

**SOME AROMA COMPOUNDS OF IMPORTANCE TO THE QUALITY OF
FERDINAND DE LESSEPS AND KERNER WINES**

by

Ilva Margaret Rogers

Thesis presented in partial fulfilment for the degree of Master of Agricultural Science at the

University of Stellenbosch



Supervisor: Prof C J van Wyk

Co-supervisor: Prof P G Goussard

Stellenbosch

March 2000

DECLARATION

I, the undersigned, hereby declare that, apart from Chapter III, the work contained in this thesis is all my original work and that I have not previously in its entirety or in part submitted it at any university for a degree. In Chapter III, I was co-responsible for the isolation, identification, odour evaluation of the medicinal compound, its evaluation in commercial and experimental wines, and the preparation of the manuscript.

SUMMARY

Ferdinand de Lesseps grapes have a distinctive fruity varietal character and were often used judiciously by wine-makers to enhance the fruity bouquet of some white table wines. Ferdinand de Lesseps grape juice was investigated to identify the main contributing compounds responsible for its unique and intense aroma. The juice was recovered from grapes under anaerobic conditions and extracted using Freon 11. The concentrated extract was analysed using a combination of capillary gas chromatography-mass spectrometry and gas chromatography-sniffing techniques. Thirty-two compounds were reported. With the aid of GC-sniffing, it was concluded that the hybrid note of the Ferdinand de Lesseps grape was most likely attributed to the presence of 2,5-dimethyl-3(2H)-furanone and o-aminoacetophenone. Esters, which featured prominently in the juice, consisted mainly of ethyl butanoate, ethyl- and methyl- 3-hydroxy butanoate, and to a lesser extent, ethyl 3-hydroxy hexanoate and ethyl-3-hydroxy propanoate. These esters are most likely responsible for the sweetish pineapple aroma. According to EEC regulations, the use of non-*Vinifera* grapes in the production of commercial wines is prohibited. The presence of 2,5-dimethyl-3(2H)-furanone in wine could therefore be interpreted as a *labrusca* indicator should it be suspected that Ferdinand de Lesseps grapes were used in the blend.

The quality of some South African Kerner table wines often is rated inferior owing to an unwanted odour described as "Elastoplast" or "medicinal". This odour is encountered occasionally in wines from other cultivars such as Gewürztraminer, Weisser Riesling, Muscat de Frontignan and Chenin blanc. The identification of the compounds responsible for the off-odour was investigated, as well as possible relationships between its occurrence and viticultural practices. The compound responsible for the "medicinal" off- odour was identified

as p-vinyl guaiacol. It is formed via decarboxylation of ferulic acid by yeast during alcoholic fermentation. Its concentration is directly related to the concentration of ferulic acid and the yeast strain. Other factors affecting p-vinyl guaiacol formation in Kerner wines are region, microclimate, exposure of grapes to sunlight and grape maturity. Wines made from grapes harvested from the warmer climatic regions and exposed to direct sunlight and increased maturity contained higher levels of p-vinyl guaiacol. Viticultural practices that can be applied to limit the formation of the p-vinyl guaiacol precursor in grapes are the use of a canopy manipulation to protect the grapes from direct sunlight and an earlier harvesting date.

Various winemaking techniques can be used to lower p-vinyl guaiacol levels in wine. Careful consideration must be given to the choice of yeast, as different yeast strains differ considerably with their ability to form p-vinyl guaiacol. Fining oxidised grape juice with phenol adsorbing agents such as activated charcoal, polyvinylpyrrolidone, casein, gelatin (particularly in combination with bentonite and "kieselsool") led to decreases in the p-vinyl guaiacol levels. Although fining with activated charcoal was the most effective must treatment for reducing the "medicinal" aroma of Kerner wines, it stripped the wines of colour and character. Excessive oxidation of must followed by fining involves extra costs and time for the winemaker and would not be used in making the reductive style of wines.

OPSOMMING

Ferdinand de Lesseps druiwe en wyne vertoon unieke vrugtige varieteitskarakter en is dikwels deur wynmakers in versnitte gebruik om die geur van sekere wit tafelwyne te verbeter. In hierdie studie is Ferdinand de Lesseps druiwe ondersoek om die belangrikste komponente wat verantwoordelik is vir die prominente, unieke aroma daarvan te identifiseer. Die sap is onder anaerobe toestande herwin en met Freon 11 ekstraheer. Die gekonsentreerde ekstrakte is met behulp van 'n kombinasie van kapillêre gaschromatografie-massaspektrometrie en gaschromatografie-snuif tegnieke analiseer. Twee-en-dertig komponente is gerapporteer. Met die behulp van GC-snuif tegnieke is bevestig dat die hibried karakter in Ferdinand de Lesseps druiwe hoofsaaklik aan die teenwoordigheid van o-amino-asetofenoon en 2,5-dimetiel-3(2H)-furanon toegeskyf kan word. Die vernaamste esters in die sap was etielbutanoaat, etiel- en metiel-3-hidroksibutanoaat en in 'n mindere mate etiel-3-hidroksiheksanoaat en etiel-3-hidroksipropanoaat. Dié esters is waarskynlik verantwoordelik vir die soet pynappel aroma. Volgens EEG regulasies word die gebruik van nie-*Vinifera* druiwe vir die produksie van komersiële wyne verbied. Die teenwoordigheid van 2,5-dimetiel-3(2H)-furanon in wyn kan dus interpreteer word as 'n *labrusca* indikator in gevalle waar gebruik van Ferdinand de Lesseps druiwe vermoed word.

Die kwaliteit van sommige Suid-Afrikaanse Kerner wyne word dikwels verlaag vanweë die teenwoordigheid van 'n geur wat beskryf word as "Elastoplast" of "medisinaal". Dié reuk word soms waargeneem in wyne van ander kultivars soos Gewürztraminer, Weisser Riesling, Muskaat de Frontignan en Chenin blanc. Die identiteit van die komponent wat vir dié ongewenste reuk verantwoordelik is, asook moontlike verwantskappe met wingerd- en wynkundige praktyke is ondersoek. Die komponent wat vir die medisinale karakter verantwoordelik is, is geïdentifiseer as para-vinielguajakol. Dit word deur dekarboksilasie van

feruliensuur deur gisselle tydens alkoholiese gisting gevorm. Die konsentrasie van para-vinielguajacol in wyn is direk verwant aan die konsentrasie feruliensuur en die gisras. Ander faktore wat para-vinielguajacol-vorming in Kerner wyn affekteer is streek, mikroklimaat, blootstelling van druiwe aan direkte sonlig en die rypheidsgraad van die druiwe. Wyne wat berei is van ryper druiwe uit warmer streke en wat direk aan sonlig blootgestel was, het hoër konsentrasies para-vinielguajacol bevat. Lowerbestuurspraktyke met behulp waarvan druiwe teen direkte sonligblootstelling beskerm word, is van die vernaamste wingerdkundige praktyke wat aangewend kan word om die vorming van die voorlopers van para-vinielguajacol in druiwe te verlaag.

Verskeie wynebereidingstegnieke kan gebruik word om die para-vinielguajacol vlakke in wyn te verlaag. Gisraskeuse is van besondere belang omdat gisrasse grootliks verskil in hul vermoë om para-vinielguajacol te vorm. Breibehandelings van geoksideerde sap met middels soos geaktiveerde koolstof, PVPP, kasseïen, gelatien (veral in kombinasie met bentoniet en "kieselol") het die vlakke van para-vinielguajacol in wyne verlaag. Hoewel geaktiveerde koolstof die doeltreffendste breimiddel vir die verlaging van para-vinielguajacol was, het dit te veel kleur en geur uit die wyn verwyder. Oormatige oksidasie van mos gevolg deur breibehandelings sal meer geld en tyd verg en word nie aanbeveel vir wyne wat in 'n reduktiewe styl berei word nie.

