

**Application of modern chromatographic technologies
for the analysis of volatile compounds in South
African wines**

by

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DECLARATION

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Summary

The present study was initiated by the wine industry of South Africa to overcome the lack of available information on the flavor and aroma of South African wines. The aim was to develop new analytical methods and improve existing ones for the analysis of volatile compounds in the South African wines. Initially a new analytical method based on stir bar sorptive extraction (SBSE) in the headspace mode for the analysis of 37 pre-selected volatile compounds was developed and validated. Consequently, the method was improved by making important modifications and increasing the number of compounds analyzed to 39. This method was successfully applied to a large number of Pinotage wines of vintages 2005 and 2006. The quantitative data of these wines were subjected to chemometric analysis in order to investigate possible co-/variances. A clear distinction was observed between the two vintages, where the 2005 wines were more characterized by wood-related compounds and the 2006 wines by the fermentation compounds. The developed method was further applied to other cultivars of vintage 2005, including two white (Sauvignon Blanc and Chardonnay) and three red (Shiraz, Cabernet Sauvignon and Merlot) cultivars. In a similar fashion, the quantitative data of the six cultivars of vintage of 2005 were analysed by chemometric methods. Significant differences were observed between the two white cultivars and among the four red cultivars. It was shown that among these cultivars, the major role-players were the wood and fermentation related volatiles. A striking observation was the confirmation of the unique character of the Pinotage wines compared to the other red cultivars, mainly influenced by the high level of isoamyl acetate and low level of isoamyl alcohol, the former being categorized as a varietal compound for Pinotage expressed by a fruity (banana) odor.

In addition, advanced chromatographic technology in the form of comprehensive two-dimensional gas chromatography (GC \times GC) coupled to time-of-flight mass spectrometry (TOFMS) was investigated for the detailed analysis of volatile compounds in young South African wines. This work focused primarily on Pinotage wines. In the first instance, solid phase micro extraction (SPME) in the headspace mode in combination with GC \times GC-TOFMS was used. Due to the high resolution and large peak capacity of GC \times GC, more than 200 compounds previously reported as wine components were identified. These compounds were dominated by the highly

volatile and less polar compounds, mainly due to the characteristics of SPME. In an attempt to further extend these results, another selective extraction method, solid phase extraction (SPE) was used in combination with GC × GC-TOFMS analysis. Using this technique, more than 275 compounds, most of them unidentified using the previous method, were detected. These groups of compounds include volatile phenols, lactones as well as mostly aromatic esters and norisoprenoids, which can potentially influence the aroma and flavor of wine. The techniques developed as part of this study have extended our knowledge of the volatile composition of South African wines.

Opsomming

Hierdie studie is geïnisieer deur die wyn industrie van Suid-Afrika om die tekort aan beskikbare inligting aangaande wyn aroma van Suid-Afrikaanse wyne te oorkom. Die doel was om nuwe analitiese metodes te ontwikkel en die huidige metodes te verbeter vir die analise van vlugtige verbindings in Suid-Afrikaanse wyne. Oorspronklik is 'n nuwe analitiese metode ontwikkel en gevalideer gebaseer is op 'stir bar sorptive extraction' (SBSE) in die gas fase vir die analise van 37 vooraf geselekteerde vlugtige verbindings. Die metode is verbeter deur belangrike modifikasies aan te bring en die hoeveelheid verbindings wat analiseer word te vermeerder na 39. Hierdie metode is suksesvol aangewend op 'n groot hoeveelheid Pinotage wyne van oesjare 2005 en 2006. Die kwantitatiewe data van hierdie wyne is onderwerp aan verskillende chemometriese analises om moontlike ko-/variasies te ondersoek. 'n Duidelike onderskeid is opgemerk tussen die twee oesjare, waar die 2005 wyne gekarakteriseer is deur hout-verwante verbindings en die 2006 wyne weer meer deur fermentasie verbindings. Die verbeterde metode is verder aangewend vir analiese van ander kultivars van oesjare 2005, wat twee wit (Sauvignon Blanc en Chardonnay) en drie rooies (Shiraz, Cabernet Sauvignon en Merlot) ingesluit het. Die kwantitatiewe data van die ses kultivars van oesjaar 2005 is op 'n soortgelyke wyse geanaliseer deur verskillende chemometriese metodes te gebruik. Beduidende verskille is opgemerk tussen die twee wit kultivars en tussen die vier rooi kultivars. Die hoof rolspelers tussen die ses kultivars was weereens die verbindings wat 'n hout en fermentasie aard het. Die unieke karakter van die Pinotage wyne in vergelyking met die ander rooi kultivars was opvallend. Hierdie wyn word gekarakteriseer deur hoë vlakke van isoamiel asetaat en lae vlakke van isoamiel alkohol, waar eersgenoemde gekatogiseer word as 'n verbinding wat 'n vrugte (piesang) geur in Pinotage uitdruk.

Verder is gevorderde chromatografiese tegnologie in die vorm van 'comprehensive two-dimensional gas chromatography' (GC x GC) gekoppel met 'time-of-flight mass spectroscopy' (TOFMS) ondersoek vir die analiese van vlugtige verbindings in jong Suid-Afrikaanse wyne. Hierdie werk het hoofsaaklik op Pinotage wyne gefokus. Eerstens is 'solid phase micro extraction' (SPME) in die gas fase gekombineer met GC x GC-TOFMS. As gevolg van die hoë resolusie en groot piek kapasiteit van GC x GC is meer as 200 verbindings wat voorheen gerapporteer is as wyn komponente

geïdentifiseer. Hierdie verbindings is gedomineer deur hoë vlugtige polêre verbindings, hoofsaaklik as gevolg van die karaktersistieke van SPME. In 'n poging om die metode verder te verbeter is 'n selektiewe ekstraksie metode naamlik 'solid phase extraction' (SPE) in kombinasie met GC x GC-TOFMS gebruik. Met hierdie tegniek is meer as 275 verbindings geïdentifiseer, waarvan die meeste nie met die vorige metode waargeneem is nie. Hierdie verbindings sluit vlugtige fenole, laktone en meestal aromatiese esters en norisoprenoïdes in, wat moontlik die reuk en smaak van wyn kan beïnvloed. Die metode ontwikkel gedurende die studie het nuwe informasie verskaf aangaande die vlugtige komponente teenwoordig in Suid Afrikaanse wyne.

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Preface

The study of wine aroma and flavor is quite a complex process, as many of the chemical constituents that are responsible for the sensory property of the wine do not come directly from the grapes; rather their formation is influenced by many other factors. In addition the number and type of compounds already reported in wine are large and certainly one can never cover all in one study. Scientists around the world have already performed numerous studies regarding wine sensory properties, but a lot remains to be done. No comprehensive studies have been carried-out on the aroma and flavor of South African wines. The objective of the current study was to develop analytical methods for the analysis of wine volatiles, and to use these methods for characterization of South African wines comprehensively based on their volatile constituents. Hence, the current study focuses mainly on the young South African wines.

The Dissertation is presented in three major categories. The first part includes the first four chapters which give a general overview on wine (including historical background, production, and flavor), chromatographic technologies, sample preparation and the use of chemometrics in characterization of wines. The second part presents the application of one-dimensional gas chromatography for the analysis of pre-selected volatiles partially responsible for the wine flavor and aroma. It includes the development and validation of analytical methods and characterization of young South African wines based on the quantitative data of the selected volatiles. This section is presented in chapters 5 – 7. The third part highlights the application of comprehensive two-dimensional gas chromatography (GC × GC) for fingerprinting and detailed characterization of young South African wines mainly focusing on the unique South African cultivar – Pinotage using volatile and semi-volatile chemical constituents. This work is highlighted in chapters 8 and 9. The last part of this Dissertation is composed of general concluding remarks and achievements as well as future work. Selected tables which are not presented either fully or partially in the previous categories are provided in the appendix.

Contents

Abbreviations i

PART I: General Overview

1. Wine	1
1.1 Historical background	2
1.2 Wine production	3
1.3 Wine flavor and aroma	6
1.3.1 Fermentation products	7
1.3.2 Storage, maturation and ageing products	11
1.4 References	16
2. Wine analysis	19
2.1 Gas chromatography	20
2.1.1 Carrier gas	21
2.1.2 Sample introduction (injector)	21
2.1.3 Thermal desorption unit (TDU)	23
2.1.4 Capillary column	24
2.1.5 The GC oven	26
2.1.6 GC Detectors	26
2.1.6.1 Quadrupole mass spectrometry (qMS)	27
2.1.6.2 Time-of-flight mass spectrometry (TOFMS)	28
2.2 Comprehensive two-dimensional gas chromatography (GC × GC)	30
2.3 References	35
3. Sample preparation	38
3.1 Solvent-based sample preparation techniques	39
3.2 Solid phase extraction (SPE)	40
3.3 Sorptive sample preparation techniques	42
3.3.1 Solid phase micro extraction (SPME)	42
3.3.2 Stir bar sorptive extraction (SBSE)	46
3.4 References	51

4.	Chemometrical data analysis	54
4.1	Analysis of variance (ANOVA)	56
4.2	Factor analysis (FA)	58
4.3	Principal component analysis (PCA)	60
4.4	Discriminant analysis (DA)	63
4.5	References	65

PART II: Application of one-dimensional gas chromatography (1D GC)

5.	Application of a headspace sorptive extraction method for the analysis of volatile components in South African wines	67
	Abstract and key words	68
5.1	Introduction	69
5.2	Material and methods	71
	5.2.1 Wine samples	71
	5.2.2 Chemicals and reagents	71
	5.2.3 Preparation of synthetic wine	71
	5.2.4 Equipment and apparatus	72
	5.2.5 Experimental conditions	72
	5.2.6 Sample preparation	73
5.3	Results and discussion	73
	5.3.1 Method optimization	73
	5.3.1.1 TDS 2 and CIS 4 conditions	74
	5.3.1.2 Influence of ionic strength (salting-out effect)	74
	5.3.1.3 Sorption time	74
	5.3.1.4 Stirring speed	75
	5.3.1.5 Effect of pH	75
	5.3.1.6 Volume (phase) ratios	76
	5.3.1.7 Extraction temperature	76
	5.3.2 Method validation	79
	5.3.3 Application to real wine samples	81
5.4	Conclusions	87
5.5	References	89

6	Analysis of volatiles in Pinotage wines by stir bar sorptive extraction and chemometric profiling	91
	Abstract and key words	92
6.1	Introduction	93
6.2	Material and methods	95
	6.2.1 Standards, reagents and equipment	95
	6.2.2 Wine samples	96
	6.2.3 Preparation of synthetic wine	97
	6.2.4 Instrumental conditions	97
	6.2.5 SBSE headspace analysis	99
	6.2.6 Statistical analysis	100
6.3	Results and discussion	100
	6.3.1 Validation of the method	100
	6.3.2 Wine analysis	104
	6.3.3 Quantitative analysis	105
	6.3.3.1 Esters	108
	6.3.3.2 Alcohols	108
	6.3.3.3 Fatty acids	109
	6.3.3.4 Volatile phenols	110
	6.3.3.5 Carbonyls	111
	6.3.3.6 Lactones	115
	6.3.4 Statistical analysis	115
	6.3.4.1 Factor analysis (FA)	115
	6.3.4.2 Advanced PCA factor analysis	118
6.4	Conclusions	123
6.5	References	125
7.	Chemometric investigation of the volatile content of young South African wines	128
	Abstract and key words	129
7.1	Introduction	130
7.2	Materials and methods	132
	7.2.1 Wine samples	132
	7.2.2 Analytical procedure	132

7.2.3	Statistical analysis	133
7.3	Results and discussion	133
7.3.1	Wine analysis	133
7.3.2	Analysis of variance (ANOVA)	134
7.3.3	Factor analysis (FA)	140
7.3.4	Principal component analysis (PCA)	142
7.3.5	Discriminant analysis (DA)	145
7.4	Conclusions	152
7.5	References	153

PART III: Application of comprehensive two-dimensional gas chromatography (GC × GC)

8.	Characterization of volatile components of Pinotage wines using comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC × GC-TOFMS)	155
	Abstract and key words	156
8.1	Introduction	157
8.2	Experimental	158
8.2.1	Samples, chemicals and materials	158
8.2.2	Instrumentation	159
8.2.3	Sample preparation	159
8.2.4	Data analysis	160
8.3	Results and discussion	160
8.4	Conclusions	180
8.5	References	182
9.	Solid phase extraction (SPE) in combination with comprehensive two-dimensional gas chromatography (GC × GC) coupled to time-of-flight mass spectrometry (TOFMS) for the detailed investigation of volatiles in South African wines	185
	Abstract and key words	186
9.1	Introduction	187
9.2	Materials and methods	189

9.2.1	Wine samples	189
9.2.2	Chemicals and reagents	189
9.2.3	Chromatographic conditions	189
9.2.4	Solid phase extraction (SPE) procedure	190
9.3	Results and discussion	191
9.4	Conclusions	213
9.5	References	214
10.	General conclusions	217
 PART IV: Appendix		
	Selected tables	221

This dissertation has been written based on the style required for the Journal of Chromatography A. It is represented as a compilation of manuscripts already published and submitted for publication. Each manuscript is a chapter of an individual entity and some repetition between chapters has, therefore, been unavoidable.

Abbreviations

Abbreviations

AED	Atomic emission detector
AIC	Analytical ion chromatogram
ANOVA	Analysis of variance
amu	Atomic mass unit
BACIS	Aroma chemical information service
BC	Before Christ
CA	Cluster analysis
CAR	Carboxen
CE	Capillary electrophoresis
CH	Chardonnay
CIS	Cooled injection system
CS	Cabernet sauvignon
CS ₂	Carbon disulfide
CW	Carbowax
DA	Discriminant analysis
DC	Direct current
d _f	Film thickness
DMS	Dimethylsulfide
DVB	Divinylbenzene
ECD	Electron capture detector
EI	Electron impact ionization
EPC	Electronic pneumatic control
eV	Electron volt
FA	Factor analysis
FFAP	Free fatty acid phase
FID	Flame ionization detector
FPD	Flame photometric detector
GC	Gas chromatography
GC × GC	Comprehensive two-dimensional GC
GLC	Gas-liquid chromatography
GSC	Gas-solid chromatography

Abbreviations

5-HMF	5-(Hydroxymethyl)furfural
HS-SBSE	Headspace SBSE
HS-SPME	Headspace SPME
HSSE	Headspace sorptive extraction
IBMP	2-Methoxy-3-isobutylpyrazine
ID	Inner diameter
IPMP	2-Methoxy-3-isopropylpyrazine
IS	Internal standard
KE	Kinetic energy
$K_{O/W}$	Octanol water partition coefficient
LAB	Lactic acid bacteria
LC	Liquid chromatography
LC × LC	Comprehensive two-dimensional LC
LD	Liquid desorption
LLE	Liquid liquid Extraction
LOD	Limit of detection
LOQ	Limit of quantification
LRI	Linear retention indices
m/z	Mass to charge ratio
MD-PCA	Multidimensional principal component analysis
M	Merlot
MLF	Malolactic fermentation
μ LLE	Micro liquid liquid extraction
MS	Mass spectrometer
MSD	Mass selective detector
NIST	National Institute of Standards
NMP	Number of modulation period
OTT	Open tubular trap
PA	Parallel analysis (statistics)
PA	Polyacrylate (polymeric coating)
PCs	Principle components
PCA	Principle component analysis
PDMS	Polydimethylsiloxane

Abbreviations

PEG	Polyethelene glycol
PI	Pinotage
PID	Photo ionization detector
PTFE	Polytetrafluoroethylene
PTV	Programmed temperature vaporizing inlet
qMS	Quadrupole mass spectrometer
RF	Radio frequency
RSD	Relative standard deviation
RT	Retention time
SA	South Africa
SB	Sauvignon blanc
SBMP	2-Methoxy-3-sec-butylpyrazine
SBSE	Stir bar sorptive extraction
SD	Standard deviation
SDME	Single-drop microextraction
SDVB	Styrenedivinylbenzene
SH	Shiraz
SIM	Selective ion monitoring
S/N	Signal to noise ratio
SPE	Solid phase extraction
SPME	Solid phase micro extraction
TD	Thermal desorption
TDS	Thermal desorption system
TDS-A	Thermal desorption system auto-sampler
TDU	Thermal desorption unit
TCD	Thermal conductivity detector
TDN	1,1,6-Trimethyl-1,2-dihydro-naphtalene
TIC	Total ion chromatogram
TOF	Time-of-flight
TPR	Templated resin
TTN	1,1,6-Trimethyl-1,2,3,4-tetrahydro-naphthalene
VCF	Volatile compounds in food database

Wine

1.1. Historical background

The history of wine is closely intertwined with the history of agriculture, cuisine, civilization and humanity. The earliest scientific evidence of grapes is the discovery of 60-million-years-old fossil vines. The earliest record in a written form, accounting of viniculture is in the Old Testament of the Bible which tells us the plantation of a vineyard and making of wine by Noah [1-3]. The Bible also mentioned that, the first miracle of Jesus Christ was the changing of water to a good quality wine. From scientific findings, the latest archeological discovery [4] on wine-making process goes back beyond 7000 years is another indication of the ancient history of wine (**Figure 1.1.**).

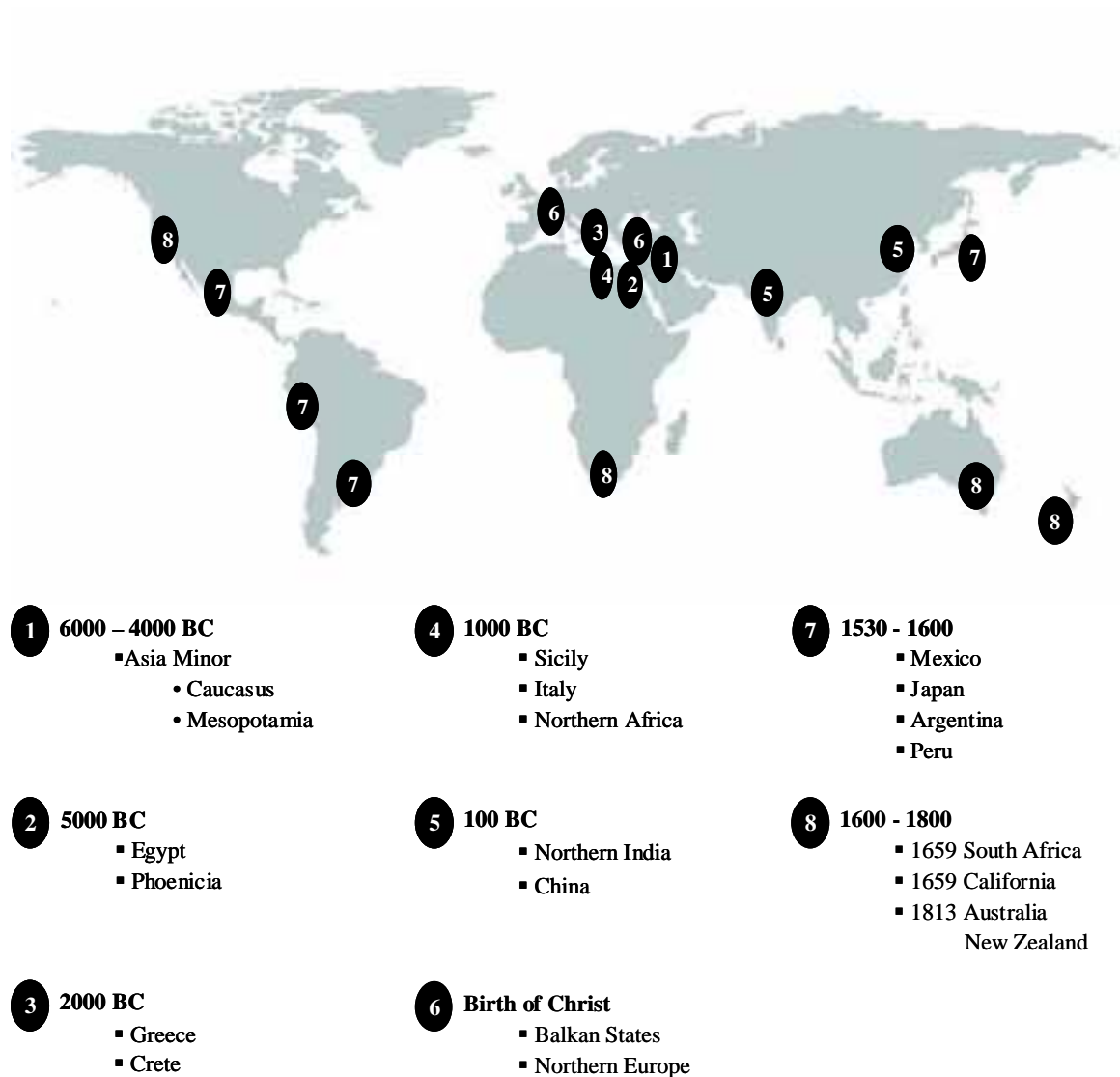


Figure 1.1. The early spreading and world distribution of the vine and wine-making technology. (Adapted from [5,6]).

A single Eurasian grape species, *Vitis vinifera* L. subspecies *sylvestris*, which grows wild in temperate zones of most wine producing continents including Europe, Asia and North America, is the source of over 99% of the current worldwide wine production. Since its introduction, wine has been loved and documented to have had a long affair with humans. In ancient times this was mainly due to its high alcohol content, which in turn could be used as an effective drug or as a disinfectant and a general remedy [3]. Wine is often associated with relaxation, communing with others, complementary to food consumption, learning about new things, and hospitality. It is also associated with the notions of well-being, contentment and classiness. Since biblical times, wine has been of significant cultural importance. It has been used in diverse societies as part of religious rituals and celebrations. The benefits to one's well-being and health in the modern era also contributed to the high consumption of wine in the 21st century [7,8].

In South Africa (SA) the first plantation of grapevines was established in 1655 by the Dutch colonizers and successful wine-making was started four years later in 1659 around the Cape area [6,9,10]. In the years to come slavery has played a vital role in shaping the wine industry in South Africa [11]. Although the wine industry had showed tremendous progress and advanced in the technology of both viticulture and enology, it did not achieve the anticipated global attention due to the sanctions by the international community during the apartheid era. The South African wine industry has started to enjoy the global market only after the fall of the apartheid system in 1994 and since then it is gaining worldwide popularity.

1.2. Wine production

Since the start of wine production in the beginning of the 17th century by Jan Van Riebeeck, the commander of the Dutch colony at the time, the SA wine industry has grown to a very competitive level globally. Due to strong competition in the market, there are diverse types of wines produced in South Africa (SA) (**Figure 1.2.**). These wines originate from different grape varieties. Most of the wines that are produced in SA are well-known worldwide. The grape varieties are the building blocks of the wines and are responsible for its full body including color. The extent to which a particular grape variety (cultivar) grows depends on many factors. These include the soil, climate, and the specific inherent qualities of individual varieties including crop

weight, resistance to diseases and extreme weather conditions as well as the inherent uniqueness in terms of aroma and flavor. Today there are a number of grape varieties grown in South Africa including the white cultivars (Chardonnay, Colombard, Chenin Blanc, Sauvignon Blanc), and the red cultivars (Shiraz, Cabernet Sauvignon and Merlot). In addition, a unique SA red cultivar, Pinotage, has been produced to a large extent. Blended wines from a combination of more than one variety are also widely produced. In SA it is widely accepted to blend up to 15% of a different variety and still name the wine as single varietal. The latest planted grape varieties were the Sauvignon Blanc and Chardonnay in the late 1980s [6].

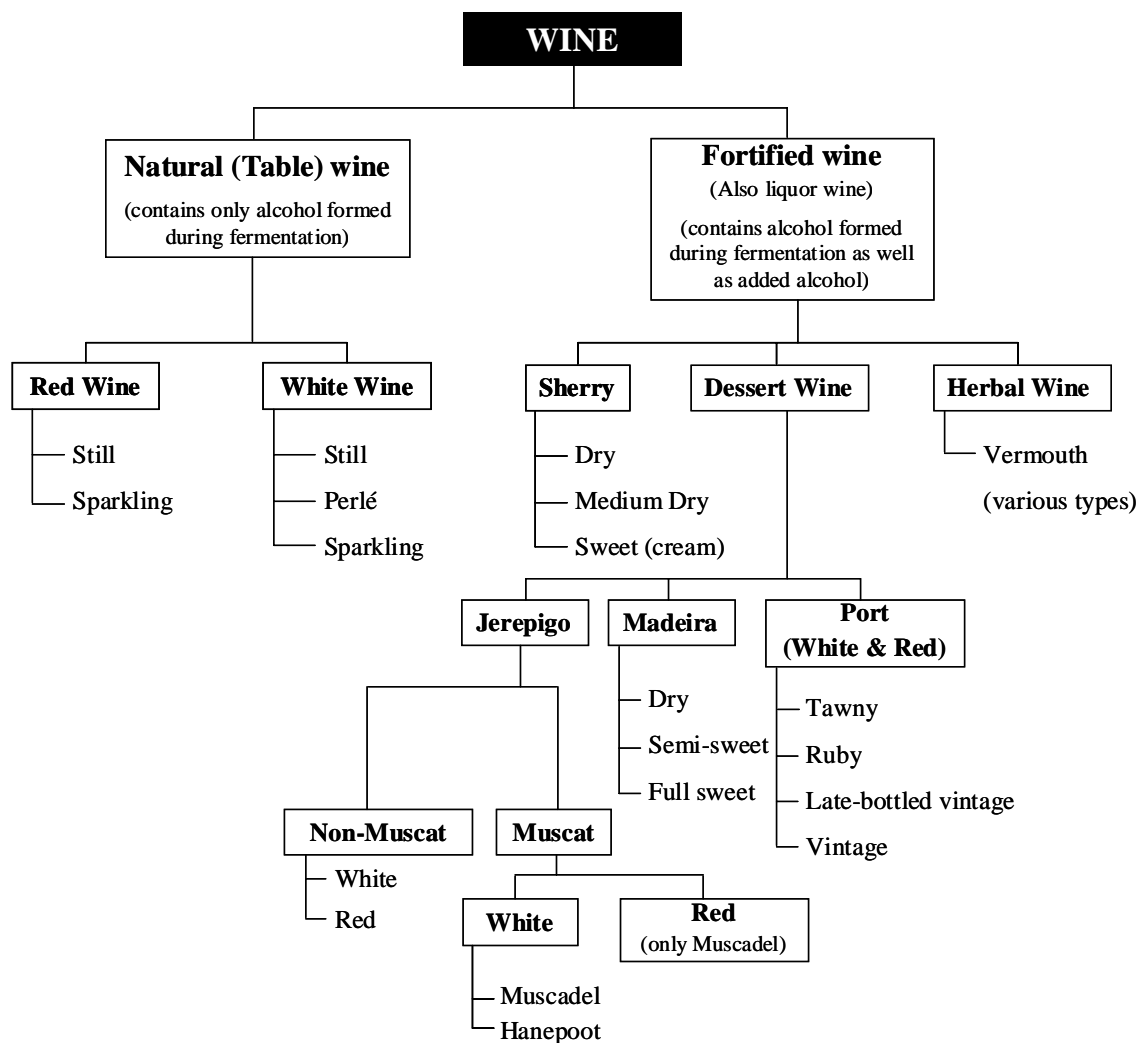


Figure 1.2. Diversity of natural (table) and fortified wines produced in South Africa. (Adapted from [5]).

Chardonnay is currently one of the most popular dry white wines in the world. It is planted almost in every wine producing country and is one of the easiest varieties to grow. It is only in the past few years that Chardonnay has begun to get recognition

and importance in South Africa. Chardonnay generally benefits from oak and is especially complex when it is barrel fermented as well as barrel aged. However, over-oaking has been a common fault for some of the first Chardonnays that were produced in the Western Cape. Wine-makers in this area are now very cautious to not let oak destroy the elegant and reviving citrus characteristics of the wine.

South Africa has recently received great attention as a world class producer of Sauvignon Blanc. There are many microclimates in South Africa ideally suited to the growing of this variety. The South African Sauvignon Blancs tend to be dry and grassy. Its plantings have increased since the mid 1980s and continue to do so. This cultivar is well-known by its vegetative, herbaceous, and green pepper aroma due to the presence of methoxy pyrazines. Pyrazines have been detected in many wine varieties but, due to their relatively high concentration, contribute to the typical aroma of Sauvignon Blanc [6,12].

Pinotage is a unique red wine cultivar resulting from a cross between *vitis vinifera* L. cv. Pinot Noir and Cinsaut in the mid 1920s in South Africa. The new vine was known for its early ripening compared to most cultivars which indicate that it can be harvested earlier than the others. Wines of this cultivar are known for their distinctive fruity character, which is expressed as plum, cherry, red berry, blackberry, and banana [13]. Its popularity around the globe is gaining momentum as more and more studies of this cultivar are carried out. Most of the studies are on volatiles and non-volatiles including the antioxidants, which are believed to be of benefit for human health [13-17]. It was previously thought to be early maturing, but it is now believed that Pinotage benefits from an extended maturation period.

Shiraz grapes (commonly known as Syrah) make a soft and rich wine often characterized by smoky and chocolaty aromas. It matures faster than Cabernet and is sometimes blended with it to speed accessibility. Recently a sesquiterpene, rotundone, was reported to contribute significantly to the peppery aroma of Shiraz wines [18].

Most of the great red wines of Bordeaux and some of the finest wines of the new world are based on Cabernet Sauvignon. It is often blended with Cabernet Franc and Merlot and its flavor is reminiscent of blackcurrants or cedar-wood. It demands ageing in small oak barrels, and the best wines require several years of bottle ageing

to reach their peak. Like Sauvignon Blanc, this cultivar is also known for its vegetative, herbaceous, and green pepper aroma due to the presence of methoxy pyrazines [6,12]. This characteristic aroma of Cabernet Sauvignon and Sauvignon Blanc is mainly due to 3-isobutyl-2-methoxypyrazine (IBMP).

The Merlot variety, next to Cabernet Sauvignon, is the most premium red wine. Merlot is fragrant and usually softer than Cabernet Sauvignon. It also shows best with oak maturation, but usually requires less bottle maturation before it is ready to drink. The growing conditions in South Africa do not require Merlot to be blended in with Cabernet. Merlot bottled as a varietal is becoming more and more commonplace in South Africa. In a recent report by Preston et al. [19], it was indicated that at low levels, vegetative aromas such as bell pepper or asparagus contribute to the distinctive varietal aromas of Merlot like Cabernet Sauvignon and Sauvignon Blanc wines. In addition, Kotseridis et al. [20] have also reported furaneol (4-hydroxy-2,5-dimethylfuran-3(2H)-one) as a caramel odor contributor to Merlot aroma.

The quality of wine is a subjective judgment and depends on many factors such as enological, viticultural, and environmental factors (**Figure 1.3.**). Good quality wine starts in the vineyard as many factors including the vine structure influence the grape composition [21]. However, physical characteristics such as color and texture also play a big role in consumer satisfaction, which, in the end sustains the wine in the market. The combination of these factors allows the creation of good quality, well-balanced and marketable wine. This indicates that it would be best to have the input of many experts from different fields. As can be seen from the chart (**Figure 1.3.**), it requires an enormous amount of work to include all the factors in a study in order to characterize wine. In this dissertation only the flavor and aroma part of wine is reviewed.

1.3. Wine flavor and aroma

The quality of wine is mainly dependent on the chemical composition, which can be classified according to volatiles and non-volatiles. The former determines wine aroma and results from a complex combination of volatiles corresponding to different classes including alcohols, esters, aldehydes, ketones, acids, volatile phenols, lactones, furans, terpenes, sulfur compounds, nitrogen-compounds and other minor components, which

gives distinctive characteristics to the wine. These compounds are already present in grapes or are produced due to fermentation and maturation process as well as storage and ageing. The combination of all of these compounds are responsible for the bouquet of wine [22,23].

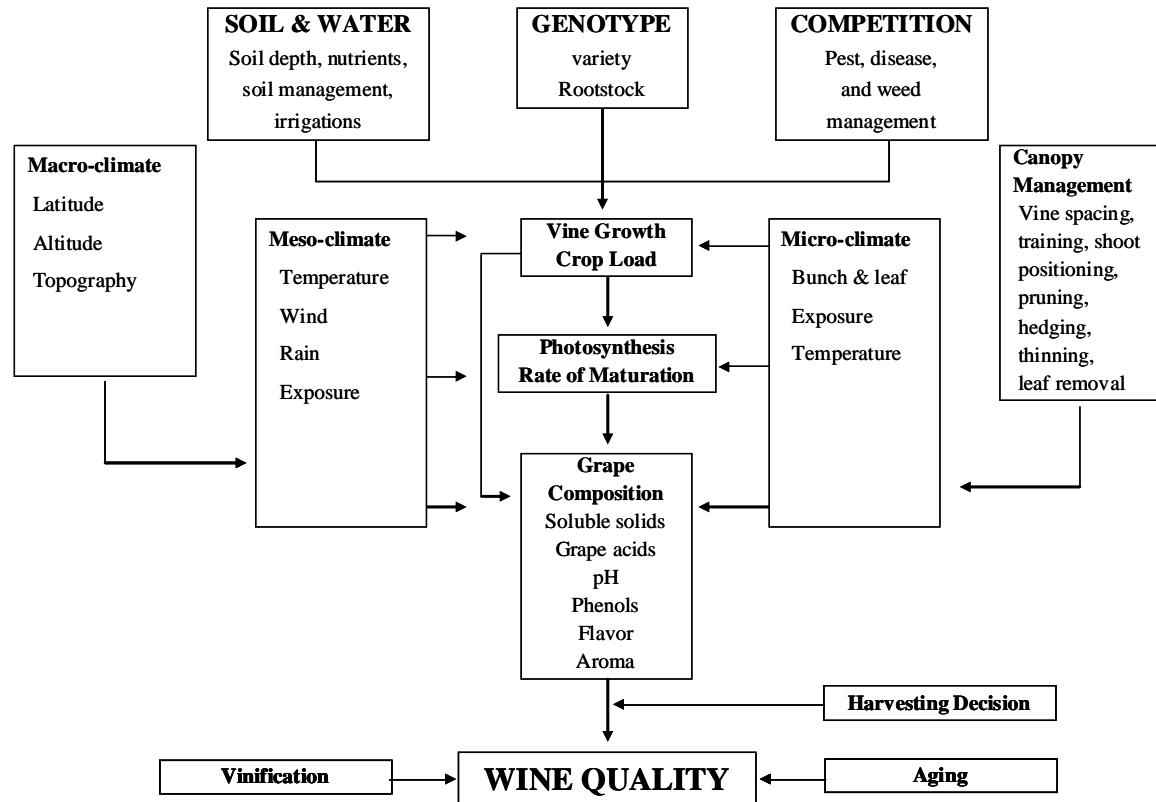


Figure 1.3. Environmental and viticultural imports into grape composition and wine. (Adapted from [21]).

1.3.1. Fermentation products

Fermentation methods can be grouped into three: natural fermentation, alcoholic fermentation and malolactic fermentation. Natural fermentation is when no yeast starter is intentionally added, as many wine-makers use different yeast to improve the wine quality. Natural fermentation in the absence of sulfur dioxide may permit the wild yeast flora to be persistent and possibly contribute to the overall sensory character of the wine. However, the impact of the natural fermentation on the wine flavor and aroma is unpredictable. Some unwanted off-flavors may be produced by wild yeast and bacteria that are difficult to remove or reduce from the final product. Furthermore, this fermentation is not predictable in terms of starting time and period. Natural or wild yeast fermentation is performed by *Saccharomyces* species [24].

Yeasts are single celled *ascomycetous* or *basidiomycetous* fungi, which grow predominantly from budding or fission. Yeast metabolism makes an important contribution to the flavor of wine. In addition to the reduction of grape sugars (glucose and fructose) to ethanol and carbon dioxide during alcoholic fermentation, the use of wine yeast, *Saccharomyces cerevisiae*, produces a number of intermediate products like acetaldehyde and several organic acids. Today, there are a number of yeast strains available commercially as well as naturally in grapes and wines [5,24,25].

Grapes of different viticultural and enological background are expected to differ in their chemical composition even if the same fermentation process is followed. This is because of the different factors that affect the grape composition. For instance, wines from cooler areas will show higher concentration of monoterpenoids [26]. In a similar way, the same fermentation process will not be suitable for grapes from different climatic regions. To overcome such problems different conditions should be applied including heat treatment, yeast strain, etc. However, precautions should also be taken as some conditions might lead to excessive levels of certain chemical constituents [24].

Apart from the conversion of sugars to ethanol and carbon dioxide, glycerol and various volatile and non-volatile compounds such as organic acids and fusel alcohols, etc. are end products of alcoholic fermentation (yeast metabolism) [24]. Alcohols in wine include mono-, di-, tri-, etc. alcohols ranging from one carbon (methanol) to larger alcohols (sugar alcohols). The amounts of alcohols with more than two carbons, commonly known as fusel alcohols (isoamyl-, active amyl-, isobutyl-, and n-propyl alcohols) are dependent on the type of yeast used during grape fermentation. Isoamyl alcohol normally accounts for more than 50% of the fusel alcohol fractions [27,28]. The total concentration of fusel alcohols in table wines is reported to range between 140 to 420 mg/L. The final concentrations of fusel alcohols depend on many factors such as yeast strain, fermentation temperature, suspended solids, oxygen levels, nutritional status and pH [21]. These alcohols have little impact on the sensory properties of wine, nonetheless, they can contribute to wine distillate because of their existence at higher levels [24]. Fusel alcohols are resulted from deamination of amino acids (**Figure 1.4.**). In addition to the given pathway (**Figure 1.4.**), fusel alcohols can

also be formed during the biosynthesis of amino acids, from an excess of keto-acid intermediates [29].

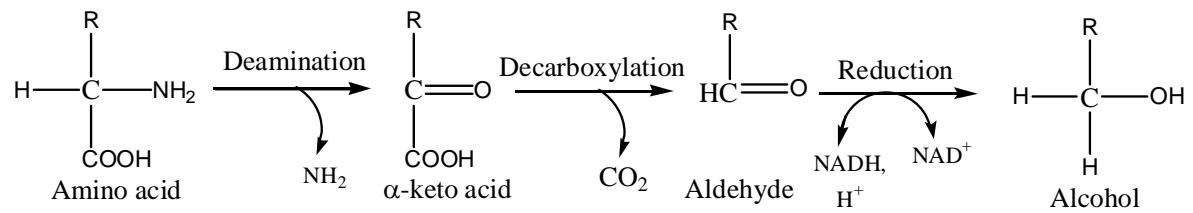


Figure 1.4. Pathway of higher alcohols formation from amino acids. (Adapted from Boulton et al. [24]).

