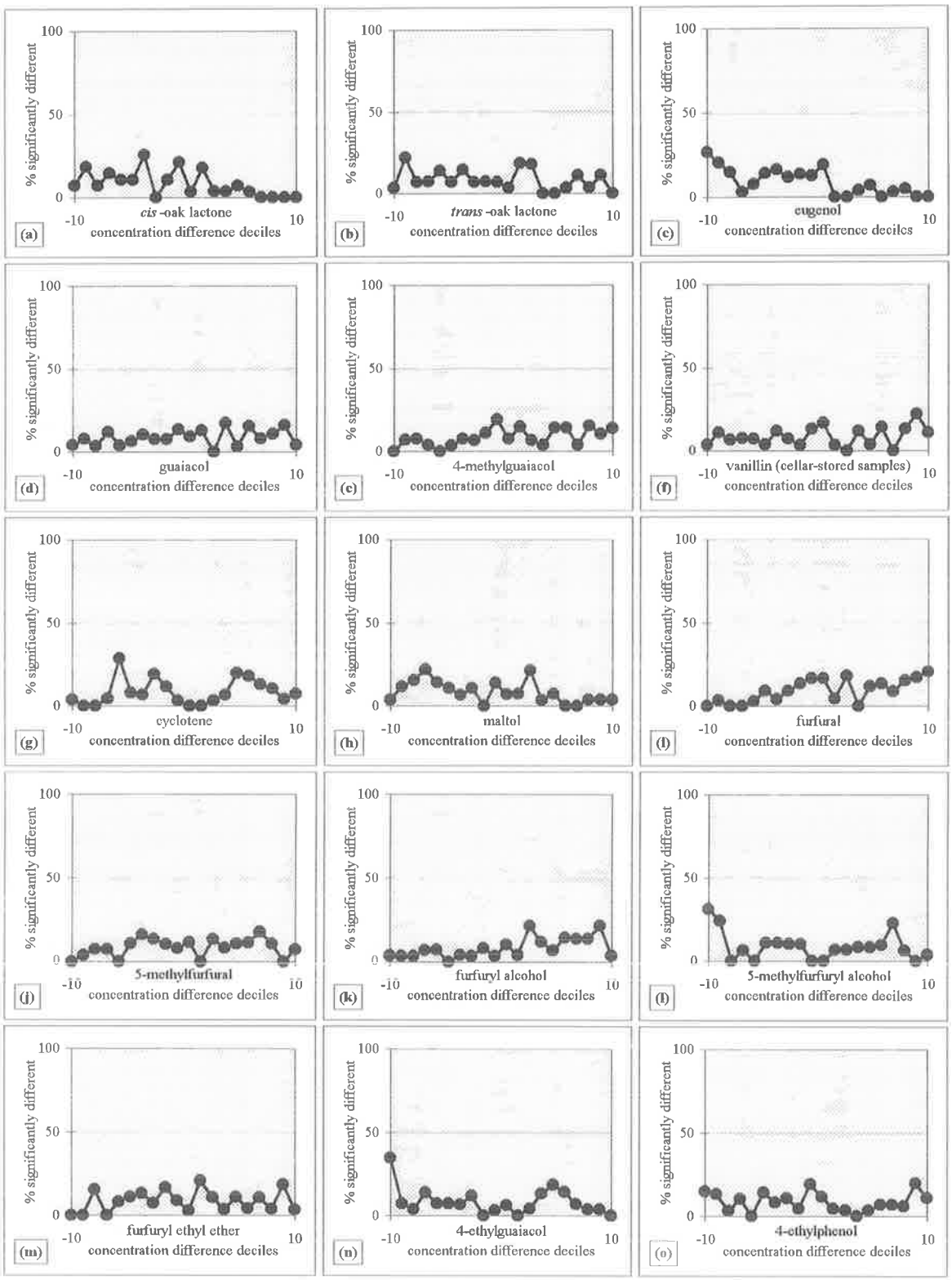
Appendix Figure J.8. 'Vanilla' aroma 'impact-pattern conformity' (IPC) test in the Cabernet Sauvignon wines.



Appendix Figure J.9. 'Coffee' aroma 'impact-pattern conformity' (IPC) test in the Cabernet Sauvignon wines.



Appendix Figure J.10. 'Dark chocolate' aroma 'impact-pattern conformity' (IPC) test in the Cabernet Sauvignon wines.



Appendix Figure J.11. 'Band-aid' (medicinal) aroma 'impact-pattern conformity' (IPC) test in the Cabernet Sauvignon wines.

Appendix K

ANOVAs for oak origin and seasoning location / cooper treatments

Appendix Table outline

Software

SYSTAT V5.0 statistical software (SYSTAT, Inc.) was used for the three–factor ANOVAs, and Microsoft Excel V5.0 spreadsheet software was used for the single– and the two–factor ANOVAs. When a mean separation procedure was required, a two–tailed Fisher's least significant difference (LSD) was calculated.

Appx. Tabs. K.1 to K.7 French oak origin and seasoning location / cooper effects

French oak origin and seasoning location sensory (aroma) effects were explored using the 36 French oak barrel–stored Chardonnay and Cabernet Sauvignon wines (Fig. 1.2). The effects for 'preference' and the six aromas common to both of the wines were explored using three–factor ANOVAs (3 x oak origins, 2 x seasoning location / cooper & 2 x wines) (Appx. Tab. K.1). The same data, along with the aromas peculiar to one of the wines were analysed in two–factor ANOVAs (3 x oak origins & 2 x seasoning location / cooper) (Appx. Tabs. K.2 & K.3).

The French oak composition effects were explored using all 48 French oak wood barrel–stored Chardonnay, Cabernet Sauvignon and model wines (Fig. 1.2). Three–factor (3 x oak origins, 2 x seasoning location / cooper & 3 x wines) unweighted means model ANOVAs (Kirby 1993 pp. 318–323) were used to accommodate the unequal cell sizes (n=2 for the model wines and n=3 for the other wines) (Appx. Tab. K.4). Each wine was also analysed in two–factor ANOVAs (3 x oak origins & 2 x seasoning location / cooper) (Appx. Tabs. K.5, K.6 & K.7).

Appx. Tabs. K.8 to K.13 American oak origin effects

The American oak wood differences, relative to the French oak woods, were analysed similarly but using two–factor or single–factor ANOVAs. Only the Australia seasoned and coopered treatments were included. The sensory (aroma) analysis results are shown in Appendix Tables K.8, K.9 and K.10, and the composition analysis results are shown in Appendix Tables K.11, K.12 and K.13. Unweighted means model ANOVAs (Kirby 1993 pp. 318–323) were used to accommodate the unequal cell sizes involved in the combined wines composition analyses (SYSTAT V5.0), and an alternate LSD formula (O'Mahony 1986, p. 164) was used when comparing means from unequal sample sizes (*i.e.* for 5–methylfurfural in Appx. Tab. K.11). The model wines were not analysed separately for the American oak origin effect exploration because the replicate numbers were small (n=2).

Appx. Tabs. K.14 & K.15 American oak seasoning location effects

The American oak wood seasoning location sensory (aroma) and composition effects are shown in Appendix Tables K.14 and K.15.

Barrel *AA34* was subject to a light toasting, relative to the other barrels, resulting in some very low 'coopering heat product' values. The seven compounds arising most substantially from coopering heat — guaiacol, 4–methylguaiacol, vanillin, cyclotene, maltol, furfural and 5–methylfurfural — are referred to as the 'coopering heat products.' The concentration values for some of these compounds, found in the model wine that was stored in this barrel, were clearly outliers. Thus, these data were excluded from the ANOVAs. Further, given

that the cause of the low values for all seven of these compounds in *AA34* was identified (low coopering heat), the data were excluded for each of the seven compounds even when the datum was not clearly an outlier, but merely the lowest value. ANOVAs were also performed on the full data sets. All ANOVA details are shown in Appendix Tables K.14 and K.15. This approach was also taken for the 'barrel fermentation effect exploration,' detailed in Appendix Table M.1. In all of these cases, the ANOVAs that were performed without *AA34* are discussed in the text, and in most cases the results of the two data set are similar.

Appx. Tabs. K.16 & K.17 Accumulation rate comparisons for French oak wood seasoned and coopered either in France or in Australia

The comparisons of the compound accumulation rates arising from the French oak wood seasoned and coopered either in France or in Australia, were explored using two–factor ANOVAs, with replication (3 x oak origins and 2 x seasoning location / cooper). The comparisons were made for the periods 0 to 55 weeks and 55 to 93 weeks, and the data used were either percentage values relative to the maximum concentration reached for each compound in each barrel (Appx. Tab. K.16) or, simply, the concentration data (Appx. Tab. K.17).

Appendix Table K.1. Oak origin and seasoning location / cooper treatment means and ANOVA *p*–values for French oak barrel wine aromas (SYSTAT V5.0, fully–crossed, fixed factors, 3–factor ANOVA: 3 x oak origins, 2 x seasoning location / cooper & 2 x wines)

	p-value							*Treatment mean**					*Origin treatment mean separation**		
	Treatment			*Interaction*				*Seasoning*		*Origin*					
Aroma	origin	seas	wine	orig x seas	orig x wine	seas x wine	orig x seas x wine	France	Aust	Lim	Tron	Vos	LSD (5%)	LSD (1%)	LSD (0.1%)
prefer	0.025	0.070	0.998	0.647	0.339	0.268	0.794	-0.276	0.276	-0.585	0.161	0.425	0.735		
smoky	0.440	0.117	1.000	0.886	0.154	0.018	0.407	-0.237	0.237	0.143	0.127	-0.269			
pncshvs	0.887	0.701	1.000	0.238	0.643	0.789	0.989	0.068	-0.068	0.114	-0.020	-0.094			
coconut	0.001	0.014	1.000	0.924	0.022	0.042	0.064	-0.297	0.297	-0.620	0.059	0.561			
caramel	0.994	0.485	1.000	0.160	0.081	0.509	0.958	-0.112	0.112	0.012	-0.025	0.013			
vanilla	0.150	0.033	1.000	0.840	0.191	0.447	0.730	-0.341	0.341	-0.413	0.097	0.317			
allspice	0.577	0.034	0.998	0.020	0.946	0.149	0.252	-0.313	0.313	0.058	-0.203	0.145			

$p<0.05$

prefer=preference; *pncshvs*=pencil shavings; seasoning=seasoning location / cooper; origin=oak origin; Aust=Australia; Lim=Limousin; Tron=Troncais; Vos=Vosges.

* Using Fisher-Yates rank transformations.

All wine type means = 0.

Appendix Table K.2. Oak origin and seasoning location / cooper treatment means and ANOVA *p*-values for French oak barrel Chardonnay wine aromas (Excel V5.0, ANOVA: 2-factor with replication: 3 x oak origins & 2 x seasoning location / cooper).

				Treatment mean*				
	p-value			Seas		Origin		
Aroma	seas	origin	interact	France	Aust	Lim	Tron	Vos
preference	0.607	0.071	0.713	-0.111	0.111	-0.612	-0.093	0.705
coconut	0.007	0.111	0.195	-0.538	0.538	-0.197	-0.340	0.537
pencil shavings	0.648	0.871	0.439	0.115	-0.115	-0.089	0.185	-0.096
caramel	0.979	0.281	0.302	-0.006	0.006	0.431	0.043	-0.474
vanilla	0.394	0.988	0.722	-0.224	0.224	-0.045	0.052	-0.007
butter	0.074	0.950	0.922	-0.453	0.453	0.013	0.084	-0.097
allspice	0.573	0.698	0.012	-0.105	0.105	-0.004	-0.189	0.193
smoky	0.591	0.346	0.849	0.133	-0.133	-0.183	0.513	-0.330
cashew nut	0.713	0.182	0.213	-0.079	0.079	0.344	-0.589	0.245
green apple	0.190	0.166	0.366	-0.287	0.287	0.498	-0.533	0.035
cinnamon	0.808	0.593	0.971	-0.064	0.064	0.337	-0.019	-0.318
aroma-PC1	0.316	0.849	0.277	0.247	0.109	0.159	0.144	0.231
aroma-PC2	0.451	0.979	0.230	-0.043	0.123	0.049	0.010	0.062
aroma-PC3	0.070	0.145	0.846	0.148	-0.224	-0.126	0.237	-0.226

: $p < 0.050$ * Using Fisher-Yates rank transformations.

seas=seasoning location / cooper; interact=interaction; Aust=Australia; Lim=Limousin; Tron=Troncais; Vos=Vosges.