CONTENTS

	PAGE
SUMMARY	i
OPSOMMING	iii
CHAPTER I	
Introduction	1
CHAPTER II	
Characterisation of the aroma of the hybrid Ferdinand de Lesseps (<i>Vitis vinifera</i> x <i>Vitis labrusca</i>)	3
ABSTRACT	3
INTRODUCTION	4
MATERIALS AND METHODS	5
RESULTS AND DISCUSSION	7
CONCLUSIONS	12
LITERATURE CITED	13
CHAPTER III	
The occurrence of a "Medicinal" off-odour in white table wines	17
ABSTRACT	17

INTRODUCTION.....	18
MATERIALS AND METHODS	19
RESULTS AND DISCUSSION	23
CONCLUSIONS.....	33
LITERATURE CITED	33

CHAPTER IV

Conclusion.....	35
------------------------	-----------

ACKNOWLEDGEMENTS

I wish to express my sincere thanks to the following people:

Prof C J van Wyk for his guidance and constructive criticism of the manuscript

Prof P G Goussard in his capacity as co-supervisor

Dr O P H Augustyn and Mr L P Ellis who acted as examiners

Miss E Carstens, Mrs D Hickman and Mrs A Louw, for their technical assistance

The departmental staff for their support and their contribution as tasting panel members

Dedicated to my family and friends

CHAPTER I

INTRODUCTION

Wine quality is related to its intrinsic organoleptic properties of which aroma is of primary importance. The aroma is determined by many factors such as grape cultivar, climate, microclimate, terroir, viticultural practices, and winemaking techniques. Wine-makers have made use of the characteristic taste and aroma qualities of certain grape varieties in the preparation of highly distinctive varietal wines. It has been possible to identify some of the essential aroma volatiles that are responsible for the characteristic aromatic nuances found in these wines, *e.g.* the contribution of terpenes to the muscat aroma, or the presence of minute quantities of methoxypyrazines in the herbaceous aroma of Sauvignon blanc wines.

Ferdinand de Lesseps has unique and intense aromatic qualities, which have been used to enhance the fruity bouquet in some South African white table wines. This American-French hybrid originated from crossbreeding 'Isabella' (*Vitis vinifera* x *Vitis labrusca*) with Royal Muscadine (*Vitis vinifera*). The results of our study into the aroma contributing compounds of Ferdinand de Lesseps are presented in Chapter II. The concentrated extract was analysed using a combination of gas chromatography-mass spectrometry and gas chromatography-sniffing which enabled us to isolate and identify the main aroma contributing compounds.

In contrast to the positive contribution of grape aroma and bouquet compounds to the character of varietal wines and ultimate wine quality, the presence of certain compounds or precursors in the grape, can have a negative impact on wine quality when present in unacceptable amounts. In Chapter III, we present the results of our investigation into the identity of an undesirable "medicinal" or "elastoplast" odour found in some South African

Kerner wines from hot regions. Kerner is known to produce high quality wines in cool regions. The fraction with the "medicinal" aroma was isolated by gas chromatography-sniffing and the corresponding compound was identified using gas chromatography-mass spectrometry. Its appearance was determined in commercial Kerner wines and experimental wines. Various vini-viticultural practices were investigated to reduce its concentration and the accompanying "medicinal" odour in wines.

The draft publications are written according to the prescriptions of the South African Journal of Enology and Viticulture and have been submitted for publication in that journal.

CHAPTER 11

Characterisation of the aroma of the hybrid Ferdinand de Lesseps

(Vitis vinifera x Vitis labrusca)

I M Rogers and C J van Wyk

Department of Viticulture and Oenology, University of Stellenbosch, Private Bag X1, 7602
Matieland (Stellenbosch), South Africa

Keywords: Ferdinand de Lesseps, American-French hybrid, grape aroma constituents, pineapple and labrusca odour

Condensed title: The aroma of Ferdinand de Lesseps grapes

ABSTRACT

Freon 11 extracted volatiles of Ferdinand de Lesseps grape juice were studied by capillary gas chromatography, capillary gas chromatography-mass spectrometry and gas chromatography-sniffing. The berries were crushed under an inert atmosphere and the juice extracted with Freon-11 for 20 hours. Thirty-two compounds were reported. The juice was quantitatively characterised mostly by esters, particularly hydroxy esters. These esters are most probably responsible for the sweetish pineapple aroma of Ferdinand de Lesseps grapes whereas, o-amino acetophenone and 2,5-dimethyl-4-hydroxy-3(2H)-furanone could contribute to its hybrid note.

INTRODUCTION

Ferdinand de Lesseps, an American-French hybrid, was exhibited at the Royal Horticultural Society Show in 1870 where it received a 1st class certificate. Royal Muscadine (or Chasselas doré) had been crossed with 'Isabella' (*Vitis vinifera* x *Vitis labrusca*), a prolific American grape to produce the new variety. It was noted that it had somewhat of Isabella's strawberry-like aroma (Perold, 1927).

In South Africa Ferdinand de Lesseps has a distinctive character and has been known as the "pineapple" or "honey" grape. It was grown on a small scale as an early table grape and also used for producing highly aromatic sweet wines. Until recently, Ferdinand de Lesseps was used in small quantities as a blending partner to enhance the fruity bouquet of some white table wines.

Several compounds have been identified that are considered to play an important role in the aroma of labrusca grapes. Large amounts of esters are found in labrusca grapes unlike in vinifera grapes. Among these are methyl anthranilate, unsaturated esters and hydroxy esters. Other compounds of interest are o-aminoacetophenone, 2,5 dimethyl-4-hydroxy-3(2H)-furanone (Acree & Lavin, 1990), its methoxy derivative (Schreier, 1980), β -damascenone (Acree, 1981, Braell *et al.*, 1986) and ethyl-2-mercaptopropionate (Kolor, 1983).

This investigation was aimed at the identification of aroma compounds that are pertinent to the fruity character of Ferdinand de Lesseps. This was achieved by extracting the grape aroma compounds with Freon-11 and submitting the concentrated extract for GC-MS identification of the compounds and for subsequent odour characterisation by GC-sniffing.

MATERIALS AND METHODS

Plant Material: Ferdinand de Lesseps grapes were harvested at 23°B from a local vineyard in Stellenbosch, South Africa.

Sample Preparation: Whole undamaged berries were selected. These were crushed by hand in plastic bags filled with nitrogen and the juice recovered and centrifuged under a nitrogen atmosphere to limit oxidation and artefact formation as far as possible.

Isolation of free volatiles: Free volatile grape aroma was extracted using a method described by Marais (1986). A 250 ml aliquot of grape juice was subjected to continuous liquid extraction with Freon 11 for 20 hours. The extracts were concentrated to approximately 100 μ l and stored at -14°C prior to analysis.

Analysis: The Ferdinand de Lesseps extracts were analysed by combined gas chromatography-mass spectrometry using a Finnegan 4600 Quadrupole mass spectrometer. The volatiles were identified by either comparing mass spectra published data or spectra obtained from standard compounds analysed under the same conditions. Relative concentrations of each compound were determined using 3-decanol as internal standard without considering the recovery of volatiles and detector response factors.

The sample was then submitted for GC-sniffing to identify compounds responsible for the varietal aroma of Ferdinand de Lesseps. An interesting Ferdinand de Lesseps-like aroma was later identified using a Finnegan GCQ.