Like alcohols, most esters are products of yeast fermentation. Esters such as acetate esters and fatty acid ethyl esters exist in all wines and contribute to the ‘fruity’ character of the wine aroma that significantly influences the quality of wine [27]. Lower temperature during fermentation favors the formation of volatile esters which could either be due to a shift in biosynthesis patterns by the yeast or prevention of hydrolysis [24].

During alcoholic fermentation, the use of wine yeast, *Saccharomyces cerevisiae*, was shown to produce a number of byproducts like alcohol acetate and ethyl esters of C₄ – C₁₀ fatty acids at increased concentration. Often it is the acetate esters formed from ethanol and higher alcohols that contribute to the aroma of freshly fermented wine. The presence of these compounds during consumption depends on their levels during production and their stability, which depends on many factors, including duration and temperature of ageing before and after bottling. They can also be formed during oxidative decarboxylation of Coenzyme A i.e. these esters are synthesized in the yeast cells by alcohol acetyltransferases (AATases), using higher alcohols and acetyl-CoA as substrates [24,25]. **Figure 1.5.** is a typical example of the this pathway.

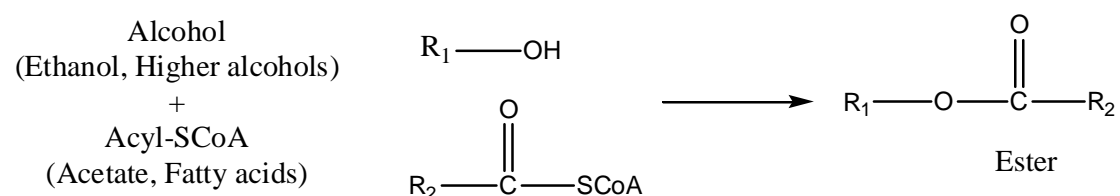


Figure 1.5. Production pathway of esters from amino acids in wine. (Adapted from Boulton et al. [24]).

The two main problems often encountered during alcoholic fermentation are sluggish fermentation and production of off-flavor, which can range from easily treatable to a

serious challenge to the production of quality wine. Sluggish fermentation is when the rate of sugar fermentation decreases significantly, leaving a high amount of sugar in the final product. It is often sourced from nutrient limitations such as nitrogen or phosphate deficiencies [24,30]. The well-known off-flavors are sulfur-containing compounds and their formation during fermentation causes a significant problem. These compounds exist at trace levels in wine but their sensory impact is detectable and harmful. The odor of these compounds can be described with expressions like cabbage, garlic, onion or rubber, which contribute to their negative effects on wine aroma. The formation of volatile sulfur compounds in wine is influenced by deficiencies in nutrients, yeast strains, fermentation temperature etc. and are often challenging for the wine-maker to control [24,31-33]. However, it must be highlighted that not all sulfur-compounds have a negative contribution to wine aroma. Sulfur compounds like dimethyl sulphide or carbon disulphide, reportedly produce satisfactory wine aromas [32,34].

In addition to alcoholic fermentation, other microbial activities which contribute to the wine quality (either positively or negatively) are associated with the wine-making process. Malolactic fermentation (MLF) is a bacterial process that usually occurs once alcoholic fermentation by yeast is complete. During MLF, apart from the conversion of malic acid to lactic acid and CO₂ by lactic acid bacteria (LAB) (**Figure 1.6.**), a lot of other changes take place which influence significantly the sensory property of the wine. The hydrolysis of non-volatile precursor glycosides during MLF can produce a large number of powerful grape-derived volatile compounds that contribute significantly to the wine aroma. These compounds including alcohols, carbonyls, C₁₃-norisoprenoids and terpene alcohols, the latter being commonly considered as varietal compounds [35-37].

MLF can happen naturally or is encouraged artificially in the wine-making process. Ugliano and Moio have indicated the influence of MLF on the levels of volatile compounds from different classes such as esters, alcohols, acids, lactones, sulfur and nitrogen compounds using commercial starters of *Oenococcus oeni* [38]. In addition to the flavor profile produced, *Oenococcus oeni* is the preferred species during MLF because of its tolerance to acids [37]. MLF starts from the moment bacteria is introduced to the wine or must and ends when the bacteria have gone through the

growth phase and entered the resting phase. MLF involves deacidification and microbial stabilization as well as improvement of the complexity of the aroma of the resulting wine. The changes in flavor resulting from MLF are complex and frequently involve changes in fruity, floral, spicy and honey notes and reduction in vegetative and herbaceous aromas, which could be associated with the release of glycosidically bound volatile compounds. When MLF occurs impulsively without any control over the strains, undesirable compounds that could diminish the quality and acceptability of the wines may be created [22,24,35,39,40].

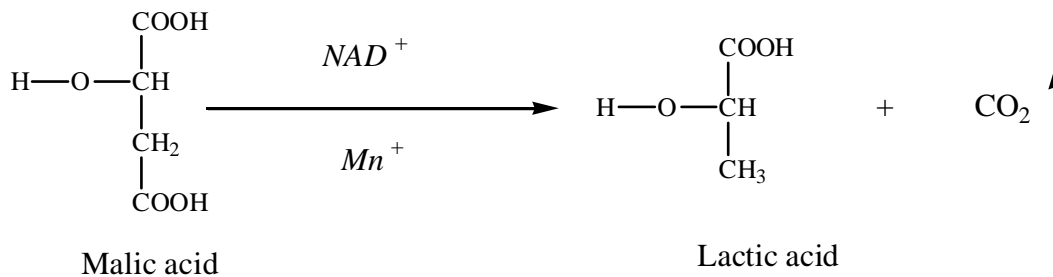


Figure 1.6. Malolactic conversion. (Adapted from Boulton et al. [24]).

1.3.2. Storage, maturation and ageing products

In general the period from the end of fermentation until bottling of the wine is known as ageing. This term is related to storage and maturation, which are linked to the wine-making processes. According to Boulton et al. [24], maturation is defined as “a bulk storage period, while bottling or its equivalent storage is known as ageing”. Depending on the final goal of the wine producer, wines can be stored in wood barrels or stainless steel or can be transferred to a bottle for further storage, maturation, and ageing. Wood ageing is a common tradition in wine production aimed at improving the sensory characteristics of wines and spirits. It is commonly used from the end of the maturation process until bottling. For instance, when wine is aged in oak barrels, it undergoes a series of transformations that cause important progress in the aroma, color, taste, and astringency. This is due to the extraction of volatile and non-volatile compounds that produce complex interactions with other wine components. In addition to the wood contribution by extraction of volatiles, interactions between volatile and non-volatile components could also impact on the aroma of wood matured wines. It is also possible that the compounds entered into the wine medium

from the wood during maturation undergo chemical transformations and so potentially modify their contribution to the wood-related aroma [41-43].

Although not fully understood yet, the process of ageing wine in oak barrels have been extensively studied [42,44-47]. Most scientific studies have focused almost exclusively on the role of oak wood as a source of extractable aromas on which only a few well-known wood odor compounds are included. This neglects the possible existence of other changes that could also be important from an aromatic point of view. The extraction of important odorants, including oak-lactones, volatile phenols, furan-derived compounds and vanillin, plays an important role in the aroma of wood-aged wines. Nevertheless, the oak cask is an active recipient from a physical, chemical, and biochemical perspective. The existence of numerous concurrent phenomena other than simple extraction acting on the aroma should also be considered. For example, Ramirez et al. [48], have demonstrated the retaining and absorbing of a significant part of the wine aroma by oak-wood.

There are two main factors that influence the level of the wood extracted compounds that enter into wine. These are the oak species and their geographic origin as well as the processing of the wood in cooperage (the method used to obtain the staves and the seasoning process applied) and the degree of oak toasting during the barrel's manufacture [45-47]. Jarauta et al. [42], have showed different concentration levels of wood compounds aged in American oak in comparison to French oak. In the same report, it was also indicated that these compounds exist in lower amounts when aged in stainless steel barrels.

The isomers of whiskey lactone (*trans*- and *cis*-oak lactone) (**Figure 1.7.**) are well known and widely reported to impact on the odor released into the wine. The ratio of *cis*- to *trans*- isomer is reported to increase with ageing in oak and the *cis* (-) is 4 – 5 times more odoriferous than the *trans* (+) isomer [42,49].

Wine

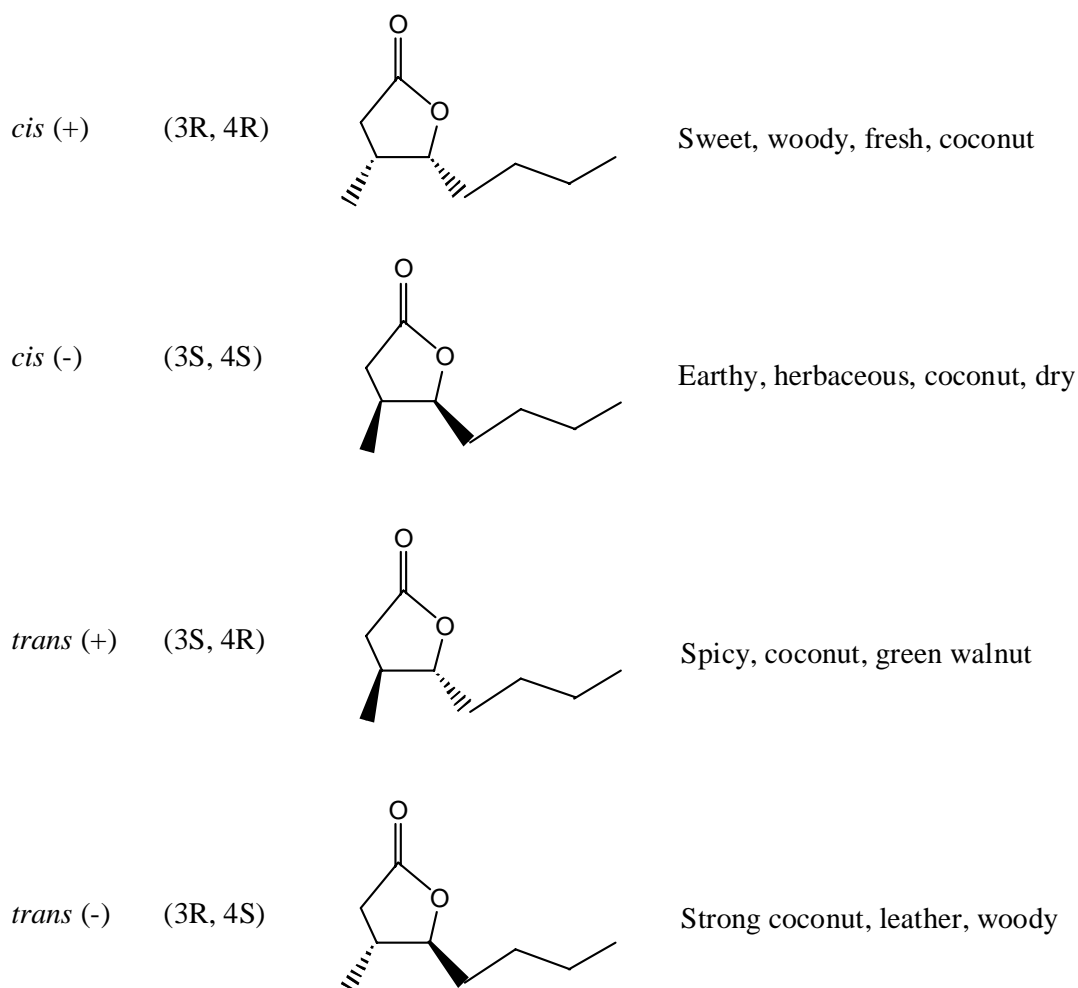


Figure 1.7. Chemical formula and aromas of various isomers of β -methyl- γ -octalactone (oak-lactone or whiskey lactone). The first three have been identified in natural oak. (Adapted from [49]).

Other compounds well known as being sourced from the wood are volatile phenols, furan-derived compounds, terpene compounds, to name only a few. These compounds are known to contribute significantly to the richness and complexity of the bouquet, as well as improving the flavor of wines. Untreated oak contains certain number of volatile substances (**Figure 1.8.**) with specific odors.

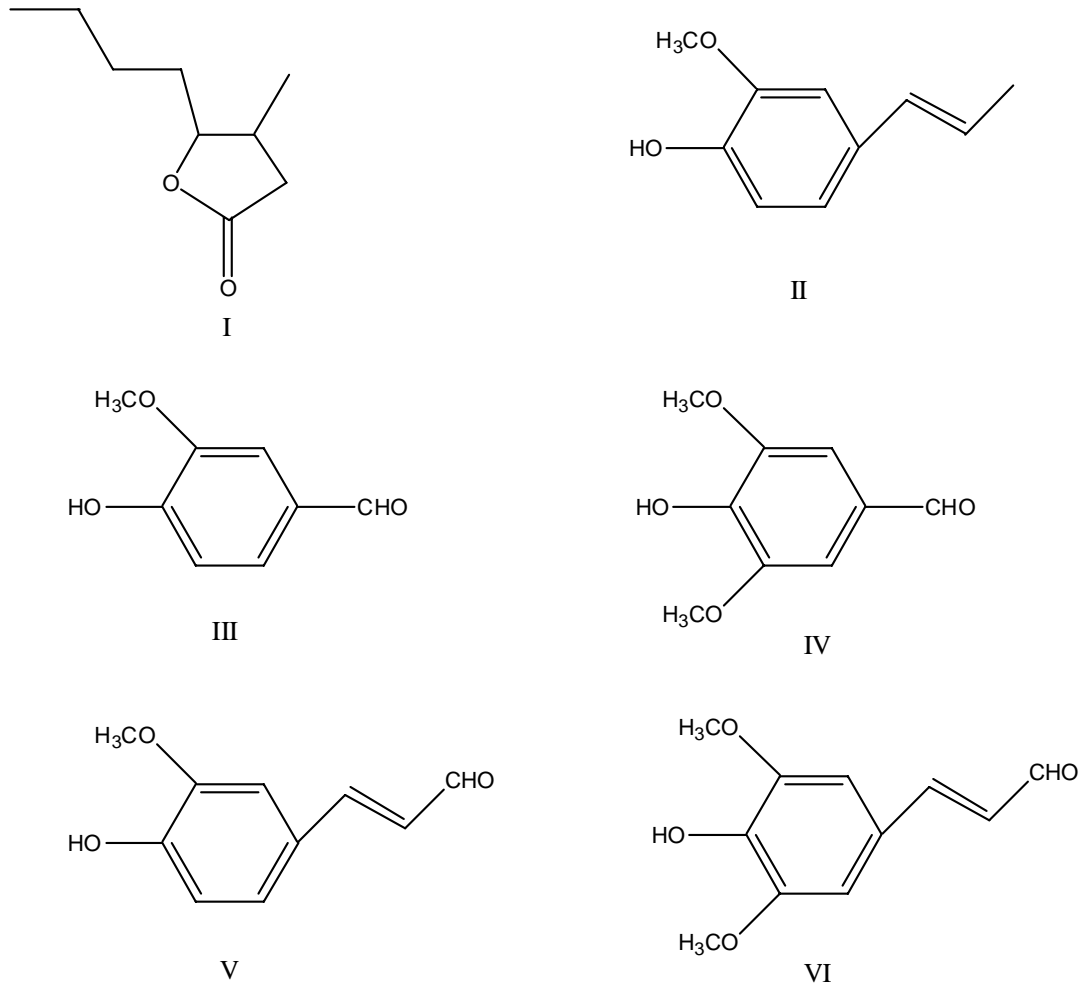


Figure 1.8. Chemical structure of main volatiles identified in extracts of non-toasted oak wood: I) methyl octalactone (methyl-4-octanolid or whiskey lactone or oak lactone), II) eugenol, III) vanillin, IV) syringaldehyde, V) coniferaldehyde, VI) sinapaldehyde. (Adapted from [49]).

During bottle ageing, wines develop in a reducing environment, giving rise to greater organoleptic quality. Apart from changes in color, this process results in an increase in the complexity and elegance of aroma. The time necessary to attain this optimum condition varies considerably with the type of wine – from a few years to several decades. Unlike the modest wines that develop their full potential within a short period of time in a bottle, great wines are generally characterized by their capacity to age for a long time.

Bottle ageing has three main stages. In the first stage, wines become mature with small changes in the quality. During the second stage, wines reach their peak and are considered fully matured. The third stage is characterized by deterioration and wines dry out and eventually become “thin”. This reduction in quality takes place at varying rates and organoleptic changes are accompanied by gradual stripping of the wine, possibly caused by precipitation in the bottle [24,49,50].

Several compounds have been reported as characteristic of bottle ageing or bottle bouquet. Perez-Prieto et al. [50], have indicated that esters and acids decrease during bottle ageing. One of the well-known bottle bouquets is dimethylsulfide (DMS). This compound occurs in grape juice, but it is highly volatile and easily lost from wine in an open container or during bulk storage. It does, however, increase with bottle ageing. Another group of compounds that increase with bottle ageing are terpenes, which contribute significantly to bottle bouquet. Vitispirane, 1,1,6-trimethyl-1,2-dihydro-naphthalene (TDN), linalool oxide, and nerol oxide are some of the terpene related compounds known to develop bottle bouquet [24].

Due to the demand for wine and its economic importance, accelerated maturation and ageing is a common practice among wine producers. This helps in rapid transformations that occur during ageing, and thereby reduces the time wines need to be stored. Standard rapid ageing processes involve oxidation within a wide range of temperature. It has been observed that wine mainly ages in summer, then makes a deposit and stabilizes in winter. Hence, the rapid ageing process should include these seasonal effects within a short period of time. The process could be a repeated cycle of saturating with air or oxygen at low temperature and then heating up to room temperature again, followed by cooling, oxygenation and subsequent heating etc. Other reported rapid maturing and ageing processes include the use of ultrasound, infrared and ultraviolet radiations, high pressure, and electrolysis to name a few [49]. Silva et al. [51], evaluated the impact of forced-ageing on Madeira wine flavor using different baking temperatures and time.

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Wine analysis

In the historic period of wine production, various analytical techniques have become important. With the development of technology and increased regulations, this has become increasingly sophisticated. Analysis of grapes and wines is always done for a number of reasons, some of which are quality control, spoilage reduction and process improvement, informatics of blending, export certification, regulatory requirements, and customer satisfaction [1].

Many scientists have been investigating different analytical techniques for the analysis of wines, varying widely based on the application, including separations like gas chromatography (GC), liquid chromatography (LC), electrophoresis, etc.; wet chemistry; and sensory evaluation [2,3].

In this review, only the former and particularly GC will be discussed. Since its invention by the Russian botanist Mikhail Tswett in 1906, chromatography has been the most extensively used separation technique.

2.1. Gas chromatography

Gas chromatography (GC) is a separation tool where compounds are separated by a series of partitions between a moving gas phase and a stationary liquid phase held in a small diameter tube (the column) after a mixture is injected as a narrow band. GC works only for analytes in a gas phase and can be grouped into gas solid chromatography (GSC) and gas liquid chromatography (GLC). The latter is the most frequently used in many fields and was first introduced in 1952 by James and Martin [4,5]. Its first application was the separation of volatile fatty acids by partition chromatography using nitrogen gas as the mobile phase and a stationary phase of silicone oil. GSC was also launched in the same year by Phillips [6].

Any chromatographic instrument consists of sample introduction, separation, detection and data collecting devices. In modern GC systems electronic pneumatic control (EPC) are included for accurate measurement of pressure and temperature, providing extremely reproducible chromatographic results. For the purpose of this dissertation a typical capillary gas chromatography (cGC) system will be discussed.

2.1.1. Carrier gas

In gas chromatography gas is passed continuously through a column and this passage promotes the elution of the components of the sample. The choice of carrier gas is associated mainly with the cost involved but to some extent with application. In GC mostly helium and hydrogen or sometimes nitrogen is used. It must be noted that a carrier gas should be inert, in that it does not react with the sample or stationary phase. The dynamic viscosity of the carrier gas is essentially independent of pressure, it does, however, vary with temperature. As temperature increases, so does the carrier gas viscosity, which is strange in that it is the opposite of what is typically encountered with liquids. Regardless of the column length and internal diameter (ID) as well as the choice of carrier gas, the pressure and linear velocity decreases as the distance from the inlet increases [7].

2.1.2. Sample introduction (injector)

There are many types of sample inlets used with GC including split/splitless injectors, programmed temperature vaporizing (PTV) injectors, on-column injectors, etc. In this study the first two were used and will briefly be reviewed. As mentioned earlier GC is a gas phase technique and all compounds need to be converted into gases in the sample inlet. Hence, a heated GC inlet is mainly used, where the temperature is controlled electronically. The most commonly used inlet is the classical split/splitless injector (**Figure 2.1.**). This injector can be operated in split or splitless mode depending on the application and the final goal. In the former mode, only a small fraction of sample (eg. 1:100) is used for analysis by splitting the gas flow – the rest is vented through the split outlet. This mode is used for highly concentrated samples in order to avoid system overloading and when sensitivity is not an issue. In the splitless mode on the other hand, in order to increase the sensitivity, the split valve is closed for a short period of time ranging from 0.5 – 2 min after injection ensuring that the entire sample is transferred for analysis [8].

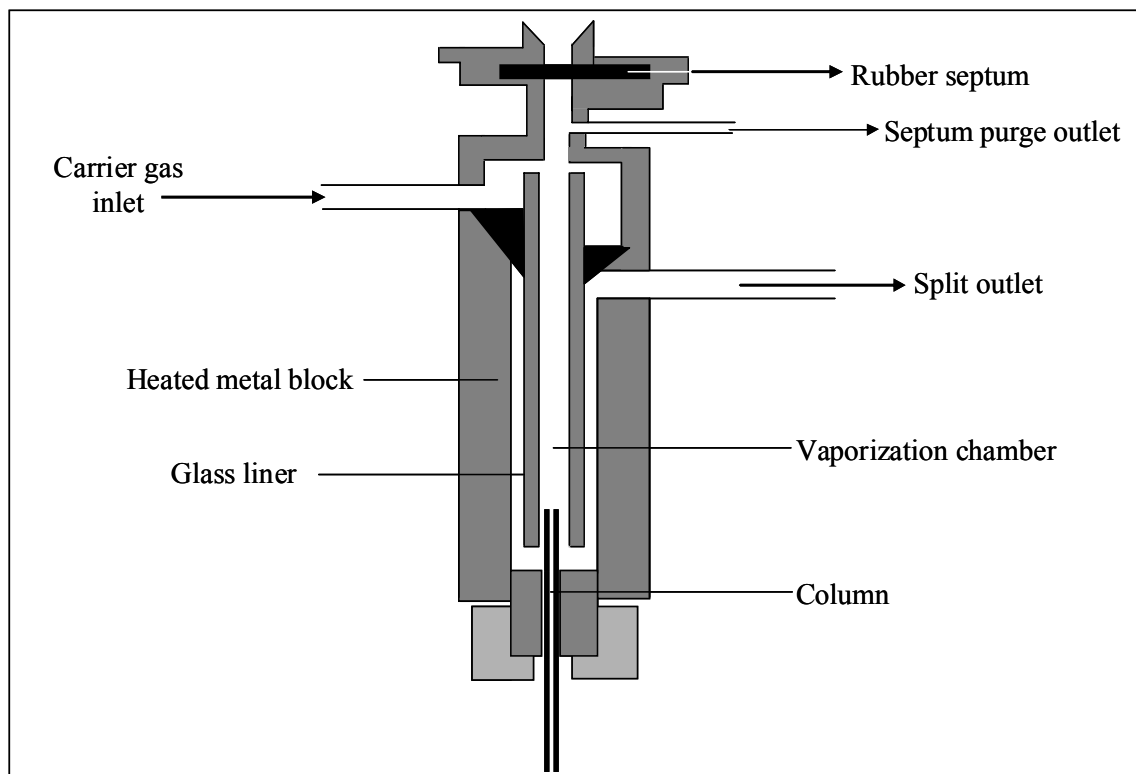


Figure 2.1. Schematic representation of the split/splitless injector. (Adapted from [9])

In contrast to the split/splitless injector, in a PTV (**Figure 2.2.**) inlet analytes are trapped at reduced temperature which commonly ranges between -150 to -50 °C. This gives some advantages to the PTV inlet over the split/splitless injector by reducing analyte discrimination during the injection step. It also shows better recovery of thermo-labile compounds and less pronounced adverse effects of non-volatile compounds present in the sample during the injection process [8]. The PTV inlet differs mainly from the classical split/splitless injector in the temperature control and also the volume. The PTV inlet can operate both in split and splitless modes [9].

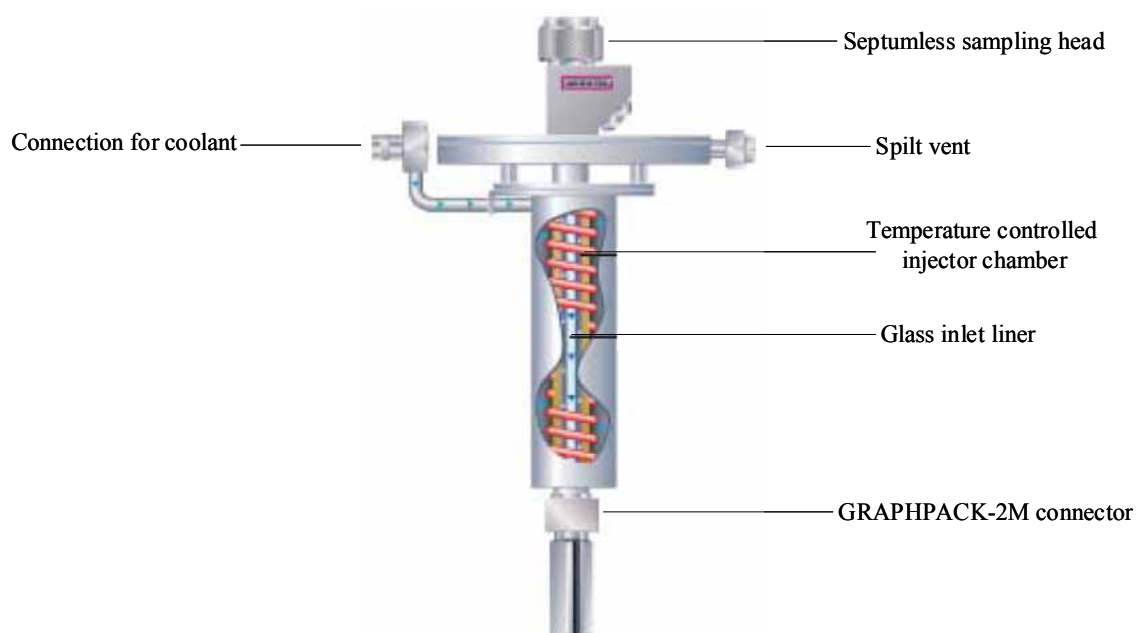


Figure 2.2. A programmed temperature vaporization (PTV) inlet operates as cooled injection system (CIS-4). (Adapted from [10]).

2.1.3. Thermal desorption unit (TDU)

One of the analytical tools extensively used in the project is the thermal desorption system (TDS) designed by Gerstel (GmbH, Germany). The TDS is commonly used to desorb compounds from solid materials. TDS is directly connected to a PTV inlet (**Figure 2.3.**) and consists of a removable desorption tube through which a carrier gas flows at a constant rate and a heating element for rapid heating of the chamber. Sampling of gases or liquids can be done by pumping or sucking the sample (off-line) through a packed bed containing either sorbents (e.g. PDMS) or adsorbents (e.g. Tenax). For the thermal re-extraction of analytes, the extraction material can be placed directly into the desorption glass tube which is cooled down to ambient temperatures in order to prevent premature desorption. After desorption at elevated temperature, the compounds are transferred to the PTV injector through a fused silica transfer column, which is kept at high temperature (≥ 300 °C) to prevent condensation of high molecular weight compounds. The solutes are then focused in the PTV inlet by selecting an appropriate low temperature (commonly ≤ -100 °C). Depending on the nature of the analytes, and (ad)sorbents materials, the desorption conditions (temperature, gas flow, and desorption mode) can be adjusted to ensure complete desorption and transfer of analytes without sample or (ad)sorbent decomposition [3,9,11].

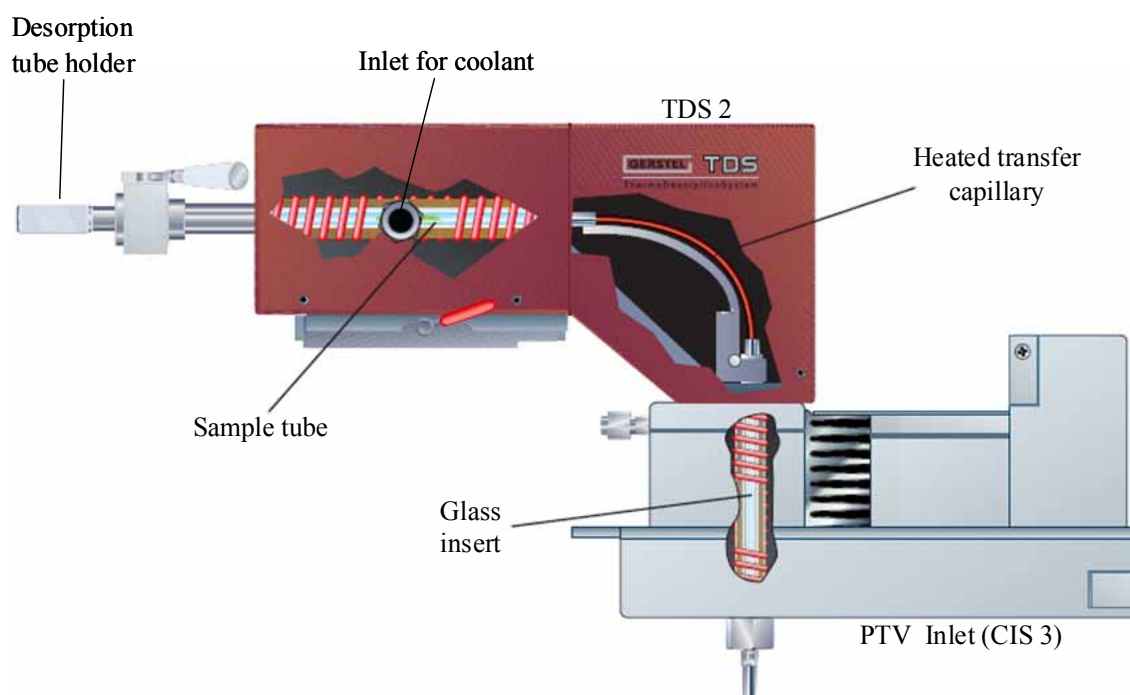
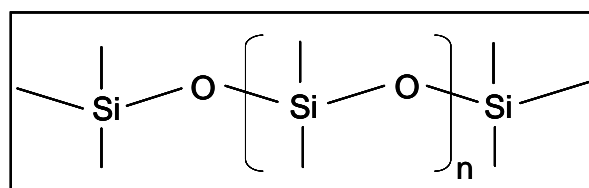


Figure 2.3. Thermal desorption system (TDS-2) coupled to a PTV injector (CIS-3). (Adapted from [12]).

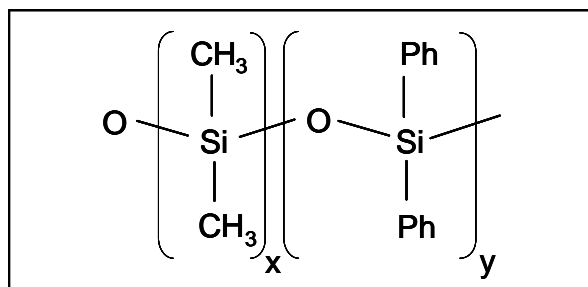
2.1.4. Capillary column

The column is at the centre of the GC and is where separation takes place. Separation occurs based on the physical and chemical properties of each analyte in the sample in relation to the stationary phase of the column. There are a wide range of capillary columns available nowadays, mainly differing in the type of their stationary phases and dimensions. The choice of column depends mainly on the type of analytes but to some extent also on the complexity of the sample and the number of analytes to be separated. The general principle of chemistry, “*like-dissolves-like*”, is applied when considering selection of stationary phases, where a phase with a polarity similar to that of the analytes of interest is usually preferred. When a non-polar stationary phase is selected, non-polar analytes would be well separated and the separation would be according to boiling point. On the contrary, when the need arise for separating polar compounds, columns with polar stationary phases should be used and the separation is then mainly be due to selective partitioning (interactions with the stationary phase). A wide range of stationary phases varying from highly polar to highly apolar are accessible for utilizing the optimal column conditions for achieving the desired separation. The most extensively used stationary phases are polydimethylsiloxane (PDMS) and polyethelene glycol (PEG, also known as Wax) phases. Substitution of the methyl group in the PDMS chain to varying degrees ranging from 5% to 50%

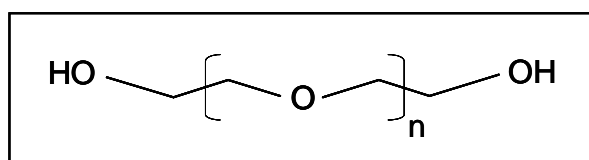
using mainly the phenyl group in order to accommodate polar molecules is also used extensively (as examples see **Figure 2.4.**) [3,5].



(a)



(a)



(c)

Figure 2.4. Chemical structures of different stationary phases used in capillary gas chromatography (cGC): (a) 100% polydimethylsiloxane (PDMS), (b) phenyl (Ph) substituted PDMS ($x = y = 50\%$), and (c) polyethylene glycol (PEG, Wax).

Numerous phases with selective applications have also been employed including free fatty acid phases (FFAP) which is a modified PEG phase designed for the analysis of fatty acids and phenols, resulting in good peak shapes for these compounds. Other selective phases include those incorporating cyclodextrins for chiral separation and siloxane phases stabilized for use at high temperatures for high-boiling analytes [3].

Concerning column size formats, one has to consider the length (L), internal diameter (ID), and the film thickness (d_f) of the stationary phase for efficient and fast separation. Generally, a longer column will give better separation leads to longer analysis time. A thicker film of stationary phase (d_f) results in an increase in the retention of analytes, thereby also increasing the analysis time. Narrow bore columns will improve separation efficiency and reduce the analysis time, but will decrease the sample capacity. Even though a wide range of column length (10 to 100 m) can be

used, the most commonly used dimension that accommodates both good efficiency, resolution, and capacity are 30 m length \times 0.25 mm I.D. \times 0.25 μm d_f [3].

2.1.5. The GC oven

The partition of analytes between the carrier gas and the stationary phase is highly dependent on temperature. GC ovens contain an electric heating element on which the column is mounted. The heat from this element is distributed in the oven uniformly as air circulation driven by a powerful fan to ensure an even temperature throughout the oven. A temperature sensor inside the oven allows oven temperature control. Typical GC ovens should operate over a fairly wide temperature range and can be quickly and precisely heated to the preferred temperature varying from -100 to 450 $^{\circ}\text{C}$ at a rate of 0.1 to 50 $^{\circ}\text{C}/\text{min}$ [13].

2.1.6. GC Detectors

Once the components of a mixture are separated using gas chromatography, they must be detected as they exit the GC column. Detectors can be grouped either on the basis of physical detection mechanisms like ionization, bulk physical properties, optical and electrical detectors, or based on the nature of the response. Detectors are broadly classified as universal, selective, or specific. Universal (non-selective) detectors respond to all chemicals differing from the carrier gas. Flame ionization (FID) and thermal conductivity (TCD) are typical examples of universal detectors. Selective detectors respond to certain compounds which have common chemical and physical properties. Detectors falling in this category include atomic emission (AED), electron capture (ECD), flame photometric (FPD), and photo ionization (PID) detectors. On the contrary specific detectors respond only to one compound. In addition to selectivity, detectors can be grouped according to their response to the concentration of analytes as mass flow and concentration dependent detectors [3,13,14]. The most important detector that provides an extra dimension of information is the mass spectrometer (MS). The mass to charge ratios (m/z) of ions resulting from breakdown of compounds are measured by mass spectrometry, which is therefore very useful for compound identification. This detector has been used extensively in the current study and will be discussed briefly.

After the determination of mass to charge ratio (m/z) of an electron, J.J. Thomson performed his first MS experiment with hydrogen, and latter with carbon, nitrogen and oxygen atoms in 1912. A few years later Thomson's student, F.W. Aston discovered the two isotopes ^{20}Ne and ^{22}Ne , which consequently led to the discovery of 212 naturally occurring isotopes. From these results, Aston formulated the so-called "Whole Number Rule", which states that when expressed in atomic weight units, the atomic weights of isotopes are very nearly whole numbers, and the deviations found in samples of elements are due to the presence of several isotopes with different weights. The use of a mass spectrometer as a detector in gas chromatography was developed in 1957 by J.C. Holmes and F.A. Morrell [3,15]. Gohlke described the direct introduction of GC effluent into a mass spectrometer in 1959, and four years latter, in 1963, detailed GC-MS analysis of natural products was reported [5].

A typical mass spectrometer consists of an ion source, mass analyzer, and a detector. Once an analyte passes through the transfer line into the ion source of the MS, it is ionized and fragmented. The produced fragments are separated based on their m/z ratios and measured. The most common and perhaps standard form of ionization used in GC technology is electron impact ionization (EI). The molecules enter into the MS where they are bombarded with free electrons emitted from a filament (70 eV). The electrons bombard the molecules causing hard ionization that fragments the molecule.

2.1.6.1. Quadrupole mass spectrometry (qMS)

Mass spectrometers are distinguished based on the type of mass analyzer. The most common type of mass analyzer associated with gas chromatography (GC) is the quadrupole (qMS, **Figure 2.5.**). A quadrupole mass analyser is a device which uses the stability of ion trajectories in an oscillating electric field to separate ions according to their m/z ratios. It is composed of four parallel hyperbolic circular rods in a square array. Those rods opposite to each other are electrically connected to a radio frequency (RF) and direct current (DC) and a voltage with opposite polarity (+/-) is applied to adjacent rods. Ions are accelerated along the z -axis between the rods out of the ion source. These ions encounter forces in the x and y axes resulting in oscillation away from the rods. When the oscillation is too large, the ions strike the rods and are lost without reaching the detector [9,14,16-19].