Appendix Table K.3. Oak origin and seasoning location / cooper treatment means and ANOVA *p*-values for French oak barrel Cabernet Sauvignon wine aromas (Excel V5.0, ANOVA: 2-factor with replication: 3 x oak origins & 2 x seasoning location / cooper).

				Treatment mean*					Origin treatment mean separation*		
	p-value			Seas		Origin			LSD	LSD	LSD
Aroma	seas	origin	interact	France	Aust	Lim	Tron	Vos	(5%)	(1%)	(0.1%)
preference	0.049	0.166	0.727	-0.441	0.441	-0.559	0.415	0.144			
coconut	0.712	0.001	0.329	-0.057	0.057	-1.043	0.458	0.585	0.799	1.120	
pencil shavings	0.935	0.661	0.538	0.021	-0.021	0.316	-0.224	-0.092			
allspice	0.030	0.771	0.662	-0.521	0.521	0.121	-0.218	0.097			
berry	0.185	0.116	0.140	-0.269	0.269	-0.600	0.182	0.418			
smoky	0.003	0.173	0.306	-0.608	0.608	0.468	-0.260	-0.208			
caramel	0.354	0.287	0.510	-0.218	0.218	-0.407	-0.093	0.500			
vanilla	0.015	0.012	0.969	-0.458	0.458	-0.782	0.142	0.640	0.866		
coffee	0.003	0.816	0.954	-0.680	0.680	-0.045	0.160	-0.115			
dark chocolate	0.152	0.379	0.756	-0.345	0.345	-0.427	0.058	0.369			
Band-aid	0.328	0.158	0.536	-0.219	0.219	0.562	-0.032	-0.530			
earthy	0.482	0.134	0.777	0.161	-0.161	0.683	-0.370	-0.313			
mint	0.286	0.388	0.428	-0.252	0.252	0.337	-0.437	0.100			
aroma PC1	0.015	0.018	0.320	-0.098	0.373	-0.244	0.230	0.427	0.444		
aroma PC2	0.059	0.036	0.571	-0.112	0.217	0.384	-0.085	-0.141	0.421		
aroma PC3	0.069	0.433	0.122	-0.028	0.240	0.224	0.006	0.088			

: $p < 0.050$ * Using Fisher-Yates rank transformations.

seas=seasoning location / cooper; interact=interaction; Aust=Australia; Lim=Limousin; Tron=Troncais; Vos=Vosges.

Appendix Table K.4. Oak origin and seasoning location / cooper treatment means and ANOVA *p* -values for French oak barrel wine composition (SYSTAT V5.0, unweighted means model ANOVA: 3 x oak origins, 2 x seasoning location / cooper & 3 x wines).

	p -value							*Treatment mean (ug/L)**					*Origin treatment mean separation**		
	Treatment			*Interaction*				*Seas*		*Origin*					
Comp	origin	seas	wine	orig x seas	orig x wine	seas x wine	orig x seas x wine	France	Aust	Lim	Tron	Vos	LSD (5%)	LSD (1%)	LSD (0.1%)
cis	0.000	0.001	0.000	0.411	0.037	0.301	0.718	271	369	191	331	438			
trans	0.002	0.794	0.000	0.015	0.034	0.305	0.305	166	166	125	195	177			
eug	0.000	0.000	0.000	0.476	0.001	0.266	0.314	30	26	21	31	33			
guaiac	0.034	0.843	0.000	0.733	0.390	0.829	0.641	17	17	13	20	18	6		
4mg	0.091	0.334	0.000	0.762	0.187	0.594	0.764	9	8	8	9	10			
van	0.992	0.000	0.000	0.986	0.171	0.000	0.462	348	429	391	389	386			
cyc	0.001	0.000	0.000	0.447	0.000	0.000	0.000	50	81	73	45	80			
malt	0.109	0.466	0.000	0.096	0.444	0.502	0.425	103	108	92	110	115			
furf	0.006	0.018	0.000	0.694	0.002	0.048	0.831	2.6	4.0	2.1	4.0	3.9			
eef	0.060	0.001	0.000	0.521	0.103	0.111	0.659	6.1	9.0	6.3	8.3	8.0			
5mf	0.001	0.015	0.000	0.495	0.000	0.023	0.891	0.37	0.48	0.31	0.47	0.50			
falc	0.943	0.019	0.000	0.557	0.450	0.038	0.403	3.5	5.0	4.2	4.4	4.1			
5mfalc	0.205	0.196	0.000	0.229	0.326	0.034	0.428	15	16	16	14	17			
valc	0.773	0.067	0.002	0.586	0.563	0.040	0.687	8	2	4	5	7			
fee	0.077	0.081	0.000	0.947	0.160	0.701	0.921	66	87	90	63	75			
5mfee	0.576	0.945	0.070	0.740	0.335	0.555	0.813	4	3	5	2	4			
vee	0.016	0.295	0.000	0.659	0.017	0.162	0.869	7	8	9	6	8			
4vg	0.001	0.731	0.000	0.372	0.000	0.877	0.399	7	8	8	5	10			
4eg	0.357	0.104	0.000	0.185	0.328	0.118	0.101	20	14	16	15	20			
4vp	0.002	0.550	0.000	0.579	0.000	0.769	0.653	19	17	17	10	26			
4ep	0.608	0.002	0.000	0.861	0.693	0.000	0.961	0.33	0.26	0.28	0.30	0.31			

: $p < 0.050$

*: mg/L for furfural, 'estimated extracted furfural,' 5-methylfurfural, furfuryl alcohol and 4-ethylphenol.

comp=compound; seas=seasoning location / cooper; orig=oak origin; Aust=Australia; Lim=Limousin; Tron=Troncais; Vos=Vosges.

cis=*cis* -oak lactone; trans=*trans* -oak lactone; eug=eugenol; guaiac=guaiacol; 4mg=4-methylguaiacol; van=vanillin; cyc=cyclotene; malt=maltol; furf=furfural; eef='estimated extracted furfural' (*i.e.* furfural plus furfuryl alcohol); 5mf=5-methylfurfural; falc=furfuryl alcohol; 5mfalc=5-methylfurfuryl alcohol; valc=vanillyl alcohol; fee=furfuryl ethyl ether; 5mfee=5-methylfurfuryl ethyl ether; vee=vanillyl ethyl ether; 4vg=4-vinylguaiacol; 4eg=4-ethylguaiacol; 4vp=4-vinylphenol; 4ep=4-ethylphenol.

Appendix Table K.5. Oak origin and seasoning location / cooper treatment means and ANOVA *p*-values for French oak barrel Chardonnay wine composition (Excel V5.0, ANOVA: 2-factor with replication: 3 x oak origins & 2 x seasoning location / cooper).

	p-value			*Treatment mean (ug/L)**					*Origin treatment mean separation**		
				Seas		*Origin*			LSD	LSD	LSD
Compound	seas	origin	interact	France	Aust	Lim	Tron	Vos	(5%)	(1%)	(0.1%)
cis-oak lactone	0.006	0.001	0.260	201	275	169	230	315	59	83	117
trans-oak lactone	0.845	0.947	0.442	129	126	129	130	124			
eugenol	0.040	0.023	0.180	20	17	16	19	22	4		
guaiacol	0.935	0.371	0.365	8	8	7	11	7			
4-methylguaiacol	0.097	0.797	0.326	5	4	5	5	4			
vanillin	0.391	0.419	0.411	278	297	307	284	271			
cyclotene	0.001	0.001	0.008	38	106	106	18	91			
maltol	0.633	0.323	0.653	51	55	49	62	47			
furfural	0.444	0.377	0.245	2.1	2.8	2.3	3.3	1.7			
'estimated extracted furfural'	0.606	0.399	0.376	5.2	5.8	5.4	6.5	4.5			
5-methylfurfural	0.850	0.580	0.231	0.25	0.26	0.25	0.31	0.22			
furfuryl alcohol	0.822	0.755	0.964	3.0	2.9	3.0	3.2	2.8			
5-methylfurfuryl alcohol	0.045	0.143	0.182	25	30	29	24	29			
furfuryl ethyl ether	0.237	0.131	0.744	87	106	118	77	94			
5-methylfurfuryl ethyl ether	0.522	0.441	0.908	7	4	10	4	3			
vanillyl ethyl ether	0.143	0.018	0.625	11	14	15	8	15	5		
4-vinylguaiacol	0.687	0.002	0.292	18	19	19	12	26	6	9	
4-ethylguaiacol	0.083	0.639	0.255	2	1	1	2	1			
4-vinylphenol	0.540	0.003	0.478	47	43	44	24	67	21	29	
composition-PC1	0.813	0.001	0.121	0.152	0.140	0.065	0.039	0.334	0.138	0.193	0.273
composition-PC2	0.670	0.444	0.220	0.048	-0.020	-0.009	0.149	-0.099			
composition-PC3	0.097	0.019	0.485	-0.042	0.162	0.182	-0.208	0.204	0.302		

: $p < 0.050$

*: mg/L for furfural, 'estimated extracted furfural' (*i.e.* furfural + furfuryl alcohol), 5-methylfurfural and furfuryl alcohol.

seas=seasoning location /cooper; interact=interaction; Aust=Australia; Lim=Limousin; Tron=Troncais; Vos=Vosges.

Appendix Table K.6. Oak origin and seasoning location / cooper treatment means and ANOVA *p*-values for French oak barrel Cabernet Sauvignon wine composition (Excel V5.0, ANOVA: 2-factor with replication: 3 x oak origins & 2 x seasoning location / cooper).

	p-value			*Treatment mean (ug/L)**					*Origin treatment mean separation**		
				Seas		*Origin*			LSD	LSD	LSD
Compound	seas	origin	interact	France	Aust	Lim	Tron	Vos	(5%)	(1%)	(0.1%)
cis-oak lactone	0.025	0.001	0.873	316	466	186	425	562	156	218	309
trans-oak lactone	0.380	0.002	0.019	220	198	128	257	243			
eugenol	0.232	0.000	0.519	38	35	25	42	43	7	9	13
guaiacol	0.822	0.317	0.665	22	21	17	26	21			
4-methylguaiacol	0.336	0.856	0.898	10.2	8.6	8.8	9.4	10.0			
vanillin	0.007	0.690	0.743	266	325	304	298	285			
cyclotene	0.669	0.669	0.364	28	25	22	30	29			
maltol	0.794	0.304	0.173	172	167	146	175	188			
furfural	0.033	0.492	0.047	0.10	0.14	0.11	0.13	0.12			
5-methylfurfural	0.126	0.010	0.002	68	94	51	120	72			
furfuryl alcohol	0.015	0.570	0.261	5.3	8.7	6.1	7.7	7.1			
5-methylfurfuryl alcohol	0.264	0.467	0.434	11	9	10	9	11			
furfuryl ethyl ether	0.137	0.602	0.833	71	102	77	82	100			
vanillyl ethyl ether	0.337	0.783	0.783	4	4	4	4	4			
4-vinylguaiacol	1.000	0.215	0.148	2	2	1	2	2			
4-ethylguaiacol	0.094	0.259	0.110	49	35	39	36	51			
4-vinylphenol	0.122	0.097	0.519	2	2	1	2	3			
4-ethylphenol	0.002	0.509	0.823	0.89	0.70	0.75	0.81	0.82			
composition-PC1	0.454	0.439	0.342	0.058	0.170	-0.019	0.207	0.155			
composition-PC2	0.010	0.274	0.087	0.115	-0.309	-0.148	-0.210	0.067			
composition-PC3	0.009	0.000	0.971	0.067	0.238	-0.196	0.311	0.342	0.146	0.204	0.289

: $p < 0.050$

*: mg/L for furfural, furfuryl alcohol and 4-ethylphenol.

seas=seasoning location / cooper; interact=interaction; Aust=Australia; Lim=Limousin; Tron=Troncais; Vos=Vosges.

Appendix Table K.7. Oak origin and seasoning location / cooper treatment means and ANOVA *p*-values for French oak barrel 93 week model wine composition (Excel V5.0, ANOVA: 2-factor with replication: 3 x oak origins & 2 x seasoning location / cooper).