Instrumental operating conditions:

Gas chromatography and gas chromatography-mass spectrometry conditions:

Finnegan 9610 gas chromatograph / 4600 Quadrupole mass spectrometer system:

Gas chromatographic parameters: Column Supelcowax 10 (60 m x 0,32 mm i.d. x 0,25 µm) fused silica capillary. Operating conditions: Injector temperature 200°C, oven temperature programme, 60°C (10 min) x 1°C/min to 190°C, carrier gas He, injection volume 1 µl, split ratio 30:1.

Mass spectrometer parameters: Source temperature, 240°C; interface temperature, 210°C; manifold temperature, 105°C; ionisation current, 0,31 amps; acceleration potential 70eV, multiplier voltage 850, scanning range 35 to 450 amu, scan time 0.95s with a 0.05s pause between scans.

Finnegan GCQ:

Gas chromatographic parameters: Column Restek Stabilowax DA (60 m x 0,32 mm i.d. x 0,25 µm) fused silica capillary. Operating conditions: Injector temperature 225°C, oven temperature programme, 60°C (5 min) x 1.5°C/min to 180°C, carrier gas He, injection volume 1 µl, split ratio 30:1.

Mass spectrometer parameters: Source temperature, 180°C; transfer line temperature, 260°C; emission current 250 micro amps, acceleration potential 70eV, electron multiplier, 1450 volts with a scanning range of 35 to 450 amu each second.

Gas chromatography-sniffing conditions:

A Carlo Erba 4200 Gas Chromatograph equipped with a sniffing port was used for the odour assessment of the GC effluent. Two experienced sniffers alternating every 15 minutes to overcome nasal fatigue assessed the GC effluent. Aroma descriptions were assigned to the identified compounds on the basis of matching relative retention times.

Gas chromatographic parameters: Column Supelcowax 10 (60 m x 0,32 mm i.d. x 0,25 µm) fused silica capillary. Operating conditions: Injector temperature 200°C, oven temperature programme, 60°C (10 min) x 1°C/min to 190°C, carrier gas He, injection volume 1 µl, split ratio 30:1.

RESULTS AND DISCUSSION

The identities of 32 volatile compounds in the Ferdinand de Lesseps grape extract were confirmed by GC-MS analysis. All of these compounds had been reported in grapes previously. Their relative concentrations and associated aromas as determined by GC and GC-sniffing respectively, are listed in Table 1.

o-Aminoacetophenone: The identity of a compound responsible for an intense Ferdinand de Lesseps grape-like aroma nosed during GC-sniffing was later verified as o-aminoacetophenone. Due to its low concentration, its presence was missed in the initial GC-MS study of the extract. o-Aminoacetophenone has been implicated as the component responsible for the "foxy" character of *labrusca* grapes (Acree *et al.*, 1990), muscadine grapes (Baek *et al.*, 1999) and as providing a *labrusca* character in Concord grapes (Shure & Acree,

TABLE 1

Volatile compounds identified by GC-MS in freon extracts of Ferdinand de Lesseps grapes.

No.	COMPOUND	CONCENTRATION relative to 3-Decanol $\mu\text{g/l}$	AROMA DESCRIPTION
1	Ethyl butanoate	57	Sweet
2	Hexanal	53	Grassy
3	n-butanol	Trace	*
4	Ethyl-2-butenolate	20	sweet, fruity
5	n-pentanol	Trace	*
6	Trans-2-hexenal	34	Grassy
7	Ethyl hexanoate	10	Sweet
8	Hexyl acetate	2	*
9	3-hydroxy-2-butanone	8	floral, sweet
10	n-hexanol	Trace	*
11	Trans-3-hexen-1-ol	5	*
12	Cis-3-hexen-1-ol	5	*
13	Nonanal	Trace	*
14	Trans-2-hexen-1-ol	60	Green
15	Cis-2-hexen-1-ol	<1	*
16	Cis-furan linalool oxide	<1	*
17	Ethyl-2-hydroxy propanoate	4	slight sweet
18	Methyl-3-hydroxy butanoate	161	sweet, fruity
19	2-ethyl-1-hexanol	<1	*
20	Ethyl-3-hydroxy butanoate ¹	320	sweet, dried fruit
21	Linalool	<1	*
22	4-terpineol	2	*
23	Ethyl-3-hydroxy hexanoate	15	sweet, fruity
24	α -terpineol	2	*
25	Trans-pyran linalool oxide	2.5	*
26	3-methyl-3-buten-2-one	2.5	*
27	Hexanoic acid	8	slight stink
28	Benzyl alcohol	9	*
29	2-phenyl ethanol ¹	43	Rose
30	Terpene-diol-1	12.5	*
31	2,5-dimethyl-4-hydroxy-3(2H)-furanone ¹	14.2	candy-floss
32	o-aminoacetophenone ¹	0.5	Ferdinand de lesseps-like

*) No perceived aroma for compound (concentration < odour threshold)

¹) Compounds identified using authentic standards

1995). In spite of its low concentration in muscadine grape juice (10 - 19 $\mu\text{g/l}$) o-aminoacetophenone gave a high flavour dilution factor *ie.* the highest dilution at which an aroma active compound could be detected. In their implication of o-aminoacetophenone being associated with the "foxy" character of labrusca grapes, Acree *et al.* (1990) measured levels of 130 – 280 ng/l . Rapp, Versini & Ullemeyer (1993) identified o-aminoacetophenone as the component responsible for an off-odour in *Vitis vinifera* cultivars of Müller-Thurgau, Riesling and Sylvaner, which was described as an atypical ageing note ("naphthalene", "hybrid" or "wet dirty towel"). In the faulty wines analysed by Dollmann *et al.* (1999) amounts of o-aminoacetophenone ranged from 0,7 to 12,8 mg/l . The "hybrid" note was recognised sensorically from a concentration of 700 ng/l and greater in fermented model wine solutions (Rapp *et al.*, 1995). Baek *et al.* (1997) used a threshold value of 400 ng/l determined in skim milk to calculate an aroma value (concentration/aroma threshold) for o-aminoacetophenone. Acree *et al.* (1990) found 130 – 280 ng/l of o-aminoacetophenone in cultivars of labrusca grapes exhibiting the foxy-like odour. The relative concentration of 500 ng/l of o-aminoacetophenone found for Ferdinand de Lesseps grape juice is in the region of the quoted threshold values and hence could be at a level where it contributes to the aroma of this cultivar.

2,5-Dimethyl-4-hydroxy-3(2H)-furanone: The presence of 2,5-dimethyl-4-hydroxy-3(2H)-furanone, (DMHF or furaneol) in Ferdinand de Lesseps aroma is of particular interest as this compound has previously been reported as responsible for a sweet candy-like aroma in cultivars derived from labrusca (Acree *et al.*, 1990) and for the "strawberry" off-flavour in berries and wines of interspecific grapevine breedings (Rapp *et al.*, 1980). Guedes de Pinho & Bertrand (1995) developed an analytical method for determining 2,5-dimethyl-4-hydroxy-3(2H)-furanone, whose presence indicated a non-*Vitis vinifera* element in the wine. 2,5-

Dimethyl-4-hydroxy-3(2H)-furanone has also been established to be a major character impact compound of pineapple flavour concentrate (Rodin *et al.*, 1965) and a contributor to pineapple flavour (Takeoka *et al.*, 1989). It exists both in the free and glycosidically bound forms and its glucoside has been identified in strawberries (Mayerl, Näf, & Thomas, 1989). Furanol was most abundant in the free and bound forms identified in Muscadine grape juice (Baek & Cadwallader, 1999). At high concentrations, furaneol has a burnt candy-like aroma and at low concentrations a pineapple- or strawberry-like aroma. The odour threshold of furaneol in water has been reported as 31 $\mu\text{g/l}$ at pH 4.5 (Buttery as quoted by Baek *et al.*, 1997). Rapp *et al.* (1995) reported the taste threshold for the recognition of the strawberry note at 80 – 150 $\mu\text{g/l}$ in wine. At a relative concentration of 14,2 $\mu\text{g/l}$ furaneol may not seem to be such an important aroma contributor to the Ferdinand de Lesseps grape aroma, but its contribution to wine aroma could be increased with the release from its glycosidically bound forms during the wine-making processes.