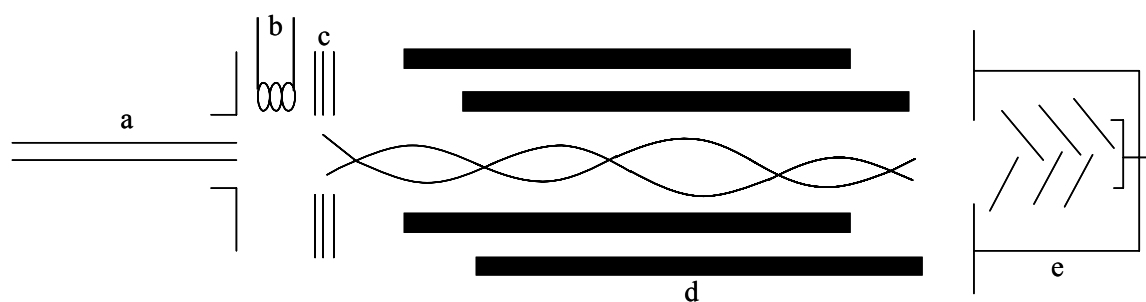


Figure 2.5. Schematic shows basic components of a quadrupole mass spectrometer: a) transfer line, b) ion source, c) focusing lenses d) quadrupole mass analyzer, and e) electron multiplier. (Adapted from [17,20])

A qMS can be operated both in scan mode, where scanning of all possible fragment ions within the specified range (eg. 35 – 350 amu) takes place, and in selected ion monitoring (SIM) mode, where only pre-selected ions will be detected. When the MS is running in the former mode, it is used as a universal detector, on the other hand the latter mode acts as a selective detector. The advantages of SIM over scan mode includes lower detection limit (10^3 fold compared to scan mode) as the instrument is only looking at a small number of fragments (e.g. three fragments) during each scan. In SIM mode more of the ions of interest reach the detector within a given time and since only a few mass fragments are being monitored, matrix interferences are low. The fewer ions used in the SIM can result in ambiguous identification, hence it is important to confirm the identity of the analyte by comparing the ratio of the ions from the various mass fragments [16-18].

2.1.6.2. Time-of-flight mass spectrometry (TOFMS)

Time-of-flight mass spectrometry (TOFMS) was first proposed by Stephens in 1946 but only became available commercially in 1955 after the design of the instrument improved by Wiley and McLaren [17,21]. Since it was perceived as having low sensitivity and resolution, TOF was never utilized widely in many major areas of mass spectrometry [21]. However, in the 1980s the demands for rapid mass scanning capabilities and wide mass ranges have sparked renewed interest in TOFMS. This can be attributed to developments in data acquisition techniques, which allowed the fast and efficient collection of large amounts of data [22,23]. In addition, developments in mass spectrometric techniques such as laser and plasma desorption, laser ionization and surface analysis [17,21] have also played important roles in the advancement of TOFMS. These techniques require the ability of TOFMS in handling an unlimited

mass range [21]. The invention of the matrix-assisted laser desorption/ionization TOF (MALDI-TOF) has opened new applications for biomolecules as well as synthetic polymers and polymer-biomolecule conjugates, which also contributed to the expansion of TOFMS [17].

A time-of-flight mass analyzer possesses a simple design used to separate ions based on their migration times. TOFMS operates on the principle that a packet of ions, with different m/z ratios but equal energy or momentum, when projected into a constant electric field will separate according to their m/z ratios (**Figure 2.6.**). Ions are formed in a short *source* (accelerating) region (s). A positive voltage (V) is applied to the backing plate imposing an electric field ($E = V/s$) across the source region, which accelerates all the ions with the same kinetic energy (KE). Then the ions pass through a much longer *drift* (field free) region (D), where they spend most of their time and separated according to their velocities before reaching the detector. Since ions with the same KE are produced, those with lower m/z arrive first followed in succession by those of higher m/z (**Figure 2.6.**) [17,20,21].

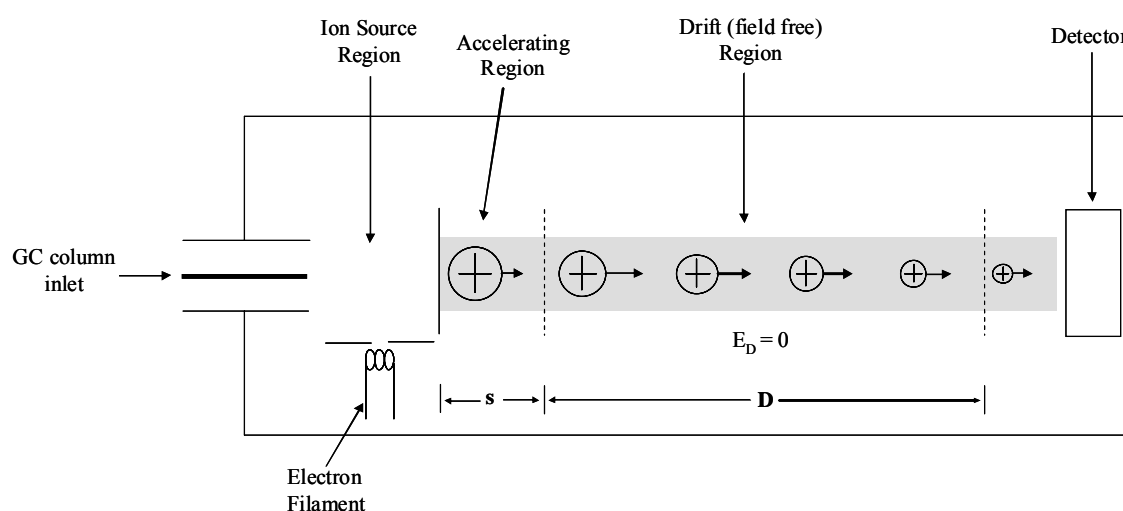


Figure 2.6. Basic components of time-of-flight mass spectrometry. (Adapted from [20,21])

In addition to the ability of handling a complete spectrum with unlimited mass range, TOFMS is capable of the highest data acquisition rate of any mass spectrometer. TOFMS data can be used for spectral deconvolution of overlapping mass spectra to yield pure chromatographic peak profiles for accurate identification or/and quantification [17,21,24]. Due to its fast data acquisition rate, TOFMS is one of the very few detectors that are fully compatible with comprehensive two-dimensional gas

chromatography (GC \times GC), in providing sufficient data density for an accurate definition of the narrow peaks [25].

2.2. Comprehensive two-dimensional gas chromatography (GC \times GC)

The idea of comprehensive chromatography was conceived in 1944 before the discovery of GC itself using 2D planar separation, consisting of two orthogonal chromatographic migrations [26]. The use of two-dimensional (2D) chromatography for separations of complex samples came to light in the 1970s [27]. Numerous efforts have been made to improve the separation power of 2D techniques. Although the 2D separations were powerful at the time of their discovery, not all samples from the 1st separation dimension were subjected to the 2nd dimension separation. These combined systems were intended to collect effluent from the 1st column and inject each aliquot onto the second column (off-line comprehensive analysis). Alternatively, heart-cutting can be performed, where only selected fractions of first column effluent are passed to the 2nd column [28,29]. The experimental realization of a comprehensive multi-dimensional system was reported for the separation of proteins using comprehensive two-dimensional liquid chromatography (LC \times LC) [30,31] and peptides using LC in combination with capillary electrophoresis (LC \times CE) [32] by Bushey and Jorgenson in 1990. Subsequently, a complete transfer of effluent from the primary column to the secondary column in an on-line comprehensive GC approach was realized by Phillips and his group in 1991 [33,34].

Comprehensive two-dimensional gas chromatography (GC \times GC) emerged as a powerful analytical technique, which is an excellent choice when the composition of complex samples has to be unraveled. GC \times GC is designed to improve the resolution of volatile compounds in a very complex mixture, compared to single column gas chromatography (1D GC). This is because of the additional dimension (2D), as every compound is separated in both dimensions (**Figure 2.7.**). This high resolving power of GC \times GC gives higher peak capacity – the overall peak capacity is roughly the product of the peak capacity of each individual dimension (**Figure 2.7.**) [35].

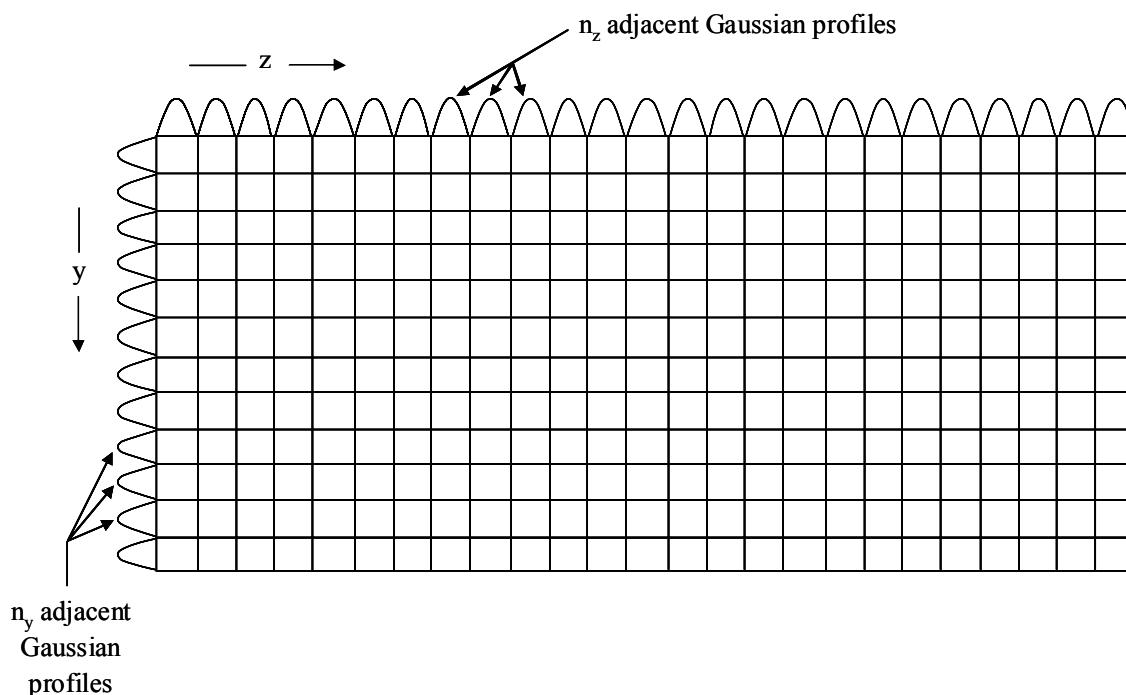


Figure 2.7. Peak capacity of a comprehensive two-dimensional system, represented by the number of boxes, is approximately equal to the product of the peak capacities of each dimension (n_z and n_y) generated along the two individual axes, as represented by the number of adjacent Gaussian profiles. (Adapted from [35]).

GC \times GC uses two columns of different characteristics, coupled in sequence through a suitable interface known as modulator (**Figure 2.8.**) that allows peaks from the primary column to be transferred onto the secondary column, so that an additional separation, and ideally complete resolution for all sample constituents, may be achieved [25]. In GC \times GC, the 1st dimension column is often non-polar and therefore separation of individual analytes is mainly achieved based on differences in boiling point. Each fraction generated by modulation goes through to the 2nd dimension short (1 – 3 m), narrow (0.1 – 0.25 mm I.D.) column for fast analysis. Normally, the 2nd dimension column is polar, therefore separation is based on polarity. This apolar-polar combination of columns is an orthogonal configuration due to different separation mechanisms [36,37]. The reverse arrangement where a long polar column in the 1st dimension and a short and narrow apolar column in the 2nd dimension are connected in sequence is also possible [38].

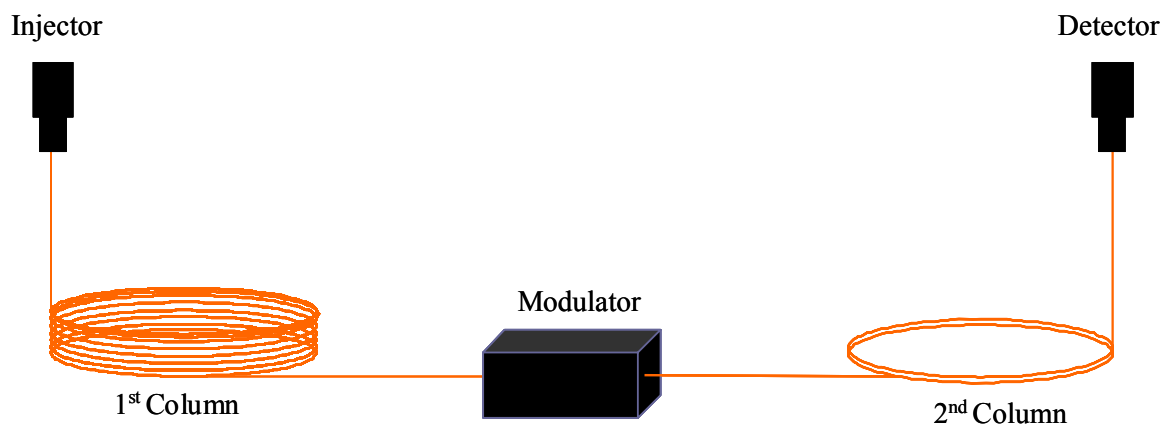


Figure 2.8. Schematic representation of comprehensive two-dimensional gas chromatographic ($GC \times GC$) system. (Adapted from [39]).

In $GC \times GC$ the modulator is the key part of the system. It helps preserve the first-dimension separation and facilitates the second-dimension separation by periodically (usually 4 – 8 seconds) collecting, focusing and transferring the effluent from the primary column onto the secondary column [36,40]. Interfaces commonly used in $GC \times GC$ can broadly be classified as thermal and valve-based modulators.

Thermal modulators are classified into two types. The first one includes those that use either a segment of a thick-film capillary column, or a cryogenically generated cold spot, to trap the effluent from the primary column. In the former, effluents are injected into the second dimension column through the application of a moving heat gradient, for instance as a rotating mechanical heater or sequentially heated segments of metal tubing. The other form of thermal modulation depends on using a liquid CO_2 or cold N_2 gas as cryogenic to cool a portion of a GC column in order to trap analytes in a cold spot, either by partitioning or freezing. When the cryogen is periodically removed, either by moving the cold spot to a different position on the capillary or by interrupting its delivery, the cold (trapping) spot is reversed to the oven temperature and thereby analytes are injected into the second column for further separation [41].

Figure 2.9. shows a two stage modulation technique.

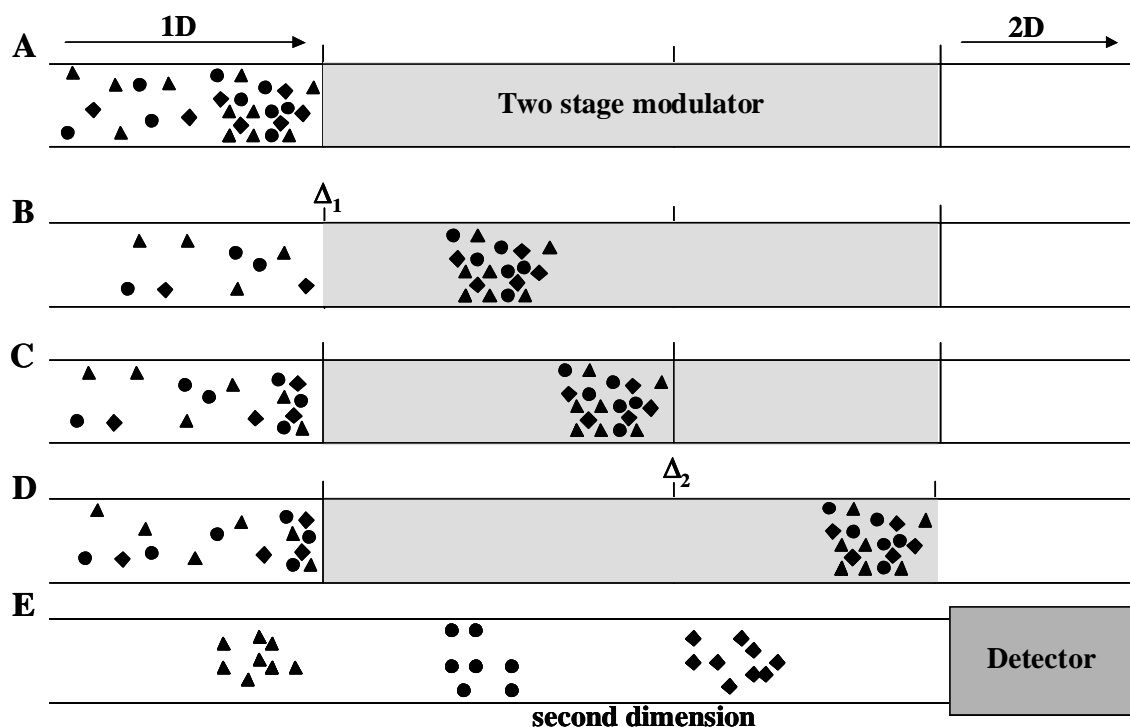


Figure 2.9. Schematic representation of thermal modulation. The different symbols (\blacktriangle \bullet \blacklozenge) represent three different compounds with different chemical and physical properties. **A:** a sharp band of 1D effluent containing three compounds enter the modulator at low temperature, **B:** movement of analytes towards the first part of the modulation tube through a rapid heating pulse (Δ_1), **C:** movement of the effluent by the mobile phase to the second portion of the modulator where they experience a cold spot while volatiles starts to collect at the head of the first part due to rapid cooling to the initial temperature, **D:** re-injection of the effluent into the 2D column through another heating pulse (Δ_2), **E:** due to the different selectivity of stationary phase in the 2D, the three compounds resolved before they reach the detector. (Adapted from [36]).

In a valve-based modulator, first the sample effluent has to pass from the primary column to a multi-port valve that is vented to the atmosphere through a sample loop, and at the same time an auxiliary gas is supplied to the second dimension column. By triggering the valve periodically, any sample found within the sample loop is directed to the second dimension column [41]. Even though the modulator prevents the breakthrough of highly volatile analytes, valve-based modulator loses a large portion of the original sample and thereby sensitivity. This drawback makes the valve-based modulator less attractive for trace analysis. In contrast, the use of thermal modulators allows the transfer of the entire sample to the 2nd dimension column, resulting in improved sensitivity, and as a result this type of modulator is very useful for trace analysis [41]. Nevertheless, thermal modulators have limitations in trapping highly volatile compounds, particularly those that use CO_2 as cryogen. Harynuk and Górecki [41], recently improved this design by using liquid N_2 as a cryogen, and have reported the analysis of highly volatile molecules such as propane and CS_2 .

Due to the fast separation in the second column, GC \times GC requires a detector with fast acquisition rates in order to provide adequate density of data for an accurate characterization of the narrow peaks. Flame ionization detector (FID) can provide high data acquisition rates of up to 200 Hz and is often used with GC \times GC. An alternative detector, providing identification capabilities, is the time-of-flight mass spectrometer (TOFMS). TOFMS collects mass spectra over a chromatographic peak, maintaining the same m/z profile in every scan without mass spectral bias (skew). Due to its high data acquisition rate (up to 500 spectra/s), and the detection of all m/z fragments simultaneously without spectral deformation, TOFMS gives better spectral deconvolution than scanning systems (like qMS), even in co-eluting situations. The combination of GC \times GC and TOFMS can provide both high resolution and good sensitivity, which are very important requirements in trace analysis. In addition, TOFMS allows compound identification power through mass spectral data [25].

Since its invention, GC \times GC has been extensively used for the analysis of volatile compounds in different food related samples including cheese [42], pepper [43], oil [44], sour cream [45], coffee beans [46], honey [47], fish [48], grapes [50-52], and wine [49] to name a few.

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Sample preparation

Despite the advanced features of state-of-the-art gas chromatographic instrumentation, direct introduction of the sample into the analytical instrument is often impossible. This is mainly due to the presence of unwanted particles or of non-volatile sample constituents which are not compatible with GC analysis. Often the concentration of the analytes are simply too low to be detected by the instrument. This is especially the case in the analysis of food related samples where enrichment is of vital importance. Samples need to undergo a series of treatments in order to make them compatible with the analytical techniques. The aim of these processing steps is to isolate the analyte(s) of interest, although often this process is complicated when interfering compounds are present or due to analyte loss. Therefore, the dictum “the best sample preparation is no sample preparation” is indeed very correct. Still sample preparation is usually unavoidable. Numerous sample preparation techniques have been developed and successfully applied for different sample matrices. Only those used for wine volatile analysis will be discussed.

3.1. Solvent-based sample preparation techniques

A number of sample cleanup procedures using organic solvents have been employed for the analysis of wine volatiles. Classical liquid liquid extraction (LLE) [1-5] is based on organic solvent extraction and normally it involves the use of large amounts of toxic solvents. The separation or extraction of the analytes from the matrix depends on their distribution coefficient (K) between the two phases (organic and aqueous). At equilibrium, an analyte x can be distributed between the two phases as:

$$K_x = \frac{C_{x,Organic}}{C_{x,Aqueous}} \quad (3.1)$$

where K_x is the equilibrium distribution coefficient of an analyte x between the two phases, $C_{x,Organic}$ and $C_{x,Aqueous}$ are the equilibrium concentrations of analyte x in the organic phase and in the sample, respectively [6]. Various organic solvents including dichloromethane, ether, pentane, etc. separately or as a mixture of two or more organic solvents have been used during extraction [2,7].

Efforts have been made to improve the classical LLE both in terms of its environmental friendliness as well as cost effectiveness. The development of a

miniaturized version of traditional LLE known as micro liquid liquid extraction (μ LLE), where the amount of solvent used is drastically reduced (μ L levels) minimizes the danger to the environment and the high solvent cost. In μ LLE the amount of solvent used is reduced, leading to an increase in the sensitivity and lower limits of detects (LODs). Other advanced solvent extraction techniques such as single-drop micro extraction (SDME), where a micro-drop of solvent suspended from the tip of a conventional microsyringe, have been developed. In SDME the solvent would be exposed to the headspace of the sample or immersed in the sample solution where extraction takes place [8,9].

3.2. Solid phase extraction (SPE)

In solid phase extraction (SPE), small cartridges filled with suitable sorbent materials are used as a stationary phase and conditioned before loading the samples. After the sample is loaded, undesired substances can be rinsed from the cartridge and the analytes of interest remain bound to the material. Thereafter, these analytes are eluted with a strong solvent. Loading, rinsing and elution are performed using either a vacuum manifold or by applying positive pressure. SPE involves different separation processes including liquid-solid partitioning, adsorption, affinity or ion exchange, which permits selection of the most suitable stationary phase, depending on the application. Broad varieties of SPE stationary phases are available including the apolar C_8 and C_{18} silica bonded phases, as well as polymeric resins such as styrene-divinyl benzene (SDVB) and polar materials such as alumina and silica. Ion-exchange adsorbents, which allow for retention of analytes based upon charge, provide permanently charged moieties across a range of solvent conditions and pHs. Mixed-mode-materials, exploiting both primary and secondary mechanisms for selective retention of analytes and some very specific selective adsorbents are also available. These different phases enable the separation of analytes based on adsorption, H-bonding, polar and apolar interactions, cation and anion exchange or size exclusion to be utilized in extraction. The most commonly used stationary phases for the analysis of organic compounds are C_{18} and polymeric styrene-divinyl benzene (SDVB). SPE offers several advantages including high sensitivity, low solvent consumption, high selectivity, and less time. In addition, SPE can give the benefits of automation and can be used in field sampling [8-11].

Figure 3.1. shows an example of a typical SPE extraction procedure. The loaded sample (black) is cleaned with different rinsing solvents in steps 1 and 2 to remove weakly retained substances, before eluting the analytes of interest using a strong solvent (step 3). In this fashion, SPE can be used as a highly selective extraction technique where unwanted substances are eliminated using different solvents in successive steps. The choice of elution solvent can be decisive, particularly if the analytes of interest are highly volatile. The solvent should be apolar and volatile enough, and it should be able to elute the analytes from the stationary phase using a small volume. If the analytes of interest are only semi-volatile, a solvent with good elution strength for most SPE adsorbents should be used. This is because the solvent forms an azeotropic mixture with water (during the transfer since small traces of water may be left in the adsorbent), which eventually evaporates smoothly [9,12]. SPE have advanced over the years and was successfully applied for the analysis of wine volatiles [7,13-18], including acids, alcohols, esters, carbonyls, lactones, terpenes, volatile phenols, methoxy pyrazines, and sulfur compounds.

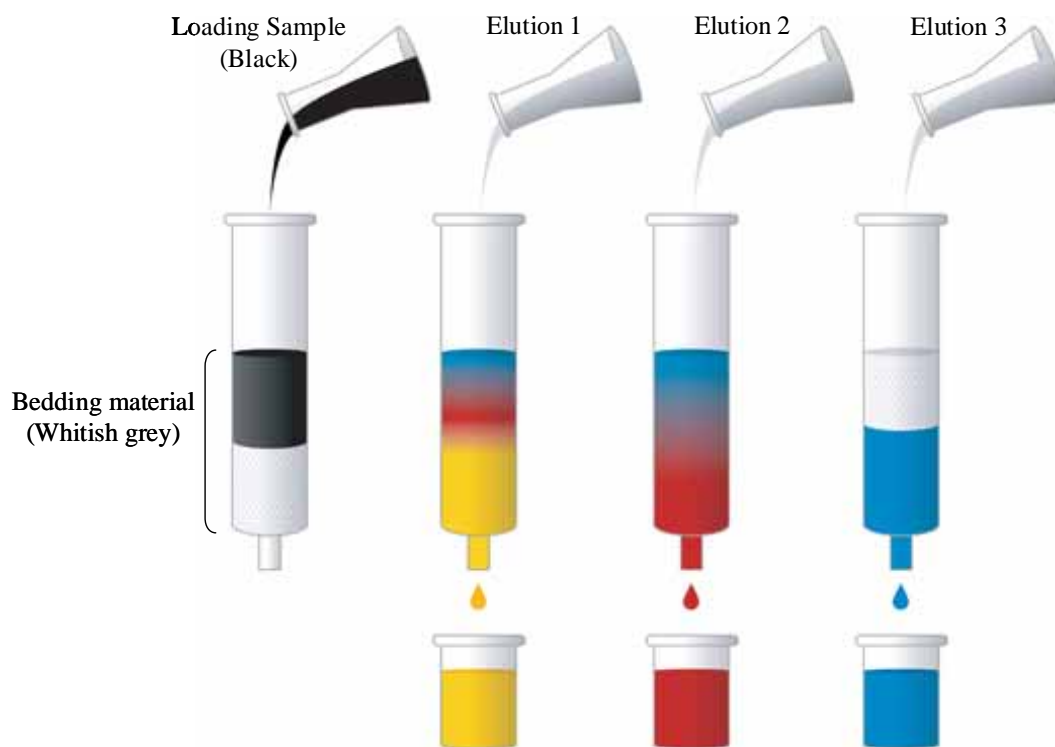


Figure 3.1. A sketch of solid phase extraction procedure: Elution 1 to 3 includes removing the unwanted substances to the collection of the analytes of interest. (Adapted from Waters' website [12]).

3.3. Sorptive sample preparation techniques

In contrast to adsorptive extraction, in sorptive extraction analytes are not bound to material but rather retained in the bulk of a polymeric stationary phase. The sorption process is more advantageous than the adsorptive process as it is a lower energy process in which surface-catalyzed reactions, which can easily occur in the adsorptive due to the strong bonding with the adsorbent material, are minimal. As a result polar compounds are more easily removed from the extraction phase. The most common sorption material used is polydimethylsiloxane (PDMS). Due to several advantages including its inert nature, which reduces the risk of analyte alteration, the relative ease with which it can be synthesized and the high temperature stability, PDMS is a very widely used sorptive material. In addition, the breakdown products of the polymer can easily be identified with the use of an MS detector, which minimizes the ambiguity during identification as a result of artifacts [8,17]. In the past three decades different sorptive extraction techniques based on PDMS, including open tubular traps (OTT's) [19], solid phase micro extraction (SPME) [20,21] and stir bar sorptive extraction (SBSE) [22] were developed. In this chapter only the latter two will be discussed.

3.3.1. Solid phase micro extraction (SPME)

Due to the drawbacks involved with solvent-based or adsorptive materials, scientists are in constant search for better sample preparation techniques. In the early 1990s Arthur and Pawliszyn developed a new PDMS based sorptive extraction technique, solid phase microextraction (SPME) [20]. It involves a small fused silica fibre (commonly 1 cm length and 0.11 mm internal diameter), coated with a polymeric phase (in the range between 10 to 150 μm). For protection, the fibre is mounted in a syringe type holder, **Figure 3.2**.

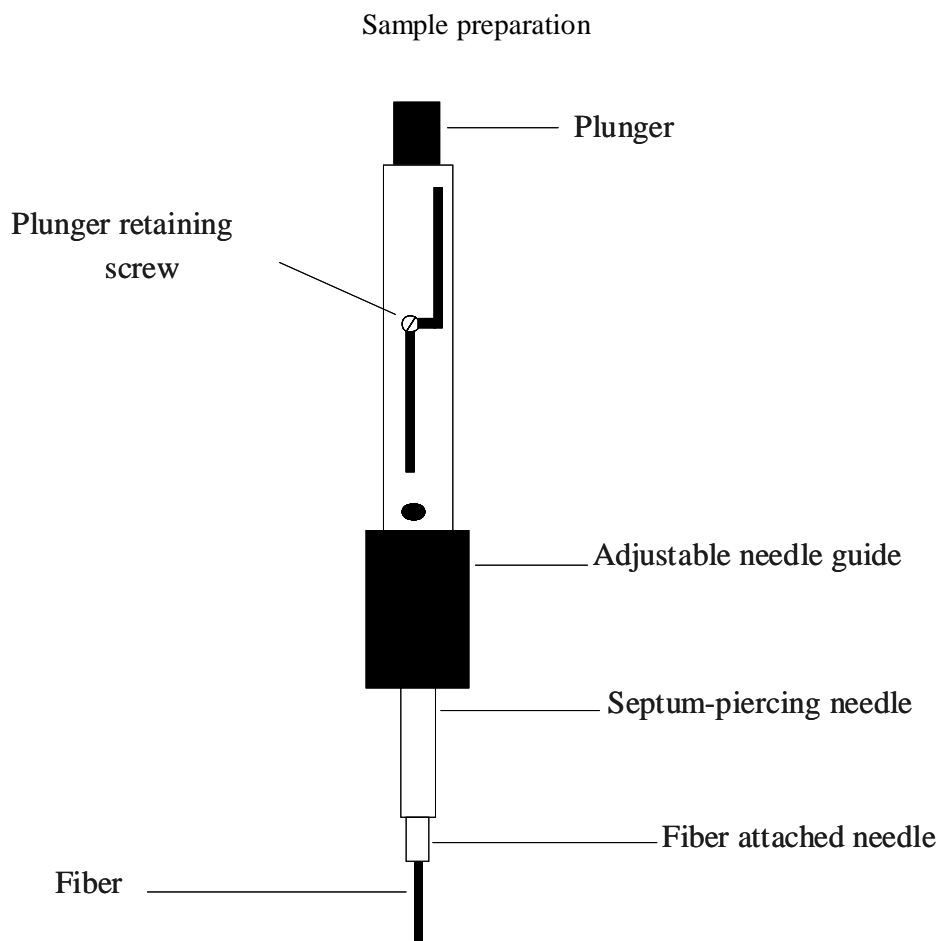


Figure 3.2. Diagram of an SPME device. (Adapted from [17]).

During SPME extraction analytes are retained by the fibre due to partition between the polymeric stationary phase and the matrix of the sample. The octanol–water partition coefficient ($K_{o/w}$) serves as a good indicator of how well, if at all, a given solute can be extracted. SPME does not require the use of organic solvents, accordingly eliminating some of the drawbacks from which LLE and SPE suffer. It is a simple, rapid and economical technique where the extraction and concentration processes are performed concurrently. Furthermore, SPME uses only small amounts of sample. After sampling, the SPME device can be coupled easily to a gas chromatography system for analysis [8,9,17,23].

There are three sampling modes in SPME: direct extraction, headspace extraction, and membrane protected extraction (**Figure 3.3**). In direct extraction, the coated SPME fiber is directly immersed in the sample and the analytes are distributed between the sample matrix and the fiber coating. In the headspace mode, the fibre is suspended in the headspace of the sample container, where the analytes need to be transported through the vapor phase above the liquid before they can reach the coating. This mode of extraction prolongs the life of the fibre. Membrane protected SPME extraction is

Sample preparation

used when highly contaminated or very dirty samples are analysed, in order to protect the fiber from damage. Membrane protected SPME is advantageous for the determination of less volatile analytes. Moreover, when using an appropriate membrane, it can be used for selective extraction of target compounds. Agitation of the sample using a magnetic stirrer or shaking can speed up the extraction process [17,24-26].

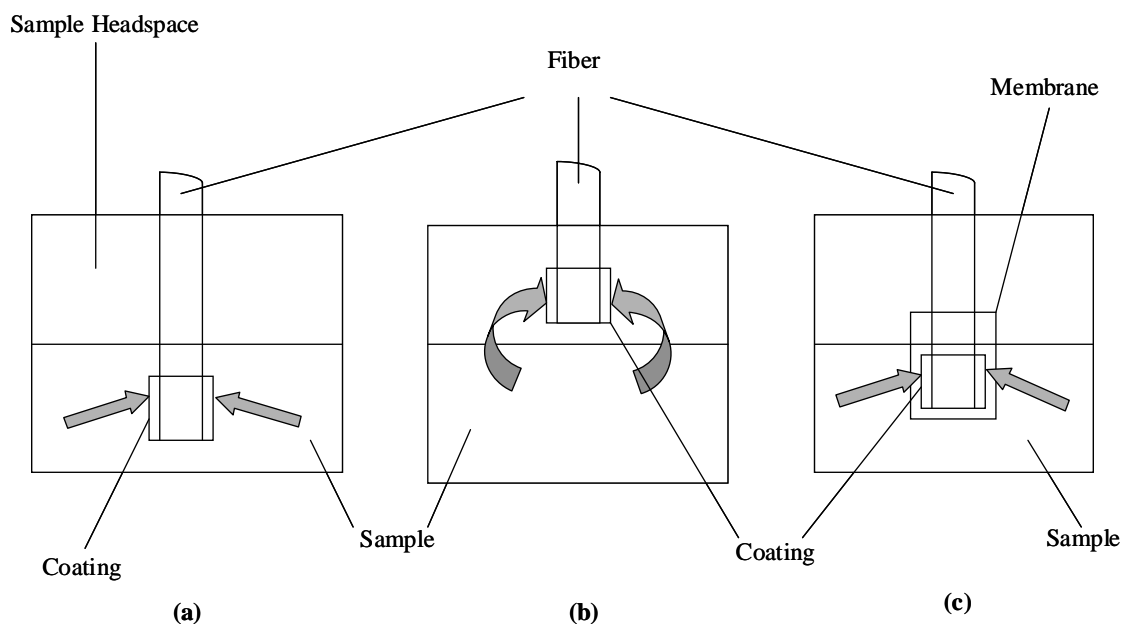


Figure 3.3. Modes of SPME process: (a) direct SPME, (b) headspace SPME, (c) membrane-protected SPME. (Taken from [25]).

The thermodynamics of SPME are affected by certain extraction conditions, through their effect on the distribution of analytes between the fibre and the matrix. These parameters include the type of coating, extraction temperature and time, ionic strength, pH, volume of the sample, volume of the head space, agitation of the sample, and shape of the sample container [27]. These parameters can have a very significant influence on the ability of SPME to extract analytes of interest from the sample matrix. If for instance the SPME is operated in the headspace mode for the extraction of volatiles from aqueous sample, adding salt would increase the ionic strength of the sample and enhance the migration of compounds to the headspace. Similarly, lowering the pH of the sample could neutralize dissociated acids by protonating, which could enhance the movement of the acids to the headspace and their interaction with the stationary phase, PDMS in particular. The type of coating is selected based on the type of analytes in the sample matrix in relation to analytes of interest. Currently, diverse stationary phases, with different polarities are commercially

available, providing different selectivity [17,28,29]. The most frequently employed phases with their respective properties are given in **Figure 3.4**.

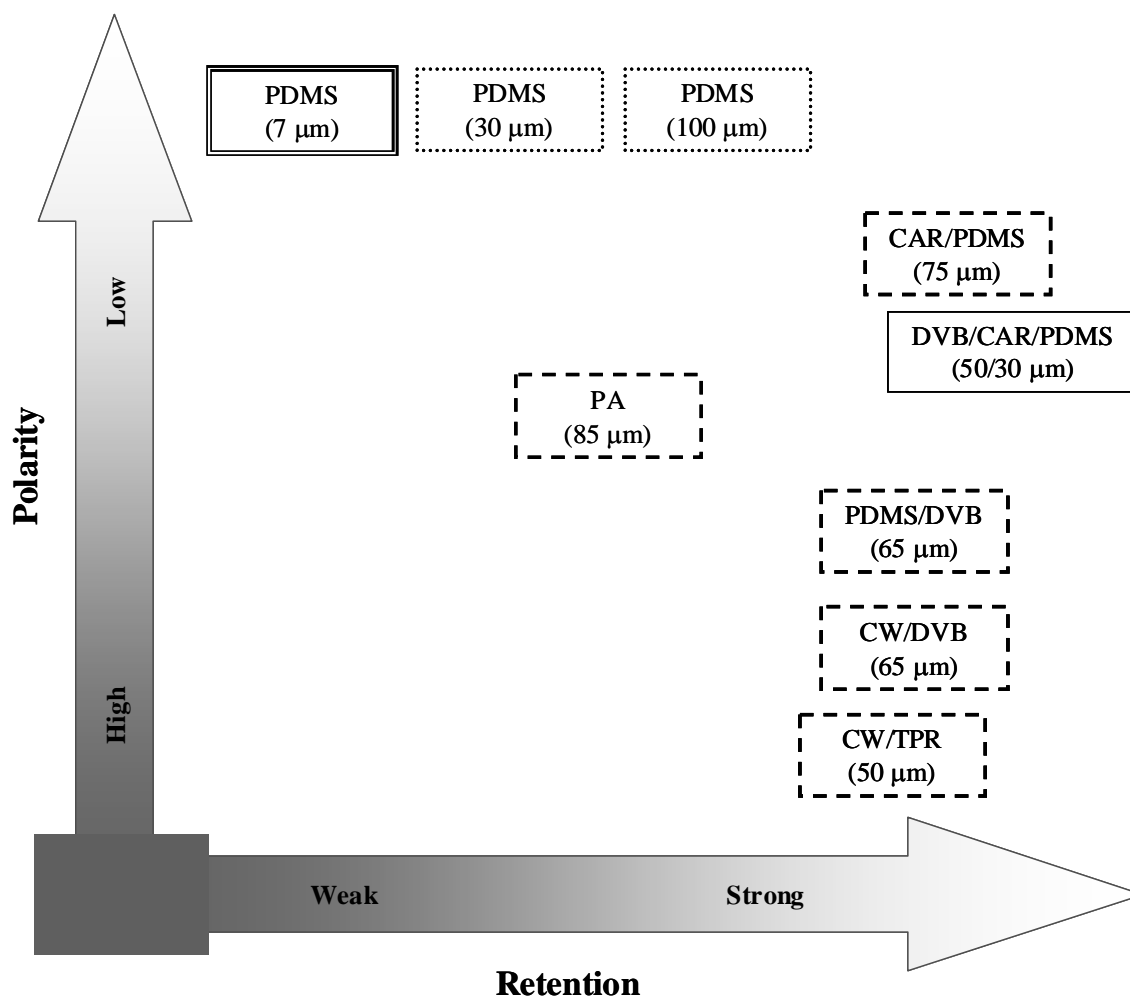


Figure 3.4. Properties of commercially available SPME fibers. Bonded, non-bonded, Partially cross-linked, highly cross-linked. Full interpretations of the abbreviations are: polydimethylsiloxane (PDMS), Carboxen (CAR), divinylbenzene (DVB), polyacrylate (PA), Carbowax (CW), and templated resin (TPR). (Reprinted from [29]).