				*Treatment mean (ug/L)**					*Origin treatment mean separation**		
	p-value			*Seas*		*Origin*			LSD	LSD	LSD
Compound	seas	origin	interact	France	Aust	Lim	Tron	Vos	(5%)	(1%)	(0.1%)
cis-oak lactone	0.198	0.016	0.212	308	364	233	340	434	117		
trans-oak lactone	0.417	0.340	0.540	140	177	116	201	159			
eugenol	0.003	0.000	0.208	34	26	21	34	36	5	7	11
guaiacol	0.665	0.189	0.678	23	25	16	26	30			
4-methylguaiacol	0.829	0.132	0.714	14	15	11	15	18			
vanillin	0.000	0.181	0.582	578	785	650	685	710			
cyclotene	0.062	0.056	0.109	102	128	98	108	139			
maltol	0.071	0.084	0.294	77	99	74	84	107			
furfural	0.139	0.081	0.979	7.3	11.5	4.6	10.7	12.9			
'estimated extracted furfural'	0.081	0.161	0.908	8.7	13.9	7.7	11.8	14.5			
5-methylfurfural	0.108	0.045	0.996	1.01	1.37	0.79	1.23	1.56	0.58		
furfuryl alcohol	0.454	0.508	0.773	1.4	2.4	3.0	1.2	1.6			
5-methylfurfuryl alcohol	0.356	0.280	0.921	5	6	4	6	6			
vanillyl alcohol	0.356	0.125	0.422	1	0	1	1	0			
furfuryl ethyl ether	0.691	0.085	0.970	27	35	68	13	11			
5-methylfurfuryl ethyl ether	0.565	0.394	0.533	4	6	4	2	10			
vanillyl ethyl ether	0.822	0.418	0.909	6	6	7	5	5			
4-vinylguaiacol	1.000	0.098	0.296	0	0	1	0	0			
4-ethylguaiacol	0.826	0.209	0.949	3	3	2	4	3			
4-ethylphenol	0.263	0.745	0.517	0	2	1	0	1			
composition-PC1	0.097	0.055	0.882	-0.126	0.211	-0.304	0.084	0.347			
composition-PC2	0.421	0.007	0.699	0.110	0.220	-0.288	0.330	0.452	0.383	0.581	
composition-PC3	0.413	0.099	0.862	-0.009	0.129	0.354	-0.091	-0.082			

: $p < 0.050$

*: mg/L for furfural, 'estimated extracted furfural' (*i.e.*, furfural + furfuryl alcohol), 5-methylfurfural and furfuryl alcohol.

seas=seasoning location / cooper; interact=interaction; Aust=Australia; Lim=Limousin; Tron=Troncais; Vos=Vosges.

Appendix Table K.8. Oak origin treatment means and ANOVA *p*-values for Australia seasoned and coopered oak barrel wine aromas (Excel V5.0, ANOVA: 2-factor with replication: 4 x oak origins & 2 x wines).

	p-value			*Treatment mean**				*Origin treatment mean separation**		
Aroma	origin	wine	interact	Amer	Lim	Tron	Vos	LSD (5%)	LSD (1%)	LSD (0.1%)
preference	0.036	1.000	0.354	-0.640	-0.427	0.521	0.546	0.981		
smoky	0.532	1.000	0.119	-0.451	0.286	0.083	0.082			
pencil shavings	0.025	1.000	0.812	-0.990	0.208	0.256	0.527	1.002		
coconut	0.000	1.000	0.352	-0.898	-0.438	0.376	0.960	0.750	1.033	1.420
caramel	0.339	1.000	0.015	0.248	0.158	0.099	-0.505			
vanilla	0.340	1.000	0.347	-0.533	-0.111	0.363	0.282			
allspice	0.148	1.000	0.265	-0.658	0.015	0.558	0.086			

: p <0.050

* Using Fisher-Yates rank transformations.

interact=interaction; Amer=American; Lim=Limousin; Tron=Troncais; Vos=Vosges.

Appendix Table K.9. Oak origin treatment means and ANOVA *p*-values for Australia seasoned and coopered oak barrel Chardonnay wine aromas (Excel V5.0, ANOVA: single factor: 4 x oak origins).

		*Treatment mean**				*Origin treatment mean separation**		
Aroma	*p*-value	Amer	Lim	Tron	Vos	LSD (5%)	LSD (1%)	LSD (0.1%)
preference	0.089	-0.455	-0.758	0.263	0.950			
coconut	0.032	-0.737	-0.438	-0.005	1.180	1.238		
pencil shavings	0.077	-1.097	0.000	0.450	0.647			
caramel	0.001	1.180	0.103	-0.103	-1.180	0.794	1.155	
vanilla	0.907	-0.101	0.012	0.352	-0.263			
butter	0.990	-0.020	0.095	0.085	-0.160			
allspice	0.558	-0.136	-0.409	0.703	-0.159			
smoky	0.670	-0.075	-0.312	0.623	-0.237			
cashew nut	0.695	-0.620	0.193	0.235	0.192			
green apple	0.037	-0.817	0.987	-0.543	0.373	1.261		
cinnamon	0.984	-0.171	0.171	0.033	-0.033			
aroma-PC1	0.136	-0.433	-0.067	0.161	0.233			
aroma-PC2	0.693	-0.002	-0.026	0.367	0.029			
aroma-PC3	0.257	0.116	-0.315	0.119	-0.477			

: p <0.050

* Using Fisher-Yates rank transformations.

Amer=American; Lim=Limousin; Tron=Troncais; Vos=Vosges.

Appendix Table K.10. Oak origin treatment means and ANOVA *p*-values for Australia seasoned and coopered oak barrel Cabernet Sauvignon wine aromas (Excel V5.0, ANOVA: single factor: 4 x oak origins).

		Treatment mean*				Origin treatment mean separation*		
Aroma	*p*-value	Amer	Lim	Tron	Vos	LSD (5%)	LSD (1%)	LSD (0.1%)
preference	0.220	-0.825	-0.095	0.778	0.142			
coconut	0.010	-1.058	-0.438	0.757	0.740	1.061	1.544	
pencil shavings	0.308	-0.883	0.415	0.062	0.407			
allspice	0.065	-1.180	0.438	0.412	0.330			
berry	0.167	-1.042	0.367	0.225	0.450			
smoky	0.076	-0.827	0.883	-0.457	0.400			
caramel	0.608	-0.685	0.213	0.302	0.170			
vanilla	0.079	-0.965	-0.235	0.373	0.827			
coffee	0.065	-1.180	0.467	0.477	0.237			
dark chocolate	0.135	-1.042	0.077	0.422	0.543			
Band-aid	0.870	0.103	0.373	-0.228	-0.249			
earthy	0.306	0.613	0.450	-0.543	-0.520			
mint	0.305	-0.742	0.215	-0.178	0.705			
aroma-PC1	0.017	-0.504	0.156	0.457	0.505	0.606		
aroma-PC2	0.370	0.013	0.461	0.051	0.139			
aroma-PC3	0.015	-0.342	0.207	0.086	0.427	0.411		

: $p < 0.050$

* Using Fisher-Yates rank transformations.

Amer=American; Lim=Limousin; Tron=Troncais; Vos=Vosges.

Appendix Table K.11. Oak origin treatment means and ANOVA *p*-values for Australia seasoned and coopered oak barrel wine composition (SYSTAT V5.0, unweighted means model ANOVA: 4 x oak origins & 3 x wines).

	p-value			Treatment mean (ug/L)*				Origin treatment mean separation*		
Compound	origin	wine	interact	Amer	Lim	Tron	Vos	LSD (5%)	LSD (1%)	LSD (0.1%)
cis -oak lactone	0.000	0.002	0.286	158	229	368	510	99	134	182
trans -oak lactone	0.000	0.010	0.366	39	156	168	174	43	59	80
eugenol	0.000	0.000	0.147	18	19	28	31	5	6	9
guaiacol	0.164	0.005	0.583	13	12	21	19			
guaiacol**	0.182	0.001	0.383	14						
4-methylguaiacol	0.014	0.000	0.230	4	7	8	10	4		
4-methylguaiacol**	0.084	0.000	0.285	5						
vanillin	0.050	0.000	0.046	344	428	432	428			
vanillin**	0.565	0.000	0.140	359						
cyclotene	0.147	0.000	0.001	76	97	54	92			
cyclotene**	0.136	0.000	0.001	82						
maltol	0.163	0.000	0.290	120	81	113	130			
maltol**	0.091	0.000	0.296	132						
furfural	0.221	0.000	0.316	2.9	2.4	5.1	4.5			
furfural**	0.162	0.000	0.227	3.2						
'estimated extracted furfural'	0.104	0.006	0.327	6.6	6.9	10.2	9.8			
'estimated extracted furfural'**	0.197	0.001	0.364	7.2						
5-methylfurfural	0.109	0.000	0.198	0.32	0.33	0.57	0.53			
5-methylfurfural**	0.034	0.000	0.055	0.36				0.20#		
furfuryl alcohol	0.479	0.000	0.100	3.6	4.5	5.2	5.3			
5-methylfurfuryl alcohol	0.241	0.000	0.237	16	18	14	17			
vanillyl alcohol	0.215	0.190	0.720	17	3	2	3			
furfuryl ethyl ether	0.299	0.001	0.492	69	101	71	88			
5-methylfurfuryl ethyl ether	0.149	0.011	0.006	11	4	1	4			
vanillyl ethyl ether	0.037	0.000	0.053	7	9	6	9	3		
4-vinylguaiacol	0.000	0.000	0.000	5	8	4	11			
4-ethylguaiacol	0.406	0.000	0.189	19	17	11	15			
4-vinylphenol	0.000	0.000	0.000	10	16	7	27			
4-ethylphenol	0.008	0.000	0.001	0.31	0.25	0.26	0.27			

: $p < 0.050$

*: mg/L for furfural, 'estimated extracted furfural' (*i.e.* furfural + furfuryl alcohol), 5-methylfurfural, furfuryl alcohol and 4-ethylphenol.

**: One outlier (*AA34*) omitted.

interact=interaction; Amer=American; Lim=Limousin; Tron=Troncais; Vos=Vosges.

#: LSD calculation based on unequal sample sizes so an alternate formula was used (O'Mahony, 1986, p. 164) and the LSD is only valid when comparing the 'Amer' mean with any of the other means.

Appendix Table K.12. Oak origin treatment means and ANOVA *p* -values for Australia seasoned and coopered oak barrel Chardonnay wine composition (Excel V5.0: ANOVA: single factor: 4 x oak origins).

		*Treatment mean (ug/L)**				*Origin treatment mean separation**		
Compound	*p* -value	Amer	Lim	Tron	Vos	LSD (5%)	LSD (1%)	LSD (0.1%)
cis -oak lactone	0.003	133	179	275	371	100	146	
trans -oak lactone	0.014	36	133	114	131	57		
eugenol	0.050	14	14	16	22	6		
guaiacol	0.466	9	6	14	5			
4-methylguaiacol	0.641	3	3	5	3			
vanillin	0.508	348	301	313	277			
cyclotene	0.008	131	179	22	116	75	109	
maltol	0.030	111	47	69	48	43		
furfural	0.489	2.8	2.0	4.8	1.6			
'estimated extracted furfural'	0.434	7.2	5.1	7.9	4.3			
5-methylfurfural	0.524	0.30	0.21	0.40	0.19			
furfuryl alcohol	0.079	4.4	3.0	3.2	2.6			
5-methylfurfuryl alcohol	0.135	28	33	24	32			
furfuryl ethyl ether	0.237	109	136	82	99			
5-methylfurfuryl ethyl ether	0.021	28	10	1	1	17		
vanillyl ethyl ether	0.041	10	17	8	16	7		
4-vinylguaiacol	0.000	10	20	9	28	6	8	12
4-ethylguaiacol	0.500	1	0	2	0			
4-vinylphenol	0.000	24	41	16	71	14	20	30
composition-PC1	0.001	-0.402	0.055	-0.037	0.401	0.256	0.372	0.559
composition-PC2	0.451	0.104	-0.147	0.320	-0.233			
composition-PC3	0.037	-0.010	0.334	-0.205	0.356	0.415		

: $p < 0.050$

*: mg/L for furfural, 'estimated extracted furfural' (*i.e.* furfural + furfuryl alcohol), 5-methylfurfural and furfuryl alcohol.

Amer=American; Lim=Limousin; Tron=Troncais; Vos=Vosges.

Appendix Table K.13. Oak origin treatment means and ANOVA *p* -values for Australia seasoned and coopered oak barrel Cabernet Sauvignon wine composition (Excel V5.0, ANOVA: single factor: 4 x oak origins).