Esters: Esters featured prominently in the Ferdinand de Lesseps' aroma profile. As a group, they contributed to 69% of the total relative concentration of the GC-registered volatiles. This may be an indication of the labrusca parentage of Ferdinand de Lesseps. Vinifera grapes differ distinctly from labrusca, with only trace amounts having been detected (Schreier, Drawert & Junker, 1976; Schreier, 1980). The high concentration of volatile esters has been used as an index of the 'fruity' character of labrusca grapes (Fuleki as quoted by Schreier, 1980). Of particular quantitative interest were the polar hydroxy esters *i.e.* ethyl-3-hydroxy butanoate, methyl-3-hydroxy butanoate and ethyl-2-butanoate and to a lesser extent ethyl-3-hydroxy hexanoate and ethyl-3-hydroxy propanoate. Most polar esters have low odour detection thresholds and contribute favourably to the fruit and flower notes of wine (Baumes *et al.*, 1986). Schreier (1980) reported that the hydroxy esters contributed to the 'fruity' character of

labrusca grapes. Methyl-3-hydroxy butanoate and ethyl-3-hydroxy hexanoate have been reported as aroma constituents in pineapple (Schreier, 1980; Rodin *et al.*, 1965). Baek (1997) reported an average concentration range of 390-550 $\mu\text{g/l}$ ethyl-3-hydroxy butanoate in muscadine grape juice, which exhibited a burnt marshmallow and muscadine-like aroma note, but it had a relatively low flavour dilution factor. Other esters present in Ferdinand de Lesseps' aroma are ethyl butanoate and ethyl hexanoate with odour thresholds of 1 $\mu\text{g/l}$ and 1,8 $\mu\text{g/l}$, respectively. These compounds have been identified as important contributors to fresh pineapple aroma (Takeoka, Buttery & Flath, 1989). The odour of ethyl hexanoate has been described as fruity with pineapple undertone (Fenaroli as quoted by Takeoka *et al.*, 1989). GC-sniffing performed in this investigation showed that the esters, specifically ethyl butanoate, ethyl-3-hydroxy butanoate and methyl-3-hydroxy butanoate contributed sweet, fruity odours to the aroma profile of the Ferdinand de Lesseps extract.

Unsaturated compounds: Schreier & Paroschy (1981) identified a number of unsaturated compounds considered to contribute to the sweet-fruity odour of certain aroma fractions of labrusca grapes. Of these, only ethyl-2-butenate, whose odour was perceived as sweet and fruity on sniffing, was identified in this study.

Alcohols: Of the small amounts of alcohols present in Ferdinand de Lesseps grape juice, quantitatively trans-2-hexen-1-ol and 2-phenyl ethanol are of interest. The odour of identity of trans-2-hexen-1-ol was perceived as green. In our study 2-phenyl ethanol exhibited a strong rose-like note with GC-sniffing. It has been implicated as a major aroma component of muscadine grapes and wine (Lamikanra, Grimm & Inyang, 1996). 2-Phenyl ethanol occurs both in free and glycosidically bound forms and has been described as having a rose-like note at high concentrations and a honey-like note at low concentrations (Baek *et al.*, 1997).

Labrusca compounds not detected in Ferdinand de Lesseps grape juice: We were unable to detect the presence of methyl anthranilate and damascenone in Ferdinand de Lesseps grape aroma when using the GCQ mass spectrometer in the Selected Ion Monitoring mode. These compounds have been reported as contributors as to the aroma of *labrusca* grapes (Acree, 1981). Damascenone, which has a pleasant floral odour and a very low threshold of 2-20 pg/g in water, is thought to contribute to the sweet perfume aroma. It is possible that it required a further fractionation of the extract for its determination.

CONCLUSIONS

The combination of sniffing of GC fractionated volatiles of a Ferdinand de Lesseps grape aroma extract with GC-MS analyses for assessing the identity of individual compounds enabled us to identify the main aroma contributing compounds of this cultivar. From our aroma description of these compounds, as well as those reported in grapes and other fruits with similar aroma tones, it appears as though the hybrid note of the Ferdinand de Lesseps could most likely be attributed to the presence of *o*-aminoacetophenone and 2,5-dimethyl-4-hydroxy-3(2H)-furanone, whereas the esters, ethyl butanoate and the ethyl and methyl esters of 3-hydroxy butanoic acid, and to a lesser extent, ethyl 3-hydroxy hexanoate and ethyl-3-hydroxy propanoate are most probably responsible for the sweetish pineapple aroma.

LITERATURE CITED

ACREE, T.E., 1981. The odour quality of *Labrusca* grapes. In: Teranishi, R. & Berrerr-Benitez, H. (eds) Quality of Selected Fruits and Vegetables of North America. ACS Symposium Series 170. American Chemical Society, Washington, DC. pp 11-19.

ACREE, T.E. & LAVIN, E.H., 1990. o-Amino acetophenone the 'foxy'-smelling component of *labruscana* grapes. In: Bessi re, Y. & Thomas, A.F. (eds) Flavour science and technology. John Wiley & Sons Ltd, Chichester. pp 49-52.

BAEK, H.H., CADWALLADER, K.R., MARROQUIN, E., & SILVA, J.L. 1997. Identification of predominant aroma compounds in Muscadine grape juice. *J. Fd. Sci.* **62**, 249-252.

BAEK, H.H. & CADWALLADER, K.R., 1999. Contribution of free and glycosidically bound volatile compounds to the aroma of Muscadine grape juice. *J. Fd. Sci.* **64**, 441-443.

BAUMES, R., CORDONNIER, R., NITZ, S. & DRAWERT, F., 1986. Identification and determination of volatile constituents in wines from different vine cultivars. *J. Sci. Food Agric.* **37**, 927-943.

BRAELL, P.A., ACREE, T.E., BUTTS, R.M. & ZHOU, P.G. 1986. Isolation of nonvolatile precursors of β -damascenone from grapes using CHARM analysis. In: Parliament, T.H. & Croteau, R. (eds) Biogenesis of aromas. American Chemical Society, Washington, DC. pp.75-84.

DOLLMANN, B., WICHMANN, D., SCHMITT, A., KOEHLER, H. & SCHREIER, P., 1996. Quantitative analysis of 2-aminoacetophenone in off-flavoured wines by stable isotope dilution assay. *J. AOAC Int.* **79**, 583-586.

GUEDES de PINHO, P. & BERTRAND, A., 1995. Analytical determination of furaneol (2,5-dimethyl-4-hydroxy-3(2H)-furanone). Application to differentiation of white wines from hybrid and various *Vitis vinifera* cultivars. *Am. J. Enol. Vitic.* **46**, 181-186.

LAMIKANRA, O., GRIMM, C.C. & INYANG, I.D., 1996. Formation and occurrence of flavor components in noble Muscadine wine. *Fd. Chem.* **56**, 373-376.

KOLOR, M.G., 1983. Identification of an important new flavour compound in Concord grape: ethyl-3-mercaptopropionate. *J. Agric. Food Chem.* **31**, 1125-1127.

MARAIS, J., 1986. A reproducible capillary gas chromatographic technique for the determination of specific terpenes in grape juice and wine. *S. Afr. J. Enol. Vitic.* **7**, 21-25.