As an apolar phase, PDMS has higher affinity for apolar compounds. However, it can be used to extract medium polar compounds. On the contrary the polar phase, polyacrylate (PA) is suitable for compounds with higher polarity. Both PDMS and PA extract analytes via sorption of analytes, which dissolve and diffuse into the coating material. In contrast, the mixed phases such as carboxen/divinylbenzene (CAR/DVB), carbowax/templated resin (CW/TPR), PDMS/CAR, and PDMS/DVB extract via adsorption of analytes on the surface of the fiber [30]. The amount of analyte extracted by the coating is directly proportional to the analyte concentration in the sample, the thickness of the polymer coating and the distribution constant of the

analytes. Since its invention SPME has extensively been applied for the analysis of wine volatiles [17,31-39].

Regardless of the numerous advantages detailed above, SPME still suffers from some drawbacks, such as:

1. The volume of the polymer extraction phase is very small, which limits the sample capacity and requires extreme precision during manufacturing of the coating.
2. The quality of the fibers depends on the manufacturer, and sometimes their performance varies from batch to batch.
3. Some level of degradation of the fiber occurs during repeated usage.
4. Fibers are fragile and can easily be broken.

3.3.2. Stir bar sorptive extraction (SBSE)

SBSE was invented in 1999 by Baltussen et al. [22,23]. SBSE is another sorptive technique, where a magnetic stir bar encapsulated in a glass sleeve and coated with PDMS (**Figure 3.5.**) is used for extraction. SBSE follows the same principle as SPME except that the former method uses larger amounts of stationary phase (PDMS) ranging from 55 to 219 μl (50 to 250 times greater than SPME), which provides higher sample capacity and improved sensitivity [40].



Figure 3.5. Diagram representing a PDMS coated stir bar. (Adapted from [24]).

SBSE can be performed either in the headspace mode (**Figure 3.6.**) or by directly immersing the PDMS coated stir bar into the aqueous matrix. In the former mode the extraction is performed by suspending the PDMS coated stir bar in the gas phase of the sample and a magnetic stirrer is used to agitate the sample in order to enhance the migration of volatile and semi-volatile compounds into the headspace where they can partition into the PDMS phase. In the direct SBSE mode of extraction, the stir bar is directly immersed into the aqueous sample and used as a stirrer while trapping the organic compounds. In spite of increasing equilibrium times compared to direct immersion SBSE, headspace extraction offers benefits in reducing the risk of

Sample preparation

contamination and increasing the lifetime of the PDMS phase (especially for very dirty or complex samples) [41-45]. After sampling, the stir bar is removed, rinsed using small amounts of deionized water, and dried gently with lint free tissue to avoid droplets of moisture and remove chemicals like proteins or sugars that can degrade the stationary phase, especially when used in the direct mode. Analytes are normally desorbed thermally. The stir bar is placed in a glass tube and positioned in the thermal desorption unit. Analytes released thermally from the polymer are typically trapped in a GC inlet at a very low temperature, commonly ranging between -100 to -50 °C. Once desorption and re-trapping process is completed, analysis and data recording will begin using a GC system.

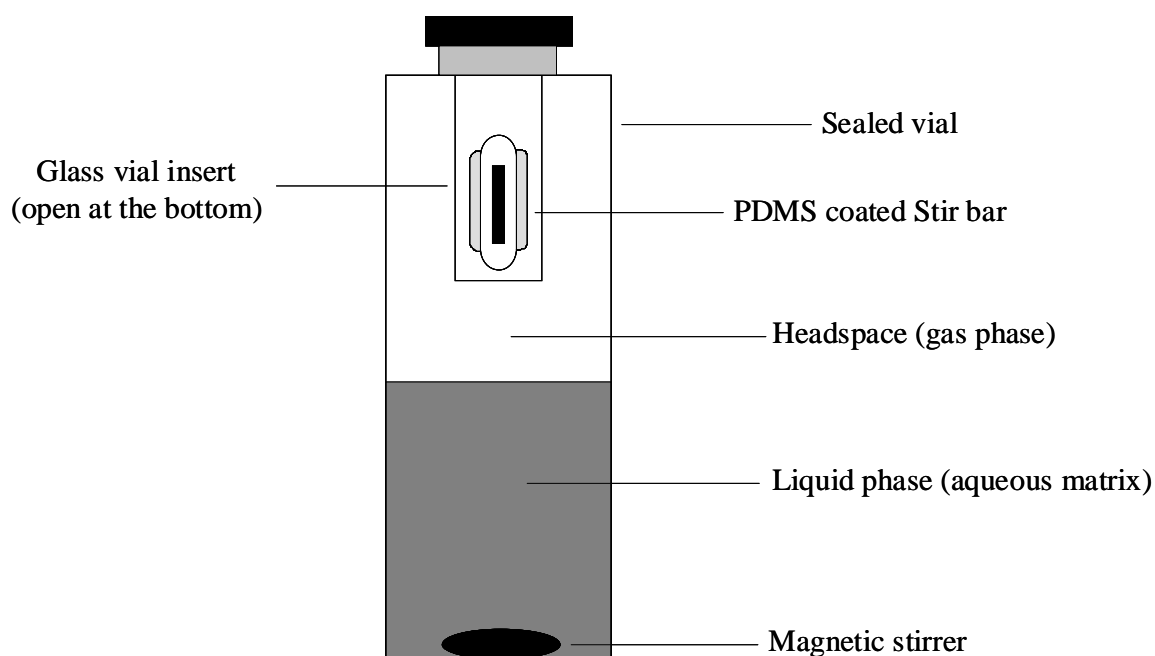


Figure 3.6. Schematic representation of headspace SBSE. (Adapted from [46]).

Recovery of a compound from a water sample by SBSE can be calculated from the sample volume, the volume of PDMS phase and the analyte's octanol-water distribution coefficient ($K_{o/w}$). In principle, SBSE follows similar thermodynamics as SPME. It is assumed that the partitioning coefficient between PDMS and water ($K_{PDMS/W}$) is roughly similar to the octanol-water partition coefficient ($K_{O/W}$), hence:

$$K_{O/W} \approx K_{PDMS/W} = \frac{C_{SBSE}}{C_W} = \frac{m_{SBSE}}{m_W} \times \frac{V_W}{V_{SBSE}} = \beta \times \frac{m_{PDMS}}{m_W} \quad (3.2.)$$

Sample preparation

where C_{SBSE} and C_W correspond to the analyte concentration in the PDMS coated stir bar and water phase, respectively. m_{SBSE} and m_W are the mass of analyte in the PDMS coated stir bar and water phase, respectively. V_{SBSE} and V_W correspond to the volume of the stir bar coating and the water phase, respectively, and β is the phase ratio, which is equal to V_W/V_{SBSE} . Using simple mathematical rearrangement, equation 3.2. can be re-written as:

$$\frac{K_{O/W}}{\beta} = \frac{m_{SBSE}}{m_W} = \frac{m_{SBSE}}{m_o - m_{SBSE}} \quad (3.3.)$$

where m_o is the total mass of the analyte originally present in the water sample. In sorptive extraction techniques, the extraction efficiency or recovery is expressed as the ratio between the extracted amount of analyte in the stationary phase (m_{PDMS}) and the initial amount of analyte originally present in the water ($m_o = m_w + m_{PDMS}$). Therefore, equation 3.3 can be rearranged for calculating recovery as:

$$\frac{m_{SBSE}}{m_o} = \frac{\left(\frac{K_{O/W}}{\beta}\right)}{1 + \left(\frac{K_{O/W}}{\beta}\right)} \quad (3.4.)$$

From the above equation, the main parameter that determines the recovery of an analyte from the sample is the ratio of the partitioning constant and the phase ratio. This implies that when the $K_{O/W}/\beta = 1$, the recovery is 0.5 (50%). As the $K_{O/W}/\beta$ decreases, the recovery becomes closer to $K_{O/W}/\beta$ and at values of $K_{O/W}/\beta$ more than 5, extraction is essentially quantitative (**Figure 3.7.**) [22].

As outlined above, the recovery of an analyte from a water sample during SBSE extraction depends on the sample volume, the volume of the stationary phase (PDMS) and the analyte's octanol–water distribution coefficient ($K_{O/W}$). In SPME the maximum volume of PDMS in the fibre is 0.5 μl for a 100 μm thick film. This shows that a sample volume of 10 ml will have a phase ratio of 2×10^4 which indicates that quantitative extraction is only achieved for analytes with $K_{O/W}$ larger than 10^5 . A very limited number of compounds reveal such high value of $K_{O/W}$. On the other hand, a stir bar coated with a 100 μl PDMS used to extract from 10 ml of water sample provides a phase ratio (β) of 100. This indicates that analytes with a $K_{O/W}$ of > 500 are

extracted quantitatively (**Figure 3.7.**). This provides simple quantification and at the same time an increase in sensitivity for compounds with $K_{O/W}$ below 10^5 [8,22,23,40,47,48]. It has also been reported that the film thickness has a more pronounced effect on the recovery compared to the length of the stir bar [49].

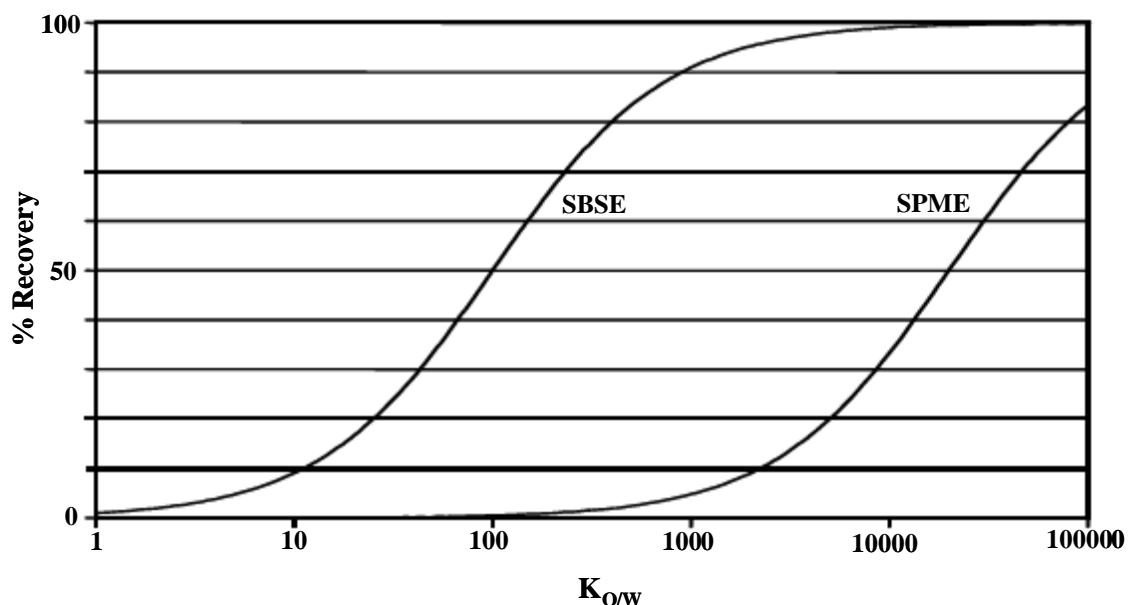


Figure 3.7. Theoretical recovery of a compound using a volume of 100 and 0.5 μl PDMS in SBSE and SPME extraction methods, respectively, from a 10 ml of water sample as a function of their octanol–water partition coefficient ($K_{o/w}$). (Adapted from [22]).

In spite of the advantages of SBSE outlined compared to SPME, the method suffers from lack of variety of stationary phases. Currently PDMS is the only commercially available phase. It is therefore difficult to extract polar compounds from the sample, which leads to low recovery. However, with some adjustments to the sample including its pH, it is possible to improve the recovery of polar analytes like acids [50,51]. Due to the availability of different stationary phases (including polar ones) as outlined above, SPME is a better choice for polar compounds. Dual-phase stir bars, the integration of PDMS with other polymers, have showed better performance but are still not available on the market [52]. Likewise, the need for a thermal desorption unit for SBSE makes this approach more costly. Liquid desorption (LD) can be an option, but only with very highly concentrated samples. Since its development, SBSE techniques have been applied extensively for the analysis of volatile compounds responsible for the aroma and flavor of wine [45,50,53-58].

Despite advancements in science and technology as well as increase the global popularity of South African wines, not much research has been performed regarding

Sample preparation

wine aroma and flavor. In an exceedingly competitive market, wine producers are investing in technology to increasing production as well as improve the quality of their product. As a result, the South African wine industry has in 2006 launched a project aimed at studying the characteristic nature of South African wines based on their chemical constituents, including their volatile content. Chapters 5 – 9 will include reports of the work done as part of this project.

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Chemometric data analysis

As mentioned in the previous chapter, the amount and nature of volatile compounds in wine can be affected by different factors including both viticultural and enological factors. In order to relate meaningfully the correlation between volatiles of wine and the different factors that influence them, a suitable statistical approach should be applied.

The intention of this chapter is not to go into the details of chemometric methods but rather to highlight the important statistical methods commonly used in classifying and categorizing food related samples.

Chemometrics has a broad definition and can span a wide area both in the social [1] and natural [2] sciences. According to Massart et al. [2], chemometrics is defined as “a chemical discipline where mathematical, statistical and related methods that use formal logic to design or select optimal measurement procedures and experiments as well as to provide maximum relevant chemical information by analyzing the chemical data”. Chemometrics link the methods used and their application in extracting useful information for a data set (raw data measurements). In the natural science field and most importantly in chemistry, chemometrics can be very useful in transforming inputs of chemical data into meaningful and more structured patterns. Inputs can be primary or secondary (even tertiary) depending on the factors that have major or minor influence on the data set [3]. For instance, spectroscopic measurements of volatile constituents in wine can be classified as primary data. The factors that have direct effect on the content of wine volatiles such as vineyard and climatic conditions as well as wine-making processes are of secondary inputs. Other factors that influence the amount of volatiles in wine indirectly can be regarded as tertiary data. For example, the concentration of amino acids in wine affect the performance of yeasts and the formation of volatile compounds during fermentation [4], and can be regarded as tertiary input [3]. These are some of the main reasons why the application of chemometrics is so important in dealing with very complex information, where it is almost impossible to bring all the information together and interpret them in a meaningful fashion.

Scientists in many fields, and those in the food and beverage industry in particular, are facing challenges in ensuring the quality of their products in meeting certain standards with increasing requirements from the regulatory agencies [5]. In addition, the need to

satisfy consumers is also another pressure that would require good quality products. Many of the quality control processes have traditionally been done by experts, who are in a position to establish a products quality by color, texture, taste, smell/aroma, and other means. It requires years of experience to obtain these skills. This is where chemometrics can be very useful as it often results in a faster and more precise evaluation of composition. The application of chemometrics can be used either to predict a property of interest or to classify the samples into one or many categories [6]. Realizing these advantages, many researchers have applied different chemometric methods for characterizing wines based on their volatile constituents. These methods include analysis of variance (ANOVA) [7-19], factor analysis (FA) [7,9,20], principal component analysis (PCA) [7-9,11,17-19,21-23], discriminant analysis (DA) [8-10,17,18,23], cluster analysis (CA) [8,19,21], etc. In this chapter only the former four methods will be highlighted.

4.1. Analysis of variance (ANOVA)

Analysis of variance (ANOVA) is a parametric statistical method that makes comparisons between two or more means and can be used to determine the existence or absence of significant relationships between variables. It can also be used to distinguish similarities and differences between variables. ANOVA estimates the effect of categorical factors by testing for a difference between categorical means in a continuous response of variables of interest. ANOVA uses the F statistic to compute the probability P of an effect at least as big as that resulting by chance from the null hypothesis. The null hypothesis, a statistical hypothesis stating there are no differences between observed and expected data, is rejected and the factor is considered to have a significant effect, if P is less than some predetermined threshold α , commonly set at 5% (0.05) [1,2,5,24].

Depending on the number of factors (variables) taken into consideration, ANOVA analysis can be categorized as one-way (only one factor or variable), two-way (two factors or variables), etc.

One-way ANOVA can be very useful in comparing means of two or more samples using the F distribution and can be used only for numerical data. It produces an F statistic which is defined as the ratio of the variance among the means to the variance

within the samples. Basically, F is a comparison of the variance amongst the different groups to the variance amongst all the individuals within those groups. A higher ratio implies a lower P -value which indicates the presence of significant differences between the groups [1,2]. A simple example would be the influence of grape variety (one factor) on the volatile composition of wine samples i.e. studying the presence of significant differences among the wine volatiles composition of different wines from different grape varieties. A significant p -value resulting from a one-way ANOVA test would indicate that a grape variety is differentially expressed in at least one of the groups analyzed. If there are more than two groups being analyzed, however, the one-way ANOVA does not specifically indicate which pair of groups exhibits statistical differences. In this case, Post Hoc (Posterior) tests can be applied in order to determine which specific pair/s are differentially expressed. Post-hoc tests (or post-hoc comparison tests) are used as a next step during ANOVA if the null hypothesis is rejected. The focal point at this stage is to identify the groups that differ significantly from others with regard to their mean values. A typical example of Post-hoc tests is the Tukey's test, where a new critical value that can be used to evaluate significant differences between any two pairs of means is calculated. The critical value is slightly different as it involves the mean difference that have to be exceeded to achieve significance. This is done by calculation of one critical value, followed by determination of differences between all possible pairs of means. Each difference is then compared to the Tukey's critical value. If the difference is larger than the Tukey's value, the comparison is significant [2].

If during the ANOVA analysis, there is another factor which can potentially influence the outcome, it can be dealt with using main effects ANOVA. It is the effect of the factor alone averaged across the levels of other factors. For example, if it was intended to do analysis of variance (ANOVA) on the volatile composition of wines based on their geographical origin, and at the same time wines from the same region could be of different grape varieties, it is then expected that the outcome would be unrealistic. This is because the effect of different grape varieties will have an influence on the volatile composition of the different wines from the same region. In this particular situation the analysis can be done using main effects ANOVA, where the influence of the other factor (in this case the grape variety) will be taken into account [9].

The effect of one variable at a time can be analyzed by ANOVA and can provide useful descriptive information, but this will be limited when it comes to relationships among variables and other important relationships in the matrix. Since univariate analyses do not take into account the effect of more than one variable, the result could be oversimplifying the model of analysis. Hence, it is vital to look at all the interactions and possible causes that will have an impact on the outcome of the result. There is a need to understand all the complicated interactions between the constituents as well as their combined effects on the whole matrix. Unlike classic univariate statistics, multivariate data analysis considers multiple variables simultaneously and has the advantage of also taking co-linearity into account. Multivariate analysis can take into consideration the variation in one or a group of variables, in terms of co-variation with other variables [5].

4.2. Factor analysis (FA)

Since its introduction by Spearman in 1904 [25], factor analysis (FA) has advanced over the years from being a controversial and difficult subject to one of the most fascinating and useful tools for data analysis. FA is a multivariate statistical mode of operation used for complex data analysis to maximize information while reducing the number of variables to a few sets of factors. FA can be very useful in chemistry and especially in separation sciences because it reduces the number of variables, which makes it easier to interpret and correlate the information. It can also look for certain qualitative and quantitative distinction in a data set. FA involves the preparation of a data matrix, reproduction of principal factors, target testing for individual factors, rotation (orthogonal or oblique transformation) for a possible clustering, combination and prediction of new data set [2,3,26-28]. Due to the rotation to achieve better clustering and easily interpretable information, FA is more desirable than principal component analysis (see section 4.3. below) for some applications [29].

How many factors to choose is somewhat an arbitrary decision. Several approaches have been proposed as guidelines and in practice they seem to give good results. The Guttman-Kaiser criterion [27,30] for instance includes all the factors that retain eigenvalues of ≥ 1 . This is equal to saying unless a factor extracts at least as much as the equivalent of one original variable, it should be ignored. Even though this method is very popular and widely used, it might show some drawbacks if the data matrix

generates too many factors with eigenvalues close to 1, such that reduction of data dimensionality fails. The Scree test method proposed by Cattell in 1966 [31] uses a simple graphic plotting of the eigenvalues. This method suggests finding the place where the smooth decrease of eigenvalues appears to level-off, where any factor to the right of this point is ignored. (Scree is a geological term referring to the debris which collects on the lower part of a rocky slope). Theoretically, these two approaches can be evaluated before deciding which one to apply on a randomly generated dataset. The first method (Kaiser criterion) sometimes retains too many factors, while the second technique (Scree test) sometimes retains too few; however, both do quite well under normal conditions, that is, when there are relatively few factors and many cases. It must be noted that the number of factors can also depend on the total variability explained (covered) in the new dimension, where higher percentage (70% and above) is always preferable, although sometimes the percentage value can be as low as 50% as the main goal remains to reduce data dimensionality (factors) [29].

Another approach for selecting the number of factors is parallel analysis (PA). In PA the actual data are factor analyzed, and separately one does a factor analysis of a matrix of random numbers representing the same number of cases and variables. For both actual and random datasets, the number of factors on the x-axis and cumulative eigenvalues on the y-axis are plotted. Where the two lines intersect determines the number of factors to be extracted. In PA, the focus is on the number of components that account for more variance than the components derived from random data [32]. The plot of PA can also be integrated with the Scree test plot for comparison [9].

Results of FA can be presented either as factor scores or factor loadings. Within a multivariate linear model, any scores that are given weights and added together are defined as factors of the resulting variables. The weights are often referred to as factor coefficients or loadings, even though both terms are occasionally used to refer to the correlation between variables and factors [27]. The values of loadings and scores can run from +1 to -1, where +1 indicates full agreement, 0 indicates no relationship and -1 indicate complete disagreement. Factor scores are the scores of each case on each factor. On the other hand, factor loadings are the correlation coefficients between the variables and factors. In confirmatory factor analysis, loadings should be at least 0.700 and above to confirm that independent variables identified a priori are

represented by a particular factor, on the rationale that the 0.700 level corresponds to about half of the variance in the indicator being explained by the factor. Nonetheless, this value of standard is high and in real-life data may well not meet this criterion. Hence, some researchers, particularly for exploratory purposes, will use lower levels including 0.400 for the central factor and 0.250 for other factors [33]. In any event, factor loadings must be interpreted in the light of theory, not by arbitrary cutoff levels. It can also depend on the number of samples.

4.3. Principal component analysis (PCA)

Principal component analysis (PCA) is by far the most common form of multivariate technique of exploratory data analysis that seeks a linear combination of variables such that the maximum variance is extracted from the variables. It then removes this variance and seeks a second linear combination which explains the maximum proportion of the remaining variance, and so on. These linear combinations (axes) of new dimensions are known as principal components (PCs). These PCs result in orthogonal (uncorrelated) axes and analyze total (common and unique) variance [2,29,34]. PCA is related to FA as both methods look for a simple structure in a set of variables by reducing dimensionality but they also differ in many scenarios. Rencher [29], has pointed out the following differences between PCA and FA:

1. In FA variables are expressed as linear combinations of factors, whereas in PCA principal components are linear functions of variables.
2. FA's effort is in explaining the co-variance, in contrast PCA attempts to explain total variance.
3. FA makes several key assumptions but PCA requires no assumption.
4. In FA factors are subject to an arbitrary rotation whereas PCA's principal components are unique with distinct eigenvalues.
5. In FA if the number of factors changed, the estimated factors are likely to change, which does not happen in PCA.

PCA is one of the oldest multivariate methods and was developed by Pearson [35] in 1901. Since then the technique has increased tremendously its popularity. The first step in PCA analysis is to start with a correlation matrix. This places the measurements on different variables on the same scale and the variances have similar magnitude. The next step would be identifying the number of PCs and this can be

done in different ways. One would consider the percentage of the cumulative proportion of total variance by defining the minimum variation desired or expected, which is the most commonly used approach in PCA analysis. The second approach is based on the magnitude of the variances explaining each PC, which measures the eigenvalues of the correlation matrix associated with each principal component. In this case it is assumed that all the standardized variables will have a variance of one and any principal component with variance < 1 is not selected as it provides insignificant information compared to the original variable/s. Since the eigenvalues are standard output of the statistical procedure, it would be easy to implement. The third approach is using the scree test plot in a similar fashion as was explained for FA. The idea behind using this plot is that in consecutive measurements the difference between successive eigenvalues becomes smaller and smaller, in turn making it easier to identify the important PCs [1,2,28,29,34].

In a similar fashion as we saw in the case of FA, in PCA analysis, data can be presented either as a scores plot of cases (samples) or a loading plot of variables using a combination of the chosen principal components. The score plot involves the projection of objects (cases/samples) as data points onto the PCs dimensions, where both x- and y-axes contains user-selected PCs. The plot contains points that represent the original data set. The score plot can be examined using combinations of either pairs of the principal components, where commonly the first two PCs represent the direction of highest fraction of the overall variability in the data set. The initial plot of the data points can lead to easy identification of outliers or nonlinearity, which can be removed if their contribution to the variability of the PCs is insignificant. However, sometimes removing an outlier can cause loss of vital information. Hence, it should be done with cautious and careful examination. Generally the first two or three PCs are sensitive to outliers that could potentially raise variances or deform covariance. At the same time, the last few PCs are equally sensitive to outliers in introducing false dimensions, or possibly hiding singularities. For these reasons, it is advisable to evaluate scores plots of at least the first two and the last two PCs in order to investigate the presence of outliers. Similar approach can be taken to loading plots of variables (see below), although a more pronounced effect can be seen in score plots. Apart from the mentioned advantages, scores plot can be very useful in revealing clustering (grouping) of points. This grouping pattern shows the multivariate normal

distribution of the data set in the new dimensions and can be interpreted based on their location in the bi-variate plots. For instance, data points (samples) that exhibit high levels of the first principal component and low levels of the second principal component are displayed in the lower right corner of the plot and vice versa. At the same time those exhibiting equal levels towards the two components lie along the diagonal of the plot.

PCA results can also be presented using loading plots of variables and interpreted accordingly. In multivariate methods such as PCA, loading plots are regarded as very important to find the relevant components and the variables significantly associated with them [36]. In the same way as score plots (plots of score vectors), loading vectors are also plotted against each other. Loadings provide information on how the original variables are related to each other and to the principal components by constituting a link between the variable space and the PCs space. It can show the variable similarities (inter-variable relationship) and also how much each variable contributes to each principal component. Similar trend as for score plots highlighted above can be followed during interpretation of variable loadings. Variables that possess high loading on the first principal component and low loading on the second principal component are displayed in the lower right corner of the loading plot and vice versa. Similarly, those variables exhibiting equal loadings towards the two components lie along the diagonal axes of the plot (between the two axes) [1,34]. These plots can also be interpreted in comparison with the score plots and can give valuable information especially in combining the information of objects (samples) and variables associated with the same PCs. In addition, both scores and loadings can be plotted together in a PCA bi-plot providing integrated information. A PCA bi-plot offers more dimensions compared to the ordinary scatter plots as both scores and loadings are visualized together, by displaying objects as data points in the two-dimensional space and variables as bi-plot axes, with a separate axis for each variable. These axes are similar to ordinary scatter plots and are calibrated based on the original scales of measurement. The axes are not perpendicular as in ordinary scatter plots but still used in a similar way to provide information on all variables in a single graph. The correlation of variables with objects (samples) and among the variables themselves can be investigated in either one or combinations of the following:

1. The trend in the weight (magnitude) of the variables
2. The size of the angles between the axes
3. The distances between axes
4. The distance between the data points (samples).

Variables with axes in close proximity are expected to be correlated considerably depending on the size of the angle formed by the axes. Regardless the type of correlation (positive or negative), axes with smaller angle have higher correlation and when they are at 90° with each other, their correlation is zero [9,28,37].

4.4. Discriminant analysis (DA)

Discriminant analysis (DA) is a statistical analysis used to predict the probability of the occurrence of an event or group separation. DA focuses mainly in separating distinctive sets of groups into two or more populations based on their similarities and/or differences. By doing so it can allocate an unknown group or object to one of the populations [28]. The main idea behind DA is to predict group membership based on a linear combination of the interval variables. The process starts with a set of observations where both group membership and the values of the interval variables are known. At the end of the process a model that allows prediction of group membership when only the interval variables are known will be created. Another reason for running DA is to understand the data set, as a careful examination of the prediction model that results from the procedure can provide insight into the relationship between group membership and the variables used to predict group membership. If one considers using DA for predicting wine samples of different grape varieties based on their volatiles, the first step would be splitting the data set randomly into two groups: the training and test sets. Pre-designed DA models will then be fitted using the training data set. The variables deemed as predictors at this stage will then be evaluated in the test set, and then be used as discriminants for unknown wine samples based on the selected predicting variables. Once this is done, variables that are considered as potential discriminants can be viewed by simple score or loading plots or, even a combination of both for specific variables [9,38].

For practical examples of the statistical methods highlighted in this chapter such as analysis of variance (ANOVA), factor analysis (FA), principal component analysis (PCA) and discriminant analysis (DA), readers are referred to **chapters 6 and 7**.

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**Application of a headspace sorptive
extraction method for the analysis
of volatile components in
South African wines[†]**

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Abstract

A headspace sorptive extraction (HSSE) in combination with thermal desorption gas chromatography-mass spectrometry (TD-GC-MS) method for the analysis of volatile components (alcohols, esters, carbonyls, acids, phenols and lactones) in wine samples was developed. Extraction conditions such as salting-out effects, sorption time, stirring speed, phase ratio, extraction temperature, and effect of pH were thoroughly evaluated as part of method validation. The method was very sensitive with LODs and LOQs between 50 pg/L to 299 µg/L and 0.2 ng/L to 0.996 µg/L, respectively. Repeatability for all the compounds was between 3 and 22%. The intermediate repeatability was obtained within the acceptable range. Out of 39 volatile compounds selected, 37 were detected and quantitated. The method was found to be simple, cost-effective, sensitive, and use a small sample volume. The method was successfully applied for the routine analysis of 79 young red and white wine samples from various South African districts.

Key words:

Stir bar, headspace extraction, CIS, TDS, volatile compounds, wine, GC, and MS.

5.1. Introduction

Wine is one of the most complex alcoholic beverages, resulting from enzymatic transformation of grape juices and its aroma responsible for much of such complexity [1,2]. Describing the wine aroma is far from a simple task for researchers because more than 800 components have been identified in the volatile fraction of wine including alcohols, esters, carbonyls, acids, phenols, lactones, acetals, thiols, terpenols, etc. It comprises different chemical characteristics covering a wide range of polarity, solubility and volatility. Furthermore, the existence of some of these constituents at a very low concentration (\leq mg/L) in wine and the unstable nature of some of these compounds giving rise to the appearance of artefacts due to oxidizing being in contact with air or degraded by heat or extreme pH, makes their analyses more complex [2-4].

Aroma of a wine is one of the major factors that determine the nature and quality of wine [3,5] and it can be influenced by the climate, soil type, geographical location, type of grape, fermentation processes, the container where fermentation and ageing takes place, to name a few [6-8]. Many of the aroma compounds in wine already exist in the grape but several are also formed during fermentation as well as maturation such as esters and higher alcohols [8-11]. Furthermore, a significant number of volatiles are formed during ageing or extraction from oak such as phenols, furans, oak-derived-vanillic compounds, to mention few [12].

Because of the complex nature of wine matrices and the low levels of some of the volatile compounds which are partially responsible for the aroma and flavor, sample enrichment is crucial for identification and quantification. This sample enrichment should allow for extraction, concentration, and isolation of analytes, thereby greatly influencing the consistent and accurate analysis of wine.

In the last few years several sample enrichment techniques have been developed that partially fulfill the above needs. Classical liquid-liquid extraction (LLE) [1-4,6,13] based on organic solvent extraction and solid phase extraction (SPE) [12,14,15] based on adsorbent materials where analytes are bound to active sites on a surface have been successfully applied to wine analyses. However, these methods may suffer from disadvantages such as time constraints, labor intensiveness and may involve multi-

step processes which may lead to analyte loss, as well as the use of toxic organic solvents.

In the early 90s a solvent free method called solid phase micro extraction (SPME) was developed by Pawliszyn and co-workers [16]. SPME has successfully been applied for the analyses of different wines [4,17-19]. Recently, a new extraction procedure for aqueous samples, named stir bar sorptive extraction (SBSE) was developed by Baltussen and Sandra [20,21]. SBSE is based on the same principle as SPME except that higher PDMS phase in SBSE (50 to 250 times greater amounts of extraction phase) [8,22] provides higher sample capacity. SBSE extraction can be done either in the headspace [23-25] or by introducing directly into the aqueous sample [5,26,27] and stirring for a given time. Regardless of the increasing in equilibrium time compared to direct SBSE, the headspace extraction is very advantageous in reducing the risk of contamination. Furthermore, it increases the lifetime of the PDMS coated stir bar when used in the headspace as a complex matrix and the presence of sugar, particularly in sweet wines, can lead to a faster degradation of the PDMS layer [28]. SBSE has been applied successfully for the analyses of aroma compounds in wine [5,8,23].

In this contribution we present a simple and cost effective extraction technique that allows analysis of a large number of volatile compounds which can potentially contribute to the aroma and flavor of wines in a single chromatographic run. This was done based on headspace stir bar sorptive extraction method. It involves sorption of volatile compounds into the PDMS phase of the stir bar from the headspace of the sample followed by desorption, cryo-trapping, and gas chromatography mass spectrometry analysis. Furthermore, given the lack of existing information, on volatile components in South African wines the developed method was applied to selected wine samples. The results are used to verify differences in the aroma constituents among similar or different cultivars according to their region and production technology. Moreover, it is important to underline that, as to the best of our knowledge the method is new with a new approach to sample preparation technique for screening volatiles in wine.

5.2. Material and methods

5.2.1. Wine samples

A total of 79 young wines of vintage 2005 were supplied by different cellars from various South African districts. The 64 are red wines of different cultivars (Pinotage, Shiraz, Cabernet Sauvignon, and Merlot), 16 from each cultivar of six different regions and produced by different cellars. The other 15 are Chardonnay white wine from different regions and produced by different cellars.

5.2.2. Chemicals and reagents

The following standards of volatile compounds and solvents were used: acetoin, *n*-propanol, *n*-butanol, isobutanol, furfural, diethyl succinate, ethyl butyrate, ethyl-D-lactate, hexyl acetate, 2-phenylethyl acetate, 2,6-dimethoxy phenol, eugenol, 5-(hydroxymethyl)furfural, propionic acid, *n*-butyric acid, isobutyric acid, *n*-valeric acid, isovaleric acid, ethyl octanoate, 4-methyl-2-pentanol (internal standard), acetone (pestanal), and NaCl (Fluka, Zwijndrecht, Netherlands); isoamyl alcohol, *n*-hexanol, 2-phenylethyl alcohol, 5-methylfurfural, ethyl hexanoate, *o*-cresol, *p*-cresol, whiskey lactone, vanillin, hexanoic acid, decanoic acid and ethyl decanoate (Aldrich, Steinheim, Germany); isoamyl acetate, methanol and absolute ethanol (HPLC grade) [(Riedel-de Haën (Steinheim, Germany))]; phenol and guaiacol (Sigma, Steinheim, Germany); acetic acid (Merck, Darmstadt, Germany), and Milli-Q water (University of Stellenbosch, Stellenbosch, South Africa) were used.

5.2.3. Preparation of synthetic wine

A global stock solution containing all the analytes was prepared in a synthetic wine matrix (12% ethanol in Milli-Q water) using different concentrations of analytes ranging from 1 mg/L for ethyl octanoate and ethyl decanoate to 1.6 g/L for acetic acid based on the collected data from different authors and VCF 2000 volatile compounds in food database [(1996–1999 Boelens, Aroma Chemical Information Service) (BACIS)] to make it as close as possible to real wine samples.

5.2.4. Equipment and apparatus

A 15 mL amber vial coupled with solid PTFE (polytetrafluoroethylene) line screw cap, (Supelco, Bellefonte, PA), 2 mL vials with green caps (Agilent, Technologies, Palo Alto, CA), 20 mL Twister headspace vials with glass inserts Twister (Gerstel, Müllheim a/d Ruhr, Germany), 20 mm magnetic aluminum crimp cap and 20 mm PTFE white silicone molded septa (Agilent Technologies, Palo Alto, CA), and JENWAY 4330 pH meter (Janway Ltd., Felsted, Dunmow, Essex, CM6 3LB, U.K.) were used.

5.2.5. Experimental conditions

The instrumental set-up was done in a similar way as described by Sandra et al. [6,9]. GC-MS analysis was carried out with an Agilent 6890 GC coupled to a 5973N MS (Agilent Technologies, Palo Alto, CA). A 30 m HP-INNOWax capillary column [(0.250 mm I.D. × 0.5 µm film thickness) (Agilent Technologies)] was used for separating the volatile compounds. The GC oven was held at 30 °C for 2 min, increased to 130 °C at a rate of 4 °C/min and then at 8 °C/min to 250 °C where it was kept for 5 min. Helium was used as the carrier gas with a flow of 1 mL/min in the constant pressure mode. The MS was operated in a scan mode with a scan range of 30 – 350 amu at 4.45 scans/sec. Spectra were recorded in the electron impact mode (EI) at 70 eV. The MS transfer line, source and quadrupole were at 250, 230, and 150 °C, respectively. Quantitation was performed with total ion chromatograms (TICs) using the sum of all ions for well-separated compounds after careful examination of the peak purity and single ion extraction was applied for closely eluting and minor peaks (**Table 5.1.**). Identification was based on comparison of mass spectra with Wiley 275 and NIST 98 libraries as well as retention times of known standards in synthetic wine for all compounds. For comparison with literature data, retention indices (RI) were experimentally determined using a mixture of *n*-alkanes (**Table 5.2.**).