		*Treatment mean (ug/L)**				*Origin treatment mean separation**		
Compound	*p* -value	Amer	Lim	Tron	Vos	LSD (5%)	LSD (1%)	LSD (0.1%)
cis -oak lactone	0.008	212	243	498	657	244	355	
trans -oak lactone	0.013	51	169	196	228	96		
eugenol	0.006	22	25	39	40	10	15	
guaiacol	0.297	13	14	27	21			
4-methylguaiacol	0.198	3.2	7.7	8.3	9.7			
vanillin	0.055	252	328	322	324			
cyclotene	0.687	22	14	29	33			
maltol	0.309	152	117	172	211			
furfural	0.005	0.05	0.09	0.17	0.16	0.06	0.09	
5-methylfurfural	0.000	20	34	183	63	47	68	103
furfuryl alcohol	0.163	5.0	6.4	9.9	9.8			
5-methylfurfuryl alcohol	0.557	11	11	8	9			
furfuryl ethyl ether	0.638	73	85	98	122			
vanillyl ethyl ether	0.441	4	4	4	4			
4-vinylguaiacol	0.268	2	2	1	2			
4-ethylguaiacol	0.210	49	44	25	37			
4-vinylphenol	0.040	3	1	1	2	1		
4-ethylphenol	0.011	0.81	0.68	0.69	0.72	0.07		
composition-PC1	0.174	-0.289	-0.117	0.307	0.321			
composition-PC2	0.034	0.322	-0.117	-0.543	-0.266	0.538		
composition-PC3	0.000	-0.397	-0.119	0.405	0.428	0.224	0.327	0.491

[ ] : $p < 0.050$

*: mg/L for furfural, furfuryl alcohol and 4-ethylphenol.

Amer=American; Lim=Limousin; Tron=Troncais; Vos=Vosges.

Appendix Table K.14. Seasoning location treatment means and ANOVA *p*-values for American oak barrel wine aromas (Excel V5.0, ANOVA: 2-factor with replication: 2 x seasoning locations & 2 x wines; then for each wine, ANOVA: single factor: 2 x seasoning locations).

	Chardonnay & Cabernet Sauvignon					*Chardonnay*			*Cabernet Sauvignon*		
	p-value			*Treat mean**			*Treat mean**			*Treat mean**	
Aroma	seas	wine	interact	Amer	Aust	*p*-value	Amer	Aust	*p*-value	Amer	Aust
preference	0.809	1.000	0.642	0.072	-0.072	0.880	-0.067	0.067	0.625	0.210	-0.210
smoky	0.824	1.000	1.000	-0.067	0.067	0.878	-0.067	0.067	0.880	-0.067	0.067
pencil shavings	0.606	1.000	0.317	-0.142	0.142	0.746	0.140	-0.140	0.281	-0.423	0.423
coconut	0.670	1.000	0.766	-0.126	0.126	0.929	-0.038	0.038	0.623	-0.213	0.213
caramel	0.900	1.000	0.550	0.037	-0.037	0.619	0.213	-0.213	0.746	-0.140	0.140
vanilla	0.940	1.000	0.300	-0.021	0.021	0.435	-0.318	0.318	0.518	0.277	-0.277
allspice	0.471	1.000	#	-0.213	0.213	0.623	-0.213	0.213	0.619	-0.213	0.213
cashew nut						0.518	-0.277	0.277			
green apple						0.928	0.038	-0.038			
butter						0.033	0.703	-0.703			
cinnamon						0.878	0.067	-0.067			
coffee									0.301	-0.423	0.423
dark chocolate									0.686	0.172	-0.172
berry									0.301	0.423	-0.423
mint									0.490	0.281	-0.281
earthy									0.301	-0.423	0.423
Band-aid									0.301	-0.423	0.423
aroma-PC1						0.422	-0.634	-0.433	0.623	-0.321	-0.504
aroma-PC2						0.510	-0.240	-0.002	0.179	-0.330	0.013
aroma-PC3						0.994	0.113	0.116	0.663	-0.295	-0.342

: $p < 0.050$

* Treatment means using Fisher-Yates rank transformations.

#: No interaction term was calculated for 'allspice': All mean values are the same. Therefore, no effective interaction.

treat=treatment; seas=seasoning location; interact=interaction; Amer=America; Aust=Australia.

All wine means = 0.

Appendix Table K.15. Seasoning location treatment means and ANOVA *p*-values for American oak barrel wine composition (first: SYSTAT V5.0, unweighted means model ANOVA: 2 x seasoning locations & 3 x wines; then for each wine: Excel V5.0, ANOVA: single factor: 2 x seasoning locations).

	Chard., Cab. Sauv. & model wines					*Chardonnay*			*Cabernet Sauvignon*		
	p-value			*Treat mean**			*Treat mean**			*Treat mean**	
Compound	seas	wine	interact	Amer	Aust	*p*-value	Amer	Aust	*p*-value	Amer	Aust
cis -oak lactone	0.394	0.053	0.702	127	158	0.338	80	133	0.505	177	212
trans -oak lactone	0.457	0.196	0.368	33	39	0.577	30	36	0.174	36	51
eugenol	0.706	0.012	0.606	18	18	0.501	12	14	0.792	23	22
guaiacol	0.765	0.136	0.993	12	13	0.670	8	9	0.715	11	13
guaiacol**	0.056	0.002	0.190		14						
4-methylguaiacol	0.839	0.109	0.912	4	4	0.725	3	3	0.500	2.2	3.2
4-methylguaiacol**	0.028	0.000	0.140		5						
vanillin	0.676	0.002	0.181	346	344	0.075	278	348	0.294	194	252
vanillin**	0.070	0.000	0.870		359						
cyclotene	0.187	0.006	0.009	45	76	0.009	26	131	0.801	17	22
cyclotene**	0.025	0.001	0.012		82						
maltol	0.504	0.144	0.278	103	120	0.089	76	111	0.316	118	152
maltol**	0.122	0.083	0.864		132						
furfural	0.536	0.002	0.204	3.5	2.9	0.252	0.2	2.8	0.345	0.07	0.05
furfural**	0.443	0.000	0.554		3.2						
'estimated extracted furfural'	0.485	0.079	0.371	7.4	6.6	0.955	7.1	7.2			
'estimated extracted furfural'**	0.733	0.006	0.932		7.2						
5-methylfurfural	0.568	0.003	0.309	0.38	0.32	0.237	0.07	0.30	0.714	0.026	0.020
5-methylfurfural**	0.254	0.000	0.585		0.36						
furfuryl alcohol	0.759	0.002	0.191	4.0	3.6	0.035	6.9	4.4	0.557	3.7	5.0
5-methylfurfuryl alcohol	0.333	0.000	0.036	14	16	0.006	21	28	0.598	9	11
vanillyl alcohol	0.220	0.360	0.529	2	17						
furfuryl ethyl ether	0.298	0.000	0.626	56	69	0.615	97	109	0.165	48	73
5-methylfurfuryl ethyl ether	0.882	0.011	0.990	10	11	0.900	26	28			
vanillyl ethyl ether	0.026	0.000	0.001	5	7	0.008	6	10	0.230	4	4
4-vinylguaiacol	0.070	0.000	0.056	4	5	0.057	8	10	0.230	2	2
4-ethylguaiacol	0.843	0.000	0.940	18	19	1.000	1	1	0.801	44	49
4-vinylphenol	0.016	0.000	0.005	7	10	0.019	15	24	1.000	3	3
4-ethylphenol	0.824	0.000	0.945	0.000	0.000				0.801	0.84	0.81
composition-PC1						0.614	-0.474	-0.402	0.621	-0.396	-0.289
composition-PC2						0.344	-0.187	0.104	0.877	0.259	0.322
composition-PC3						0.001	-0.348	-0.010	0.495	-0.517	-0.397

*: mg/L for furfural, 'estimated extracted furfural' (*i.e.* furfural + furfuryl alcohol), 5-methylfurfural, furfuryl alcohol and 4-ethylphenol.

**: with *AA34* outlier omitted.

Chard.=Chardonnay; Cab. Sauv.=Cabernet Sauvignon; treat=treatment; : $p < 0.050$

seas=seasoning location; interact=interaction; Amer=America; Aust=Australia.

Appendix Table K.16. *p*-Values for compound accumulation rate comparisons (data as the % of the maximum concentration reached in each barrel) for the French oak barrel-stored model wines (Excel V5.0 ANOVA: 2-factor with replication: 3 x oak origin treatments, 2 x seasoning/cooper treatments; *n* =2).

The maximum for each barrel was determined from two sampling times - 55 and 93 weeks.

	p-value					
	0 to 55 week change			*55 to 93 week change*		
Compound	seas/coop	origin	interaction	seas/coop	origin	interaction
cis -oak lactone	0.778	0.459	0.093	0.778	0.459	0.093
trans -oak lactone	0.636	0.664	0.481	0.636	0.664	0.481
eugenol	0.141	0.400	0.221	0.141	0.400	0.221
guaiacol	0.040	0.049	0.275	0.040	0.049	0.275
4-methylguaiacol	0.002	0.937	0.373	0.000	0.088	0.016
vanillin	0.021	0.595	0.531	0.021	0.595	0.531
cyclotene	0.021	0.085	0.032	0.012	0.034	0.015
maltol	0.679	0.242	0.833	0.548	0.663	0.441
furfural	0.228	0.076	0.632	0.342	0.094	0.756
'estimated extracted furfural'	0.011	0.017	0.078	0.011	0.017	0.078
5-methylfurfural	0.022	0.075	0.419	0.022	0.075	0.419

: p <0.050

Appendix Table K.17. *p*-Values for compound accumulation rate comparisons (concentration data) for the French oak barrel-stored model wines (Excel V5.0 ANOVA: 2-factor with replication: 3 x oak origin treatments, 2 x seasoning/cooper treatments; *n* =2).

	p-value					
	0 to 55 week change			*55 to 93 week change*		
Compound	seas/coop	origin	interaction	seas/coop	origin	interaction
cis -oak lactone	0.259	0.028	0.228	0.119	0.008	0.039
trans -oak lactone	0.343	0.352	0.430	0.785	0.332	0.914
eugenol	0.028	0.004	0.317	0.070	0.092	0.354
guaiacol	0.944	0.215	0.607	0.081	0.041	0.350
4-methylguaiacol	0.152	0.128	0.312	0.000	0.024	0.005
vanillin	0.001	0.961	0.503	0.666	0.263	0.627
cyclotene	0.088	0.006	0.233	0.044	0.301	0.097
maltol	0.008	0.052	0.289	0.478	0.798	0.567
furfural	0.235	0.133	0.829	0.165	0.092	0.879
'estimated extracted furfural'	0.334	0.380	0.814	0.030	0.060	0.954
5-methylfurfural	0.318	0.089	0.986	0.039	0.032	0.991

: p <0.050

Appendix L

Baked grape marc — a source of 'coopering heat products'

When subjected to a temperature of 240 °C for 30 minutes, a post–fermentation, 1994 Clare Valley (South Australia) Shiraz grape marc was reduced in mass by two–thirds, from 150 g to 47 g. The baked marc appeared dry and dark in colour and smelled similar to coffee. It was steeped in wine made from the same grapes. The proportion of liquid and solid was equivalent to that initially in the grapes. The bottle was N_2 gas sparged for 30 seconds, 50 mg/L of SO_2 was added, and the bottle was sealed, mixed and stored at room temperature (approx. 20 °C). After 11 days, the solids were separated by sieving, pressing, centrifuging and decanting, and a further 50 mg/L of SO_2 was added.

After this treatment, guaiacol, cyclotene, maltol, furfural and 5–methylfurfural were found in concentrations higher than the mean concentrations found in the 93 week new oak barrel–stored Cabernet Sauvignon wines (Appx. Tab. L.1). Vanillin and 4–methylguaiacol did not seem to be produced by the treatment, although vanillin was quantified with low precision (Freon extraction method, Tab. 2.4) and a treatment effect for it, if one existed, was not detected.

A personal informal sensory evaluation indicated that the wine possessed a baked bread crust aroma, a burnt toast flavour and a moderately bitter taste. The aroma was acceptable in general wine character terms but the palate was unpleasant. Consequently, the process may have no potential as a wine production technique, although better results may be obtained by varying the treatment.

Appendix Table L.1.
Oak wood-derived compounds yielded by baked Shiraz grape marc.