MAYERL, F., NÄF, R. & THOMAS, A.F., 1989. 2,5-Dimethyl-4-hydroxy-3(2H)-furanone glucoside: Isolation from strawberries and synthesis. *Phytochem.* **28**, 631-633.

PEROLD, A.I., 1927. *Treatise on Viticulture*. Macmillan and Co., Ltd., London.

RAPP, A., KNIPSER, W., ENGEL, L., ULLEMEYER, H. & HEIMANN, W., 1980. Off-flavour compounds in the berry and wine aroma of grapevine hybrids. I. The strawberry-like flavour. *Vitis* **19**, 13-23.

RAPP, A., VERSINI, G. & ULLEMEYER, H., 1993. 2-Aminoacetophenone: causal component of 'untypical ageing flavour' ('naphthalene' note, 'hybrid' note) of wine. *Vitis* **32**, 61-62.

RAPP, A., VERSINI, G. & ENGEL, L., 1995. Determination of 2-aminoacetophenone in fermented model wine solutions. *Vitis* **34**, 193-194.

RODIN, J.O., HIMEL, C.M., SILVERSTEIN, R.M., LEEPER, R.W. & GORTNER, W.A., 1965. Volatile flavour and aroma components of pineapple. I. Isolation and tentative identification of 2,5-dimethyl-4-hydroxy-3(2H)-furanone. *J. Fd. Sci.* **30**, 280-285.

SCHREIER, P., DRAWERT, F. & JUNKER, A., 1976. Identification of volatile constituents from grapes. *J. Agric. Food Chem.* **24**, 331-336.

SCHREIER, P., 1980. Volatile constituents in different grape species. In: Grape and wine centennial symposium proceedings. University of California, Davis. pp. 317-321.

SCHREIER, P. & PAROSCHY, J.H., 1981. Volatile constituents from Concord, Niagara (*Vitis labrusca*, L.) and Elvira (*V.labrusca*, L. x *V.riparia*, M.) grapes. *Can.Inst.Food Sci.Technol.J.* **14**, 112-118.

SHURE, K.B. & ACREE, T.E., 1995. *In vivo* and *in vitro* flavour studies of *Vitis labruscana* Cv. Concord. In, Rouseff, R.L. & Leahy, M., (eds) *Fruit flavors – Biogenesis, Characterization and Authentication*. ACS Symposium Series 596, American Chemical Society, Washington. pp. 127-133.

TAKEOKA, G., BUTTERY, R.G., FLATH, R.A., TERANISHI, R., WHEELER, E.L., WIECZOREK, R.L. & GUENTERT, M., 1989. Volatile constituents of pineapple (*Ananas cosmosus* [L.] Merr.). In: Bessi re, Y. & Thomas, A.F. (eds) Flavour science and technology, John Wiley & Sons Ltd, Chichester. pp. 223-237.

CHAPTER III

The Occurrence of a "Medicinal" Off-odour in White Table Wines

C J van Wyk and I M Rogers

Department of Viticulture and Oenology, University of Stellenbosch, Private Bag X1, 7602
Matieland (Stellenbosch), Republic of South Africa

Keywords: Medicinal off-odour, white wines
Condensed title: Medicinal off-odour in white wines

ABSTRACT

The quality of some Kerner table wines often is rated inferior owing to the presence of an objectionable odour designated as "medicinal" or "elastoplast" (band-aid). Occasionally this odour is also encountered in wines from other cultivars such as Gewürztraminer, Weisser Riesling, Muscat de Frontignan and Chenin blanc. The objectives of this study were to identify the compounds responsible for the off-odour and to establish possible relationships between their occurrence and vini-viticultural procedures. The component predominantly responsible for the "medicinal" odour was identified as p-vinyl guaiacol. This compound is known to be formed during alcoholic fermentation via decarboxylation of ferulic acid. Yeast strains, however, differed appreciably with respect to their ability to produce p-vinyl guaiacol. Most, if not all, of this component is apparently formed during alcoholic fermentation of grape juice. Only in one exceptional case, was the presence of this odour detected in grapes

from a crossing of Cruchen blanc x Servan blanc, which also contained relatively high levels of p-vinyl guaiacol. Wines made from grapes harvested at an advanced degree of maturity and in particular those exposed to sunlight, contained higher levels of p-vinyl guaiacol than did those from shaded grapes. Oxidation and subsequent treatment of grape juice with phenol-absorbing fining agents such as activated charcoal, polyvinyl polypyrrolidone, casein and gelatine (in combination with "kieselsool" and bentonite) led to decreases in the p-vinyl guaiacol levels and the concomitant "medicinal" odour intensity.

INTRODUCTION

The cultivar Kerner is well known for its premium quality table wines in Europe and South Africa. However, Kerner grapes from certain estates in the wine region Paarl, South Africa, consistently produced lower quality wines. Such wines were usually rejected for purposes of certification as wines of origin and cultivar by panels of wine connoisseurs because of the presence of an unacceptable "medicinal" or "elastoplast" aroma, the origin of which has been erroneously associated with some form of contamination. Occasionally this odour is also encountered in wines from other cultivars such as Gewürztraminer, Weisser Riesling, Muscat de Frontignan and Chenin blanc. However, none of a large number of German Kerner wines and a few from South Africa exhibited this obtrusive odour. The inconsistent occurrence of the "medicinal" odour as well as the negative impact on wine quality prompted us to investigate its origin and identity as well as possible factors responsible for its erratic occurrence. These factors include region, degree of ripeness, exposure of grapes to sunlight, yeast strain, fermentation temperature, free amino nitrogen content, oxidation of grape juice, phenol adsorbing agents such as activated carbon, polyvinylpolypyrrolidone, casein and gelatin and the addition of ferulic acid.

MATERIALS AND METHODS

Identification and quantification of off-odour components:

Freon extraction: Grape juices and wines were extracted with Freon 11 using the apparatus and extraction technique described by Marais (1986). 3-Decanol (100 µg/l) was used as an internal standard. After final concentration, the extracts were stored at -23°C prior to analysis.

Gas chromatography and gas chromatography-mass spectrometry conditions:

Carlo Erba HRGC:

Gas chromatographic parameters: Column Quadrex/BTR (60 m x 0,32 mm x 0,25 µm) fused silica capillary. Operating conditions: Injector temperature 200°C, oven temperature programme 60°C (5 min) x 1,5°C/min to 190°C, detector temperature 240°C, carrier gas He injection volume 1µl, split ratio 30:1. For GC-sniffing, the eluent was split 1:1 for simultaneous flame ionisation detection and odour assessment.

Finnegan 9610 gas chromatograph / 4600 Quadrupole mass spectrometer system:

Gas chromatographic parameters: Column Quadrex/BTR (60 m x 0,32 mm x 0,25 µm) fused silica capillary. Operating conditions: Injector temperature 200°C, oven temperature programme 60°C (5 min) x 1,5°C/min to 190°C, carrier gas He, injection volume 1µl, split ratio 30:1.

Mass spectrometer parameters: Source temperature, 240°C; interface temperature, 210°C; manifold temperature, 105°C; ionisation current, 0,31 amps; acceleration potential 70eV,

multiplier voltage 850, scanning range 35 to 450 amu, scan time 0.95s with a 0.05s pause between scans.

Quantitation of p-vinyl guaiacol using an internal standard method: A chemically pure standard solution of p-vinyl guaiacol (Oxford Chemicals, UK.) was prepared and extracted with Freon-11 using 3-Decanol (TCI, Tokyo) as internal standard. The extract was analysed using the Carlo Erba GC operating conditions. An average response factor for p-vinyl guaiacol was calculated and used in the quantitative determination of the samples.