The TDS 2 was carried out with a temperature program from 30 °C held for 1 min and raised at 20 °C/min to 260 °C where it was held for 10 min. It was operated in solvent vent mode with a purging time of 3 min and equilibrium time of 1 min. The heated transfer line was set at 300 °C. After desorption, the analytes were cryofocused in a programmed temperature vaporizing (PTV) injector at -100 °C using liquid nitrogen

prior to injection. An empty baffled glass liner was used in the PTV. Solvent vent injection with splitless time of 2 min and purge time of 0.1 min was performed by ramping the PTV from -100 to 270 °C at 12 °C/sec and held for 10 min.

5.2.6. Sample preparation

One mL of wine, 100 µL (1.7 mg/L) of 4-methyl-2-pentanol (internal standard), and 1.5 g NaCl was transferred into a 20 mL headspace vial. The volume was made to 6 mL with ultra-pure water of 12% ethanol mixture. The pH was adjusted to 3.2 using a formate buffer. A glass coated magnetic stirrer was added to the mixture. A preconditioned SBSE stir bar Twister (Gerstel, Müllheim a/d Ruhr, Germany) of 10 mm length coated with a 0.5 mm PDMS layer (25 µL) was suspended in the headspace using a glass insert. The vial was sealed with 20 mm aluminum crimp cap and PTFE/silicone molded septa using a hand crimper. The mixture was stirred for 1 hour at room temperature and 1200 rpm. Then the vial was left standing for 3 hours at room temperature. After sampling, the stir bar was removed, dried gently with lint free tissue, and placed in a glass tube of 187 mm length, 6 mm o.d. and 4 mm i.d., which then was placed in the TDS-A auto-sampler tray (Gerstel, Müllheim a/d Ruhr, Germany). It was followed by thermal desorption, cryo-trapping, and gas chromatography-mass spectrometry analysis. The stir bars were re-conditioned for 30 min at 280 °C under a nitrogen stream flow, and no carry-over was observed. Regularly system blanks were run to confirm cleanliness of the system.

5.3. Results and discussions

5.3.1. Method optimization

To characterize the aroma and flavoring compounds in South African wines using HSSE-TD-GC-MS, standard parameters such as ionic strength, sorption time, stirring speed, pH, sample volume, extraction temperature, TDS 2 (desorption), and CIS 4 (cryo-trapping) conditions were thoroughly investigated to evaluate sorption and desorption conditions of the method as well as separation of the analytes. The synthetic wine was used to get the optimum conditions that give an adequate number of chromatographic peaks and quantifiable peak areas in a single run.

5.3.1.1. TDS 2 and CIS 4 conditions

Taking the number of chromatographic peaks and total chromatographic areas as an experimental response for optimization [30], TDS-2 and CIS-4 (Gerstel, Müllheim a/d Ruhr, Germany) working conditions were thoroughly investigated. Among the many parameters investigated, purging time, desorption time, desorption temperature, as well as inlet initial and final temperatures have showed significant influence on the quality of the analyses. As a result, the above-mentioned desorption and cryo-trapping conditions were selected.

5.3.1.2. Influence of ionic strength (salting-out effect)

Salting-out effect on the extraction of flavoring compounds at various concentration levels were described by many authors [12,31]. Based on the data gathered from different authors [17,18,28] only sodium chloride (NaCl), the most common salt used in sample enrichment, was examined at different concentration levels (from 0% to saturation) such as 0.5, 1, 1.5, and 2 g. As the amount of salt increases the peak areas increased proportionally except for acetoin and acids which could be due to their high ionization properties. Since addition of 2 g NaCl saturated the solution and started to negatively affect the early eluting compounds, 1.5 g NaCl was selected as an optimum concentration.

5.3.1.3. Sorption time

The amount of analytes from the aroma and flavor of wine samples that can be extracted by HSSE is determined by two partition coefficients [8]. The partition coefficient of the analytes between the headspace and the PDMS coated stir bar as well as between the headspace and the sample matrix. Therefore, sorption of analytes into the stir bar was investigated by two parameters .i.e. stirring time where the time the headspace vial stirred and standing time where after stirring the vial stands at room temperature. In the former, five different times, namely 30, 60, 90, 120, and 150 min, were tested. Stirring time beyond 1 hour showed a decrease in peak areas for acetoin, ethyl-D-lactate, isobutyric acid, *o*-cresol, phenol, 4-ethylguaiaicol, octanoic acid, and *p*-cresol. This behavior could probably be due to them being released from the PDMS phase of the stir bar after being initially sorbed and then replaced by less

volatile but more apolar compounds that require a considerably longer time to reach equilibrium between liquid phase and the headspace since equilibrium is not yet reached. Hence, 1 hour stirring time was chosen as the optimum to encompass average sensitivity for all compounds. The second sorption parameter (standing time), showed a dramatic improvement on the extraction for most of the compounds. This could be due to the time required for the analytes that have already migrated to the headspace of the sample to be fully sorbed into the PDMS coating of the stir bar [8]. Hence, eight different standing times ranging from 30 min to 12 hours (30 min, 1, 2, 3, 4, 6, 8, and 12 hours) were tested. Beyond 3 hours, the peak areas of the lower alcohols and esters start to decrease as the time increases, probably due to them being released from the PDMS layer to the headspace [32]. For the rest of the compounds, no significant increase was achieved beyond 4 hours. Consequently, a time of 3 hours that satisfies the sensitivity of all the compounds was selected as an optimum time for the sorption process. Thus, by combining the two parameters discussed, a total of 4 hours were taken as an optimum sorption time.

5.3.1.4. Stirring speed

Stirring speed of the sample (solution) during extraction where it gives rapid equilibrium between the liquid sample and the gas phase was the most influential variable. Stirring speeds of 500, 900, 1100, and 1200 rpm were tested. With the exception of few compounds such as the lower alcohols (isobutanol, 1-butanol, and isoamyl alcohol), C₂ to C₅ acids and acetoin, increasing stirring speed showed good improvement on the peak areas of all analytes. Increasing stirring speed not only increases extraction efficiency but also lowers the equilibrium time. Thus, 1200 rpm was selected as an optimum stirring speed for extraction.

5.3.1.5. Effect of pH

Compounds can exist in solution as either neutral or charged species depending on the pH of the solution [33]. As the pH of wine samples range between 2.8 and 4.0 [34], it was decided to adjust the pH to 3.2 [17]. Hence, HCl and format buffer were investigated. Both HCl (1 M, drop-wise) and a formate buffer (400 µL, 1 M, pH 4.1) showed good improvement of extraction mainly for the acids, although a decrease in extraction efficiency was measured for the lower alcohols and esters. The evident

effect on the acids is mainly due to protonation changing them from ionized to neutral species [33] which allow their migration to the gas phase as well as their interaction with the PDMS phase of the stir bar. Since adding a fixed amount of formate buffer adjusts the sample pH to the desired value while eliminating the need for continuous pH measurements, this method is less labor-intensive and was therefore selected.

5.3.1.6. Volume (phase) ratios

During the extraction three different total volumes of samples, namely 3, 6, and 9 mL, were investigated. Beyond 6 mL increasing the volume of sample and decreasing the headspace volume shows no significant improvement for any of the analytes, as previously reported [30]. Moreover, increasing the sample volume decreases the stirring power of the magnetic stirrer, thus increasing the equilibration time. In addition the probability the matrix coming in contact with the PDMS coated stir bar suspended in a headspace was high. As a result, 6 mL was selected as an optimum volume for the sample.

5.3.1.7. Extraction temperature

The effect of extraction temperature on analytical response was evaluated at three levels: room temperature, 40, and 50 °C. Increasing extraction temperature showed a negative effect for most of the analytes, this could be due to shifting of the equilibrium between the gas phase and the PDMS favoring the former [4] or between the sample matrix and the headspace.

Although initially 39 volatile compounds were selected for this work, two of them (methanol and 1-propanol) were excluded at the end of the method optimization because under all the conditions examined during extraction, they failed to be detected. This could be due to the high solubility in water which keeps them from migrating to the headspace [1] or lack of interaction with the PDMS phase of the stir bar due to their very low partition coefficients. For this reason, subsequent work was done based on the thirty seven remaining compounds (**Table 5.1**).

Headspace sorptive extraction (HSSE)

Table 5.1. Method validation data obtained by HSSE-TD-GC-MS. (Conditions see text).

No	Compounds	($y = mx + c$) ^a	(R^2) ^b	LODs (ng/l)	LOQs (ng/l)	Repeatability ^c	Intermediate Repeatability ^d	Relative %Recovery ^e	Quantitative Signal ^f
1	Ethyl butyrate	$y = 1.6075x + 1.063$	0.9710	10.50	34.90	12	5	94	TIC ⁱ
2	Isobutanol	$y = 0.011x + 0.0138$	0.9911	126.20	420.80	4	10	108	TIC ⁱ
3	Isoamyl acetate	$y = 2.7946x + 1.3326$	0.9929	1.90	6.40	13	8	70	TIC ⁱ
4	n-Butanol	$y = 0.02x + 0.0202$	0.9919	0.60	2.10	5	8	73	TIC ⁱ
5	Isoamyl alcohol	$y = 0.0933x + 0.1105$	0.9938	43.50	145.10	8	7	43	TIC ⁱ
6	Ethyl hexanoate	$y = 13.561x + 0.9322$	0.9946	0.80	2.60	3	6	67	TIC ⁱ
7	Hexyl acetate	$y = 12.985x + 0.2265$	0.9991	0.70	2.20	5	5	72	TIC ⁱ
8	Acetoin	$y = 0.0013x + 0.0001$	0.9949	9.54 ^g	31.78 ^h	5	16	42	45 ^j
9	Ethyl-D-lactate	$y = 0.0056x + 0.0571$	0.9934	2.42 ^g	8.06 ^h	12	24	91	TIC ⁱ
10	1-Hexanol	$y = 0.3255x + 0.4976$	0.9899	1.80	6.00	5	7	94	TIC ⁱ
11	Ethyl octanoate	$y = 134.01x + 0.0557$	0.9995	0.08	0.30	6	4	54	TIC ⁱ
12	Acetic acid	$y = 0.0023x + 0.2174$	0.9900	5.40	17.80	8	7	91	TIC ⁱ
13	Furfural	$y = 0.0482x + 0.0466$	0.9964	2.10	7.00	20	15	90	TIC ⁱ
14	Propionic acid	$y = 0.0046x + 0.0033$	0.9992	2.80	9.40	8	13	126	74 ^j
15	Isobutyric acid	$y = 0.0355x - 0.0087$	0.9994	5.80	19.50	21	11	95	TIC ⁱ
16	5-Methylfurfural	$y = 0.2064x + 0.012$	0.9996	2.00	6.70	13	14	99	TIC ⁱ
17	n-Butyric acid	$y = 0.0239x + 0.0147$	0.9996	3.00	10.10	11	14	120	60 ^j
18	Ethyl decanoate	$y = 88.471x - 0.0148$	0.9999	0.05	0.20	16	20	34	88 ^j
19	Isovaleric acid	$y = 0.0559x + 0.0304$	0.9996	7.40	24.60	9	19	121	60 ^j
20	Diethyl succinate	$y = 0.1362x + 0.3446$	0.9924	39.10	130.30	13	11	95	101 ^j
21	n-Valeric acid	$y = 0.0676x - 0.0009$	0.9998	2.60	8.60	16	17	92	TIC ⁱ
22	2-Phenethyl acetate	$y = 1.0095x + 0.235$	0.9914	5.10	17.00	13	21	58	104 ^j
23	Hexanoic acid	$y = 0.5482x + 0.0251$	0.9997	0.40	1.20	12	12	24	60 ^j
24	Guaiacol	$y = 0.3434x + 0.0314$	0.9957	4.10	13.80	19	20	102	109 ^j
25	<i>trans</i> -oak-lactone	$y = 0.2291x + 0.0039$	0.9994	0.35 ^g	1.16 ^h	12	9	87	99 ^j
26	2-Phenylethyl alcohol	$y = 0.0989x + 0.0676$	0.9965	18.50	61.60	15	5	88	TIC ⁱ
27	<i>cis</i> -oak-lactone	$y = 0.1258x + 0.0134$	0.9997	0.64 ^g	2.15 ^h	18	18	109	99 ^j
28	o-Cresol	$y = 0.3668x + 0.0131$	0.9995	13.10	43.70	20	18	97	TIC ⁱ
29	Phenol	$y = 0.0746x - 0.0034$	0.9997	3.70	12.50	13	14	125	TIC ⁱ
30	4-Ethylguaiacol	$y = 0.6471x + 0.0328$	0.9991	38.80	129.20	10	7	123	137 ^j
31	Octanoic acid	$y = 0.2385x + 0.0726$	0.9918	0.30	0.90	19	21	80	TIC ⁱ
32	p-Cresol	$y = 0.3182x + 0.001$	0.9986	0.37 ^g	1.25 ^h	18	18	97	TIC ⁱ

Headspace sorptive extraction (HSSE)

33	Eugenol	$y = 0.4531x + 0.034$	0.9991	110.80	369.30	15	16	115	164 ^j
34	Decanoic acid	$y = 0.1034x + 0.0179$	0.9977	9.80	32.70	19	20	103	60 ^j
35	2,6-Dimethoxy phenol	$y = 0.0094x + 0.0043$	0.9933	19.00 ^g	62.00 ^h	22	22	82	154 ^j
36	5-(Hydroxymethyl)furfural	$y = 0.0008x + 0.0002$	0.9958	299.00 ^g	996.00 ^h	5	26	68	126 ^j
37	Vanillin	$y = 0.0019x + 0.0002$	0.9978	31.00 ^g	103.00 ^h	18	6	94	151 ^j

^a Regression equation where y = the relative peak area, m = slope and c = intercept. ^b Regression coefficient. ^c Repeatability (n = 8) and ^d Intermediate Repeatability (n = 4) both in terms of %relative standard deviations. ^e Relative Recovery (%). ^f Quantitative Signal where ⁱ TIC (total mass) and ^j Single ion extract used for quantitation. ^g LODs and ^h LOQs: concentrations presented in µg/L.

5.3.2. Method validation

As the importance of method validation is a requisite for a good method, the optimized method was validated thoroughly using the synthetic wine. The calibration lines of each compound were prepared by dilution of the global stock solution to different concentrations ranging between 8.3 ng/L and 333 mg/L for esters, 250 ng/L and 667 mg/L for acids, 625 ng/L and 31.25 mg/L for alcohols, 125 ng/L and 25 mg/L for phenols, 167 ng/L and 33.3 mg/L for furans, 250 ng/L and 280.3 mg/L for carbonyls, as well as 420 ng/L and 20.8 mg/L for lactones. After the addition of 1.7 mg/L internal standard (4-methyl-2-pentanol) for each of the above calibration concentrations, the previously mentioned HSSE extraction procedure and TD-GC-MS conditions were applied.

Each concentration level used for calibration was repeated four times (four replicates). The average peak areas relative to internal standard obtained against the different concentrations used were applied to construct the calibration curves. From each calibration curve, the regression coefficient (R^2), linearity and other analytical characteristics were calculated. The regression coefficient (R^2) for most of the compounds was greater than 0.99 except for ethyl butyrate (0.9710) (**Table 5.1.**). A wide range of linearity ($\approx 10^5$) was obtained for most of the compounds.

The limit of detections (LODs) and limit of quantitations (LOQs) were calculated from the calibration graphs constructed for each volatile compound as 3 and 10 times the signal to noise ratio (S/N), respectively [4]. The method proved very sensitive, achieving low LODs ranging between 50 pg/L to 299 μ g/L and low LOQs between 0.2 ng/L to 996 μ g/L for ethyl decanoate and 5-(hydroxymethyl)furfural, respectively, (**Table 5.1.**).

The repeatability was evaluated using eight replicates of a synthetic wine of the same batch using different stir bars assuming all PDMS coated stir bars are the same and calculated as percent relative standard deviation (%RSD). The repeatability was between 3 and 22% for ethyl hexanoate and 2,6-dimethoxy phenol, respectively (**Table 5.1.**). The intermediate repeatability was evaluated by analyzing four replicates of different batches using different stir bars and calculated in terms of %RSD. With the exception of ethyl lactate (24%), 2-phenylethyl acetate (21%),

octanoic acid (21%), 2,6-dimethoxy phenol (22%), and 5-(hydroxymethyl)furfural (26%), it was within the acceptable range ($\leq 20\%$) [35] (**Table 5.1.**).

In an extraction based on sorptive techniques the recovery, expressed as the ratio of the extracted amount of solute (m_{PDMS}) over the original amount of solute in the water ($m_o = m_w + m_{\text{PDMS}}$), is dependent upon the distribution coefficient [22]. Since the developed method involves three phases (liquid, gas, and PDMS), it is expected for the analytes to experience different partition properties among the different phases [8]. Hence, it was impossible to calculate the absolute recovery as the original concentration of the analytes is distributed among the three phases. Nevertheless, the relative recovery (**Table 5.1.**) was carried out from a spiked synthetic wine. i.e. spiked with known amount and recovery calculated in a similar fashion as reported [1].

The method was very selective and applicable to compounds that can migrate to the headspace of the vial (volatile and semi-volatiles) as well as compounds that can have good interaction with the PDMS phase of the stir bar.

In all the parameters tested, isoamyl acetate, isoamyl alcohol, ethyl hexanoate, hexyl-acetate, ethyl octanoate, ethyl decanoate, diethyl succinate, and 2-phenylethyl acetate were easily and efficiently extracted. This could be due to their higher distribution coefficient ($K_{o/w}$) compared to the rest of the compounds [22]. Moreover, even with the increase the concentrations, the peak intensity of the lower alcohols (isobutanol, and 1-butanol), acetoin, C_3 to C_5 acids, 5-(hydroxymethyl)furfural, and vanillin remain very small, whereas their area increases proportionally. For the rest of the compounds, the analytical responses were proportional to their concentrations and relatively good. This variation might be related to the response factor of each compound.

It is also essential to highlight that the very low detection and quantitation limits of almost all the analytes using MS detector makes the technique suitable for sample screening and multi-compound analysis.

5.3.3. Application to real wine samples

After optimizing and validating the method thoroughly, it was applied to the analysis of 64 red wine samples from four different red wine cultivars (Pinotage, Shiraz, Cabernet Sauvignon, and Merlot, 16 samples of each cultivar), and 15 Chardonnay wines, all of vintage 2005. A typical chromatogram of Pinotage wine is presented in **Figure 5.1**.

Headspace sorptive extraction (HSSE)

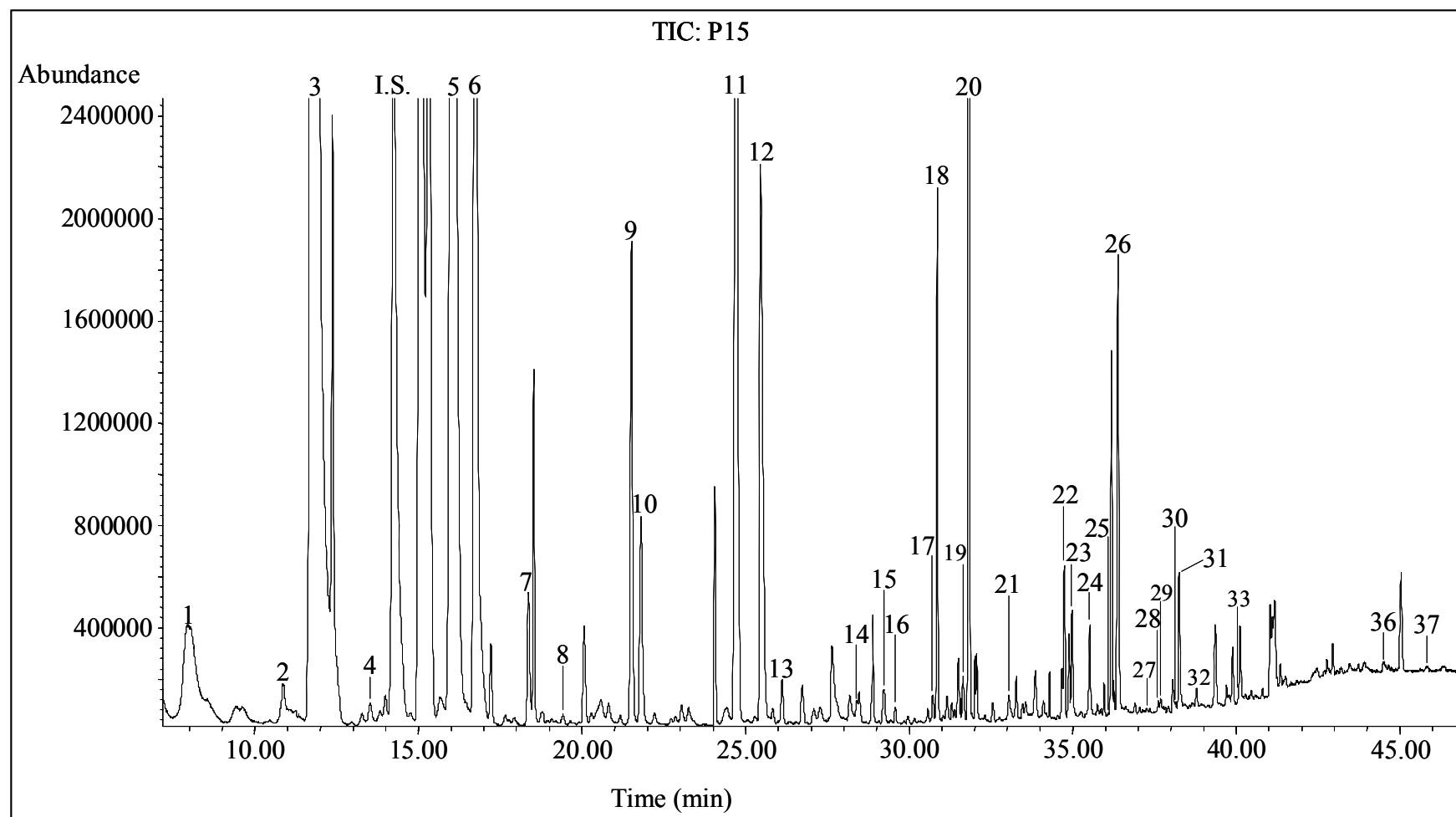


Figure 5.1. A TIC Chromatogram of Pinotage wine vintage 2005 obtained using the optimized method HSSE-TD-GC-MS. Compound identification see **Table 5.1.** and Quantitative information see **Table 5.2.** Concentration of I.S. was 1.7 mg/l. (Conditions see text).

Headspace sorptive extraction (HSSE)

The summary of all the volatile components identified in the wine samples are presented in **Table 5.2**. These compounds mainly belong to esters, alcohols, lower acids, and furans as well as other compounds in lesser amounts belonging to carbonyls, lactones, and phenols. With the current method decanoic acid and 2,6-dimethoxy phenol were unable to identify in all the samples. Moreover, *p*-cresol was below the LOD in all wines of Shiraz, Cabernet Sauvignon, and Merlot cultivars. The *trans*-oak-lactone was unidentified in the white wines. Furthermore, it was not detected in all the samples of Cabernet Sauvignon cultivars except in one. Its racemic isomer, *cis*-oak-lactone, was not determined in all the samples of Cabernet Sauvignon, Merlot, and Chardonnay cultivars.

Headspace sorptive extraction (HSSE)

Table 5.2. Average concentration (mg/l \pm SD) of volatile compounds obtained in 79 young South African wine samples of vintage 2005 using the validated method HSSE-TD-GC-MS. (Conditions see text).

Compounds	Pinotage	Shiraz	Cabernet Sauvignon	Merlot	Chardonnay	RI ^c
	Average ^a \pm SD ^b	Average ^a \pm SD ^b	Average ^a \pm SD ^b	Average ^a \pm SD ^b	Average ^a \pm SD ^b	
Ethyl butyrate	1.04 \pm 0.73	0.60 \pm 0.17	0.75 \pm 0.24	0.79 \pm 0.27	2.09 \pm 0.59	990
Isobutanol	23.64 \pm 11.68	38.87 \pm 15.79	36.32 \pm 12.74	45.53 \pm 29.89	9.61 \pm 5.52	1072
Isoamyl acetate	6.27 \pm 2.88	4.00 \pm 1.55	4.25 \pm 1.70	3.65 \pm 1.88	10.34 \pm 3.53	1098
n-Butanol	3.13 \pm 0.80	6.81 \pm 3.54	6.22 \pm 3.01	5.01 \pm 2.34	10.09 \pm 5.66	1145
Isoamyl alcohol	183 \pm 36.96	207 \pm 23.13	268 \pm 44.62	264 \pm 69.38	159 \pm 24.92	1216
Ethyl hexanoate	0.45 \pm 0.26	0.30 \pm 0.08	0.36 \pm 0.10	0.34 \pm 0.14	1.14 \pm 0.37	1233
Hexyl acetate	0.01 \pm 0.01	0.01 \pm 0.01	0.006 \pm 0.004	0.003 \pm 0.003	0.12 \pm 0.05	1278
Acetoin	19.71 \pm 10.25	28.51 \pm 16.38	19.93 \pm 12.12	21.87 \pm 11.72	26.54 \pm 21.20	1307
Ethyl-D-lactate	230 \pm 62.73	184 \pm 72.89	220 \pm 74.73	208 \pm 75.80	51.81 \pm 69.80	1364
1-Hexanol	3.55 \pm 2.97	4.15 \pm 0.98	4.79 \pm 1.02	4.06 \pm 1.78	6.31 \pm 13.49	1372
Ethyl octanoate	0.04 \pm 0.03	0.02 \pm 0.01	0.024 \pm 0.01	0.023 \pm 0.01	0.12 \pm 0.04	1455
Acetic acid	996 \pm 999	1344 \pm 846	1395 \pm 763	1509 \pm 1014	901 \pm 499	1476
Furfural	3.73 \pm 1.99	7.90 \pm 4.15	7.68 \pm 3.81	10.39 \pm 4.13	15.54 \pm 6.29	1495
Propionic acid	6.30 \pm 3.99	9.33 \pm 6.82	17.02 \pm 7.59	23.85 \pm 10.49	28.44 \pm 10.88	1570
Isobutyric acid	0.56 \pm 0.34	0.64 \pm 0.20	0.59 \pm 0.20	0.89 \pm 0.39	0.29 \pm 0.09	1597
5-Methylfurfural	0.14 \pm 0.09	0.18 \pm 0.06	0.20 \pm 0.10	0.24 \pm 0.08	0.28 \pm 0.08	1610
n-Butyric acid	2.40 \pm 4.95	0.99 \pm 0.52	1.00 \pm 0.44	1.28 \pm 0.60	1.40 \pm 0.38	1659
Ethyl decanoate	0.01 \pm 0.01	0.006 \pm 0.003	0.006 \pm 0.003	0.005 \pm 0.003	0.03 \pm 0.01	1665
Isovaleric acid	1.03 \pm 0.54	1.33 \pm 0.52	2.17 \pm 0.97	2.02 \pm 0.70	0.37 \pm 0.09	1707
Diethyl succinate	17.38 \pm 8.15	24.61 \pm 7.37	28.14 \pm 11.88	22.83 \pm 8.91	2.06 \pm 1.03	1716
n-Valeric acid	0.44 \pm 0.22	0.32 \pm 0.30	0.24 \pm 0.19	0.26 \pm 0.21	0.20 \pm 0.19	1772
2-Phenethyl acetate	0.16 \pm 0.10	0.23 \pm 0.16	0.20 \pm 0.11	0.12 \pm 0.06	0.21 \pm 0.15	1863
Hexanoic acid	0.24 \pm 0.09	0.16 \pm 0.08	0.18 \pm 0.06	0.16 \pm 0.06	0.47 \pm 0.16	1876
Guaiacol	0.21 \pm 0.15	0.13 \pm 0.04	0.20 \pm 0.08	0.14 \pm 0.05	0.014 \pm 0.01	1909
<i>trans</i> -oak-lactone	0.01 \pm 0.003	0.01 \pm 0.01	0.01	0.02 \pm 0.01	nd ^e	1949
2-Phenylethyl alcohol	13.80 \pm 4.11	36.72 \pm 14.37	67.05 \pm 45.20	49.82 \pm 19.25	6.89 \pm 2.35	1968
<i>cis</i> -oak-lactone	0.08 \pm 0.04	0.07 \pm 0.05	nd ^e	nd ^e	nd ^e	2030
o-Cresol	0.03 \pm 0.02	0.04 \pm 0.03	0.053 \pm 0.03	0.07 \pm 0.03	0.005 \pm	2053
Phenol	0.20 \pm 0.10	0.30 \pm 0.09	0.29 \pm 0.08	0.32 \pm 0.10	0.24 \pm 0.07	2059
4-Ethylguaiacol	0.013 \pm 0.01	0.02 \pm 0.01	0.02 \pm 0.02	0.015 \pm 0.01	0.009 \pm 0.01	2090
Octanoic acid	0.92 \pm 0.34	0.72 \pm 0.45	0.87 \pm 0.45	0.97 \pm 0.31	3.01 \pm 1.30	2097

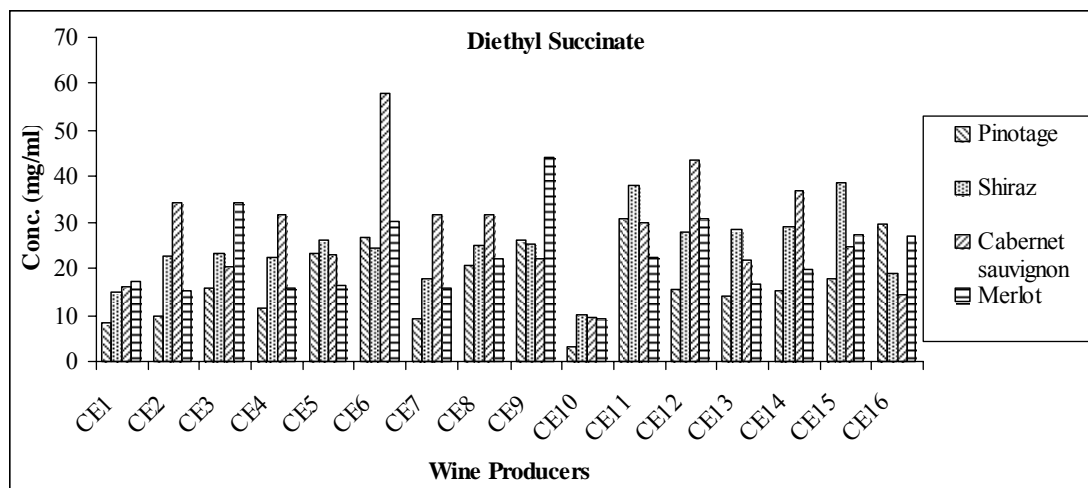
Headspace sorptive extraction (HSSE)

p-Cresol	0.09 ± 0.07	nd ^e	nd ^e	nd ^e	0.007 ± 0.01	2134
Eugenol	0.05 ± 0.03	0.05 ± 0.02	0.07 ± 0.04	0.05 ± 0.03	0.008 ± 0.01	2225
Decanoic acid	nd ^e	nd ^e	nd ^e	nd ^e	nd ^e	2255 ^d
2,6-Dimethoxy phenol	nd ^e	nd ^e	nd ^e	nd ^e	nd ^e	2274 ^d
5-(Hydroxymethyl)furfural	56.98 ± 19.37	111 ± 47.31	113 ± 64.23	114 ± 56.37	154 ± 62.81	2528
Vanillin	47.35 ± 27.63	55 ± 31.89	92.83 ± 56.53	34.19 ± 17.73	47.46 ± 23.74	2568

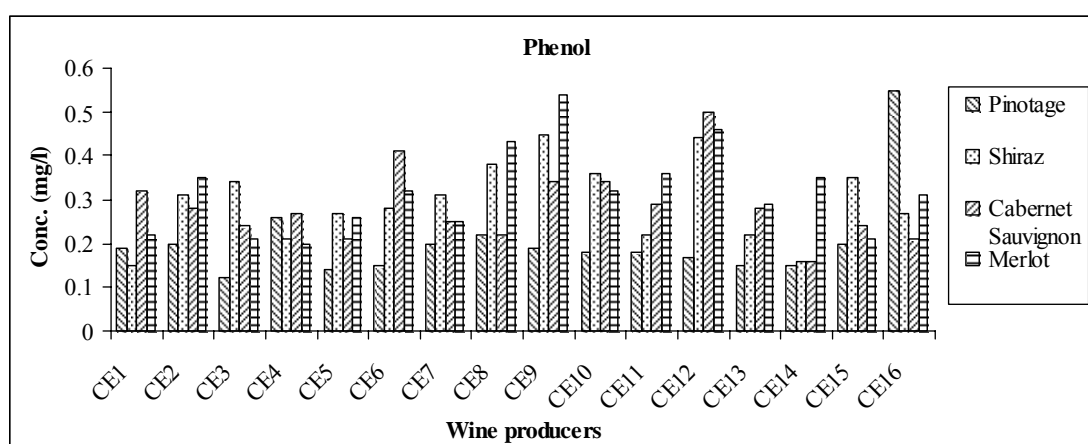
^a Average: Average of the detected values only. ^b SD: Standard Deviation of the determined values only. ^c RI: Retention indices from real wine samples and ^d RI from synthetic wine calculated on HP-INNOWax column. ^e nd: not detected.

Small, but in some cases observable, differences were found in the measured amounts of the analytes in wines, even among those from the same cultivar, producer, and region. For instance in **Figure 5.2.**, the amount of diethyl succinate and phenol measured in four different red wine cultivars is presented for sixteen different producers in South Africa. From this **figure** it would seem that wine-making procedures, geographical origin, and cultivar plays a more detrimental role in the quality of the wines and not the age since all the wines analyzed were from the 2005 vintage. The data in **Figure 5.2.** suggests that the method and data generated would prove useful to study the volatile composition of wines and possibility to classify them according to certain criteria such as geographical origin, production technology, or grape variety. This will be the focus of subsequent statistical investigations in future.

Headspace sorptive extraction (HSSE)



(a)



(b)

Figure 5.2. Chart representation of **a)** Diethyl Succinate and **b)** phenol measured in Pinotage, Shiraz, Cabernet Sauvignon and Merlot wine samples, 16 from each cultivar obtained by HSSE-TD-GC-MS. (Conditions see text).

NB: CE1 to CE16 = Cellar 1 to Cellar 16 suppliers of the wine samples. Each cellar represents same region but different cultivar.

5.4. Conclusions

In conclusion the developed analytical technique based on stir bar technology was found very sensitive and suitable for the analysis of trace and ultra-trace compounds. HSSE extraction was very advantageous in reducing the risk of contamination and increasing the lifetime of the PDMS coated stir bar.

The overall results are satisfactory for the analysis of volatile compounds in wine responsible for its aroma achieving low detection and quantification limits. The methodology proposed in this paper allowed us to determine the 37 most important volatile compounds partially responsible for the aroma of wines in a relatively quick and easy procedure with a low sample volume and cost effectively.

Headspace sorptive extraction (HSSE)

Although SBSE is a very sensitive technique, PDMS, a non-polar phase, is the only polymer at present adopted as coating of stir bars. This results in poor recoveries of polar compounds with low octanol–water partition coefficients ($K_{o/w}$). This was improved by pH adjustment especially for the organic acids. However, a dual-phase twister approach could bring some solution to the limitation of the current stir bar technology by utilizing a material which retains both polar and non-polar compounds.

5.5. References

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**Analysis of volatiles in Pinotage
wines by stir bar sorptive extraction
and chemometric profiling[†]**

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Abstract

A fast, simple, cost-effective, and reliable method based on stir bar sorptive extraction (SBSE) in the headspace mode was used for the analysis of 39 volatile components in Pinotage wines. The method was sensitive with LODs ranging from 50.0 pg/L to 281 ng/L and LOQs between 180 pg/L and 938 ng/L. Precision was between 6 and 20%. The intermediate precision was within the acceptable range. Moreover, good calibration curves with $R^2 > 0.99$ for all compounds were achieved. The method was successfully applied for the analysis of 87 young Pinotage wines of vintages 2005 and 2006 collected from various South African regions. To characterize the results based on vintage and origin, the obtained concentrations of the compounds were subjected to chemometric analysis. Exploratory factor analysis (FA), principal component analysis (PCA), and analysis of variance (one-way ANOVA) were consecutively done. The chemometrics approach revealed a reasonable correlation among the volatile components of these wines, as well as with respect to their year of production.

Key words:

SBSE, headspace extraction, volatile compounds, Pinotage, wine, GC, MS.

6.1. Introduction

Pinotage is a unique South African red wine cultivar that was bred in 1924 from Pinot Noir and Cinsaut Noir varieties. Pinotage wine is known for its distinctive fruity character, which is expressed as plum, cherry, red berry, blackberry, and banana [1,2]. As the demand for Pinotage wine is growing both locally and internationally [1], the industry is putting huge efforts and money into research to enhance the production of good quality wine. Aroma and flavor are some of the important factors that establish wine character and quality [3,4]. The profile of wine aromas has a well-known contribution to create the existing relationship between a product's chemical composition in odorants and its sensorial attributes [5] and is determined through the combined effects of several hundreds of chemically different compounds [6], which correspond to different chemical classes such as alcohols, esters, carbonyls, acids, phenols, lactones, acetals, thiols, and terpenols [4,7]. The combination of all these compounds composes the character of wine and distinguishes one wine from another. Many of these classes of compounds already exist in the grape, however, several are also produced during fermentation and maturation, such as esters and higher alcohols [7,8]. Moreover, a considerable number of volatiles are formed during aging as well as extraction from oak wood [9].

To satisfy the needs of wine consumers, it is very important to have a good quality wine that can be sustained in the market. The sustainability of the wine can be achieved by having a good understanding of the chemical, physical, and/or sensorial parameters that express differences in composition based on geographical origin, climatic conditions, soil, grape ripeness and variety, aging, manufacturing techniques, and commercial type [7,10]. Hence, it is necessary to investigate reliable analytical techniques to establish criteria for determining the quality of wine.

The gas chromatographic (GC) analysis of volatile organic compounds in wine is a very important tool for wine classification, and it has attracted many researchers in the past [11,12]. However, the wine matrix is complex in nature, and some of the volatile compounds that are responsible for the aroma and flavor exist at low levels, mostly below the detection ability of the instrument. Hence, sample preparation that allows the extraction, concentration, and separation of the analytes without affecting their chemical and/or physical nature prior to analysis is necessary.