Compound	*Treatment concentration (ug/L)** Grape marc treatment: Control	No heat	60 min 160 °C	30 min 240 °C	*Mean of the 95% confidence intervals (ug/L)**	*Mean concentration in the Cabernet Sauvignon after 93 weeks (ug/L)**
cis -oak lactone	0	0	0	0	17	342
trans -oak lactone	1	1	1	1	16	168
eugenol	2	2	1	2	1	33
guaiacol	7	10	9	179	3	19
4-methylguaiacol	1.5	2.3	0.7	0.0	0.8	7.7
vanillin (Freon extraction)*	0.0	0.0	0.1	0.1	0.2	277#
cyclotene	31	5	40	103	21	25
maltol	12	15	50	707	21	161
furfural*	0.00	0.00	0.00	0.17	0.34	0.10
'estimated extracted furfural'*	0.0	0.0	0.0	1.7	1.7	6.4
5-methylfurfural	0	0	1	122	71	66
furfuryl alcohol*	0.0	0.0	0.0	1.6	1.3	6.3
5-methylfurfuryl alcohol	1	1	1	3		10
vanillyl alcohol	13	102	56	17	51	14
furfuryl ethyl ether	0	0	0	1		80
5-methylfurfuryl ethyl ether	0	0	0	6		0
vanillyl ethyl ether	2	3	2	3	18	4
4-vinylguaiacol	8	10	9	9	7	2
4-ethylguaiacol	0	0	0	0		43
4-vinylphenol	5	8	3	4		2
4-ethylphenol*	0.00	0.00	0.00	0.02	0.11	0.80

: Concentration exceeds mean concentration in the 93 week barrel stored Cabernet Sauvignon wines.

*: mg/L for vanillin, furfural, 'estimated extracted furfural,' furfuryl alcohol and 4-ethylphenol.

#: Mean vanillin concentration among the Cabernet Sauvignon wines expressed in ug/L.

Appendix M

Microbiological experiment materials, methods and results

Appendix outline

M.1 Alcoholic fermentation effects

The treatment means and the ANOVA *p*–values for this comparison are shown in Appendix Table M.1.

M.2 Malolactic fermentation effects

1994 Adelaide Hills (South Australia) Pinot Noir grapes were crushed, fermented, pressed after 6 days, fermented to dryness and cold settled before being subjected to the following preparative treatments and then to malolactic fermentation (MLF). The results are discussed in Chapter 7.

Preparative treatments
(1) No treatment: 30 mg/L of SO_2 was added to the wine.
(2) Sterilised (DMDC): 0.15 mL of dimethyldicarbonate (DMDC) in 1.5 mL of ethanol per L of wine was added, along with 30 mg/L of SO_2.
(3) Denatured (boiled): The wine was brought to boiling (92 °C) and then cooled. During this treatment, the wine volume dropped by 8 % and the alcohol concentration dropped from 12.5 % to 10.0 %. Consequently, 95 % aqueous ethanol was added to correct the concentration, and 30 mg/L of SO_2 was added after cooling.

Treatments (1), (2) and (3) were poured to three, four and five sterilised 750 mL bottles, respectively. Appendix Table M.2 illustrates the scheme. An ethanol solution (1.5 mL) of 15 purified oak wood–derived or associated compounds (*i.e.*, the 20 compounds except for 5–methylfurfuryl alcohol, furfuryl ethyl ether, 5–methylfurfuryl ethyl ether, 4–ethylguaiacol and 4–vinylphenol) ('standards mix') was added to some of the bottles; the other bottles received an equivalent amount of ethanol without the compounds. All of the wines were sparged with CO_2 gas. The wines not to undergo MLF received 100 mg/L of SO_2 and were crown sealed. The other wines were inoculated with the manufacturer's recommended

dosage of a dry culture of *Leuconostoc oenos* (Viniflora Oenos, Chr. Hansen's Laboratory, Denmark) for MLF. The bottles were mixed and covered with aluminium foil. The free and total SO_2 concentrations in the inoculated wines were approximately 1 and 15 mg/L, respectively, immediately prior to the inoculation. All 12 bottles were kept at 25 °C for approximately four weeks, over which time MLF had proceeded to completion (Appx. Tab. M.2).

The samples were kept at 2 °C until volatile compound quantification by GC–MS, as described for the Cabernet Sauvignon wines in the main experiment (Chapter 2). There was approximately one month between MLF and extraction/quantification. The analytical precision was comparable to that for the Cabernet Sauvignon wines (Tab. 2.4). The treatments were not replicated sufficiently to warrant analyses of variance of the compound concentrations. The data are shown in Appendix Table M.2, and a summary of these data is shown in Table 7.2.

M.3 Denatured microbial cell effects

Aliquots (440 mL) of the Chardonnay control wine were measured to ten 750 mL bottles. The two 'denatured yeast' and two 'denatured lactic acid bacteria' treatment replicates received inocula to densities of 10^8 cells/mL. An active dried wine yeast, Maurivin AWRI 796 strain (*Saccharomyces cerevisiae*), was rehydrated according to the manufacturer's specifications (in 10 times the yeasts' mass of 40 °C water for 15 minutes) before addition to the wine. A lactic acid bacteria strain, Viniflora Oenos (*Leuconostoc oenos*) was added without reactivation, as is specified by the manufacturer.

All bottles were then autoclaved (42 minute cycle peaking at 121 °C). After cooling to 15 °C, 50 mg/L of SO_2 was added to each bottle, and the two 'activated yeast' and two 'activated lactic acid bacteria' treatment replicates received inocula to densities of 10^8 cells/mL. All ten lots were then transferred to 375 mL bottles and equal amounts of the 'standards mix' (similar to that used in the MLF experiment) were delivered to each.

Alcohol lost during autoclaving (4 %) was added, the bottles were sparged with CO_2 for one minute, crown sealed, thoroughly mixed, and stored for five days at 15 °C with daily mixing to resuspend the cells. After the last mixing, the samples were poured to 500 mL centrifuge tubes with 20 mg/L of SO_2, centrifuged at 10,000 rpm and 4 °C for 10 minutes, and the supernatant carefully decanted from the sediment.

The samples were kept at 2 °C until volatile compound quantification by GC–MS, as described for the Chardonnay wines in the main study (Chapter 2). The analytical precision may be comparable to that for the Chardonnay wines (Tab. 2.1) except that each replicate was quantified using only one sample instead of the two used in the main study. The treatments were not replicated sufficiently to warrant analyses of variance of the compound concentrations. The data are shown in Appendix Table M.3.

M.4 Chardonnay wine aroma associations with MLF

Appendix Table M.4 and Appendix Figure M.1 show the associations between malate consumption and the Chardonnay wine aroma.

Appendix Table M.1. Barrel fermentation effects: Chardonnay and model wine composition means and ANOVA *p*-values for selected treatments at racking (11 weeks) (SYSTAT V5.0, unweighted means model ANOVA: 2 x wines & 3 x treatments).

Compound	*p-value*			*Wine mean (ug/L)**		*Chardonnay control (ug/L)**
	wine	treat	interact	model	Chardonnay	
cis -oak lactone	0.586	0.004	0.232	102	110	21
trans -oak lactone	0.031	0.002	0.810	40	68	4
eugenol	0.390	0.002	0.319	12	11	1
guaiacol	0.229	0.122	0.361	11	9	0
guaiacol**	0.065	0.385	0.701	13		
4-methylguaiacol	0.032	0.010	0.102	9	6	0
4-methylguaiacol**	0.015	0.063	0.329	11		
vanillin	0.000	0.718	0.743	229	86	51
vanillin**	0.000	0.035	0.265	252		
cyclotene	0.012	0.331	0.768	11	22	2
cyclotene**	0.039	0.462	0.888	12		
maltol	0.549	0.795	0.233	45	59	4
maltol**	0.792	0.679	0.424	52		
furfural	0.003	0.380	0.467	5.4	1.4	0.0
furfural**	0.000	0.024	0.099	6.3		
'estimated extracted furfural'	0.003	0.780	0.129	5.7	11.0	0.0
'estimated extracted furfural'**	0.006	0.793	0.046	6.6		
5-methylfurfural	0.113	0.233	0.313	0.55	0.37	0.00
5-methylfurfural**	0.001	0.026	0.108	0.65		
furfuryl alcohol	0.000	0.290	0.139	0.3	9.6	0.0
5-methylfurfuryl alcohol	0.000	0.464	0.883	2	24	6
vanillyl alcohol	0.010	0.896	0.896	0	78	1
furfuryl ethyl ether	0.000	0.271	0.049	5	42	0
5-methylfurfuryl ethyl ether	0.000	0.147	0.147	0	34	0
vanillyl ethyl ether	0.000	0.885	0.885	0	10	1
4-vinylguaiacol	0.000	0.603	0.593	0	100	188
4-ethylguaiacol	0.757	0.009	0.225	2	2	0
4-vinylphenol	0.000	0.180	0.180	0	610	992
4-ethylphenol	0.458	0.569	0.569	0	0	0

[ ]: p <0.050

*: mg/L for furfural, 'estimated extracted furfural' (furfural + furfuryl alcohol), 5-methylfurfural and furfuryl alcohol.

**: One outlier (*AA34*) omitted.

treat = treatment; interact = interaction.

Appendix Table M.2. Malolactic fermentation experiment results.

		Treatment summary												
Sample number:		1	2	3	4	5	6	7	8	9	10	11	12	
Initial treatment:		*No treatment*			*Sterilised (DMDC)*				*Denatured (boiled)*					
'Standards mix' added?:				Yes		Yes	Yes	Yes		Yes	Yes	Yes	Yes	
MLF induced?:			Yes				Yes	Yes				Yes	Yes	
Final malate (g/L)**:		4.2	0.1	4.2	4.1	4.3	0.0	0.1	4.9	4.4	4.5	0.1	0.1	*Mean of the 95 % confidence intervals*
Compound (ug/L)*	*Addition (ug/L*)*													
cis -oak lactone	391^	0	2	206	1	201	223	243	1	210	210	222	228	16
trans -oak lactone	^	1	1	143	1	141	145	162	1	142	148	151	151	16
eugenol	10	3	3	12	3	12	14	14	3	12	12	12	13	1
guaiacol	11	1	1	11	1	12	12	11	1	11	10	12	11	1
4-methylguaiacol	5	0.0	0.0	4.6	0.0	4.7	4.5	4.7	0.0	4.6	4.2	4.8	4.7	0.8
vanillin (Freon extract)*	0.392	0.0	0.0	0.1	0.0	0.1	0.1	0.1	0.0	0.2	0.1	0.1	0.1	0.2
cyclotene	91	5	13	88	3	95	91	100	39	146	126	131	144	21
maltol	80	10	18	92	19	109	124	135	18	121	105	130	132	16
furfural*	4.002	0.01	0.00	0.20	0.02	3.78	0.01	0.01	0.03	3.16	2.25	0.01	0.01	0.34
5-methylfurfural	484	0	1	303	1	478	0	0	1	406	289	1	1	70
furfuryl alcohol*	8.434	0.0	0.0	11.0	0.0	8.5	8.7	10.8	0.0	6.8	5.8	10.6	10.2	1.3
5-methylfurfuryl alcohol		1	2	2	2	2	2	3	6	6	6	6	5	n.dn.
vanillyl alcohol	95	49	19	59	26	32	20	46	27	47	34	26	48	52
furfuryl ethyl ether		0	0	14	0	17	20	20	0	13	12	15	14	n.dn.
5-methylfurfuryl ethyl ether		0	0	1	0	0	15	16	0	0	0	16	15	n.dn.
vanillyl ethyl ether	226	3	3	148	3	137	89	115	4	163	165	127	148	17
4-vinylguaiacol	144	5	7	5	5	5	6	6	5	5	4	6	6	7
4-ethylguaiacol		0	0	0	0	0	0	0	0	0	0	0	0	n.dn.
4-vinylphenol		1	2	1	1	0	1	1	1	0	0	1	1	n.dn.
4-ethylphenol*	0.509	0.00	0.00	0.43	0.00	0.42	0.36	0.37	0.00	0.44	0.43	0.45	0.46	0.11

*: mg/L for vanillin (Freon extraction method), furfural, furfuryl alcohol and 4-ethylphenol.

**: Malate prior to MLF: No treatment = 4.4 g/L; Sterilised (DMDC) = 4.6 g/L; Denatured (boiled) = 4.6 g/L.

^: One addition (391 ug/L) of a racemic mixture of the oak lactones was made.

DMDC = dimethyldicarbonate; n.dn. = not determined.

: Compound not included in the 'standards mix.'

Appendix Table M.3. Denatured microbial cell effects.