Sensory Evaluation: A panel of five trained judges rated the "medicinal" aroma-intensity of the commercial and experimental wines on a structured 9-point scale according to which 1 = not noticeable, 3 = weak, 5 = medium, 7 = strong and 9 = very strong. Similar "medicinal" odour free wines to which 250, 500, 750 and 1000 $\mu\text{g/l}$ of p-vinyl guaiacol had been added respectively, were available to the panel during sensory sessions in order to enable panel members to familiarise themselves with this particular aroma at different concentrations in different wines.

Wines: Commercial Kerner wines exhibiting high and low intensities of the "medicinal" odour were collected for analysis. In addition, juice and wine samples of a new crossing (Cruchen blanc x Serval blanc) exhibiting a strong "medicinal" odour were collected. In the course of this study the "medicinal" off-odour was also periodically detected in wines from other cultivars *viz.* Gewürztraminer, Weisser Riesling, Muscat de Frontignan and Chenin blanc. Such wines were also collected for analysis.

Experimental wines were made in duplicate according to standard small scale wine-making practices. These included cooling freshly picked grapes to 5°C before crushing, low

temperature (10°C) skin contact in presence of limited sulphur dioxide (25 mg/l) and the grape juice clarification by settling using a pectolytic enzyme preparative (Ultrazyme). Fermentation was conducted at 15°C using a commercial pure yeast culture (Anchor Yeast Vin 7). After fermentation, SO₂ (50 mg/l) and a sodium bentonite suspension (0,75 g/l) were added to each wine, which was then cold stabilised and finally racked and filtered. These wines were kept at 15°C until analysed for p-vinyl guaiacol and rated for "medicinal" aroma intensity.

For the purpose of studying the effect of shading of bunches by leaves as well as grape maturity, Kerner grapes from Lievland, Paarl were harvested at 18,8°B and at 23,5°B. Insufficient shading at the second picking did not permit harvesting of a properly shaded sample. At Grondves, Stellenbosch, both sun-exposed and shaded grape bunches were harvested at two maturity levels (20°B and 21,2 - 21,4°B). It should be noted that the Lievland vineyard is situated in a hotter region than the Grondves vineyard.

In order to provide sufficient quantities of Kerner juice for additional studies on the formation p-vinyl guaiacol and the intensity of the "medicinal" odour, batches of Kerner juice from different origins were recovered and stored at -4°C. Before using such samples, their capacity to yield wine with the medicinal off-odour was established by fermenting samples and sensorically testing such wines.

To test the effects of juice oxidation and phenol adsorbing agents, one half of a juice which had been tested positively for its potential to produce the "medicinal" aroma was aerated to promote oxidation whilst the other half was protected from oxidation. Aeration was performed by intermittently pouring the juice (at 15°C) from one container to another until the brown colour of the juice reached a maximum. Both reduced and oxidised samples were

treated with relatively high doses of phenol adsorbing agents *e.g.* gelatin (20g/hl in combination with 2g/hl kieselsol and 75g/hl bentonite), casein (100g/hl), PVPP (100g/hl) and activated carbon (150g/hl). The fining agents were regularly resuspended and finally allowed to settle overnight at 15°C after which they were removed by centrifugation. Fermentation was conducted in the same way as described above using a freshly rehydrated pure yeast culture, Anchor Yeast WE 372, which had been selected for its efficiency to produce p-vinyl guaiacol. The wines were treated as before.

In view of the fact that p-vinyl guaiacol is formed by yeast during alcoholic fermentation, the ability of a number of locally available commercial *Saccharomyces cerevisiae* strains was tested by fermenting samples of Kerner juice which had tested positively for its potential to produce the "medicinal" aroma. These strains are listed in Table 5.

In order to confirm the role of the concentration of ferulic acid, the p-vinyl guaiacol precursor in grape juice, different levels (2,0 and 10,0 mg/l) of ferulic acid (Sigma) was added to Kerner juice recovered from grapes produced at two locations in Paarl (KWV and Lievland). The two samples from Lievland were fermented with the pure yeast cultures Lalvin L 2056 and Blastocel MW respectively, whereas the samples from the 1992 vintage (KWV and Lievland) were all fermented with Anchor Yeast WE 372.

The effect of fermentation temperature and the free amino nitrogen level of the juice was studied by fermenting two different Kerner juice samples at two temperatures (15°C and 25°C) and at two levels of free amino nitrogen as obtained by addition of 750mg/l diammonium phosphate. The two Kerner samples had been selected for the "medicinal" odour potential, one low and the other high. Anchor Yeast WE 372 was used as the pure yeast culture.

RESULTS AND DISCUSSION

Identification of off-odorous component in Kerner wine extracts: Gas chromatographic fractionation of Freon II extracts permitted sniffing of volatile fractions as they emerged from the GC column. Only one fraction exhibited the typical "elastoplast" or "medicinal" odour. The mass spectrum of the main component present in the "medicinal"-containing fraction matched that of p-vinyl guaiacol. GC-MS analysis of an authentic sample confirmed this identification.

According to Dubourdieu *et al.* (1989) p-vinyl guaiacol is formed via enzymatic decarboxylation of ferulic acid by yeast during alcoholic fermentation. However, Peleg *et al.* (1992), utilising a model solution simulating orange juice provided evidence that p-vinyl guaiacol is also formed from ferulic acid in the absence of yeast and alcoholic fermentation. Although the production of p-vinyl guaiacol via chemical decarboxylation in Kerner grapes or wine can as yet not be ruled out, it is highly unlikely that such a contribution would be significant. Firstly, the "medicinal" odour as such could not be detected in juice, whereas it was prominently displayed in the freshly made corresponding wines. Secondly, no increase in the intensity of this aroma was noted during storage of any of the experimental Kerner wines.

Identification of off-odorous components in Cruchen blanc x Servan blanc crossing: The grapes of an experimental crossing, *Vitis vinifera* L. cvs. Cruchen blanc x Servan blanc made at the Department of Viticulture, University of Stellenbosch, in the early 1970's, exhibited an unusual, unmistakable, medicinal flavour taste never before encountered in South African grapes, and hence did not receive a cultivar status. The GC-MS analysis of Freon 11 extract of the juice not only confirmed the identity of p-vinyl guaiacol, but also of 4-allyl-2-methoxy phenol which has a spicy aroma and is the main aroma constituent of cloves. A high

concentration of p-vinyl guaiacol (1988 $\mu\text{g/l}$) was determined in wine of this variety. Although the presence of p-vinyl guaiacol in wine can normally be attributed to the enzymatic decarboxylation of ferulic acid during fermentation, there is now conclusive evidence that p-vinyl guaiacol also occurs at relatively high levels in the berries of at least one grape variety.

Perception concentration of p-vinyl guaiacol in white wine: By adding increasing increments of authentic p-vinyl guaiacol to young Weisser Riesling and Chenin blanc wines which did not display any "medicinal" aroma, it was assessed that at an addition of 1000 $\mu\text{g/l}$ this compound was consistently recognised for its typical "medicinal" aroma. However, when added to bottle-aged Weisser Riesling and Kerner wines with more complex aromas than young white wines, larger doses were required to allow recognition of the typical aroma, apparently because of the masking effect of maturation bouquet compounds.

p-Vinyl guaiacol in other varietal wines: The occurrence of p-vinyl guaiacol was, however, not limited to Kerner wines only. In fact, it is present in most wines, but seldom at levels high enough to display a "medicinal" aroma. In a few commercial wines produced in relatively hot regions of South Africa from other cultivars, particularly Gewürztraminer, Weisser Riesling, Muscat de Frontignan and Chenin blanc, the typical "medicinal" odour was also very pronounced. The p-vinyl guaiacol contents of such samples fell within the range of concentrations found in Kerner wines with pronounced medicinal contents.