Liquid liquid extraction (LLE) based on organic solvent extraction has been successfully applied for the analysis of volatile compounds in wine [4,8,12,13]; however, it is a time consuming and labor intensive technique, involving multi-step procedures subject to analyte loss and usually requires toxic organic solvents. Solid phase extraction (SPE) [9], in which analytes are bound to active sites on a surface, also suffer from similar drawbacks. Hence, finding an alternative that is fast, simple, inexpensive, and environmentally friendly is important.

Pawliszyn and co-workers developed a solvent-free extraction method in the early 1990s called solid phase micro extraction (SPME) [14]. It involves no solvent consumption, which has an important effect on analytical costs and the environment [15]. SPME can be very selective and can result in the production of clear chromatograms from complex matrices such as wine, depending on the type of fiber used. The application of SPME for wine analysis has increased tremendously since its invention [7,11]. However, due to the smaller sample capacity of SPME and the low concentrations of some of the volatile compounds in wine, a better enrichment probe is often desirable [7].

More recently, a new extraction procedure for aqueous samples, named stir bar sorptive extraction (SBSE), was developed by Baltussen and Sandra [16]. The theory of SBSE is very similar to that of SPME, where the efficiency of analytes partitioning into the polydimethylsiloxane (PDMS) phase of the stir bar at the equilibrium can roughly be predicted by the octanol–water partition coefficients [16]. SBSE offers higher sample capacity (50 – 250 times higher) due to the greater amounts of PDMS phase (24 – 126 μL) [6,15] in which the amount of the analyte is extracted. The extraction efficiency is proportional to the coating thickness, resulting in lower detection limits. This can be very useful for trace and ultra-trace analysis [7]. SBSE extraction can be done either in the headspace mode [17] or by introducing the stir bar directly into the aqueous sample [4] and stirring for a given time. SBSE has been applied successfully for the analyses of aroma compounds in wine [4,6,8].

The concentration and type of flavoring compounds in wine are greatly influenced by many viticultural and enological factors [19]. Despite the complexity of factors influencing the formation of volatiles in wine, a correlation between the concentration of wine volatiles and grape variety [20], wine-making practice [21] and ageing [9]

was evident. However, obtaining feasible information from wine analysis may result in a difficult task due to the multiple sources of variation stated above. As a result, the application of chemometrics to wine data has grown tremendously in the past few years because it provides fast and more precise assessment of composition. For example, Martí et al. [22] evaluated the classification and differentiation of wines based on grape varieties, origin, and ageing using principal component analysis (PCA). Other authors applied discriminant analysis (DA) to classify wines according to grape variety [23]. Similarly PCA [10,22,23], cluster analysis [22], and DA [10,21] have been applied to characterize wines. By relating all of the components to the different factors that affect the quality of wine some control can be exercised on the conditions for producing a well-balanced good quality wine from one production year to the next.

The previously reported method [24] based on headspace stir bar sorptive extraction (HS-SBSE) in combination with thermal desorption gas chromatography-mass spectrometry (TD-GC-MS) was modified in the current study. The method was employed for screening of 39 major volatile compounds in 87 Pinotage wines of vintages 2005 and 2006 produced by different cellars and obtained from various South African districts. Given the lack of existing information, the first objective of this study was to identify and quantify the major volatile components present in the young Pinotage wines of the two vintages. Because there are no previous studies that relate aroma profiles of Pinotage wines, the results obtained were extensively studied using a variety of chemometric techniques. The quantitative values of the volatile components were subjected to exploratory factor analysis (FA), PCA, and analysis of variance (one-way ANOVA) to classify as well as characterize the wines according to vintage and geographic origin.

6.2. Material and methods

6.2.1. Standards, reagents and equipment

Standards of ethyl acetate, ethyl butyrate, 1-propanol, isobutanol, *n*-butanol, hexyl-acetate, acetoin, ethyl-D-lactate, ethyl octanoate, furfural, diethyl succinate, 2-phenylethyl acetate, 2,6-dimethoxyphenol, eugenol, 5-(hydroxymethyl)furfural, propionic acid, *n*-butyric acid, isobutyric acid, *n*-valeric acid, isovaleric acid, and 4-

methyl-2-pentanol (internal standard), as well as solvent acetone (pestanal grade) and NaCl were purchased from Fluka (Zwijndrecht, The Netherlands). Isoamyl acetate, isoamyl alcohol, 1-hexanol, 2-phenylethyl alcohol, 5-methylfurfural, ethyl hexanoate, *o*-cresol, *p*-cresol, whiskey lactone (4-hydroxy-3-methyloctanoic acid lactone, also called oak lactone), vanillin, hexanoic acid, octanoic acid, decanoic acid, ethyl-decanoate, phenol, guaiacol, 4-ethylguaiacol, and solvents methanol and absolute ethanol (HPLC grade) were supplied by Aldrich (Steinheim, Germany). Acetic acid (Merck, Darmstadt, Germany), tartaric acid (Analar, the British drug Houses Ltd. England), and ultra-pure water purified by a Milli-Q water purification system (Millipore, Bedford, MA) were used.

A 15 mL amber vial coupled with a solid polytetrafluoroethylene (PTFE) lined screw cap (Supelco, Bellefonte, PA), 2 mL vials with green caps (Agilent, Technologies, Palo Alto, CA), 20 mL Twister headspace vials with glass inserts, Twister (Gerstel, Müllheim a/d Ruhr, Germany), 20 mm magnetic aluminum crimp cap, 20 mm PTFE white silicone molded septa (Agilent Technologies), and a JENWAY 4330 pH-meter (Jenway Ltd., Felsted, Dunmow, Essex, U.K.) were used.

6.2.2. Wine samples

A total of 87 young Pinotage wines (47 from the 2005 vintage and 40 from the 2006 vintage) were supplied by the Young Wine Show collected from different producers. These wines were from various South African districts: Worcester (W), Stellenbosch (S), Paarl (P), Swartland (SW), Robertson (RO), Olifant River (OR), and Klein Karoo (KK) (**Table 6.1**). The wine samples were 1-year-old when supplied to our laboratory, that is, the vintages 2005 and 2006 arrived in our laboratory in 2006 and 2007, respectively. The wines were stored at 4 °C and then analyzed within 3 months of receipt.

Table 6.1. Pinotage wine samples analyzed (for Conditions, see text).

Vintage	Samples ^a	Region ^b	Sample ^c	Wine suppliers ^d
2005	14	W	P1 to P14	C1 to C14
	10	S	P15 to P24	C15 to C24
	10	P	P25 to P34	C25 to C34
	5	SW	P35 to P39	C35 to C39
	4	RO	P40 to P43	C40 to C43
	4	OR	P44 to P47	C44 to C47
Total				47
2006	11	W	P48 to P58	C48 to C58
	9	P	P59 to P67	C59 to C67
	7	RO	P68 to P74	C68 to C74
	5	SW	P75 to P79	C75 to C79
	4	KK	P80 to P83	C80 to C83
	4	S	P84 to P87	C84 to C87
Total				40
Total no. of samples (vintages 2005 and 2006)				87

^aNumber of samples from each region. ^bCodes given to the different regions from where the samples were collected: W, Worcester; S, Stellenbosch; P, Paarl; SW, Swartland; RO, Robertson; OR, Olifant-River; KK, Klein Karoo. ^cCode given to each sample. ^dCode given to each wine producer (supplier).

6.2.3. Preparation of synthetic wine

A global stock solution containing all of the analytes was prepared in a synthetic wine matrix (12% ethanol, 2 g/L tartaric acid in Milli-Q water) using different concentration ranges of analytes varying from 1.00 mg/L for ethyl octanoate and ethyl decanoate to 1.60 g/L for acetic acid on the basis of data collected from different authors as well as VCF 2000 volatile compounds in food database [1996-99 Boelens, Aroma Chemical Information Service (BACIS)] to make it as close as possible to the real wine samples.

6.2.4. Instrumental conditions

The instrumental conditions previously reported [24] were slightly modified as follows. The GC-MS analysis was carried out with an Agilent 6890 GC coupled to a 5973N MS (Agilent Technologies). A 30 m HP-INNOWax capillary column [0.250 mm i.d. × 0.5 µm film thickness (Agilent Technologies)] was used for separating the volatile compounds. The GC oven was held at 30 °C for 2 min and increased to 130 °C at a rate of 4 °C/min and then at 8 °C/min to 250 °C, at which it was kept for 5 min. Helium was used as the carrier gas with a flow of 1 mL/min in the constant pressure mode. The MS was operated in a scan mode with a scan range of

30 – 350 amu at 4.45 scans/s, for peak identification, ion selection, and locating the compounds in the TIC plot. However, for quantitation purposes the MS was operated in the selected ion monitoring (SIM) mode. Three ions with a dwell time of 50 ms for each compound (one quantitative or target ion and two qualitative ions) were selected (**Table 6.2.**). Spectra were recorded in the electron impact mode (EI) at 70 eV. The MS transfer line, source, and quadrupole were at 250, 230, and 150 °C, respectively. Identification was based on comparison of mass spectra with Wiley 275 and NIST 98 libraries as well as retention times of known standards in synthetic wine for all compounds. As a complementary identification, linear retention indices (LRI) were experimentally determined using a mixture of *n*-alkanes and compared with literature values (**Table 6.3.**).

The TDS 2 was carried out with a temperature program from 30 °C held for 1 min and raised at 20 °C/min to 260 °C, at which it was kept for 10 min. It was operated in solvent vent mode with a purging time of 3 min and equilibrium time of 1 min. The heated transfer line was set at 300 °C. After desorption, the analytes were cryofocused in a programmed temperature vaporizing (PTV) injector at -100 °C using liquid nitrogen prior to injection. An empty baffled glass liner was used in the PTV. Solvent vent injection with a splitless time of 2 min and a purge time of 0.1 min was performed by ramping the PTV from -100 to 270 at 12 °C/s and held for 10 min.

Table 6.2. Selected ions for SIM mode and method linearity data (n = 3) obtained by headspace SBSE-TD-GC-MS (for conditions, see text).

No.	Compound	Selected ions	y-intercept	slope	R ²
1	Ethyl acetate	<u>61</u> , 70, 88	0.0007	0.0092	0.9983
2	Ethyl butyrate	72, 101, <u>116</u>	-0.0004	0.1387	0.9997
3	1-Propanol	<u>31</u> , 33, 34	-0.0002	0.0035	0.9985
4	Isobutanol	31, <u>33</u> , 40	0.0046	0.0035	0.9991
5	Isoamyl acetate	69, 71, <u>87</u>	-0.0006	0.532	0.9999
6	<i>n</i> -Butanol	<u>31</u> , 33, 45	0.0118	0.0107	0.9985
7	Isoamyl alcohol	31, <u>39</u> , 69	0.0009	0.0204	1.0000
8	Ethyl hexanoate	100, <u>101</u> , 116	0.0004	3.4658	1.0000
9	Hexyl acetate	<u>56</u> , 61, 84	-0.0016	6.389	0.9998
10	Acetoin	<u>45</u> , 46, 88	0.0029	0.0005	0.9921
11	Ethyl-D-lactate	<u>45</u> , 47, 75	0.0043	0.0053	0.9994
12	1-Hexanol	68, <u>69</u> , 84	0.0078	0.0898	0.9991
13	Ethyl octanoate	83, <u>127</u> , 172	-0.003	23.9 ⁹ 6	0.9992
14	Acetic acid	47, <u>60</u> , 61	0.0828	0.0007	0.9920
15	Furfural	95, <u>96</u> , 97	0.057	0.0271	0.9954
16	Propionic acid	30, 31, <u>74</u>	0.0065	0.0025	0.9956

Characterization of Pinotage wines

17	Isobutyric acid	41, 60, <u>88</u>	0.00008	0.0006	0.9998
18	5-Methylfurfural	81, 109, <u>110</u>	0.0027	0.0438	0.9999
19	<i>n</i> -Butyric acid	37, 38, <u>60</u>	0.0019	0.0043	0.9999
20	Ethyl decanoate	<u>155</u> , 157, 200	0.0032	3.6248	0.9990
21	Isovaleric acid	<u>60</u> , 87, 100	-0.0008	0.0126	0.9991
22	Diethyl succinate	<u>128</u> , 130, 174	-0.0038	0.0116	0.9980
23	<i>n</i> -Valeric acid	<u>60</u> , 74, 87	-0.0007	0.0086	0.9987
24	2-Phenethyl acetate	78, <u>104</u> , 105	0.0395	0.1754	0.9985
25	Hexanoic acid	<u>60</u> , 74, 87	-0.0012	0.0127	0.9967
26	Guaiacol	81, <u>109</u> , 124	0.0005	0.0358	0.9992
27	<i>trans</i> -oak-lactone	96, <u>99</u> , 100	-0.0024	0.028	0.9976
28	2-Phenylethyl alcohol	<u>92</u> , 122, 123	-0.0009	0.0069	0.9994
29	<i>cis</i> -oak-lactone	<u>99</u> , 100, 114	-0.0009	0.0112	0.9977
30	<i>o</i> -Cresol	90, 107, <u>108</u>	-0.0025	0.0441	0.9978
31	Phenol	66, 93, <u>94</u>	0.0015	0.0197	0.9994
32	4-Ethylguaiacol	121, <u>137</u> , 152	-0.0028	0.1016	0.9970
33	Octanoic acid	<u>60</u> , 84, 115	-0.0009	0.0191	0.9935
34	<i>p</i> -Cresol	77, <u>107</u> , 109	-0.0002	0.0169	0.9986
35	Eugenol	121, <u>131</u> , 164	-0.0003	0.0078	0.9963
36	Decanoic acid	<u>60</u> , 143, 172	-0.0005	0.0115	0.9967
37	2,6-Dimethoxy phenol	<u>93</u> , 96, 140	0.00007	0.00005	0.9945
38	5-(Hydroxymethyl)furfural	<u>97</u> , 109, 126	0.0051	0.0044	0.9946
39	Vanillin	81, <u>151</u> , 152	0.0031	0.0004	0.9949

Underscoring indicates the quantitative (target) ion.

6.2.5. SBSE headspace analysis

A 0.5 mL of wine, 50 μ L (1.7 mg/L) of 4-methyl-2-pentanol (internal standard), and 1.5 g of NaCl were transferred to a 20 mL headspace vial. The volume was made up to 6 mL with a blank model wine (a mixture of 12% ethanol in 2 g/L tartarate solution of pH 4.2), which brought the pH of the sample to 3.2. A glass-coated magnetic stirrer was added to the mixture. A preconditioned SBSE stir bar of 10 mm length, coated with a 0.5 mm PDMS layer (25 μ L), Twister (Gerstel), was suspended in the headspace using a glass insert, Twister[®]. The vial was sealed with a 20 mm aluminum crimp cap and a PTFE/silicone molded septum using a hand crimper. The mixture was stirred for 1 h at 1200 rpm and controlled room temperature (23 ± 1 °C). After sampling, the stir bar was removed, dried gently with a lint-free tissue and placed in a glass tube of 187 mm length, 6 mm o.d., and 4 mm i.d., which then was placed in the TDS-A auto-sampler tray (Gerstel). It was followed by thermal desorption, cryo-trapping and gas chromatography-mass spectrometric analysis. The stir bars were reconditioned for 30 min at 280 °C under a nitrogen stream and no carry-over was observed. Regular system blanks were run to confirm the cleanliness of the system.

6.2.6. Statistical analysis

The quantitative chemical data obtained were used as variables for object description. The objects were young Pinotage wines of two vintages produced by different wine-makers from seven regions (**Table 6.1.**). The measured amount of the 39 analytes obtained from each wine was used for computerized multivariate analysis of data, as exploratory factor analysis (FA), principal component analysis (PCA), and ANOVA by the software package Statistica 8 (2007) from StatSoft, Inc. (Tulsa, OK). A 5% significance level ($p < 0.05$) was used as a guideline for determining significant differences.

6.3. Results and discussion

6.3.1. Validation of the method

The calibration curves were prepared for each volatile compound from a stock solution with all 39 volatiles in 12% ethanol by dilution using hydro-alcoholic solution (12% ethanol and 2 g/L tartaric acid) to different concentration levels. After the addition of 1.7 mg/L internal standard (4-methyl-2-pentanol) to each of the calibration concentrations, the previously mentioned HS-SBSE extraction procedure and TD-GC-MS conditions were applied. Each concentration level for the calibration was repeated three times (three replicates) and the average peak area ratios (peak area of a compound to the internal standard) against the known concentrations of standards used were applied to construct the calibration curves for each volatile compound. From each curve, the regression coefficient (R^2), linearity, and other analytical characteristics were calculated. The regression coefficient (R^2) was > 0.99 for all of the analytes (**Table 6.2.**).

The limits of detection (LODs) and limits of quantitation (LOQs) (**Table 6.3.**) were calculated from the calibration graphs constructed for each volatile compound as 3 and 10 times the signal-to-noise ratio (S/N), respectively [7]. Low LODs and LOQs ranging between 50.0 pg/L to 281 ng/L and between 180 pg/L to 938 ng/L, respectively, were achieved. The wide range of LODs and LOQs observed is related to the difference in chemical and physical properties of each compound. As a result,

the different classes of compounds were affected differently, especially during sample preparation.

The precision (repeatability) of the method was evaluated with a synthetic wine of the same batch using different stir bars, presuming all PDMS coated stir bars are the same and following the previously mentioned HS-SBSE procedure and TD-GC-MS analysis. It was estimated as percent relative standard deviation (%RSD) of the relative peak areas for seven replicates ($n = 7$) and varied between 6 and 20% (**Table 6.3.**), with an average of 13%. The intermediate precision (intermediate repeatability) was examined by analyzing five replicates ($n = 5$) of different batches using different stir bars and calculated in terms of %RSD (**Table 6.3.**). The results indicated fluctuations between 2 and 20% with a mean %RSD of 13%.

In the sorptive extraction procedure recovery should be expressed as the ratio of the extracted amount of solute into the PDMS phase (m_{PDMS}) over the original amount of solute in the water phase ($m_o = m_w + m_{\text{PDMS}}$), which depends on the partition coefficient [15]. However, headspace SBSE involves three phases (liquid, gas, and PDMS), and analytes experience different distribution properties among the different phases [6]. As a result, it was not practical to calculate the absolute recovery because the original concentration of the analytes was dispersed among the three phases. Even so, the relative recovery (**Table 6.3.**) was carried out from a spiked wine at different concentrations and was varied between 24 and 112% for all of the analytes.

Characterization of Pinotage wines

Table 6.3. Method validation data obtained using headspace SBSE-TD-GC-MS (for conditions, see text).

Compound	LODs ^a (ng/L)	LOQs ^b (ng/L)	Precision ^c	Intermediate precision ^d	Relative % recovery	LRI _{Cal.} ^e	LRI _{Lit.} ^f	ΔLRF ^g
Ethyl acetate	24.7	82.4	8	6	69	900	899 [25]	1
Ethyl butyrate	210 ^h	710 ^h	6	6	42	1044	1046 [25]	2
1-Propanol	281	938	10	17	27	1046	1051 [25]	5
Isobutanol	2.74	9.14	13	13	93	1103	1105 [25]	2
Isoamyl acetate	21.4	71.2	7	18	46	1128	1127 [25]	1
<i>n</i> -Butanol	530 ^h	1.75	16	12	24	1155	1155 [25]	0
Isoamyl alcohol	104	347	6	14	80	1220	1221 [25]	1
Ethyl hexanoate	1.06	3.55	7	16	66	1245	1242 [25]	3
Hexyl acetate	810 ^h	2.70	7	16	52	1285	1269 [25]	16
Acetoin	18.3	61.1	16	20	68	1302	1291 [3]	11
Ethyl-D-lactate	38.2	128	18	14	73	1357	1353 [3]	4
1-Hexanol	8.97	29.9	6	14	112	1365	1362 [25]	3
Ethyl octanoate	60.0 ^h	190 ^h	10	14	53	1448	1444 [5]	4
Acetic acid	460 ^h	1.53	16	15	47	1463	1461 [5]	2
Furfural	50.0 ^h	180 ^h	15	11	101	1483	1474 [25]	9
Propionic acid	380 ^h	1.25	15	20	24	1554	1554 [25]	0
Isobutyric acid	1.41	4.69	17	10	43	1582	1584 [3]	2
5-Methylfurfural	60.0 ^h	200 ^h	11	3	97	1597	1591 [25]	6
<i>n</i> -Butyric acid	2.19	7.29	17	9	79	1643	1646 [5]	3
Ethyl decanoate	1.81	6.04	11	15	83	1653	1647 [25]	6
Isovaleric acid	2.77	9.22	16	9	77	1690	1687 [5]	3
Diethyl succinate	46.2	154	17	9	74	1701	1690 [3]	11
<i>n</i> -Valeric acid	4.03	13.4	20	5	71	1755	1755 [26]	0
2-Phenethyl acetate	1.65	5.49	12	11	98	1845	1830 [5]	15
Hexanoic acid	2.63	8.76	19	14	68	1857	1857 [27]	0
Guaiacol	360 ^h	1.20	12	18	92	1899	1880 [5]	19
<i>trans</i> -oak-lactone	14.1	47.1	19	7	77	1925	1933 [28]	8
2-Phenylethyl alcohol	6.82	22.7	18	17	105	1944	1942 [5]	2
<i>cis</i> -oak-lactone	10.1	33.6	17	16	87	2006	1993 [27]	13
<i>o</i> -Cresol	2.39	7.96	11	13	70	2030	2017 [28]	13
Phenol	3.80	12.7	15	16	52	2035	2039 [29]	4
4-Ethylguaiacol	1.75	5.83	12	2	69	2068	2055 [27]	13
Octanoic acid	3.68	12.3	10	17	61	2077	2072 [26]	5

Characterization of Pinotage wines

<i>p</i> -Cresol	2.95	9.82	10	11	99	2112	2103 [26]	9
Eugenol	10.9	36.2	18	18	68	2211	2215 [27]	4
Decanoic acid	13.3	44.3	13	18	79	2281	2294 [26]	13
2,6-Dimethoxy phenol	7.68	25.6	7	13	40	2298	2307 [5]	9
5-(Hydroxymethyl)furfural	410 ^h	1.36	11	9	63	2527	2526 [30]	1
Vanillin	720 ^h	2.41	18	18	49	2598	2581 [27]	17

^a Limits of detection, ^b Limits of quantitation, ^c Precision (n = 7), ^d Intermediate precision (n = 5), ^e LRI_{Cal}: Calculated linear retention indices using *n*-alkanes on HP-INNOWax column, ^f LRI_{Lit}: Linear retention indices obtained from literature, ^g ΔLRI: Difference between the calculated and literature values of the linear retention indices. ^h LODs and LOQs expressed in pg/L.

6.3.2. Wine analysis

To the best of our knowledge this is the largest survey of South African Pinotage wine to date, which includes large numbers of major volatiles classified under different classes. The survey was done for 87 young wines from 2005 and 2006 vintages. Moreover, the wines were from seven different regions (districts) and produced by different wine-makers (**Table 6.1.**). This paper indicates a large number of compounds, and it correlates the concentrations obtained among the different classes of volatiles as well as to their respective year and area of production.

Figure 6.1. is an example of an ion monitoring chromatogram of a typical aroma profile of a Pinotage wine from the vintage 2006 obtained by headspace SBSE in combination with TD-GC-MS. Identification of analytes was carried out using mass spectra from Wiley 275 and NIST 98 libraries, retention times of known standards in synthetic wine, and linear retention indices (LRI) (**Table 6.3.**).

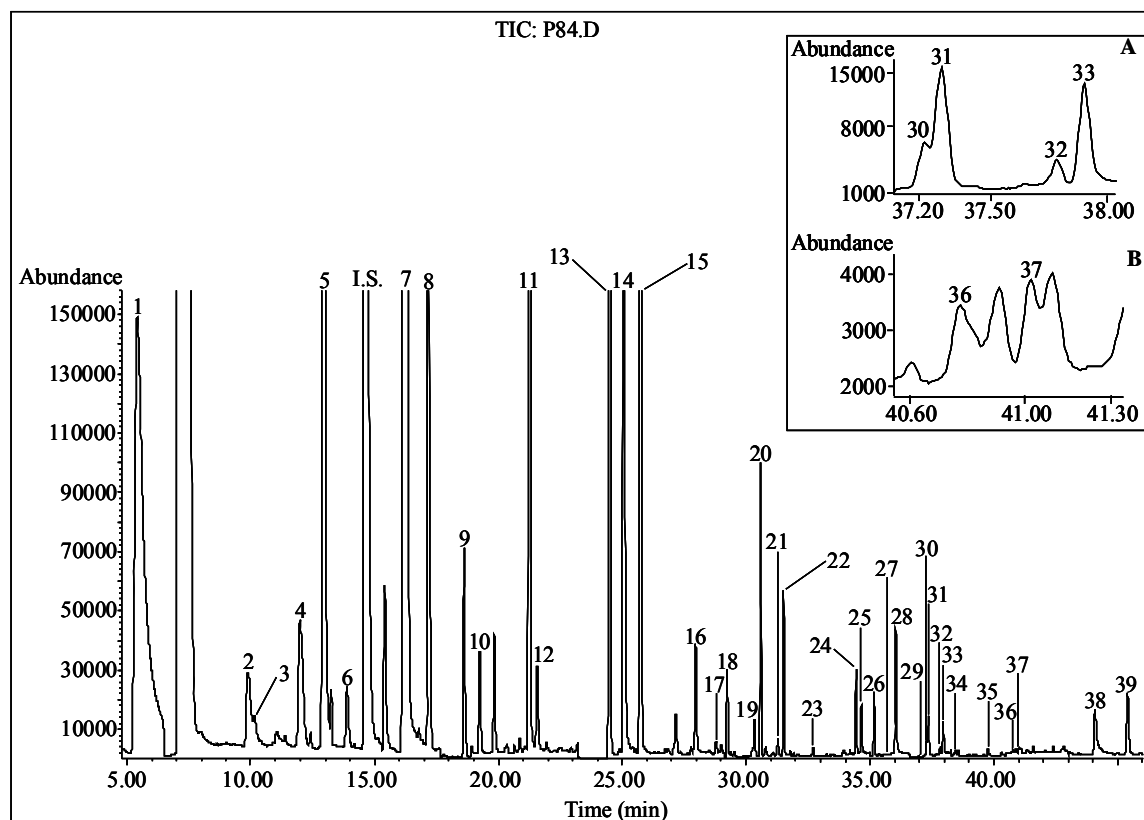


Figure 6.1. GC-MS ion monitoring chromatogram of young Pinotage wine from 2006 vintage: (A) inlay of peaks 30 – 33; (B) inlay of peaks 36 and 37. Concentration of I.S. was 1.7 mg/L. Peak identity is given in **Table 6.2.** and quantitation in **Table 6.4.** and **6.5.** For conditions, see text.

6.3.3. Quantitative analysis

The quantitative value of each analyte was calculated from the calibration curves using peak area ratio of the analytes to that of the internal standard (4-methyl-2-pentanol) as reported previously, due to unavailability of certain reference standards [7,24]. Efforts to find additional suitable internal standards for each of the different classes of compounds were not successful due to the failure to achieve sufficient separation for the complex wine extracts obtained by HS-SBSE. Hence, 4-methyl-2-pentanol was selected as an I.S. due to the fact that no discrimination was observed for any of the compounds. It also elutes close to the middle of the chromatogram.

It could be observed that the free aroma compounds from the Pinotage wine samples are predominantly composed of esters and alcohols. Even though the wine was diluted 12 times (0.5 mL in 6 mL) prior to analysis, the analytical response for esters and alcohols remains significantly large. However, further dilution to minimize the analytical response for these compounds could result in losing sensitivity for some compounds, especially C₄–C₁₀ acids and volatile phenols.

The mean, maximum and minimum values for the volatile compounds determined in the Pinotage wines over the two vintages studied are presented in **Table 6.4**. Most of these compounds are major volatiles and have been identified in all of the wines.

Characterization of Pinotage wines

Table 6.4. Average \pm Standard Deviation (SD), minimum and maximum concentrations (mg/L) of volatiles in Pinotage wines from vintages 2005 and 2006 obtained by headspace SBSE-TD-GC-MS (for conditions, see text).

Compound	Pinotage vintage 2005 (n = 47)			Pinotage vintage 2006 (n = 40)		
	Average \pm SD	Maximum	Minimum	Average \pm SD	Maximum	Minimum
Ethyl acetate	142 \pm 43	223	71.7	191 \pm 35	277	124
Ethyl butyrate	300 \pm 90*	530*	130*	360 \pm 90.0*	620*	210*
1-Propanol	60.5 \pm 39	211	15.7	50.4 \pm 34	152	15.4
Isobutanol	54.5 \pm 23	118	230*	49.5 \pm 16	86.3	7.63
Isoamyl acetate	4.49 \pm 2.3	9.59	420*	5.84 \pm 2.9	10.9	400*
<i>n</i> -Butanol	7.47 \pm 3.5	11.8	30.0*	8.03 \pm 4.0	29.7	10.0*
Isoamyl alcohol	160 \pm 21	201	122	152 \pm 18	192	117
Ethyl hexanoate	210 \pm 90*	550*	60.0*	430 \pm 120*	830*	280*
Hexyl acetate	20.0 \pm 10*	50.0*	10.0*	30.0 \pm 20*	90.0*	4.00*
Acetoin	57.4 \pm 42	176	1.84	68.9 \pm 52	219	3.15
Ethyl-D-lactate	294 \pm 133	915	3.94	295 \pm 97	486	1.04
1-Hexanol	573 \pm 318*	1.03	50.0*	610 \pm 390*	1.62	500**
Ethyl octanoate	30.0 \pm 10*	90.0*	10.0*	120 \pm 40*	220*	60.0*
Acetic acid	847 \pm 440	2.63x10 ³	314	666 \pm 470	2.64x10 ³	188
Furfural	15.7 \pm 7.4	34.9	690*	10.0 \pm 6.9	21.5	250*
Propionic acid	19.4 \pm 10	47.6	6.80	15.2 \pm 9.4	39.3	1.36
Isobutyric acid	1.73 \pm 0.89	5.36	400*	2.52 \pm 1.2	7.14	1.25
5-Methylfurfural	430 \pm 270*	840*	10.0*	320 \pm 210*	830*	30.0*
<i>n</i> -Butyric acid	3.13 \pm 1.71	5.14	40.0*	2.20 \pm 1.8	5.27	80.0*
Ethyl decanoate	10.0 \pm 2.0*	10.0*	70.0**	40.0 \pm 30*	110*	4.00*
Isovaleric acid	1.65 \pm 0.45	4.46	1.27	1.60 \pm 0.13	2.00	1.35
Diethyl succinate	9.63 \pm 2.4	15.4	5.30	9.33 \pm 3.0	17.1	4.60
<i>n</i> -Valeric acid	1.59 \pm 0.35	3.80	1.30	1.63 \pm 0.14	1.90	1.38
2-Phenethyl acetate	200 \pm 130*	560*	40.0*	300 \pm 200*	1.04	30.0*
Hexanoic acid	3.50 \pm 0.62	5.66	2.43	4.13 \pm 0.78	6.38	2.89
Guaiacol	450 \pm 260*	1.14	40.0*	470 \pm 220*	1.26	90.0*
<i>trans</i> -oak-lactone	1.04 \pm 0.01	1.05	1.03	1.04 \pm 0.004	1.04	1.03
2-Phenylethyl alcohol	16.4 \pm 6.5	36.8	8.47	13.4 \pm 4.3	24.3	6.76

Characterization of Pinotage wines

<i>cis</i> -oak-lactone	1.00 ± 0.02	1.04	980*	980 ± 10*	1.00	970*
<i>o</i> -Cresol	850 ± 60*	1.00	770*	830 ± 30*	910*	740*
Phenol	1.02 ± 0.72	3.27	190*	740 ± 350*	1.55	200*
4-Ethylguaiacol	360 ± 10*	390*	340*	370 ± 50*	700*	340*
Octanoic acid	1.62 ± 0.42	3.33	1.04	1.90 ± 0.37	3.08	1.28
<i>p</i> -Cresol	290 ± 50*	430*	220*	280 ± 20*	350*	250*
Eugenol	650 ± 100*	950*	510*	635 ± 85*	952*	496*
Decanoic acid	730 ± 190*	1.91	600*	780 ± 80*	1.02	690*
2,6-Dimethoxy phenol	12.5 ± 12	53.7	3.40	9.69 ± 6.9	37.0	1.72
5-(Hydroxymethyl)furfural	7.05 ± 6.8	27.8	590*	2.33 ± 3.0	12.9	70.0*
Vanillin	40.9 ± 25	141	14.9	43.7 ± 50	237	3.98

n: number of samples. * measured in µg/L. ** measured in ng/L.

6.3.3.1. Esters

Young Pinotage wines are characterized by relatively higher concentration of esters, particularly isoamyl acetate [2]. Between the two vintages, 2006 showed higher levels of esters, although the value for ethyl butyrate was reasonably constant across the various regions, and, in fact, slight differences were insignificant. The observed differences between the two vintages can be ascribed to variation in grape composition during harvest, resulting from differences in climatic conditions and grape maturity [1]. For the ethyl esters the mean values of ethyl lactate and diethyl succinate were significantly higher in vintage 2006. Similar trends have been reported by Falqué et al. [20] for white wines. The acetate esters revealed comparatively higher values in vintage 2006. Isoamyl acetate, which gives a pleasant banana-like aroma to wine, was reported to exist at a relatively higher concentration in young Pinotage wines. However, at a very high level it can reveal a negative (nail polish) character [1,2].

Small variations in the values obtained were also observed among different regions (**Table 6.5**). For instance, isoamyl acetate content was highest in samples obtained from the OR region and lowest in region RO for the 2005 vintage. Ethyl lactate was highest in region SW in 2005. Ethyl acetate, diethyl succinate and 2-phenylethyl acetate were comparatively higher in regions SW, OR and S, respectively, in 2005. On the other hand, hexyl acetate levels were lowest in region RO. Ethyl acetate and 2-phenylethyl acetate were higher in regions P, OR, and RO, respectively. On the contrary, the level of diethyl succinate of the same vintage 2006 for the former two regions was lower. It should be noted that the use of nitrogen-containing fertilizers can have a significant effect on the amount of esters in the wine [33]. The mean concentrations of C₆ – C₁₀ ethyl esters were significantly lower relative to the rest and were higher in the 2006 compared to 2005. Although slightly lower, these levels are in general agreement with the values found by Alves et al. [6] in Madeira wine.

6.3.3.2. Alcohols

The mean values of most of the alcohols investigated between the two vintages were comparable. The fusel alcohols (1-propanol, isobutanol, isoamyl alcohol, and 2-phenylethyl alcohol) were present at highest concentrations. These alcohols are

believed to be formed as secondary products of metabolism by yeast [13]. 1-Propanol and isobutanol levels appear to be slightly higher in 2005 compared to 2006. The average content of isoamyl alcohol was the highest of all the alcohols in all wines, whereas the average level of 1-hexanol was the lowest in both vintages, which seems to coincide with the previous result for Mencia wines [12]. In a similar fashion as detailed for the esters, variations in the mean values among few regions were evident (**Table 6.5.**). The highest mean concentration of 1-propanol was measured in region SW of 2005 vintage. 2-Phenylethyl alcohol, which has an aromatic description of “rose” [3] and may contribute to the floral nuance of the wines [20], appeared to be present in high concentration next to isoamyl alcohol, 1-propanol and isobutanol. These values show similarity to those reported by Selli et al. [3] and Calleja et al. [12] for other red cultivars. The above variations among the wines mentioned could be due to either their geographical origin or wine-making practice such as yeast strains used during fermentation [34]. The rest of the alcohols among the wines of the different regions and vintages showed comparable concentrations.

6.3.3.3. Fatty acids

Acids are normally derived from grape must and yeast fermentation. The mean concentration of isovaleric, valeric and decanoic acids were balanced among the regions and vintages and showed no significant variations (**Table 6.5.**). Similar circumstances were observed for hexanoic acid (vintage 2006) and octanoic acid (vintage 2005). Acetic acid, commonly known by its vinegar odor [27], was present at the highest concentrations of all acids. The mean concentration of acetic acid in vintage 2005 was higher, whereas no significant variation was observed among the regions. For region S (vintage 2006) the highest level of all wines amounting to a mean value of 1.48×10^3 mg/L was recorded, which is also higher than the mean value of vintage 2005 of the same region. This was as a result of the higher concentration of acetic acid (2.64×10^3 and 2.13×10^3 mg/L, respectively) obtained from samples P85 and P87, which were supplied by cellars C85 and C87, respectively. Moreover, a value of 2.63×10^3 mg/L was measured in sample P7 supplied by cellar C7 from region W (2005). This variation could be due to relatively higher oxidation of esters and alcohols [35]. Despite their contribution to volatile acidity, higher amounts of acids could also indicate bacterial spoilage [5]. Reynolds et

al. [36] have indicated that the use of yeasts with lower ethanol formation can result in a higher concentration of acetic acid. Similar results of acetic acid for cultivars of Cabernet Sauvignon and Merlot were reported [8].

The next highest level of acid recorded was propanoic acid, with a mean concentration ranging between 12.5 and 23.2 mg/L for regions KK and S of vintages 2006 and 2005, respectively. This is similar to values obtained by Lilly et al. [37] for white wines. Generally speaking, the mean concentrations obtained were comparable among the different regions of the two vintages. Isobutyric and butyric acids are characterized by a fatty and cheesy smell [10]. The mean concentrations of isobutyric acid from vintage 2005 were very similar to butyric acid in vintage 2006 samples. A similar trend was observed for isobutyric acid of 2006 and butyric acid of 2005. Octanoic acid, described as being responsible for a fatty and unpleasant odor [5], showed slightly higher concentrations in region P of 2006 in comparison to the rest of wine samples. Very similar contents of C₆, C₈, and C₁₀ fatty acids were reported by Falqué et al. [20]. On average, the values for all the acids among the different regions of the vintages were similar.

6.3.3.4. Volatile phenols

Volatile phenols originate from the thermal degradation of lignin from oak wood during the toasting of the staves, but some of them are also present in the wood itself [38]. The mean concentrations of phenol compounds studied in this work (guaiacol, *o*-cresol, phenol, 4-ethylguaiacol, *p*-cresol and eugenol) were between 0.110 and 1.36 mg/L, with similar values among all of the regions. Eugenol, with its clove-like odor, was reported as an important contributor to the aroma of wine [38]. Contrary to the other volatile phenols, 2,6-dimethoxyphenol displayed a distinct result for most of the regions, which varied between 6.68 mg/L for region S, being the lowest, and 14.8 mg/L for region W of the 2005 vintage. The mean concentration 28.2 mg/L of 2,6-dimethoxyphenol for the sample obtained from region OR was slightly higher when compared to the other regions. In the 2006 vintage, however, the values were slightly different, ranging between 5.48 mg/L in region W and 22.7 mg/L for region S. 4-Ethylguaiacol, which is responsible for the spicy and clove-like aroma in a wine, was observed to have very similar values among all of the regions as well as the

vintages. This compound results from enzymatic decarboxylation and reduction of ferulic acid [39].