Compound	*Treatment mean (ug/L)**					*Mean of 95% CIs for the Chardonnay wines** (ug/L)**
		Yeast		LAB		
	control	activ.	denat.	activ.	denat.	
cis -oak lactone	223	220	256	221	222	106
trans -oak lactone	175	177	197	174	179	79
eugenol	12	12	14	12	12	6
guaiacol	9	10	9	12	12	4
4-methylguaiacol	5	5	4	6	5	2
vanillin (Freon extraction)*	0.05	0.02	0.05	0.04	0.02	0.5
cyclotene	136	148	119	168	178	101
maltol	82	78	66	75	89	35
furfural*	1.7	1.2	2.7	2.0	3.0	1.6
5-methylfurfural*	0.22	0.19	0.18	0.20	0.29	0.29
furfuryl alcohol*	3.6	5.5	4.6	6.8	5.6	2.1
5-methylfurfuryl alcohol	16	20	11	25	29	n.dn.
vanillyl alcohol	23	15	27	16	12	41
furfuryl ethyl ether	2	2	1	3	3	n.dn.
5-methylfurfuryl ethyl ether	0	6	0	22	0	n.dn.
vanillyl ethyl ether	200	190	206	187	179	16
4-vinylguaiacol	158	157	179	153	146	27
4-ethylguaiacol	0	0	0	0	0	n.dn.
4-vinylphenol	84	134	102	81	75	n.dn.
4-ethylphenol*	0.45	0.44	0.52	0.47	0.47	n.dn.

: treatment mean greater than one 95 % confidence interval from the control.

*: mg/L for Vanillin (Freon extraction method), furfural, 5-methylfurfural, furfuryl alcohol and 4-ethylphenol.

**: 95 % confidence intervals from Table 2.1.

LAB = lactic acid bacteria; activ. = activated; denat. = denatured; CIs = confidence intervals; n.dn. = not determined.

: Compound not included in the 'standards mix.'

Appendix Table M.4. Associations between aromas and malate consumption for 24 Chardonnay barrel wines at 55 weeks

(Spearman's rank-order and Pearson's product-moment correlation coefficients).

malate	*preference*	*coconut*	*pencshavs*	*caramel*	*vanilla*	*butter*	*allspice*	*smoky*	*cashew*	*grnapple*	*cinnamon*
Spearman's	-0.345	-0.187	-0.708	0.639	-0.012	0.391	-0.300	-0.268	-0.294	-0.147	-0.020
Pearson's	-0.358	-0.179	-0.595	0.679	-0.115	0.629	-0.426	-0.246	-0.385	-0.059	0.055

pencshavs = pencil shavings; cashew = cashew nut; grnapple = green apple.

: significant correlation, $p<0.05$ or stronger.

Critical values for Spearman's 2-tailed test of correlation,

$n = 24$, from O'Mahony (1986).

If rho > or = 0.407, significant correlation, $p<0.05$.

If rho > or = 0.521, significant correlation, $p<0.01$.

If rho > or = 0.608, significant correlation, $p<0.002$.

Critical values for Pearson's 2-tailed test of correlation,

$n = 24$, d.f. $= n - 2 = 24 - 2 = 22$.

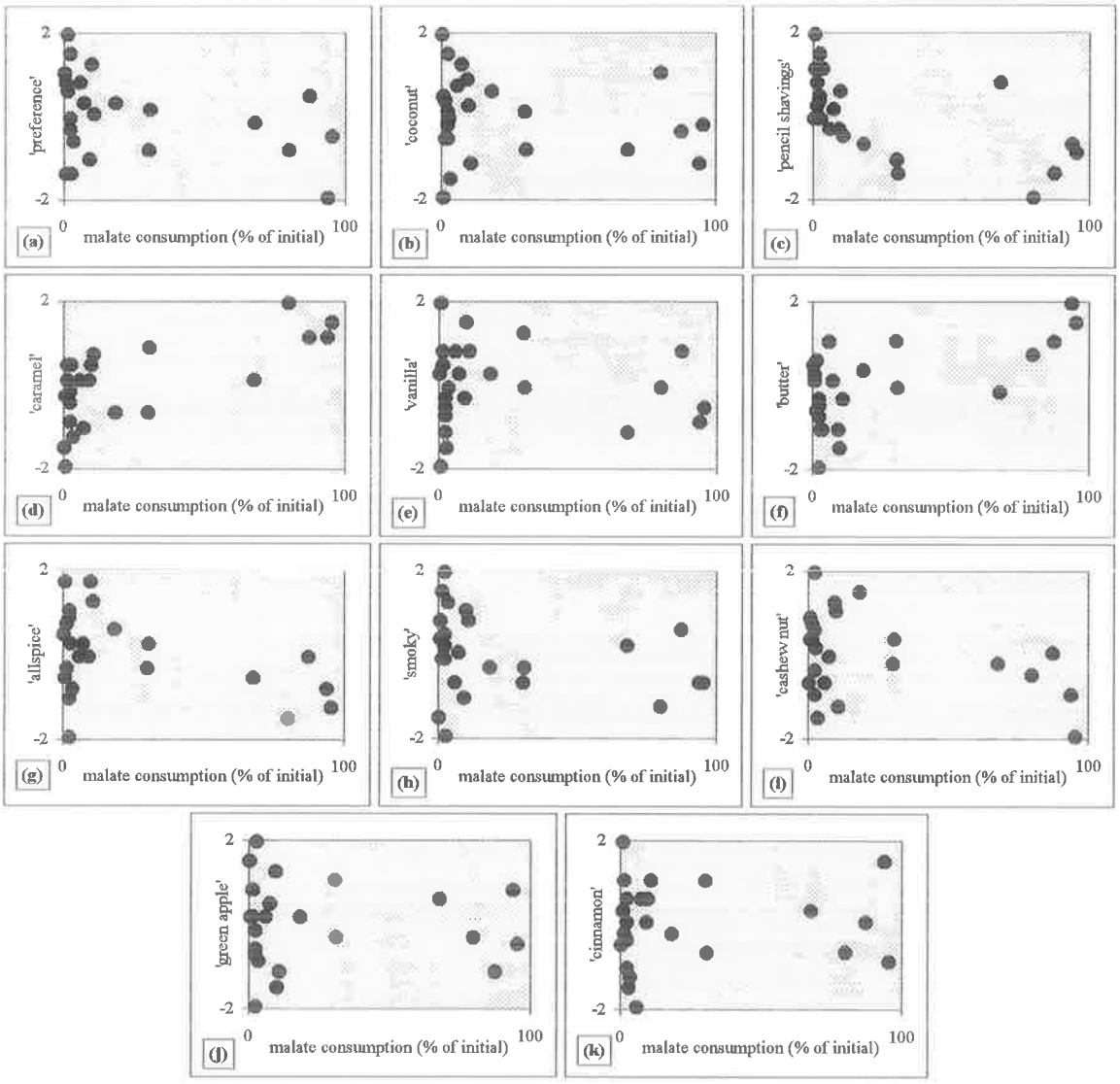
If r > or = 0.404, significant correlation, $p<0.05$.*

If r > or = 0.515, significant correlation, $p<0.01$.*

If r > or = 0.6524, significant correlation, $p<0.001$.**

*: from Snedecor and Cochran (1967), 22 *d.f.*

**: from O'Mahony (1986), 20 *d.f.*



Appendix Figure M.1. Scatter plots of the Chardonnay wine Fisher-Yates aroma rank transformations versus the extent of malate consumption.

Appendix N

Compound accumulation ANOVAs

Eight American and Limousin barrel–stored model wines were sampled for volatile compound quantification at 6, 11, 32, 55 and 93 weeks. Two replicates each of four treatments were involved (Appx. Tab. A.10). Microbial activity was kept to a minimum (Appx. Tab. A.9). Compound quantifications were as described for the model wines in Chapter 2, and accuracy and precision estimates are summarised in Table 2.6.

Two different analysis of variance (ANOVA) models were used in the data analyses. The repeated measures aspect of the design, involving the comparison of concentrations at different times in the same barrel, was accommodated by two–factor ANOVAs, without replication (repeated measures). Accompanying these analyses were two–factor ANOVAs, with replication, to test for interaction effects. The *p*–values for these analyses are listed in Appendix Table N.1.

When a multiple comparison was required, a two–tailed Fisher's least significant difference (LSD) ($p<0.05$) was performed to separate the means. Microsoft Excel V5.0 spreadsheet software was used for all statistical analyses.

Appendix Table N.1. ANOVA *p* -values for a comparison of compound concentrations in the barrel-stored model wine at different sampling times (Two Microsoft Excel V5.0 ANOVA models were used: a 2-factor, repeated measures ANOVA, without replication, 5 sampling times x 8 barrels; and a 2-factor ANOVA, with replication; 5 sampling times x 4 treatments, *n* =2).

	p-values for ANOVA, without replication		*p-values for ANOVA, with replication*		
Compound	*sampling time*	*barrel*	*sampling time*	*treatment*	*interaction*
cis -oak lactone	0.000	0.000	0.000	0.000	0.067
trans -oak lactone*	0.000	0.000	0.000	0.000	0.000
American/America (2 brls)	0.004	0.015			
American/Australia (2 brls)	0.073	0.007			
Limousin/France (2 brls)	0.016	0.209			
Limousin/Australia (2 brls)	0.000	0.007			
eugenol	0.000	0.000	0.000	0.007	0.796
guaiacol	0.000	0.000	0.077	0.979	0.993
4-methylguaiacol	0.025	0.000	0.287	0.016	0.859
vanillin	0.000	0.000	0.000	0.000	0.992
cyclotene	0.000	0.000	0.000	0.131	0.980
maltol	0.000	0.000	0.018	0.214	0.977
'estimated extracted furfural'	0.015	0.000	0.194	0.054	0.891
5-methylfurfural	0.388	0.000	0.843	0.112	0.975

: $p < 0.050$

These data are used in Table 8.1.

*: Due to a significant interaction effect for *trans*- oak lactone, data subsets were analysed separately for this compound by 2-factor, repeated measures ANOVA, without replication (5 sampling times x 2 barrels).

Literature cited

Abbott N.A., Coombe B.G., Sefton M.A., Williams P.J. 1989 The composition of Shiraz grapes in relation to the quality of table wines. *Proceedings 4ème Symposium, International d'Oenologie: Actualites oenolgiques*, 15–17 June 1989, Bordeaux, France.

Aiken J.W., Noble A.C. 1984 Composition and sensory properties of Cabernet Sauvignon wines aged in French versus American oak barrels. *Vitis*, **23**: 27–36.

Altner H. 1986 Physiology of olfaction. In: *Fundamentals of sensory physiology, 3rd edition.* Ed. Schmidt R.F. Springer–Verlag, Berlin, pp. 247–255.

Ames J.M. 1990 Control of the Maillard reaction in food systems. *Trends in Food Science and Technology*, December, pp. 150–154.

Anon. 1986 Barrel fermentation, an ancient technique becomes modern technology. *Australian Wine Industry J.*, **1**: 10–13.

Anon. *circa* 1989 Chart of French wood types and utilization according to varietal or alcohol. Tonnellerie Vicard, Cognac, France.

Anon. 1995 Full–bodied Cabernet Sauvignon — a varietal report. *Australian & New Zealand Wine Industry J.* **10**: 24–31.

Baldwin S., Black R.A., Andreasen A.A., Adams S.L. 1967 Aromatic congener formation in maturation of alcoholic distillates. *J. Agric. Food Chem.*, **15**: 381–385.

Baltes W. 1988 Investigations on thermal aroma formation. *Proceedings — Frontiers of flavor, 5th International Flavour Conference,* 1–3 July 1987, Porto Karros, Chalkidiki, Greece. Elsevier Science Publishers B.V., Amsterdam.

Bertuccioli M., Viani R. 1976 Red wine aroma: identification of headspace constituents. *J. Sci. Food Agric.*, **27**: 1035–1038.

Boidron J.–N., Chatonnet P., Pons M. 1988 Influence du bois sur certaines substances odorantes des vins. *Connaissance Vigne Vin,* **22**: 275–294.

Broschat T.K. 1979 Principal component analysis in horticultural research. *Hortscience,* **14**: 114–117.

Calleja G.B. 1987 Cell aggregation. In: *The Yeasts, vol. 2 — Yeasts and the environment, 2nd edition.* Eds. Rose A.H., Harrison J.S. Academic Press, London, pp. 165–238.

Cattell R.B. 1966 The scree test for the number of factors. *Multivariate behavioral research,* **1**: 245–276.

Cavin J.F., Andioc V., Etievant P.X., Divies C. 1993 Ability of wine lactic acid bacteria to metabolize phenol carboxylic acids. *Am. J. Enol. Vitic.*, **44**: 76–80.