Factors affecting p-vinyl guaiacol formation:

Wine type and region: Kerner wines from Germany whose vineyards are known to be cooler than most in South Africa did not exhibit any recognisable "medicinal" odour, whereas several from South African producers, in particular those from two estates viz. Lievland and

Backsberg in one specific macroclimatic region often contained relatively high p-vinyl guaiacol (PVG) levels and were rated high in "medicinal" odour intensity (Table1).

TABLE 1

"Medicinal" intensity ratings of South African Kerner wines.

Origin	Rating ¹⁾	PVG (µg/l) ²⁾
Nederburg Kerner 1983	4	61
Nederburg Kerner (SLH) ³⁾ 1989	2	136
Lievland Kerner (SLH) 1989	4	316
KWV Kerner 1981	5	456
Backsberg Kerner 1989	6	818
Lievland Kerner SLH 1986	7	1 287

¹⁾Median of 10 ratings on a 9 point scale where 1 = not noticeable and 9 = very strong

²⁾Average of duplicate analyses

³⁾Special late harvest

Microclimate, exposure of grapes to sunlight, and grape maturity: The p-vinyl guaiacol content of wines made from shaded grapes at 18,8°B from the hotter location (Lievland) was practically of the same order of magnitude as that of the cooler location (Grondves, Stellenbosch) as seen in Table 2. However, wines made from sun-exposed early-harvested grapes at 18,8°B contained almost twice as much p-vinyl guaiacol in case of the hotter Lievland vineyard. The median "medicinal" aroma intensity ratings were also higher for the latter wines. These differences were similar, but more marked in wines made from more matured sun-exposed grapes from the second picking. The effect of climate on p-vinyl guaiacol content appears to be of particular interest. Whereas wines made from the hotter

Lievland vineyards showed a marked increase from the early to the late harvested sun-exposed grapes, no marked change was noted in the case of the cooler Grondves vineyards. Although the difference in the degree of ripeness could have had an effect, it would appear as though wines made from Kerner grapes in the cooler location contained lower p-vinyl guaiacol levels and accordingly were rated lower in "medicinal" odour intensity. This observation probably offers an explanation for the absence of the "medicinal" odour in Kerner wines from the cool vineyards of Germany.

TABLE 2

Effect of region, degree ripeness, exposure to sunlight on "medicinal" intensity ratings and p-vinyl guaiacol (PVG) concentrations in Kerner wines.

Region	Sugar °Balling	Exposure to sunlight	"Medicinal" intensity rating ¹⁾	PVG (µg/l) ²⁾
Lievland	18.8	Shade	4	191
	18.8	Sun	5	852
	23.5	Sun	8	1 003
KWV (Grondves)	20.0	Shade	3	226
	20.0	Sun	4	483
	21.2	Shade	4	361
	21.4	Sun	2	405

¹⁾Median of 10 ratings

²⁾Average of duplicate analyses

Oxidation: Since grape juice oxidation normally gives rise to the polymerisation and concomitant precipitation of phenols, it was anticipated that such a treatment could possibly

lead to reduced p-vinyl guaiacol content in wines. However, the opposite effect was noted as is shown in Table 3. In every case, aeration followed by oxidation led to an increase in the p-vinyl guaiacol content. These increases varied according to the origin of the grapes as well as the particular yeast strain, and in the case of the Lievland Kerner were of sufficient magnitude to have caused increases in the "medicinal" aroma intensity with concomitant adverse quality effects. It therefore seems advisable to limit excessive aeration and oxidation of grape juice in case of Kerner and other cultivars which under specific conditions tend to yield wines containing relatively high levels of p-vinyl guaiacol.

TABLE 3

Effect of oxidation on Kerner grape juice on p-vinyl guaiacol (PVG) content of wine.

Vineyard	Yeast strain	PVG ($\mu\text{g/l}$) ¹⁾		
		Control	Oxidised	Increase
Grondves	Anchor Yeast WE 228	670	740	70
Grondves	Anchor Yeast WE 372	962	1103	141
Lievland	Lalvin 2056	560	794	234
Lievland	Blastocel K	563	989	426

¹⁾Average of duplicate analyses

Phenol adsorbing fining agents: Since activated carbon and polyvinylpyrrolidone (PVPP) are insoluble in wine and casein becomes insoluble as it is added to juice or wine, these agents ought to be the most efficient adsorbents for the removal of low molecular weight phenolic

compounds such as ferulic acid. Gelatin, being soluble in white wine, ought to be co-precipitated by fining agents such as kieselsol and bentonite in order to remove low molecular weight phenols effectively.

Although the activated carbon treatment of the juice reduced the p-vinyl guaiacol content and the "medicinal" aroma intensities of the wines (Table 4) very effectively in both non-oxidised and oxidised samples, the corresponding wines were colourless and neutral. For this reason, much lower but less efficient dosages ought to be used in practice. The reduction in p-vinyl guaiacol levels and "medicinal" aroma intensity by PVPP and casein was not according to expectations although relatively high doses of these agents had been used. Rather surprising was the marked reduction in the "medicinal" aroma intensity and the p-vinyl guaiacol levels by the gelatine-kieselsol-bentonite fining at standard dosages.

From the percentage reduction in p-vinyl guaiacol content it is clear that PVPP, casein and the combined fining of gelatine, kieselsol and bentonite were much more effective in removing the precursors of p-vinyl guaiacol in the oxidised juice than the unoxidised juice. This may be ascribed to the fact that phenol oligomers formed by oxidative polymerisation upon aeration were more readily adsorbed than the unoxidised monomeric forms such as e.g. ferulic acid. Since oxidation of grape juice without subsequent fining gave rise to higher p-vinyl guaiacol levels in wine, oxidised juice from grapes with a high p-vinyl guaiacol potential ought to be fined before fermentation.

It is clear that the efficiency of fining agents for removal of p-vinyl guaiacol precursors from the grape juice should not be evaluated without also taking into account the effect on wine quality. At this stage, it appears as though the activated carbon is most efficient, but

unfortunately not with respect to wine quality. Other fining agents e.g. gelatine-kieselsool-bentonite will be more appropriate from a wine quality point of view.

TABLE 4

Effect of oxidation and fining of juice with phenol adsorbents on p-vinyl guaiacol (PVG) content and "medicinal" aroma intensity of wine.

Treatment¹⁾ Oxidised juice	PVG ($\mu\text{g/l}$)²⁾	% Reduction in PVG concentration	"Medicinal" aroma intensity³⁾
Control	1103	-	7
Activated Carbon	120	89	1
PVPP	711	36	4
Cassein	531	52	6
Gelatin + Kieselsol + Bentonite	486	56	2
Unoxidised juice			
Control	962	-	3
Activated Carbon	70	93	1
PVPP	903	6	5
Cassein	894	7	4
Gelatin + Kieselsol + Bentonite	835	13	2

¹⁾Dosage levels (g/hl): cassein and PVPP 100, activated carbon 150, gelatin 20, kieselsol 2, bentonite 75. Yeast strain: WE 372.

²⁾Average of duplicate analyses

³⁾Medians of 10 ratings

Yeast strain and fermentation conditions: The different capacities of various yeast strains to decarboxylate ferulic acid to p-vinyl guaiacol are clearly reflected by the p-vinyl guaiacol

TABLE 5

Effect of yeast strain on "medicinal" intensity ratings and p-vinyl guaiacol (PVG) concentrations in Kerner wines.