6.3.3.5. Carbonyls

The carbonyl compounds dealt with in this study were acetoin, furfural, 5-methylfurfural, 5-(hydroxymethyl)furfural (5-HMF) and vanillin. The last four aldehydes are believed to be derived from wood cooperage [40]. Acetoin was estimated at higher concentration values with slight differences among the regions. This is in agreement with the previously reported values for red and white wines [41]. However, a significant gap between the lowest and highest mean concentration values in wines from vintage 2005 compared to 2006 was visible, which could be related to the wine-making practice (especially the yeast strain) [42]. Vanillin, commonly associated with vanilla flavor [3], is related to the lignin of wood [32] and has the next highest mean concentration. Region S in 2006 showed the highest mean value of 92.3 mg/L. According to Morales et al. [40] the use of oak chips is a valuable alternative to oak barrels in order to increase the concentration of vanillin in wine. Comparable results among the different regions and vintages were obtained for furfural and 5-methylfurfural, the latter being the lowest mean concentration of all carbonyls. The mean concentration of 5-HMF for samples from the majority of the regions displayed between 1.25 mg/L (region P) and 13.0 mg/L (region S). However, some discrepancy in regions W and KK was evident, showing mean values of 0.760 and 0.390 mg/L, respectively.

Characterization of Pinotage wines

Table 6.5. Mean \pm SD (mg/l) of volatiles in Pinotage 2005 and 2006 vintages collected from various South African regions obtained using headspace SBSE-TD-GC-MS (for conditions, see text).

Compound	Mean \pm SD (mg/l) of various regions					
	Vintage 2005					
	P (n = 10)	S (n = 10)	W (n = 14)	RO (n = 4)	OR (n = 4)	SW (n = 5)
Ethyl acetate	161 \pm 40	134 \pm 43	134 \pm 45	137 \pm 38	116 \pm 33	172 \pm 47
Ethyl butyrate	280 \pm 80*	250 \pm 40*	340 \pm 110*	200 \pm 60*	350 \pm 73*	350 \pm 80*
1-Propanol	63.6 \pm 42	64.9 \pm 35	62.3 \pm 24	43.1 \pm 12	42.0 \pm 20	78.3 \pm 86
Isobutanol	51.5 \pm 12	57.9 \pm 27	53.5 \pm 23	81.9 \pm 31	52.5 \pm 8.3	36.1 \pm 21
Isoamyl acetate	4.45 \pm 2.1	5.56 \pm 2.3	4.64 \pm 1.9	1.36 \pm 0.60	6.27 \pm 1.9	3.12 \pm 2.9
<i>n</i> -Butanol	7.83 \pm 2.6	7.87 \pm 3.6	8.36 \pm 3.3	4.72 \pm 3.5	5.61 \pm 5.8	7.15 \pm 3.6
Isoamyl alcohol	145 \pm 15	158 \pm 26	164 \pm 18	175 \pm 23	173 \pm 18	162 \pm 16
Ethyl hexanoate	190 \pm 70*	150 \pm 30*	250 \pm 130*	150 \pm 30*	270 \pm 60*	230 \pm 60*
Hexyl acetate	10.0 \pm 10*	20.0 \pm 10*	20.0 \pm 10*	10.0 \pm 2.0*	20.0 \pm 10*	10.0 \pm 5.0*
Acetoin	57.5 \pm 57	55.9 \pm 35	53.6 \pm 41	81.8 \pm 60	42.7 \pm 27	62.9 \pm 28
Ethyl-D-lactate	264 \pm 110	337 \pm 82	259 \pm 93	241 \pm 115	316 \pm 90	389 \pm 302
1-Hexanol	655 \pm 305*	570 \pm 320*	570 \pm 330*	340 \pm 400*	650 \pm 300*	560 \pm 340*
Ethyl octanoate	30.0 \pm 10*	30.0 \pm 10*	40.0 \pm 20*	20.0 \pm 10*	40.0 \pm 10*	30.0 \pm 10*
Acetic acid	634 \pm 232	973 \pm 381	875 \pm 657	842 \pm 340	973 \pm 161	848 \pm 309
Furfural	14.6 \pm 7.5	18.7 \pm 2.1	15.0 \pm 10	13.3 \pm 8.9	12.0 \pm 8.0	18.4 \pm 2.1
Propionic acid	17.0 \pm 7.3	23.2 \pm 11	19.1 \pm 13	20.8 \pm 15	17.1 \pm 5.1	18.7 \pm 9.4
Isobutyric acid	1.32 \pm 0.56	2.11 \pm 1.3	1.31 \pm 0.49	2.13 \pm 0.57	2.64 \pm 0.82	1.96 \pm 0.80
5-Methylfurfural	470 \pm 270*	490 \pm 290*	370 \pm 280*	370 \pm 340*	490 \pm 70*	400 \pm 320*
<i>n</i> -Butyric acid	2.60 \pm 1.8	3.58 \pm 1.5	3.45 \pm 1.7	2.56 \pm 1.6	3.03 \pm 2.1	2.92 \pm 2.3
Ethyl decanoate	10.0 \pm 2.0*	10.0 \pm 3.0*	10.0 \pm 2.0*	4.00 \pm 2.0*	10.0 \pm 3.0*	10.0 \pm 2.0*
Isovaleric acid	1.50 \pm 0.14	1.92 \pm 0.91	1.60 \pm 0.19	1.54 \pm 0.05	1.72 \pm 0.17	1.61 \pm 0.12
Diethyl succinate	9.69 \pm 1.7	9.59 \pm 2.3	9.47 \pm 2.7	7.07 \pm 1.1	11.42 \pm 1.83	10.6 \pm 2.6
<i>n</i> -Valeric acid	1.48 \pm 0.09	1.76 \pm 0.73	1.58 \pm 0.13	1.55 \pm 0.12	1.61 \pm 0.10	1.53 \pm 0.15
2-Phenethyl acetate	150 \pm 80*	320 \pm 160*	170 \pm 90*	110 \pm 80*	300 \pm 150*	160 \pm 110*
Hexanoic acid	3.19 \pm 0.44	3.56 \pm 0.87	3.52 \pm 0.51	3.05 \pm 0.52	4.06 \pm 0.32	3.88 \pm 0.46
Guaiacol	420 \pm 200*	500 \pm 270*	450 \pm 310*	460 \pm 270*	370 \pm 180*	460 \pm 300*
<i>trans</i> -oak-lactone	1.04 \pm 0.01	1.04 \pm 0.004	1.04 \pm 0.01	1.04 \pm 0.01	1.04 \pm 0.01	1.04 \pm 0.004
2-Phenylethyl alcohol	13.3 \pm 4.1	21.2 \pm 9.1	14.2 \pm 5.0	18.5 \pm 4.6	17.7 \pm 4.3	16.1 \pm 6.3
<i>cis</i> -oak-lactone	1.01 \pm 0.02	990 \pm 8.0*	1.00 \pm 0.02	1.00 \pm 0.01	990 \pm 10*	990 \pm 10*
<i>o</i> -Cresol	840 \pm 50*	850 \pm 60*	850 \pm 70*	870 \pm 80*	830 \pm 50*	840 \pm 60*

Characterization of Pinotage wines

Phenol	910 ± 450*	830 ± 620*	1.09 ± 0.89	1.36 ± 0.55	1.31 ± 1.2	920 ± 620*
4-Ethylguaiacol	360 ± 10*	360 ± 10*	360 ± 10*	360 ± 10*	360 ± 10*	360 ± 10*
Octanoic acid	1.43 ± 0.27	1.86 ± 0.64	1.56 ± 0.30	1.36 ± 0.36	1.77 ± 0.36	1.79 ± 0.25
<i>p</i> -Cresol	280 ± 50*	280 ± 50*	300 ± 60*	280 ± 40*	290 ± 26*	300 ± 40*
Eugenol	620 ± 90*	670 ± 100*	660 ± 120*	650 ± 70*	620 ± 80*	650 ± 130*
Decanoic acid	670 ± 50*	840 ± 390*	(690 ± 50)*	670 ± 60*	730 ± 70*	730 ± 80*
2,6-Dimethoxy phenol	8.62 ± 6.1	6.68 ± 3.4	14.8 ± 17	11.3 ± 4.1	28.2 ± 10	13.8 ± 7.4
5-(Hydroxymethyl)furfural	1.25 ± 0.88	13.0 ± 8.3	8.11 ± 6.2	6.34 ± 6.8	6.85 ± 4.1	4.48 ± 3.2
Vanillin	32.2 ± 14	37.9 ± 24	42.5 ± 33	45.9 ± 12	45.9 ± 7.4	52.0 ± 36
Vintage 2006						
	P (n = 9)	S (n = 4)	W (n = 11)	RO (n = 7)	KK (n = 4)	SW (n = 5)
Ethyl acetate	215 ± 41	194 ± 48	190 ± 26	174 ± 34	177 ± 12	181 ± 28
Ethyl butyrate	420 ± 110*	330 ± 70*	390 ± 80*	300 ± 60*	350 ± 50*	330 ± 70*
1-Propanol	58.8 ± 28	61.8 ± 61	62.1 ± 43	45.7 ± 22	34.9 ± 7.5	35.0 ± 14
Isobutanol	44.3 ± 19	52.5 ± 25	45.6 ± 11	57.6 ± 22	48.3 ± 6.7	55.1 ± 10
Isoamyl acetate	6.06 ± 3.0	7.13 ± 4.1	6.53 ± 2.1	5.31 ± 3.3	5.12 ± 2.3	4.18 ± 3.5
<i>n</i> -Butanol	7.01 ± 3.1	8.73 ± 1.8	9.66 ± 6.9	7.09 ± 1.1	7.28 ± 1.5	7.67 ± 2.0
Isoamyl alcohol	149 ± 18	148 ± 14	148 ± 11	169 ± 21	149 ± 22	145 ± 21
Ethyl hexanoate	500 ± 150*	390 ± 50*	430 ± 100*	380 ± 110*	350 ± 60*	510 ± 160*
Hexyl acetate	30.0 ± 20*	40.0 ± 20*	40.0 ± 20*	30.0 ± 20*	20.0 ± 10*	40.0 ± 30*
Acetoin	77.5 ± 66	77.8 ± 92	52.1 ± 25	76.8 ± 48	67.4 ± 74	73.1 ± 26
Ethyl-D-lactate	335 ± 89	319 ± 57	288 ± 82	275 ± 128	235 ± 160	296 ± 68
1-Hexanol	640 ± 320*	500 ± 280*	620 ± 570*	780 ± 170*	510 ± 460*	450 ± 350*
Ethyl octanoate	140 ± 40*	110 ± 40*	110 ± 30*	100 ± 30*	100 ± 30*	120 ± 40*
Acetic acid	622 ± 294	1.48x10 ³ ± 1.1x10 ³	494 ± 223	748 ± 229	452 ± 202	533 ± 228
Furfural	9.68 ± 7.8	13.7 ± 7.6	10.9 ± 7.4	8.14 ± 5.4	10.9 ± 6.7	7.69 ± 7.5
Propionic acid	16.4 ± 6.9	22.3 ± 17	12.8 ± 7.5	15.2 ± 11	12.5 ± 2.4	15.0 ± 12
Isobutyric acid	2.60 ± 1.5	2.29 ± 0.60	1.87 ± 0.35	3.28 ± 2.0	2.59 ± 0.51	2.84 ± 0.88
5-Methylfurfural	330 ± 220*	390 ± 310*	370 ± 260*	240 ± 190*	200 ± 150*	310 ± 80*
<i>n</i> -Butyric acid	1.44 ± 1.3	3.43 ± 1.9	2.86 ± 1.7	1.66 ± 2.1	1.30 ± 1.1	2.62 ± 2.4
Ethyl decanoate	60.0 ± 30*	40.0 ± 20*	30.0 ± 20*	30.0 ± 25*	20.0 ± 10*	41.2 ± 40*
Isovaleric acid	1.58 ± 0.13	1.69 ± 0.10	1.55 ± 0.12	1.71 ± 0.15	1.61 ± 0.12	1.54 ± 0.11
Diethyl succinate	8.30 ± 2.4	12.6 ± 3.4	8.79 ± 2.0	10.8 ± 4.0	8.54 ± 3.2	8.37 ± 2.2
<i>n</i> -Valeric acid	1.63 ± 0.14	1.65 ± 0.21	1.60 ± 0.12	1.60 ± 0.12	1.67 ± 0.19	1.65 ± 0.19
2-Phenethyl acetate	260 ± 130*	370 ± 240*	260 ± 90*	410 ± 340*	290 ± 190*	230 ± 190*
Hexanoic acid	4.51 ± 0.98	3.98 ± 0.59	4.02 ± 0.63	4.18 ± 0.12	3.96 ± 0.70	3.88 ± 0.61

Characterization of Pinotage wines

Guaiacol	560 ± 220*	740 ± 360*	360 ± 140*	430 ± 190*	420 ± 150	430 ± 130*
<i>trans</i> -oak-lactone	1.03 ± 0.004	1.04**	1.04 ± 0.004	1.04 ± 0.0003	1.04**	1.04**
2-Phenylethyl alcohol	11.6 ± 3.4	15.1 ± 4.3	11.4 ± 1.8	18.7 ± 4.7	12.1 ± 2.6	13.2 ± 4.7
<i>cis</i> -oak-lactone	980 ± 10*	980 ± 20*	980 ± 10*	980 ± 10*	980 ± 10*	980 ± 10*
<i>o</i> -Cresol	840 ± 30*	850 ± 40*	810 ± 40	830 ± 30*	820 ± 20*	840 ± 30*
Phenol	830 ± 340*	1.33 ± 0.29	560 ± 250*	690 ± 330*	640 ± 200*	690 ± 240*
4-Ethylguaiacol	360 ± 10*	370 ± 10*	350 ± 10*	360 ± 10*	360 ± 10*	420 ± 150*
Octanoic acid	2.11 ± 0.50	1.80 ± 0.26	1.83 ± 0.30	1.90 ± 0.47	1.72 ± 0.18	1.90 ± 0.27
<i>p</i> -Cresol	290 ± 20*	300 ± 40*	270 ± 20*	280 ± 20*	280 ± 20*	280 ± 30*
Eugenol	670 ± 90*	2.59 ± 3.7	600 ± 50*	620 ± 80*	610 ± 70*	620 ± 20*
Decanoic acid	820 ± 100*	780 ± 30*	760 ± 50*	780 ± 90	730 ± 60*	790 ± 100*
2,6-Dimethoxy phenol	10.2 ± 4.8	22.7 ± 10	5.48 ± 3.0	9.00 ± 3.6	11.3 ± 6.3	7.39 ± 6.4
5-(Hydroxymethyl)furfural	2.44 ± 4.0	6.27 ± 4.6	760 ± 430*	2.07 ± 1.1	390 ± 520*	4.38 ± 1.8
Vanillin	58.5 ± 54	92.3 ± 99	27.3 ± 26	50.5 ± 50	25.3 ± 27	19.2 ± 17

n = number of samples analyzed from each region. P, S, W, RO, OR, SW and KK are the codes given to the different regions (full descriptions of the regions refer to the text and footnote of **Table 6.1.**). * measured in µg/L. ** Identified only in one sample. SD: Standard deviation.

6.3.3.6. Lactones

The two main wood lactones, *trans*- and *cis*-oak lactones, commonly known as whiskey lactone, were investigated in this study. These racemic isomers, which emanate from oak wood [31] and add a coconut flavor to the wine [32], were not detected in some of the wines. From the 2005 vintage, *trans*- and *cis*-oak lactones were below the detection limit in 13 and 5 wines, respectively. On the other hand, they were identified and measured only in 13 and 25 samples of vintage 2006, respectively. As these compounds are extracted from wood, the observation could be related to wine-making practice [40]. Jarauta et al. reported that qualitative and quantitative detection of the *trans*- and *cis*-oak lactones can be affected by the storage material (oak wood/stainless steel) and origin of oak wood [31]. In a similar way, Díaz-Maroto et al. [32] have shown the variation in concentration of these two isomers based on origin and type (toasted vs non-toasted) as well as length of storage time in the oak wood. In the rest of the samples the calculated mean concentration of the *trans*- and *cis*-isomers of whiskey lactone among all of the regions of the two vintages were very similar.

6.3.4. Statistical analysis

The concentration levels determined for the volatiles in the 87 Pinotage wine samples of vintages 2005 and 2006 from various South African regions were subjected to statistical analysis. Exploratory FA, PCA and one-way ANOVA were applied to characterize and examine the relationships among the variables as well as to determine if there are considerable differences among the volatile components with respect to their origin and vintages.

6.3.4.1. Factor analysis (FA)

FA is a method of multivariate analysis that linearly transforms one set of variables into another set of fewer variables (factors) that conserve the information of the original set, searches for associations among the variables, and is able to detect natural groups present in the samples (unsupervised method) [13]. FA was done using the independent variables (concentration of volatiles) with respect to the dependent variables (two vintages and seven regions). As mentioned above, *trans*- and *cis*-

isomers of whiskey lactone were unidentified in some samples largely in the 2006 vintage wines. Hence, the two isomers of whiskey lactone were removed from the statistical analysis, reducing the number of variables to 37.

Table 6.6. Results of FA using 37 volatile components and 87 samples.

Factors	Eigen value	Cumulative Eigen value	% Total Variance	Cumulative %
Factor 1	8.15	8.15	22.03	22.03
Factor 2	6.41	14.57	17.33	39.37
Factor 3	3.25	17.82	8.79	48.15
Factor 4	2.13	19.95	5.77	53.92
Factor 5	1.98	21.93	5.35	59.27

Even though selection of factors that can explain > 75% of the total variability is preferable, this could only be achieved from 10 factors with eigen values > 1. However, only the first five factors that cover 59.27% of the total variance (**Table 6.6.**) were selected because it was evident from the analysis that increasing the number of factors adds only a very small percentage to the total variability, as well as reduces the number of components loaded to each factor.

Table 6.7. presents the loading of each variable to the selected factors. To simplify the presentation of the results, loading variables with absolute coefficient values of ≥ 0.30 were selected.

Factor 1 explained 22% of the total variance. The highest numbers of variables were associated with this factor. Lower acids of C₂ and C₃ showed positive correlation with factor 1. On the contrary, ethyl acetate was observed to have a high negative correlation. This behavior could be related to the oxidation of ethyl acetate into acetic acid [35]. Most volatile phenols, which are believed to originate from thermal degradation of the lignin of oak wood during the toasting of the staves [38], showed higher positive association to factor 1. Other wood related compounds with high positive association to factor 1 were furfural, 5-methylfurfural, 5-HMF and vanillin. 2-Phenylethyl alcohol was also highly associated with this factor.

Compounds formed during alcoholic fermentation such as ethyl and acetate esters [43] proved to have high positive correlation with factor 2. Even though low, compounds related to usage of oak wood during wine processing, particularly the furfural-derived compounds [40], were negatively correlated to factor 2. Because the

association of C₆ and C₈ acids demonstrated a positive sign to factor 2, they must have evolved in a similar way to the esters.

Moreover, the fatty acids, except butyric acid, showed high positive correlation to factor 3. Generally speaking, factors 1, 2 and 3 were associated with compounds that evolved due to microbiological processes during fermentation and storage such as esters, acids, and higher alcohols as well as compounds released from wood and transferred to the wine during ageing in the barrels.

Table 6.7. Loadings of the variables to the selected factors.

Variables	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Ethyl acetate	-0.42	0.52	–	–	-0.41
Ethyl butyrate	–	0.70	–	–	–
1-Propanol	–	–	–	0.76	–
Isobutanol	-0.32	–	–	–	0.62
Isoamyl acetate	–	0.76	–	–	–
<i>n</i> -Butanol	–	–	–	0.41	–
Isoamyl alcohol	–	–	–	–	0.76
Ethyl hexanoate	–	0.81	–	–	–
Hexyl acetate	–	0.79	–	–	–
Acetoin	0.42	–	–	0.37	–
Ethyl-D-lactate	–	–	0.40	0.42	–
1-Hexanol	–	–	–	–	–
Ethyl octanoate	–	0.83	–	–	–
Acetic acid	0.84	–	–	0.32	–
Furfural	0.32	-0.43	–	–	–
Propionic acid	0.86	–	–	–	–
Isobutyric acid	–	–	0.46	-0.30	–
5-Methylfurfural	0.49	–	–	–	–
<i>n</i> -Butyric acid	–	–	–	0.42	–
Ethyl decanoate	–	0.81	–	–	–
Isovaleric acid	–	–	0.84	–	–
Diethyl succinate	–	–	–	–	–
<i>n</i> -Valeric acid	–	–	0.87	–	–
2-Phenethyl acetate	–	0.64	–	–	0.41
Hexanoic acid	–	0.54	0.65	–	–
Guaiacol	0.86	–	–	–	–
2-Phenylethyl alcohol	0.31	–	0.35	–	0.66
<i>o</i> -Cresol	0.89	–	–	–	–
Phenol	0.85	–	–	–	–
4-Ethylguaiacol	0.31	0.32	–	–	–
Octanoic acid	–	0.46	0.73	–	–
<i>p</i> -Cresol	0.77	–	–	–	–
Eugenol	–	–	–	0.53	–
Decanoic acid	–	–	0.90	–	–
2,6-Dimethoxy phenol	0.77	–	–	–	–
5-(Hydroxymethyl)furfural	0.72	–	–	–	–
Vanillin	0.69	–	–	–	–

Higher alcohols such as 1-propanol, isobutanol, *n*-butanol, isoamyl alcohol and 2-phenylethyl alcohol, which enter the wine medium as secondary products of yeast metabolism [13], were positively associated with factors 4 and 5. Another compound

positively associated with factor 4 was eugenol. It should be noted that there were other compounds associated with each factor, but their value was not considered because the loading was small.

Factors 1 and 2 covered the highest percentage of the total variance of the data in comparison with the other factors, hence, only these two factors had clear enological importance and therefore will be discussed.

6.3.4.2. Advanced PCA factor analysis

PCA studies were carried out on the basis of the factors selected above as components for the PCA. The percentage of the total variability captured was 59.27% (**Table 6.6**). The interpretation of the volatile pattern and wine characteristics was mainly based on the representation of information contained in factors 1 and 2. Plots of other combinations of factors (components) were also examined (graphs not shown here), even though they did not bring additional information of interest in the wine characterization. It should be noted that, however, these interpretations have to be cautious as the percentage of variance retained with these two factors was quite limited.

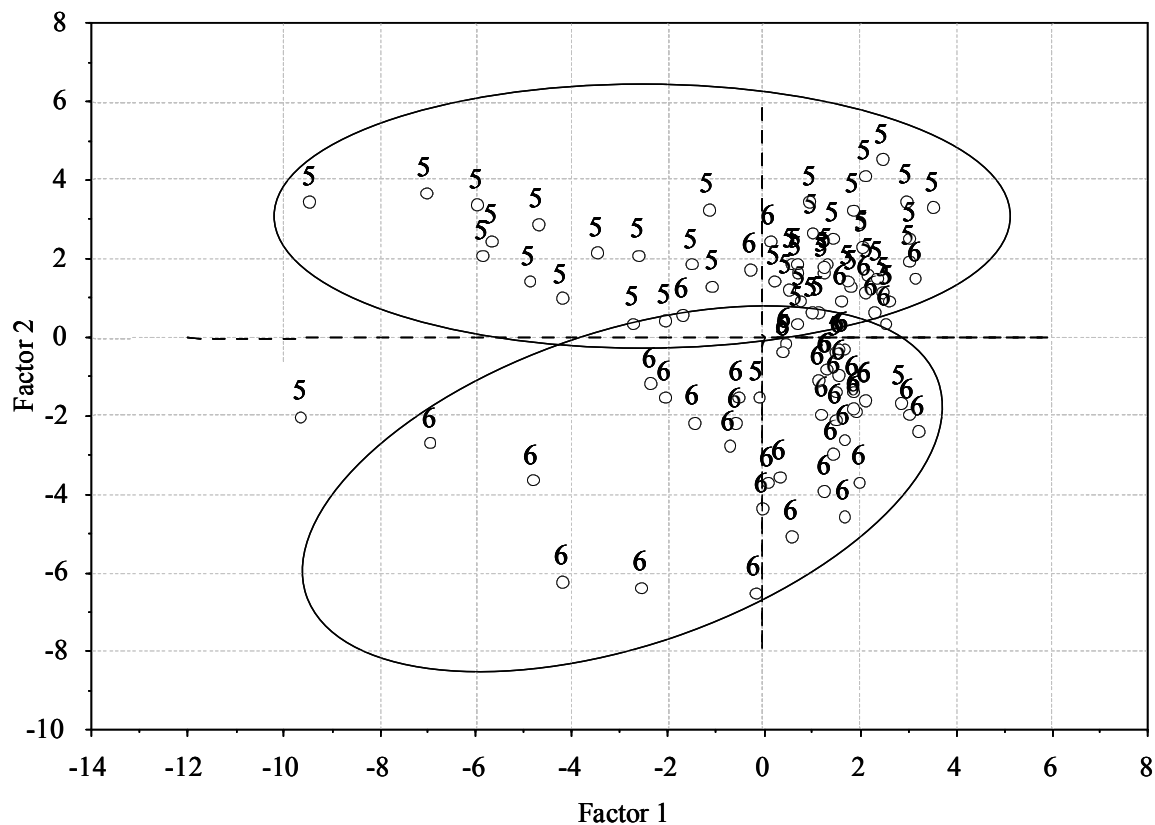


Figure 6.2. Distribution of Pinotage wines studied in the plane defined by factors 1 and 2 according to vintage. 5 and 6 represent vintages 2005 and 2006, respectively.

The variation in volatile compounds between the two vintages (2005 and 2006) was already highlighted by the plane-defined PCA plot (**Figure 6.2.**). The wines of vintage 2005 were mainly situated at the top zone of the graph, whereas the 2006 vintage wines appeared at the bottom. This observation could be due to seasonal differences during harvesting of the grapes. Obviously, this behavior could not be understood conclusively as some samples appeared in the intermediate zones and certain mixing of samples was observed. The study of the distribution of samples according to their geographical origin did not show relevant pattern (**Figure 6.3.**). Unlike the observed grouping between the vintages, the PCA plots did not conform to groupings based on their geographic characteristics of the wines, as they are scattered all over the plane, mostly around the origin of the graph. Hence, the regional classification did not bring additional or complementary conclusions of interest in wine description and characterization as the regions were widely spread with no predominant areas.

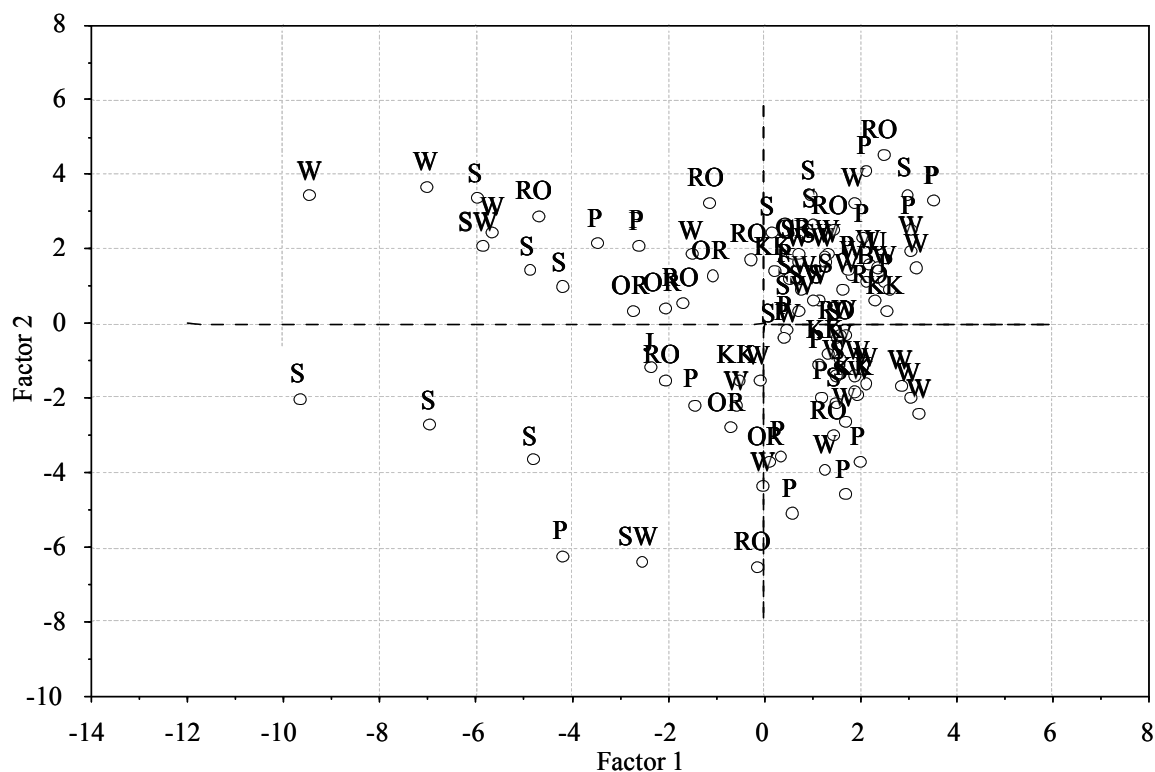


Figure 6.3. Distribution of Pinotage wines studied in the plane defined by factors 1 and 2 according to their geographic origin. P, S, W, RO, OR, SW and KK are the codes given to the different regions (full descriptions of the regions, refer to the text and footnote of **Table 6.1.**).

PCA of loadings of the variables based on the first two factors using the concentrations of volatile compounds obtained was also performed (**Figure 6.4.**). This figure shows clearly the association of the compounds with each other as well as with the first two factors. With the exception of a few discrepancies, it revealed some relevant pattern of the volatiles. As can be seen from the graph, the wood-related compounds appear on the top left area of the loading plot. On the other hand, the esters appear on the bottom right of the plot. This indicates that these two groups of compounds are negatively correlated with one another. In a similar way, alcohols are situated on the top right part of the plot, but acids appear on the bottom left of the loading plot. A very similar correlation could be drawn for these two classes of compounds as well.

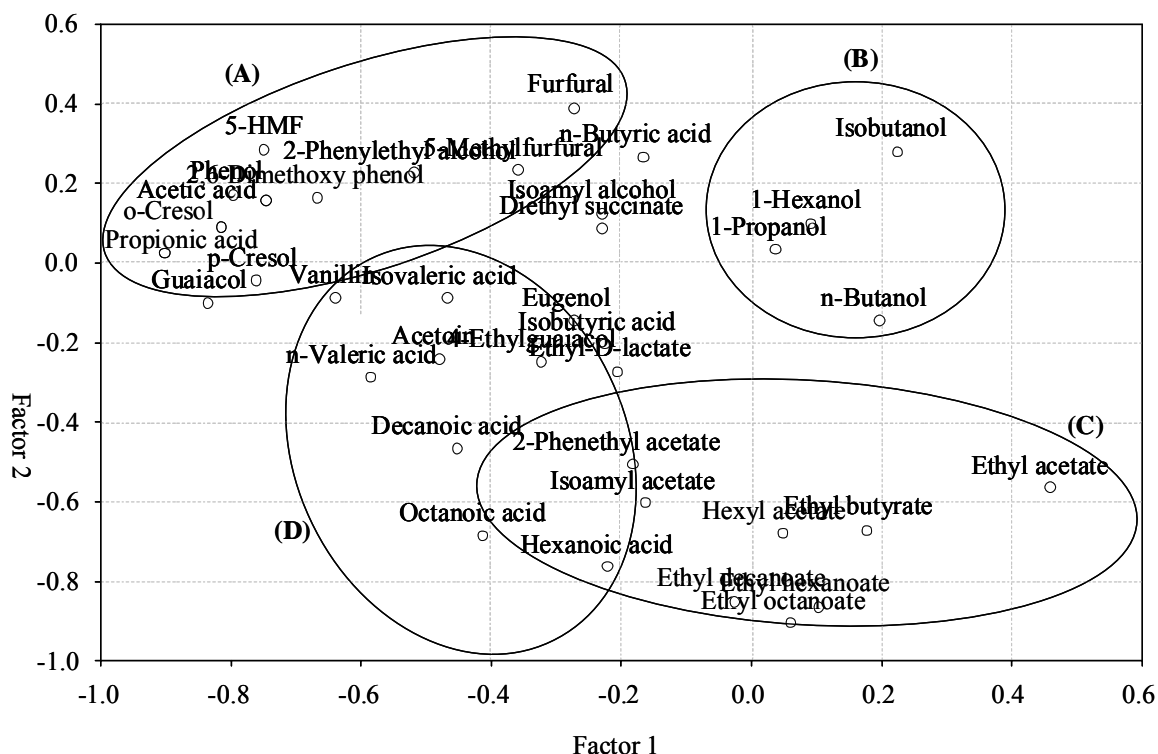


Figure 6.4. Distribution of volatile components in the plane defined by factors 1 and 2: A, wood-related compounds; B, alcohols; C, esters; D, acids.

As an alternative way of comparison among the obtained results, one-way ANOVA was performed. The data analyzed for each vintage and regions correspond to the mean concentration obtained for each compound studied. **Table 6.8.** presents the volatile components that showed significant and non-significant differences among the various regions and vintages. As a counter check for the ANOVA *p*-value obtained in determining the difference of the mean value of each compound between the two vintages, a Mann-Whitney U (nonparametric) method was applied, whereas for the different regions, the Kruskal-Wallis (nonparametric) method was used. The *p*-values obtained by the nonparametric methods for both vintages and regions were in agreement with the ANOVA *p*-values. However, in specific cases, where it was found that the ANOVA assumptions were violated, the nonparametric *p*-values were reported (**Table 6.8.**).

Characterization of Pinotage wines

Table 6.8. One-Way ANOVA carried out on quantitative data to analyze the variation of the mean concentration of volatile components among regions and vintages.

Compound Name	Vintages ^a		Regions ^b	
	F-value	P-value	F-value	P-value
Ethyl acetate	36.020	0.000 ^c	1.463	0.201
Ethyl butyrate	11.119	0.001 ^c	2.904	0.013 ^c
1-Propanol	1.996	0.161	1.070	0.387
Isobutanol	1.098	0.297	1.456	0.203
Isoamyl acetate	6.413	0.013 ^c	1.673	0.138
<i>n</i> -Butanol	0.574	0.451	1.187	0.322
Isoamyl alcohol	3.454	0.066	2.811	0.015 ^c
Ethyl hexanoate	104.230	0.000 ^c	1.385	0.230
Hexyl acetate	24.792	0.000 ^c	0.386	0.886
Acetoin	1.535	0.219	0.492	0.813
Ethyl-D-lactate	0.377	0.541	1.670	0.139
1-Hexanol	0.003	0.958	0.609	0.723
Ethyl octanoate	258.540	0.000 ^c	0.671	0.673
Acetic acid	4.334	0.040 ^c	2.213	0.050
Furfural	11.905	0.001 ^c	1.250	0.290
Propionic acid	3.642	0.060	1.074	0.385
Isobutyric acid	13.298	0.000 ^c	3.136	0.008 ^c
5-Methylfurfural	3.525	0.064	0.869	0.521
<i>n</i> -Butyric acid	5.156	0.026 ^c	2.250	0.046 ^c
Ethyl decanoate	79.238	0.000 ^c	0.659	0.683
Isovaleric acid	0.276	0.601	1.573	0.165
Diethyl succinate	0.000	0.988	0.964	0.455
<i>n</i> -Valeric acid	0.501	0.011 ^d	0.677	0.669
2-Phenethyl acetate	6.971	0.010 ^c	1.867	0.096
Hexanoic acid	18.856	0.000 ^c	0.488	0.816
Guaiacol	0.305	0.582	0.763	0.601
2-Phenylethyl alcohol	4.673	0.033 ^c	4.926	0.000 ^c
<i>o</i> -Cresol	3.379	0.069	0.403	0.875
Phenol	4.042	0.047 ^c	0.381	0.889
4-Ethylguaiacol	0.758	0.386	1.214	0.307
Octanoic acid	14.109	0.000 ^c	0.579	0.746
<i>p</i> -Cresol	0.503	0.480	0.114	0.995
Eugenol	0.934	0.336	1.093	0.374
Decanoic acid	3.954	0.050	0.726	0.630
2,6-Dimethoxy phenol	2.405	0.124	1.381	0.232
5-HMF	16.331	0.000 ^c	4.453	0.000 ^c
Vanillin	0.246	0.621	0.535	0.780

^a Vintages 2005 and 2006. ^b Applied to seven regions P, S, W, RO, OR, KK and SW (for full descriptions of the regions, refer to the text and footnote of **Table 6.1**). ^c $p < 0.05$, significant difference. ^d Significant difference confirmed by the nonparametric Mann-Whitney U method.

One-way ANOVA revealed samples with high value among the different wines. For instance, in sample 23 (P23) the concentration of acids was relatively higher in comparison with the rest of the samples. However, for isovaleric, valeric, and decanoic acids the increase in concentration was > 2-fold (**Figure 6.5**). The higher value of the acids in this particular sample could be related to the wine-making practice of that particular supplier, as using different yeasts in the presence of water can promote the production of free fatty acids in wine [35]. The observed differences mentioned for the acids were confirmed by running a residual plot and test of homogeneity of variance.