Chang R. 1988 *Chemistry, 3rd edition.* McGraw–Hill Publishing Company, New York.

Chatonnet P. 1989 Origines et traitements des bois destinés à l'élevage des vins de qualité. *Revue des Oenologues,* **52**: 21–25.

Chatonnet P., Boidron J.–N., Pons M. 1989 Incidence du traitment thermique du bois de chêne sur sa composition chimique, 2^{e} partie: évolution de certains composés en fonction de l'intensité de brûlage. *Connaissance Vigne Vin*, **23**: 223–250.

Chatonnet P., Boidron J.–N. 1990 Thermal treatment of wood cooperage: effect on the extractable components of oak and on the sensory characters of wines. *Proceedings 7th Australian wine industry technical conference*, Adelaide, South Australia. Winetitles, Adelaide, p. 272.

Chatonnet P., Boidron J.–N., Pons M. 1990 Maturation of red wines in oak barrels: evolution of some volatile compounds and their aromatic impact. *Sciences des Aliments*, **10**: 565–587.

Chatonnet P., Dubourdieu D., Boidron J.–N. 1991 Effects of fermentation and maturation in oak barrels on the composition and quality of white wines. *Australian & New Zealand Wine Industry J.*, **6**: 73–84.

Chatonnet P., Dubourdieu D., Boidron J.–N. 1992a Le caractère phénolé des vins rouges: caractérisation, origine et moyens de lutte. *Rev. Fran. Oenol.*, **138**: 21–24.

Chatonnet P., Dubourdieu D., Boidron J.–N., Pons, M. 1992b The origin of ethylphenols in wines. *J. Sci. Food Agric.*, **60**: 165–178.

Chatonnet P., Dubourdieu D., Boidron J.–N. 1992c Incidence of fermentation and ageing conditions of dry white wines in barrels on their composition in substances yielded by oak wood. *Sci. des Aliments,* **12**: 665–685.

Chatonnet P., Dubourdieu D., Boidron J.–N., Lavigne, V. 1993 Synthesis of volatile phenols by *Saccharomyces cerevisiae* in wines. *J. Sci. Food Agric.*, **62**: 191–202.

Chatonnet P., Boidron J.–N., Dubourdieu D., Pons, M. 1994a Évolution des composés polyphénoliques du bois de chêne au cours de son séchage — premiers résultats. *J. International des Sciences de la Vigne et du Vin,* **28**: 337–357.

Chatonnet P., Boidron J.–N., Dubourdieu D., Pons, M. 1994b Évolution de certains composés volatils du bois de chêne au cours de son séchage — premiers résultats. *J. International des Sciences de la Vigne et du Vin,* **28**: 359–380.

Chatonnet P., Dubourdieu D., Boidron J.–N. 1995 The influence of *Brettanomyces/Dekkera* sp. yeasts and lactic acid bacteria on the ethylphenol content of red wines. *Am. J. Enol. Vitic.*, **46**: 463–468.

Conner J.M., Paterson A., Piggott J.R. 1993 Changes in wood extractives from oak cask staves through maturation of scotch malt whisky. *J. Sci. Food Agric.*, **62**: 169–174.

Connors W.J., Johanson L.N., Sarkanen K.V., Winslow P. 1980 *Holzforschung*, **34**: 29. Cited in Wittkowski *et al.* 1992.

Deschamps A.M. 1989 Microbial degradation of tannins and related compounds. In: *Plant cell wall polymers — biosynthesis and degradation, ACS Symposium Series 399.* Eds. Lewis N.G., Paice M.G, Washington, DC.

Deves M. 1988 Cooperage. *Australian & New Zealand Wine Industry J.*, **3**: 33–41.

Douglas L.J. 1987 Adhesion to surfaces. In: *The Yeasts, vol. 2, Yeasts and the environment, 2nd edition.* Eds. Rose A.H., Harrison J.S. Academic Press, London, 239–280.

Dubois P. 1983 Volatile phenols in wines. In: *Flavour of distilled beverages — origin and development.* Ed. Piggott J.R. Ellis Horwood, Chichester, pp. 110–119.

Dubois P. 1989 Apports du fût de chêne neuf à l'arôme des vins. *Rev. Fr. Oenol.*, **120**: 19–24.

Dugelay I., Baumes R., Günata Z., Razungles A., Bayonove C. 1995 Evolution de l'arôme au cours de la conservation du vin: formation de 4–(1–éthoxyéthyl)–phénol et 4–(1–éthoxyéthyl)–gaïacol. *Sci. des Aliments*, **15**: 423–433.

Federer W.T., McCulloch C.E., Miles–McDermott N.J. 1987 Illustrative examples of principal components analysis. *J. Sensory Studies*, **2**: 37–54.

Fiddler W., Parker W.E., Wasserman A.E., Doerr R.C. 1967 *J. Agric. Food Chem.*, **15**:757. Cited in Wittkowski *et al.* 1992.

Fisher R.A., Yates F. *1963 Statistical tables for biological, agricultural and medical research, 6th edition.* Oliver & Boyd, Edinburgh.

Fors S. 1983 Sensory properties of volatile Maillard reaction products and related compounds — a literature review. In: *The Maillard reaction in foods and nutrition, ACS Symposium Series 215.* Eds. Waller G.R., Feather M.S., Washington, DC.

Francis I.L., Sefton M.A., Williams P.J. 1992 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**: 23–30.

Gatfield I.L., Sommer H. 1993 The enzymatic and fermentative production of lactones and their use in natural flavors. *Proceedings — Recent developments in flavor and fragrance chemistry, 3rd international Haarmann and Reimer symposium.* VCH Verlagsgesellschaft, Weinheim.

Gladstones J.S. 1992 *Viticulture and environment.* Winetitles, Adelaide.

Guadagni D.G., Buttery R.G., Okano S., Burr H.K. 1963 Additive effect of sub–threshold concentrations of some organic compounds associated with food aromas. *Nature,* **200**: 1288–1289.

Guichard E., Fournier N., Masson G., Puech J.–L. 1995 Sterioisomers of β–methyl–γ–octalactone. I. Quantification in brandies as a function of wood origin and treatment of the barrels. *Am. J. Enol. Vitic.,* **46**: 419–423.

Günther C., Mosandl A. 1986 Stereoisomere Aromastoffe: XII: 3–methyl–4–octanolid — — "Quercuslacton, whiskylacton" — Structur und Eigenschaften der Stereoisomeren, *Liebigs Ann. Chem.,* 2112–2122.

Harayama K., Hayase F., Kato H. 1995 Contribution to stale flavor of 2–furfuryl ethyl ether and its formation mechanism in beer. *Biosci. Biotech. Biochem.,* **59**: 1144–1146.

Henick–Kling T., Acree T., Gavitt B.K., Krieger S.A., Laurent M.H. 1993 Sensory aspects of malolactic fermentation. *Proceedings 8th Australian wine industry technical conference.* Winetitles, Adelaide, pp. 148–152.

Hodge J. 1967 In: *Chemistry and physiology of flavors.* Eds. Schultz H.W., Day E.A., Libbey L.M. AVI Publishing Co., Westport, Connecticut, pp. 465–491. Cited in Fors 1983.

Hoey A.W. 1986 Why be concerned about oak barrel maturation. *Australian Grapegrower & Winemaker,* **275**: 14–20.

Hoey A.W., Codrington I.D. 1987 Oak barrel maturation of table wine. *Proceedings 6th Australian wine industry technical conference*, 14–17 July 1986, Adelaide, South Australia. Australian Industrial Publishers, Adelaide, pp. 261–266.

Johnson R.R., Alford E.D., Kinzer G.W. 1969 Formation of sucrose pyrolysis products. *J. Agric. Food Chem.*, **17**: 22–24.

Keith E.S., Powers J.J. 1968 Determination of flavor threshold levels and sub–threshold, additive, and concentration effects. *J. Food Sci.,* **33**: 213–218.

Kempler G.M. 1983 Production of flavor compounds by microorganisms. *Adv. Appl. Microbiol*, **29**: 29–51.

King B.M., Arents P., Moreau N. 1995 Cost/efficiency evaluation of descriptive analysis panels — I. Panel size. *Food Quality and Preference,* **6**: 245–261.

Kinsella J.E. 1990 Flavor perception and binding. *International news on fats, oils and related material,* 1: 215–226.

Kirby K.N. 1993 *Advanced data analysis with SYSTAT.* Van Nostrad Reinhold, New York.

Leisola M.S.A., Fiechter A. 1985 New trends in lignin biodegradation. In: *Advances in biotechnological processes 5.* Alan R. Liss, Inc., pp. 59–89.

Lesschaeve I., Issanchou S. 1996 Could selection tests detect the future performance of descriptive panellists? *Food Quality and Preference,* **7**: 177–183.

Litchev V. 1989 Influence of oxidation processes on the development of the taste and flavour of wine distillates. *Am. J. Enol. Vitic.*, **40**: 31–35.

Lubbers S., Voilley A., Feuillat M., Charpentier C. 1994 Influence of mannoproteins from yeast on the aroma intensity of a model wine. *Lebensm.–Wiss. u.–Technol.* **27**: 108–114.

Maarse H., van den Berg F. 1989 Current issues in flavour research. In: *Distilled beverage flavour — Recent developments.* Eds. Piggott J.R., Paterson A. Ellis Horwood, Chichester, pp. 1–15.

Maga J.A. 1985 Flavor contribution of wood in alcoholic beverages. *Proceedings — Progress in flavour research, 4th Weurman flavour research symposium,* 9–11 May 1984, Dourdan, France. Ed. Adda J. Elsevier Science Publishers, Amsterdam, pp. 409–416.

Maga J. A. 1989a Formation and extraction of *cis*– and *trans*–β–methyl–γ–octalactone from *Quercus alba.* In: *Distilled beverage flavour — Recent developments.* Eds. Piggott J.R., Paterson A. Ellis Horwood, Chichester, pp. 171–176.

Maga J. A. 1989b The contribution of wood to the flavor of alcoholic beverages. *Food Reviews International,* **5**: 39–99.

Marche M., Joseph E. 1975 Etude theorique sur le cognac, sa composition et son vieillisement naturel du cognac en barriques, et sur la formation du "Rancio Charentais." *Rev. Fran. Oenol.*, **57**: 18–33.

Marco J., Artajona J., Larrechi M.S., Rius F.X. 1994 Relationship between geographical origin and chemical composition of wood for oak barrels. *Am. J. Enol. Vitic.*, **45**: 192–200.

Marsal F. 1987 Évolution des substances du bois au cours de la fermentation alcoolique et de l'élevage des vins blancs secs. *Connaissance Vigne Vin, Special issue, Le bois et la qualité des vins et des eaux–de–vie,* pp. 93–99.

Marsal F., Sarre Ch. 1987 Etude par chromatographie en phase gazeuse de substances volatiles issues du bois de chêne. *Connaissance Vigne Vin*, **21**: 71–80.

Marsal F., Sarre Ch., Dubourdieu D., Boidron J.–N. 1988 Role de la levure dans la transformation de certains constituents volatils du bois de chêne au cours de l'élaboration en barrique des blancs secs. *Connaissance Vigne Vin*, **22**: 33–38.

Masson G., Guichard E., Fournier N., Puech J.–L. 1995 Stereoisomers of β–methyl–γ–octalactone. II. Contents in the wood of French (*Quercus robur* and *Quercus petraea*) and American (*Quercus alba*) oaks. *Am. J. Enol. Vitic.*, **46**: 424–428.

Masuda M., Nishimura K. 1971 Branched nonalactones of some *Quercus* species. *Phytochemistry*, **10**: 1401–1402.

Masuda M., Nishimura K. 1981 Absolute configuration of *Quercus* lactones (3S, 4R)– and (3S, 4S)–3–methyl–4–octanolide, from oak wood and chiroptical properties of mono–cyclic γ–lactones. *Chemistry letters,* pp. 1333–1336.

McDaniel M., Henderson L.A., Watson Jr. B.T., Heatherbell D. 1987 Sensory panel training and screening for descriptive analysis of the aroma of Pinot Noir wine fermented by several strains of malolactic bacteria. *J. Sensory Studies,* **2**: 149–167.

Meilgaard M.C. 1989 Use of the taste panel in flavour stability studies: organisation, threshold, synergism, antagonism. *Louvain Brew. Lett.,* **2**: 3–11.

Meilgaard M., Civille G.V., Carr B.T. 1991 *Sensory evaluation techniques, 2nd edition.* CRC Press, Inc., Boca Raton.