Species	Strain	"Medicinal" Intensity rating ¹⁾	PVG ($\mu\text{g/l}$) ²⁾
<i>Saccharomyces cerevisiae</i>	Hefix 1000	5	890
	Anchor Yeast N96	5	813
	Lalvin 734	4	734
	Anchor Yeast (Vin 11)	3	616
	Blastocel MW	6	591
	Lalvin L 2506	5	575
	Lalvin L 2506	9	843
	Anchor Yeast (WE 14)	3	509
	Blastocel Kappa	1	-
	Anchor Yeast (WE 372)	7	-
	Anchor Yeast (WE 228)	3	-
	Zymaflor VL 1	2	35
	71 B	8	954
	Anchor Yeast (Vin 7)	8	659
	EC 118	7	843
<i>Saccharomyces bayanus</i>	Hefix 2000	5	823
	Blastocel V5	5	810
	AEB	4	616
<i>Saccharomyces bayanus/cerevisiae</i>	Oenol Vit Bc	6	764

¹⁾Median of 10 ratings

²⁾Average of duplicate analyses

concentrations as well as the "medicinal" aroma-intensity ratings as presented in Table 5. These results emphasise the significance of interactions between yeast strain and grape variety, which may affect wine quality appreciably. It would therefore make sense to select a

yeast strain with a low p-vinyl guaiacol forming potential e.g. Anchor Yeast Vin 11, Anchor Yeast WE 14 and Zymaflor VL 1 to ferment grape juice from Kerner and other high ferulic acid-containing cultivars, particularly those from the hot regions. Strains such as 71 B, EC 118, Anchor Yeast WE 372 and Lalvin 2056 should preferably not be used in the latter cases.

TABLE 6

Effect of ferulic acid addition²⁾ to Kerner grape juice and yeast strain on p-vinyl guaiacol (PVG) content of wine.

Vineyard	Yeast Strain	PVG ($\mu\text{g/l}$) ¹⁾		
		Control	Control + Ferulic Acid (FA) ²⁾	Increase per mg/l FA added
Lievland '90	Lalvin L2056	794	833	19.5
Lievland '90	Blastocel MW	989	1 062	36.5
KWV Paarl '92	Anchor Yeast WE 372	1 103	6 326	522.3
KWV Paarl '92	Anchor Yeast WE 372	962	5 791	482.9
Lievland '92	Anchor Yeast WE 372	1 092	5 962	487.0

¹⁾Average of duplicates

²⁾Ferulic acid dosage: 2 mg/l in case of 1990 samples, 10 mg/l in case of 1992 samples.

Effect of ferulic acid: p-Vinyl guaiacol is known to be formed by enzymatic decarboxylation of ferulic acid during alcoholic fermentation (Dubourdieu *et al.* 1989). This was confirmed by

addition of different levels of ferulic acid to Kerner grape juice samples from two origins and two vintages and fermentation with different yeast strains. According to Table 6, all of the wines obtained from juice samples, to which 10 mg/l ferulic acid had been added, received the maximum "medicinal" odour intensity ratings of 9 points each. The variation in increases of p-vinyl guaiacol per mg/l ferulic acid added clearly reflects the decarboxylation potential of different yeast strains. This is also demonstrated by the p-vinyl guaiacol concentrations as well as "medicinal" odour ratings of wines made from the same juice, but fermented by different yeast strains (Table 5).

TABLE 7

The effect of fermentation temperature and free amino nitrogen.

Low FAN must ¹⁾		Fermentation Temperature (°C)	PVG (µg/l) ²⁾	"Medicinal" intensity rating ³⁾
A	No DAP added	15	686	8
B		15	23	2
A		25	453	7
B		25	23	2
A	DAP added ⁴⁾	15	559	8
B		15	40	3
A		25	600	8
B		25	32	5

¹⁾FAN = Free amino nitrogen

²⁾Mean of duplicates

³⁾Median of 10 ratings

⁴⁾Diammonium phosphate dosage: 750 mg/l

Fermentation temperature and free amino nitrogen: Increases in fermentation temperature from 15° to 25°C and free amino nitrogen content by addition of 750 mg/l diammonium phosphate to Kerner juice prior to fermentation did not result in consistent changes in "medicinal" odour intensity and the p-vinyl guaiacol concentration (Table 7).

CONCLUSIONS

The main factors affecting p-vinyl guaiacol levels of wines and the recognisable concomitant "medicinal" off-odour are climate, cultivar, yeast strain, exposure of grapes to sunlight and grape maturity. Therefore, in relatively hot regions protection of grape clusters from exposure to sunlight by proper viticultural practices together with early harvesting have merits to produce wines with relatively low p-vinyl guaiacol contents and less pronounced "medicinal" odours. However, strong consideration should be given towards planting Kerner vines in relatively cool regions only. The treatment of juice with phenol-adsorbing fining agents (with the exception of activated carbon) cannot be recommended for reduction of the p-vinyl guaiacol levels unless the juice is strongly oxidised. Apart from the time and cost involved, winemakers in their attempts to make highly reductive fruity wines normally would, however, not apply deliberate oxidation of grape juice.

LITERATURE CITED

DUBOURDIEU, D., DARRIET, P., CHATONNET P. & BOIDRON, J.N., 1989. Intervention de systems enzymatiques de *Saccharomyces cerevisiae* sur certains precurseurs d'aromes du Raisin. In: Ribereau-Gayon P. & Lonvaud A. (eds) Actualités Oenologiques 89. Proceedings of the Fourth International Oenological Symposium, 15-17 June 1989. pp151-159. Bordeaux, France. 567pp.

PELEG, H., HAIM, M., ZEHAVI, U., ROUSEFF, R.L. & NAGY, S., 1992. Pathways of 4-vinyl guaiacol formation from ferulic acid in model solutions of orange juice. *J. Agric. Food Chem.* **40**, 764-767.

MARAIS, J., 1986. A reproducible capillary gas chromatographic technique for the determination of specific terpenes in grape juice and wine. *S. Afr. J. Enol. Vitic.* **7**, 21-25.

CHAPTER 1V

CONCLUSION

The application of capillary gas chromatography-mass spectrometry coupled with capillary gas chromatography-sniffing enabled us to identify the key aroma components of Ferdinand de Lesseps grape juice and the objectionable "medicinal" odour of Kerner wines.

Ferdinand de Lesseps' *labrusca* parentage is reflected in its grape volatile composition. The presence of 2,5-dimethyl-4-hydroxy-3 (2H)-furanone and o-aminoacetophenone are most likely responsible for the hybrid note of the Ferdinand de Lesseps, whereas the esters, ethyl butanoate, ethyl and methyl 3-hydroxy butanoate, and to a lesser extent, ethyl 3-hydroxy hexanoate and ethyl-3-hydroxy propanoate are most probably responsible for the sweetish pineapple aroma.

The hybrid cultivars are becoming extinct as a result of government regulations. Most European countries (except for a few cases in France) forbid the commercialization of wines made from such grapes. The fraudulent use of Ferdinand de Lesseps in a blended white wine can be detected with aid of gas chromatography/mass spectrometry. The presence of 2,5-dimethyl-4-hydroxy-3 (2H)-furanone would be an indicator of a *labrusca* contribution to the wine.

The unpopularity of the "medicinal" odour found in some South African Kerner wines led to the demise of the cultivar. However, the results of our study have shown that the main factors affecting the p-vinyl guaiacol levels of wines are climate, exposure of grapes to direct sunlight, grape maturity and yeast strain. Certain steps can be taken to limit the presence of p-

vinyl guaiacol in wine. These are to plant the vines in cool regions, to protect the grapes from direct sunlight using a suitable canopy management system and to harvest the grapes at an earlier ripening stage.

Lower p-vinyl guaiacol levels in Kerner wines can also be obtained by selecting a yeast strain with a low p-vinyl guaiacol forming potential and with the careful treatment of grape juice prior to fermentation with phenol-adsorbing fining agents.