Miller D.P., Howell G.S., Michaelis C.S., Dickmann D.I. 1992 The content of phenolic acid and aldehyde flavour components of white oak as affected by site and species. *Am. J. Enol. Vitic.,* **43**: 333–338.

Miller J.C., Miller J.N. 1988 *Statistics for analytical chemists, 2nd edition.* Ellis Horwood Ltd., Chichester.

Mosedale J.R. 1995 Effects of oak wood on the maturation of alcoholic beverages with particular reference to whisky. *Forestry,* **68**: 203–230.

Mosedale J.R., Savill P.S. 1996 Variation of heartwood phenolics and oak lactones between the species and phenological types of *Quercus petraea* and *Q. robur. Forestry,* **69**: 47–55.

Moutounet M., Rabier Ph., Puech J.–L., Verette E. and Barillere J.–M. 1989 Analysis by HPLC of extractable substances in oak wood — Application to a Chardonnay wine. *Sciences des Aliments,* **9**: 35–51.

Nabeta K., Yonekubo J., Miyake M. 1986 Analysis of volatile constituents of European and Japanese oaks. *Mokuzai Gakkaishi,* **32**: 921–927.

Nicolini G., Mattivi F., Dalla Serra A. 1991 Must hyperoxidation: analytical and sensorial effects in 1989 vintage wines. *Rivista di Viticoltura e di Enologia,* **44**: 45–56.

Nishimura K., Ohnishi M., Masuda M., Koga K., Matsuyama R. 1983 Reactions of wood components during maturation. In: *Flavour of distilled beverages — origin and development.* Ed. Piggott J.R. Ellis Horwood, Chichester, pp. 241–255.

Nykänen L., Nykänen I., Moring M. 1985 Aroma compounds dissolved from oak chips by alcohol. *Proceedings — Progress in flavour research, 4th Weurman flavour research symposium,* 9–11 May 1984, Dourdan, France. Ed. Adda J. Elsevier Science Publishers B.V., Amsterdam.

O'Mahony M. 1986 *Sensory evaluation of food — statistical methods and procedures.* Marcel Dekker, Inc., New York, pp. 327–332.

Omori T., Yamamoto A., Yasui H. 1968 Studies on the flavors in saké. Part X. Confirmation of demethoxylation of vanillin by yeasts. *Agr. Biol. Chem.*, **32**: 539–548.

Onishi M., Guymon J.F., Crowell E.A. 1977 Changes in some volatile constituents of brandy during aging. *Am. J. Enol. Vitic.*, **28**: 152–158.

Peng S., Scalbert A., Monties B. 1991 Insoluble ellagitannins in *Castanea sativa* and *Quercus petraea* woods. *Phytochemistry*, **30**: 775–789.

Peynaud E. 1981 *Knowing and making wine*. English trans. by A. Spencer, 1984, John Wiley & Sons, New York.

Pocock K.F., Strauss C.R., Somers T.C. 1984 Ellagic acid deposition in white wines after bottling: a wood–derived instability. *Australian Grapegrower & Winemaker*, **244**: 87.

Pocock K.F., Sefton M.A., Williams P.J. 1994 Taste thresholds of phenolic extracts of French and American oak wood: Influence of oak phenols on wine flavour. *Am. J. Enol. Vitic.*, **45**: 330–334.

Puech J.–L. 1981 Extraction and evolution of lignin products in Armagnac matured in oak. *Am. J. Enol. Vitic.*, **32**: 111–114.

Puech J.–L. 1987 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**: 236–238.

Puech J.–L., Moutounet M. 1990 Oak wood chemistry and extractable substances. *Proceedings Aviemore conference on malting, brewing & distilling*, **3**: 209–225.

Puech J.–L., Moutounet M. 1992 Phenolic compounds in an ethanol–water extract of oak wood and in a brandy. *Lebensm.–Wiss. u.–Technol.*, **25**: 350–352.

Quinn M.K., Singleton V.L. 1985 Isolation and identification of ellagitannins from White Oak wood and an estimation of their roles in wine. *Am. J. Enol. Vitic.*, **36**: 148–155.

Reazin G.H. 1981 Chemical mechanisms of whiskey maturation. *Am. J. Enol. Vitic.*, **32**: 283–289.

Reazin G.H., Baldwin S., Scales H.S., Washington H.W., Andreasen A.A. 1976 Determination of the congeners produced from ethanol during whisky maturation. *J. Assoc. Offic. Agric. Chem.*, **59**: 770–776.

Ribéreau–Gayon P., Glories Y. 1987 Phenolics in grapes and wines. *Proceedings 6th Australian wine industry technical conference*, 14–17 July 1986, Adelaide, South Australia. Australian Industrial Publishers, Adelaide, pp. 247–256.

Ribéreau–Gayon P., Pontallier P., Glories Y. 1983 Some interpretations of colour changes in young red wines during their conservation. *J. Sci. Food Agric.* **34**: 505–516.

Romano P., Suzzi G. 1993 Sulfur dioxide and wine microorganisms. In: *Wine microbiology and biotechnology.* Ed. Fleet, G.H. Harwood Academic Publishers, Switzerland, pp. 380–382.

Rous C., Alderson B. 1983 Phenolic extraction curves for white wine aged in French and American oak barrels. *Am. J. Enol. Vitic.*, **34**: 211–215.

Salo P., Nykänen L., Suomalainen H. 1972 Odor thresholds and relative intensities of volatile aroma components in an artificial beverage imitating whisky. *J. Food Sci.*, **37**: 394–398.

Schahinger G., Rankine B. 1992 *Cooperage for winemakers — a manual on the construction, maintenance and use of oak barrels.* Ryan Publications, Adelaide.

Schmidt O., Kerner–Gang W. 1986 Natural Materials. In: *Biotechnology, a comprehensive treatise in 8 volumes, vol. 8.* Series Eds. Rehm H.–J., Reed G., Volume Ed. Schönborn W. VCH Verlagsgesellschaft mbH, D–6940, Weinheim.

Schreier P. 1984 Chromatographic studies of biogenesis of plant volatiles. In: *Chromatographic methods.* Heidelberg: Hüthig, pp. 120–125.

Sefton M.A. 1991 How does oak barrel maturation contribute to wine flavour? *Pract. Winery Vineyard*, **12**: 17–20.

Sefton M.A., unpublished data, 1997. The Australian Wine Research Institute, PO Box 197, Glen Osmond, SA 5042, Australia.

Sefton M.A., Skouroumounis G.K., Massy–Westropp R.A., Williams P.J. 1989 Norisoprenoids in *Vitis vinifera* white wine grapes and the identification of a precursor of damascenone in these fruits. *Australian J. Chem.*, **42**: 2071–2084.

Sefton M.A., Francis I.L., Williams P.J. 1990a Volatile flavour components of oakwood. *Proceedings 7th Australian wine industry technical conference*, Adelaide, South Australia. Winetitles, Adelaide, pp. 107–112.

Sefton M.A., Francis I.L., Williams P.J. 1990b Volatile norisoprenoid compounds as constituents of oak woods used in wine and spirit maturation. *J. Agric. Food Chem.*, **38**: 2045–2049.

Sefton M.A., Francis I.L., Pocock K.F., Williams P.J. 1993a The influence of natural seasoning on the concentrations of eugenol, vanillin, and *cis*– and *trans*–β–methyl–γ–octalactone extracted from French and American oakwood. *Sciences des Aliments,* **13**: 629–643.

Sefton M.A., Spillman P.J., Pocock K.F., Francis I.L., Williams P.J. 1993b The influence of oak origin, seasoning, and other industry practices on the sensory characteristics and composition of oak extracts and barrel–aged white wines. *Australian Grapegrower & Winemaker,* **355**: 17–25.

Shigematsu H., Shibata S., Kurata T., Kato H., Fujimaki M. 1975 *J. Agric. Food Chem.*, **23**: 233–237. Cited in Fors 1983.

Singleton V.L. 1974 Some aspects of the wooden container as a factor in wine maturation. In: *Chemistry of winemaking, ACS Advances in chemistry series 137.* Ed. Webb A.D. Washington, DC, pp. 254–277.

Singleton V.L. 1990 An overview of the integration of grape, fermentation, and ageing flavours in wines. *Proceedings 7th Australian wine industry technical conference*, Adelaide, South Australia. Winetitles, Adelaide, pp. 96–106.

Singleton V.L. 1995 The 1994 James F. Guymon Lecture — Maturation of wines and spirits: comparisons, facts and hypotheses. *Am. J. Enol. Vitic.*, **46**: 98–115.

Snedecor G.W., Cochran W.G. 1967 *Statistical methods, 6th edition.* The Iowa State University Press, Ames, Iowa.

Spillman P.J. 1995 Non–adhesion of oak flavour compounds to microbial cells. *Australian Grapegrower & Winemaker*, **379**: 19–22; and *errata* in *ibid*, **380**:11.

Spillman P.J., Pollnitz A.P., Liacopoulos D., Skouroumounis G.K., Sefton M.A. 1997 Accumulation of vanillin during barrel–aging of white, red and model wines. *J. Agric. Food Chem.*, **45**: 2584–2589.

Spillman P.J., Pollnitz A.P., Liacopoulos D., Pardon K.H., Sefton M.A. 1998 Formation and degradation of furfuryl alcohol, 5–methylfurfuryl alcohol, vanillyl alcohol, and their ethyl ethers in barrel–aged wines. *J. Agric. Food Chem.*, **46**: 657–663.

Sponholz W.–R. 1993 Wine spoilage by microorganisms. In: *Wine microbiology and biotechnology.* Ed. Fleet G.H. Harwood Academic Publishers, Switzerland, p. 400.

Steinke R.D., Paulson M.C. 1964 The production of steam–volatile phenols during the cooking and alcoholic fermentation of grain. *J. Agric. Food Chem.*, **12**: 381–387.

Towey J.P., Waterhouse A.L. 1996 The extraction of volatile compounds from French and American oak barrels in Chardonnay during three successive vintages. *Am. J. Enol. Vitic.*, **47**: 163–172.

Viriot C., Scalbert A., Lapierre C., Moutounet M. 1993 Ellagitannins and lignins in aging of spirits in oak barrels. *J. Agric. Food Chem.*, **41**: 1872–1879.

Viriot C., Scalbert A., Hervé du Penhoat C.L.M., Moutounet M. 1994 Ellagitannins in woods of sessile oak and sweet chestnut dimerization and hydrolysis during wood ageing. *Phytochemistry*, **36**: 1253–1260.

Vivas N. 1993 Les phénomènes liés à la maturation du bois de chêne pendant son séchage. *Revue des Oenologues*, **70**: 17–21.

Vivas N., Glories Y. 1996 Role of oak wood ellagitannins in the oxidation process of red wines during aging. *Am. J. Enol. Vitic.*, **47**: 103–107.

Wackerbauer K., Kossa T., Tressel R. 1978 Die bildung von phenolen durch hefen. *Monatschrift fur brauerie,* **31**: 52–55.

Waterhouse A.L., Towey J.P. 1994 Oak lactone isomer ratio distinguishes between wines fermented in American and French oak barrels. *J. Agric. Food Chem.*, **42**: 1971–1974.

Weil W.J., personal communication, *circa* 1992. Canton Wood Products Company, USA.

Wilson B., Strauss C.R., Williams P.J. 1984 Changes in free and glycosidically bound monoterpenes in developing Muscat grapes. *J. Agric. Food Chem.*, **32**: 919–924.

Winterhalter P., Sefton M.A., Williams P.J. 1990 Two–dimensional GC–DCCC analysis of the glycoconjugates of monoterpenes, norisoprenoids and shikimate–derived metabolites from Riesling wine. *J. Agric. Food Chem.*, **38**: 1041–1048.

Wittkowski R., Ruther J., Drinda H., Rafiei–Taghanaki F. 1992 Formation of smoke flavor compounds by thermal lignin degradation. In: *Flavour precursors — thermal and enzymatic conversion, ACS Symposium Series 490.* Eds. Teranashi R., Takeoka G.R., Günert M., Washington, DC.

Yates F. 1970 *Experimental design — selected papers of Frank Yates, C.B.E., F.R.S.* Griffin, London.

Yoshizawa K., Momose H., Hasuo T. 1981 Studies on aging of whiskey. I. Substances evaporated through barrel during aging of whiskey. *Nippon Nogei Kagaku Kaishi,* **55**: 1063–1068. Cited in Maga 1989b.