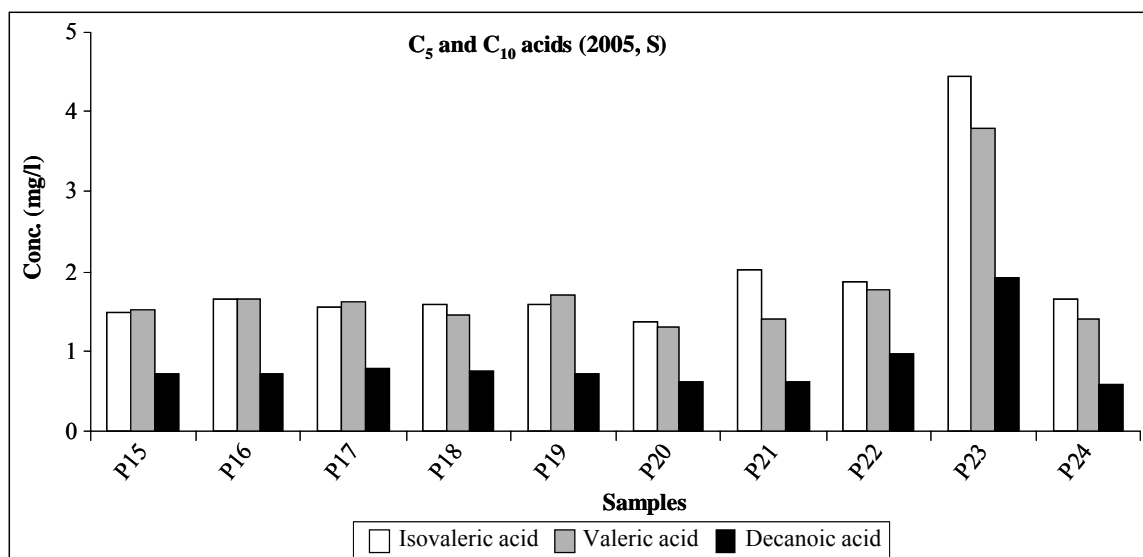


Figure 6.5. Absolute concentration of isovaleric, valeric and decanoic acids in 10 samples (P15 – P24) from region S of vintage 2005.

6.4. Conclusions

The SBSE method was fast, simple, cost-effective, and reliable for the analysis of the 39 volatile components in Pinotage wines, achieving low LODs and LOQs. The precision obtained for the method was within the acceptable range. Moreover, good calibration curves with a wide linearity range of concentrations for each analyte were obtained. The method proposed here for the characterization of wines managed to pull out relevant information on the samples analyzed as well as motivating relationships among concentrations of major wine volatiles and certain wine features such as vintages were deduced.

Simple chemometric techniques such as FA, PCA and One-Way ANOVA were used for processing the data. The role of volatile profiles in the characterization of wine origin was limited. Contents of certain volatiles were somewhat characteristic of a given vintage. The relationship between volatile components and the vintages was certainly substantial. Comparatively, esters were higher in vintage 2006. On the other hand, their corresponding acids were higher in vintage 2005. Volatile phenols showed very comparable results between the two vintages. The aromatic aldehydes, furfural and 5-methylfurfural, which are primarily formed in wood during the toasting process, were slightly lower in 2005 vintage compared to 2006. However, whiskey lactone, especially the cis-isomer, was lower in vintage 2006. Even though there is no clear conclusion, the above observations could be due to variation in either geographical

Characterization of Pinotage wines

origin or the wine-making practice. Because we do not have the detailed history of the wines, we were unable to make a correlation among the different volatiles and their method of production. The statistical approach taken for characterizing the wine samples in terms of their volatiles provides insight for further study.

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Chemometric investigation of the volatile content of young South African wines[†]

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Abstract

The content of major volatiles of 334 wines of six different cultivars (Sauvignon Blanc, Chardonnay, Pinotage, Shiraz, Cabernet Sauvignon and Merlot) and vintage 2005 was used to investigate the aroma content of young South African wines. Wines were sourced from six different regions and various producers. 39 Volatile components partially responsible for the flavour of wine were quantified. In order to investigate possible correlation between volatile content and grape variety and/or geographical origin, analysis of variance, factor analysis (FA), principal component analysis (PCA) and discriminant analysis (DA) were used. Significant differences in the levels of certain volatiles were observed as a function of region and cultivar, with the latter factor proving to be more influential. A few volatile compounds were identified as potential predictors of the white wine cultivars. Prediction for red wine cultivars was poor, with the exception of Pinotage wines, for which four compounds (isoamyl acetate, isoamyl alcohol, ethyl octanoate, and diethyl succinate) were identified as accurate predictors. The importance of these four volatile compounds in distinguishing young Pinotage wines are discussed, and possible reasons for the unique levels in wines of this cultivar are highlighted.

Keywords:

Volatile compounds; Wine; Chemometrics; Pinotage; Cultivar.

7.1. Introduction

In an exceedingly competitive international market, wine producers need to invest in technology to improve production and product quality to remain competitive. As the market is the main driving force behind wine research, it is essential to understand consumer preferences. Human physiology and psychology is associated with the behavioral response of people, who may have certain preferences regardless of their knowledge of the chemical composition of wine. However, it is equally important to understand the relationship between the chemical nature and sensory properties of wines, and by extension the enological and viticultural practices influential determining the chemical content of wine.

Wine aroma is one of the most influential properties when it comes to consumer preference, and is mainly determined by the volatile compounds. Certain volatiles, referred to as impact odorants, are characteristics for particular wine varieties. For instance, norisoprenoid compounds contribute to the varietal character of Chardonnay wines [1], methoxypyrazines contribute to distinctive Sauvignon Blanc and Cabernet Sauvignon aroma [2], and isoamyl acetate was reported as an important aroma constituent of young Pinotage wines [3]. Similarly, furaneol (4-hydroxy-2,5-dimethylfuran-3(2H)-one) has been reported as a caramel odor contributor to Merlot aroma [4], while a sesquiterpene, rotundone, has recently been shown to be responsible for the pepper aroma associated with Shiraz wines [5]. Aside from these impact odorants, the common and universal pattern of wine volatiles is dominated by the major fermentation products such as alcohols, esters and fatty acids [6].

The flavor of young wines results from a series of different biochemical and technological processes. Formation of volatile compounds begins in the grape, while during juice production, fermentation, maturation, ageing and storage the chemical composition continues to change. The amount and type of chemicals that influence wine flavor therefore depend on many factors including the origin of the grapes, grape varieties and ripeness, soil and climate, yeast used during fermentation and a variety of other wine-making practices [7-9].

Taking into consideration the diverse factors that affect the level of each volatile compound in wine, it is often difficult to meaningfully interpret volatile data and establish a relationship between the chemical constituents and particular sensory properties or manufacturing processes. Chemometric methods, in particular multivariate data analysis methods, have proven particularly useful in studies involving the evaluation of food quality and/or authenticity, and indeed their application to wine characterization and classification has increased in recent years. Principal component analysis (PCA) and discriminant analysis (DA) in particular have extensively been applied to characterize wines based on their volatile content [8-12]. Cluster analysis (CA) has been used to categorize wines based on their volatile composition [13,14]. Sivertsen et al. [15] have classified French red wines according to their geographical origin based on sensory and chemical data. Analysis of variance (ANOVA), PCA, CA, and DA have been used to classify South African wines according to cultivar based on volatile [16] and non-volatile content [17,18]. Recently we have used the levels of major volatiles to classify Pinotage wines according to vintage using PCA [19].

The present report aims to extend previous work on the volatile content of South African wine to a much larger and statistically more significant number of samples. The goal was to further investigate the variation in volatile content of young South African wines as a function of specifically region of origin and grape variety. To this end, 334 wine samples from six different cultivars (Sauvignon Blanc, Chardonnay, Pinotage, Shiraz, Cabernet Sauvignon and Merlot) of vintage 2005 were analysed for their content of 39 major volatile compounds using the method reported previously [19]. The choice of a single vintage further reduces the impact of wine age on volatile composition. ANOVA, factor analysis (FA) and multidimensional principal component analysis (MD-PCA) were used. In addition, the predictive method discriminant analysis (DA) was used to identify potentially influential compounds capable of differentiating between wine samples of different cultivar.

7.2. Materials and methods

7.2.1. Wine samples

334 Young South African wines (65 Sauvignon Blanc (SB), 45 Chardonnay (CH), 41 Pinotage (PI), 64 Shiraz (SH), 60 Cabernet Sauvignon (CS) and 59 Merlot (M)) from vintage 2005 were obtained from the South African Young Wine Show. The wines originated from most of the important South African wine producing regions including Paarl (P), Stellenbosch (S), Worcester (W), Robertson (RO), Olifants River (OR), and Swartland (SW). Results for the Pinotage wines of vintage 2005 previously reported [19] are also included in this work for comparison and correlation purposes.

7.2.2. Analytical procedure

A 0.5 ml wine, 50 μ l internal standard solution (1.7 mg/L of 4-methyl-2-pentanol in blank model wine (12% ethanol and 2 g/L tartaric acid in Milli-Q water)) and 1.5 g NaCl were transferred to a 20 ml headspace vial. The volume was adjusted to 6 ml using a blank model wine, and a glass coated magnetic stirrer was added to the mixture. A TwisterTM stir bar (Gerstel[®], Müllheim a/d Ruhr, Germany) was suspended in the headspace using a glass insert (Gerstel), and the vial was sealed using a hand crimper. The mixture was stirred for 1 hour at 1200 rpm and room temperature (23 ± 1 °C).

Following sampling and gentle drying using lint free tissue, the stir bar was placed in a TDS-A auto-sampler tray (Gerstel, Müllheim a/d Ruhr, Germany). Desorption was performed in a TDS 2 thermal desorption unit according to the following temperature program: 30 °C for 1 min, ramped at 20 °C/min to 260 °C, and held for 10 min. Analytes were trapped in a programmed temperature vaporizing (PTV) inlet at -100 °C using liquid nitrogen prior to injection. The PTV was operated in splitless mode for 2 min and heated for injection from -100 to 270 °C at 12 °C/s, kept for 10 min.

An Agilent 6890 GC coupled to a 5973N MS (Agilent Technologies, Palo Alto, CA) equipped with an HP-INNOWax column (30 m \times 0.250 mm i.d. \times 0.5 μ m d_f , Agilent Technologies) was used for volatile separation with helium as carrier gas at a flow

rate of 1 ml/min in constant pressure mode. Compound identification was based on comparison of mass spectra with Wiley 275 and NIST 98 spectral libraries, as well as comparison of retention times of authentic standards. Experimentally calculated linear retention indices (LRI) were used as additional identification criterion. Quantitation was performed using a single (target) ion, in selected ion monitoring (SIM) mode. For further details on the analytical method, the reader is referred to [19].

7.2.3. Statistical analysis

The measured concentrations of 37 volatile compounds in each wine were used for multivariate data analysis following standardization of all variables to 0 mean and 1 standard deviation. Analysis of variance (both one-way and main effects ANOVA), factor analysis, and discriminant analysis were performed using STATISTICA v8 (StatSoft, Inc., Tulsa, OK, USA). PCA bi-plots were constructed using a statistical package written in-house in the R statistical programming language (BiplotGUI). A 5% significance level ($p = 0.05$) was used as a guideline for determining significant differences.

7.3. Results and discussion

7.3.1. Wine analysis

The validated analytical method provided limits of detection (LODs) and limits of quantitation (LOQs) in the range of 0.050 – 281 ng/L and 0.180 – 938 ng/L, respectively. Repeatability for the method was between 6 and 20% [19]. All 39 volatile components studied were identified in most of the wines, with the exception of *trans*- and *cis*-oak-lactones. In the samples where these two isomers were identified and quantified, their mean values were observed to vary between 0.980 and 1.13 mg/L. The variable levels of these compounds are presumably related to differences in levels of wood contact between the wines [20]. Since these two compounds were not quantified in all samples, they were excluded from the statistical analysis.

7.3.2. Analysis of variance (ANOVA)

As a first step, main effects ANOVA was performed using the measured concentrations of the 37 quantified volatile compounds for samples grouped according to region of origin. Main effects ANOVA was applied since variation in volatile content among wines from different regions could potentially be overshadowed by the effect of grape variety. Only eight compounds showed significant differences in mean levels among the six regions (**Table 7.1.**). These include the alcohols isoamyl alcohol, 1-hexanol and phenethyl alcohol; the esters ethyl butyrate and phenethyl acetate; isbutyric acid, acetoin and 5-hydroxymethyl furfural.

Characterization of South African wines

Table 7.1. Main effects ANOVA results for the mean values (mg/L) of volatile compounds between wines from the 6 different regions.

No.	Compounds	Regions ^a						p-value
		Mean ± SD (mg/L) ^b						
		P (n = 72)	S (n = 63)	W (n = 83)	RO (n = 77)	OR (n = 21)	SW (n = 18)	
1	Ethyl acetate	134 ± 35.1	124 ± 34.5	122 ± 35.9	126 ± 38.7	118 ± 35.2	138 ± 40.0	0.11
2	Ethyl butyrate	0.296 ± 0.124	0.278 ± 0.126	0.318 ± 0.129	0.325 ± 0.161	0.323 ± 0.127	0.329 ± 0.108	0.05*
3	1-Propanol	35.3 ± 29.6	32.1 ± 24.1	39.1 ± 20.7	29.2 ± 23.2	28.2 ± 10.3	37.5 ± 33.6	0.33
4	Isobutanol	58.4 ± 33.2	64.6 ± 34.9	59.5 ± 30.8	59.4 ± 38.8	61.8 ± 32.8	55.5 ± 30.8	0.17
5	Isoamyl acetate	2.67 ± 2.28	2.60 ± 2.38	3.15 ± 2.40	3.23 ± 2.59	3.40 ± 2.45	2.58 ± 2.46	0.64
6	<i>n</i> -Butanol	6.84 ± 3.52	7.00 ± 3.80	7.66 ± 3.74	6.39 ± 4.13	6.24 ± 4.25	6.43 ± 3.52	0.36
7	Isoamyl alcohol	172 ± 49.0	188 ± 49.7	179 ± 48.5	177 ± 57.3	186 ± 45.5	170 ± 34.1	< 0.01*
8	Ethyl hexanoate	0.287 ± 0.236	0.250 ± 0.216	0.319 ± 0.243	0.357 ± 0.263	0.281 ± 0.177	0.262 ± 0.154	0.32
9	Hexyl acetate	30.0 ± 37.0 ^c	32.3 ± 41.9 ^c	32.5 ± 38.2 ^c	35.8 ± 37.1 ^c	27.2 ± 31.8 ^c	22.6 ± 29.8 ^c	0.15
10	Acetoin	43.0 ± 33.0	53.2 ± 38.0	40.2 ± 29.2	47.1 ± 29.4	32.7 ± 16.1	48.8 ± 23.6	0.04*
11	Ethyl-D-lactate	165 ± 122	173 ± 123	173 ± 117	117 ± 110	193 ± 120	206 ± 132	0.25
12	1-Hexanol	0.605 ± 0.568	0.586 ± 0.353	0.763 ± 0.493	0.896 ± 0.661	0.586 ± 0.390	0.702 ± 0.789	< 0.01*
13	Ethyl octanoate	41.9 ± 39.8 ^c	35.8 ± 34.9 ^c	41.5 ± 33.3 ^c	54.0 ± 44.7 ^c	40.8 ± 33.0 ^c	34.9 ± 25.3 ^c	0.79
14	Acetic acid	486 ± 280	470 ± 289	456 ± 274	470 ± 348	489 ± 245	552 ± 288	0.77
15	Furfural	13.4 ± 5.50	13.3 ± 6.02	12.2 ± 6.44	12.0 ± 6.32	12.3 ± 6.54	12.4 ± 6.84	0.67
16	Propionic acid	11.5 ± 7.79	13.4 ± 7.90	11.6 ± 8.43	9.52 ± 7.78	11.9 ± 6.69	12.5 ± 7.11	0.33
17	Isobutyric acid	1.73 ± 1.13	2.05 ± 1.40	1.67 ± 1.13	2.24 ± 1.81	2.02 ± 1.13	1.48 ± 1.37	< 0.01*
18	5-Methylfurfural	0.446 ± 0.215	0.384 ± 0.233	0.348 ± 0.231	0.354 ± 0.247	0.431 ± 0.236	0.402 ± 0.248	0.08
19	<i>n</i> -Butyric acid	3.20 ± 1.48	3.11 ± 1.51	2.98 ± 1.60	2.80 ± 1.53	2.96 ± 1.54	3.18 ± 1.52	0.54
20	Ethyl decanoate	6.08 ± 5.17 ^c	6.78 ± 5.99 ^c	6.53 ± 4.27 ^c	6.63 ± 4.77 ^c	6.80 ± 5.02	5.77 ± 3.05 ^c	0.72
21	Isovaleric acid	1.73 ± 0.401	1.79 ± 0.473	1.71 ± 0.348	1.81 ± 0.530	1.70 ± 0.299	1.63 ± 0.230	0.06
22	Diethyl succinate	8.89 ± 3.47	8.73 ± 3.50	8.74 ± 3.63	7.53 ± 3.84	9.82 ± 4.36	10.0 ± 3.63	0.09
23	<i>n</i> -Valeric acid	1.57 ± 0.347	1.51 ± 0.149	1.58 ± 0.274	1.56 ± 0.297	1.54 ± 0.165	1.52 ± 0.147	0.56
24	2-Phenethyl acetate	0.244 ± 0.198	0.335 ± 0.185	0.229 ± 0.154	0.243 ± 0.146	0.204 ± 0.104	0.241 ± 0.223	< 0.01*
25	Hexanoic acid	3.53 ± 1.18	3.53 ± 1.02	3.71 ± 0.975	4.12 ± 1.42	3.72 ± 0.941	3.79 ± 1.17	0.17
26	Guaiacol	0.308 ± 0.181	0.312 ± 0.188	0.304 ± 0.197	0.293 ± 0.185	0.259 ± 0.148	0.259 ± 0.215	0.42
27	2-Phenylethyl alcohol	26.3 ± 20.6	30.8 ± 21.1	22.1 ± 16.4	27.4 ± 23.6	23.9 ± 15.6	24.0 ± 13.8	< 0.01*
28	<i>o</i> -Cresol	809 ± 49.9 ^c	811 ± 46.8 ^c	814 ± 48.5 ^c	809 ± 50.3 ^c	804 ± 48.4 ^c	806 ± 44.5 ^c	0.96
29	Phenol	0.670 ± 0.424	0.665 ± 0.436	0.658 ± 0.432	0.670 ± 0.433	0.572 ± 0.331	0.599 ± 0.438	0.69

Characterization of South African wines

30	4-Ethylguaiacol	351 ± 8.90 ^c	351 ± 8.82 ^c	351 ± 9.32 ^c	350 ± 8.26 ^c	352 ± 12.3 ^c	348 ± 10.2 ^c	0.77
31	Octanoic acid	1.71 ± 0.652	1.85 ± 0.814	1.75 ± 0.677	1.96 ± 0.890	1.78 ± 0.781	1.79 ± 0.633	0.57
32	<i>p</i> -Cresol	258 ± 39.5 ^c	262 ± 43.1 ^c	267 ± 39.9 ^c	258 ± 40.2 ^c	256 ± 32.5 ^c	255 ± 38.3 ^c	0.66
33	Eugenol	602 ± 67.7 ^c	602 ± 66.8 ^c	600 ± 65.0 ^c	598 ± 67.8 ^c	585 ± 63.1 ^c	576 ± 90.4 ^c	0.37
34	Decanoic acid	726 ± 108 ^c	730 ± 107 ^c	730 ± 124 ^c	734 ± 109 ^c	720 ± 80.1 ^c	715 ± 57.8 ^c	0.99
35	2,6-Dimethoxy phenol	5.78 ± 4.13	5.81 ± 5.07	6.12 ± 4.69	5.97 ± 3.64	7.94 ± 8.99	7.44 ± 5.59	0.54
36	5-(Hydroxymethyl)furfural	3.31 ± 3.57	4.78 ± 3.98	4.64 ± 3.72	4.35 ± 3.24	5.15 ± 4.30	3.75 ± 2.26	0.05 [*]
37	Vanillin	22.2 ± 14.9	26.5 ± 21.5	20.6 ± 17.9	21.1 ± 20.8	24.2 ± 17.7	25.2 ± 25.6	0.12

n: number of samples involved in the analysis from each region. ^a Paarl (P), Stellenbosch (S), Worcester (W), Robertson (RO), Olifants River (OR), and Swartland (SW). ^b Mean ± standard deviation. ^c values in µg/L. ^{*} Significance difference ($p \leq 0.05$) among regions.

One-way ANOVA was used to identify differences in volatile content between wines of different cultivars. Results for the means among the six cultivars (two white and four red) showed significant differences for all compounds, with the exception of *n*-butanol, *n*-butyric acid, acetoin, and furfural (results not shown). In order to obtain more meaningful information, one way ANOVA was performed for the white wines and red wines separately (**Table 7.2.**). More than half the variables displayed significant differences between the two white wines cultivars. Among the fusel alcohols, isoamyl alcohol concentrations were higher in Sauvignon Blanc (SB) wines, whereas 1-propanol, isobutanol and *n*-butanol levels were higher in Chardonnay (CH) wines. According to Nykänen [21], higher levels of isoamyl alcohol is formed under anaerobic fermentation conditions, so that different fermentation practices between SB and CH may be responsible for these variations. Average concentrations of ethyl-lactate were two times higher in CH than SB wines, an observation that is probably related to different mean levels of lactic acid between South African wines of these cultivars [16,22]. CH wines contained higher amounts of wood-derived compounds such as 4-ethyl guaiacol, eugenol, 2,6-dimethoxy phenol and vanillin, although some discrepancies were observed. These variations could be related to the higher incidence of wood maturation of CH compared to SB wines which is common practice in South Africa [16]. On the contrary, mean levels of furan derivatives were observed to be higher in SB wines.

Characterization of South African wines

Table 7.2. One-way ANOVA results for two white cultivars and four red cultivars obtained from volatile data (mg/L).

No.	Compounds	White cultivars ^a		p-value	Red cultivars ^c				p-value
		Mean ± SD (mg/L) ^b			Mean ± SD (mg/L) ^b				
		SB (n = 65)	CH (n = 45)		PI (n = 41)	SH (n = 64)	CS (n = 60)	M (n = 59)	
1	Ethyl acetate	113 ± 29.3	134 ± 34.9	< 0.01	146 ± 40.0	133 ± 32.7	106 ± 28.7	135 ± 39.4	< 0.01
2	Ethyl butyrate	406 ± 92.4 ^d	529 ± 89.7 ^d	< 0.01	302 ± 88.7 ^d	227 ± 53.0 ^d	198 ± 57.3 ^d	236 ± 56.1 ^d	< 0.01
3	1-Propanol	21.7 ± 10.7	34.8 ± 27.5	< 0.01	57.8 ± 33.5	39.5 ± 27.6	29.5 ± 15.9	28.5 ± 15.7	< 0.01
4	Isobutanol	24.8 ± 12.5	28.0 ± 16.1	0.25 [*]	56.0 ± 22.9	81.6 ± 24.3	83.0 ± 31.4	79.8 ± 24.8	< 0.01
5	Isoamyl acetate	3.29 ± 3.36	5.71 ± 1.75	< 0.01	4.49 ± 2.33	2.07 ± 0.984	1.53 ± 1.16	1.76 ± 0.816	< 0.01
6	<i>n</i> -Butanol	6.85 ± 3.73	7.32 ± 3.59	0.51 [*]	7.59 ± 3.47	6.48 ± 4.41	6.40 ± 2.95	7.19 ± 4.42	0.36 [*]
7	Isoamyl alcohol	130 ± 13.7	117 ± 11.8	< 0.01	160 ± 21.9	199 ± 32.7	223 ± 30.5	224 ± 36.4	< 0.01
8	Ethyl hexanoate	0.559 ± 0.146	0.622 ± 0.125	0.02	208 ± 94.8 ^d	140 ± 43.9 ^d	172 ± 202 ^d	148 ± 47.2 ^d	0.02
9	Hexyl acetate	86.8 ± 31.5 ^d	64.2 ± 30.2 ^d	< 0.01	15.7 ± 10.3 ^d	9.87 ± 3.98 ^d	7.50 ± 3.40 ^d	6.17 ± 2.10 ^d	< 0.01
10	Acetoin	38.4 ± 18.9	42.1 ± 19.1	0.31 [*]	51.5 ± 40.9	47.4 ± 34.3	43.7 ± 30.1	47.7 ± 39.0	0.75 [*]
11	Ethyl-D-lactate	15.1 ± 10.2	27.7 ± 45.8	0.03	280 ± 95.6	226 ± 79.8	214 ± 72.7	220 ± 68.9	< 0.01
12	1-Hexanol	0.545 ± 0.352	0.618 ± 0.374	0.30 [*]	0.613 ± 0.504	0.995 ± 0.795	0.808 ± 0.577	0.630 ± 0.413	< 0.01
13	Ethyl octanoate	90.0 ± 25.5 ^d	95.4 ± 19.0 ^d	0.22 [*]	30.2 ± 14.6 ^d	15.0 ± 6.28 ^d	16.0 ± 7.29 ^d	18.0 ± 6.77 ^d	< 0.01
14	Acetic acid	280 ± 165	499 ± 247	< 0.01	744 ± 303	434 ± 224	448 ± 250	561 ± 369	< 0.01
15	Furfural	13.0 ± 6.19	12.9 ± 5.81	0.96 [*]	14.6 ± 6.89	12.4 ± 4.01	12.3 ± 5.94	11.1 ± 7.60	0.05
16	Propionic acid	14.8 ± 7.42	7.77 ± 5.74	< 0.01	17.4 ± 8.53	8.69 ± 5.37	6.92 ± 4.62	14.5 ± 9.22	< 0.01
17	Isobutyric acid	1.41 ± 1.36	1.07 ± 0.692	0.12 [*]	1.56 ± 0.653	2.11 ± 1.30	2.40 ± 1.72	2.55 ± 1.40	< 0.01
18	5-Methylfurfural	0.366 ± 0.241	0.427 ± 0.232	0.19 [*]	0.424 ± 0.260	0.454 ± 0.192	0.352 ± 0.240	0.307 ± 0.225	< 0.01
19	<i>n</i> -Butyric acid	3.25 ± 1.54	3.26 ± 1.45	0.98 [*]	3.03 ± 1.77	3.00 ± 1.02	2.96 ± 1.58	2.65 ± 1.77	0.53 [*]
20	Ethyl decanoate	11.5 ± 7.10 ^d	8.34 ± 4.77 ^d	< 0.01	5.60 ± 2.29 ^d	3.52 ± 1.65 ^d	5.23 ± 2.59 ^d	4.57 ± 2.33 ^d	< 0.01
21	Isovaleric acid	1.46 ± 0.408	1.48 ± 0.446	0.81 [*]	1.59 ± 0.173	1.80 ± 0.288	2.00 ± 0.307	2.07 ± 0.397	< 0.01
22	Diethyl succinate	4.43 ± 0.318	4.74 ± 0.470	< 0.01	9.48 ± 2.40	10.5 ± 2.23	12.0 ± 3.71	10.1 ± 2.29	< 0.01
23	<i>n</i> -Valeric acid	1.57 ± 0.439	1.52 ± 0.313	0.55 [*]	1.53 ± 0.121	1.51 ± 0.192	1.55 ± 0.218	1.62 ± 0.138	< 0.01
24	2-Phenethyl acetate	0.375 ± 0.188	0.353 ± 0.212	0.57 [*]	203 ± 130 ^d	204 ± 0.135 ^d	232 ± 155 ^d	159 ± 79.5 ^d	0.02
25	Hexanoic acid	4.84 ± 1.04	5.03 ± 1.22	0.38 [*]	3.42 ± 0.536	2.89 ± 0.528	3.05 ± 0.645	3.38 ± 0.654	< 0.01
26	Guaiacol	0.204 ± 0.122	0.285 ± 0.145	< 0.01	0.405 ± 0.223	0.241 ± 0.155	0.253 ± 0.166	0.448 ± 0.181	< 0.01
27	2-Phenylethyl alcohol	8.72 ± 2.92	8.84 ± 2.32	0.82 [*]	15.9 ± 6.34	30.8 ± 17.3	44.5 ± 18.3	41.5 ± 18.1	< 0.01
28	<i>o</i> -Cresol	807 ± 60.8 ^d	806 ± 45.8 ^d	0.98 [*]	835 ± 50.2 ^d	792 ± 40.9 ^d	800 ± 37.8 ^d	829 ± 38.0 ^d	< 0.01
29	Phenol	0.554 ± 0.387	0.566 ± 0.376	0.87 [*]	0.860 ± 0.522	0.596 ± 0.340	0.555 ± 0.351	0.862 ± 0.463	< 0.01

Characterization of South African wines

30	4-Ethylguaiacol	347 ± 6.94 ^d	351 ± 7.27 ^d	< 0.01	357 ± 10.1 ^d	346 ± 8.69 ^d	350 ± 9.57 ^d	356 ± 7.21 ^d	< 0.01
31	Octanoic acid	2.78 ± 0.858	2.27 ± 0.675	< 0.01	1.59 ± 0.348	1.33 ± 0.276	1.50 ± 0.368	1.40 ± 0.231	< 0.01
32	<i>p</i> -Cresol	242 ± 43.0 ^d	257 ± 38.1 ^d	0.06	282 ± 46.0 ^d	253 ± 37.4 ^d	255 ± 26.9 ^d	283 ± 30.6 ^d	< 0.01
33	Eugenol	573 ± 46.9 ^d	616 ± 58.2 ^d	< 0.01	629 ± 83.0 ^d	569 ± 61.9 ^d	581 ± 66.0 ^d	638 ± 59.5 ^d	< 0.01
34	Decanoic acid	819 ± 145 ^d	733 ± 96.1 ^d	< 0.01	698 ± 76.1 ^d	697 ± 75.6 ^d	721 ± 97.2 ^d	687 ± 64.6 ^d	0.12 [*]
35	2,6-Dimethoxy phenol	4.04 ± 1.87	6.16 ± 3.76	< 0.01	10.1 ± 7.75	5.36 ± 2.96	5.49 ± 3.14	7.17 ± 6.47	< 0.01
36	5-(Hydroxymethyl)furfural	6.39 ± 3.92	4.35 ± 3.01	< 0.01	5.70 ± 5.17	4.32 ± 3.44	2.16 ± 1.79	3.11 ± 2.22	< 0.01
37	Vanillin	5.99 ± 5.12	14.0 ± 11.8	< 0.01	37.4 ± 20.5	25.4 ± 13.5	22.0 ± 16.0	35.1 ± 23.2	< 0.01

n: number of samples involved in the analysis. ^a Sauvignon blanc (SB) and Chardonnay (CH). ^b Mean ± standard deviation. ^c Pinotage (PI), Shiraz (SH), Cabernet Sauvignon (CS) and Merlot (M). ^d measurement in µg/L. ^{*} no significant difference (p > 0.05) between cultivars.

Except for four compounds (*n*-butanol, acetoin, *n*-butyric acid, and decanoic acid) all of the variables showed significant differences among the four red wine cultivars (PI, SH, CS, and M). The first three compounds contain a common C₄-skeleton with different constituents, and are derived from pyruvate in the presence of Acetyl CoA through different biosynthetic routes [23]. The absence of significant difference for these volatiles might be related to their similar formation and persistence in red wines. The observed significant differences with regard to the rest of the volatiles among the red cultivars can primarily be attributed to the Pinotage variety, as the mean levels of most volatile constituents measured in this cultivar were higher. Most of the fermentation products such as alcohols, esters and acids were present in higher levels in Pinotage wines. The exception is isoamyl alcohol, where lower levels in this cultivar might be linked to the higher level of isoamyl acetate, due to the esterification of the former compound [24]. This observation correlates with previous reports on the contribution of isoamyl acetate to the characteristic fruity character of young Pinotage wines developed during fermentation [3]. In addition, the wood-related compounds eugenol, 2,6-dimethoxy phenol, 5-hydroxymethyl furfural (5-HMF) and vanillin were quantitatively higher in Pinotage wines. Volatile phenols like eugenol are formed as by-products of lignin breakdown during wood toasting [25], implying higher incidence of wood contact for young Pinotage wines.

7.3.3. Factor analysis (FA)

FA was performed following varimax normalization of the quantitative volatile data. Based on the scree test method proposed by Cattell in 1966 [26], a total of four factors were selected as optimal for explaining the total variability in the volatile data. In addition, a parallel plot of simulated and re-sampled data was drawn with the actual data to identify the important factors [27] (**Figure 7.1**). Based on these two approaches, four factors were selected which explained 58% of the total variation in the data. During successive steps of FA, several loadings were eliminated because they did not contribute to a simple factor structure and failed to meet a minimum criteria of an absolute loading value of 0.300 or above.

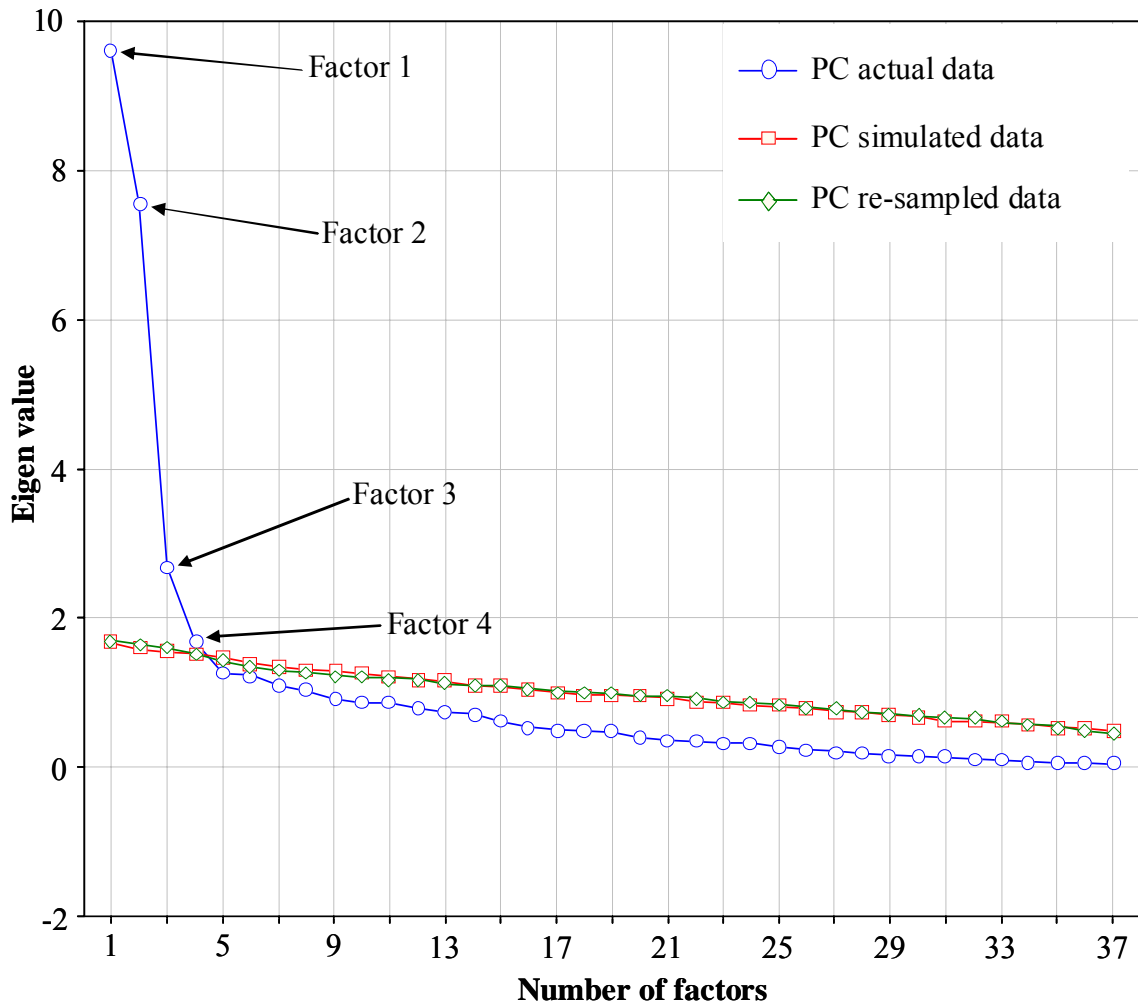


Figure 7.1. A scree plot with parallel analysis used for selection of optimal number of factors in factor analysis.

The first factor explains the highest percentage of the variability in the data set. More than half the variables were associated with factor 1 (F1). These compounds were mainly volatile fermentation products, including some compounds potentially responsible or varietal aroma like isoamyl acetate. Some compounds associated with wood ageing also showed relatively high loadings on this factor (5-HMF and vanillin), although lower than the alcohols and esters. Ethyl and acetate esters, partially responsible for the fruity flavor of wines [28], showed high negative correlation with F1. On the contrary, the organic acid-derived esters (ethyl lactate and diethyl succinate) as well as fusel alcohols (isobutanol, isoamyl alcohol, and 2-phenylethyl alcohol) displayed high positive correlation with F1. The branched aliphatic acids (isobutyric and isovaleric acids) showed positive correlation with F1, while C₆, C₈, and C₁₀ straight chain acids were negatively correlated to this factor. F1

therefore seems to describe primarily the influence of fermentation on the levels of volatile compounds studied here.

Factor 2 (F2) was associated with compounds that are released from wood during fermentation or maturation in barrels [20], including the volatile phenols (guaiacol, *o*-cresol, phenol, 4-ethyl guaiacol, *p*-cresol, eugenol, and 2,6-dimethoxy phenol), vanillin and 5-hydroxymethyl furfural. All these compounds presented high positive loadings on F2. C₂, C₃, and C₅ straight chain aliphatic acids and acetoin, both formed during fermentation, were also positively associated with this factor. F2 therefore seems to mainly describe the influence of wood contact on the major volatile composition.

Most acids (C₄ – C₁₀) as well as 2-phenylethyl acetate and 2-phenylethyl alcohol were positively associated with F3. Ethyl acetate and 1-propanol showed negative correlation with F3. Furan derived compounds such as furfural, 5-methylfurfural, and 5-hydroxymethyl furfural were negatively correlated with Factor 4, whereas isoamyl-alcohol and 2-phenylethyl alcohol showed positive correlation with this factor. Note that cross loading of some variables (loading of a variable in more than one factor) was observed. For instance, the branched C₄ and C₅ acids displayed positive association with both F1 and F3, whereas C₆, C₈, and C₁₀ acids showed negative correlation with F1 and positive correlation with F3. Similar trends were observed for other volatiles as well.

In conclusion, FA indicates that the principal variation in major volatile composition appear to be due to fermentation practices and wood ageing. This seems reasonable, considering the origin of most of these compounds, and indeed is in agreement with the previous report dealing with major volatile composition of a smaller number of South African wines of different vintages [16].

7.3.4. Principal component analysis (PCA)

PCA involves transformation of set of variables to a new coordinate system, in which the new axes are the principal components, following the direction of highest variance in the data set. These principal components are orthogonal to one another and constructed in such a manner that the amount of residual variation decreases with increasing number of principal components. Visualization of PCA results may be

performed as a score plot of the cases (samples) or a loading plot of the variables (volatile compounds). Both scores and loadings may be visualized together in a PCA bi-plot. In this form of presentation, multidimensional observations are displayed by points in the two-dimensional space by interpolation, whereas variables are represented as bi-plot axes, with a separate axis for each variable. A PCA bi-plot constructed using the measured values of 37 variables in the 334 South African young wines of vintage 2005 are shown in **Figure 7.2**.

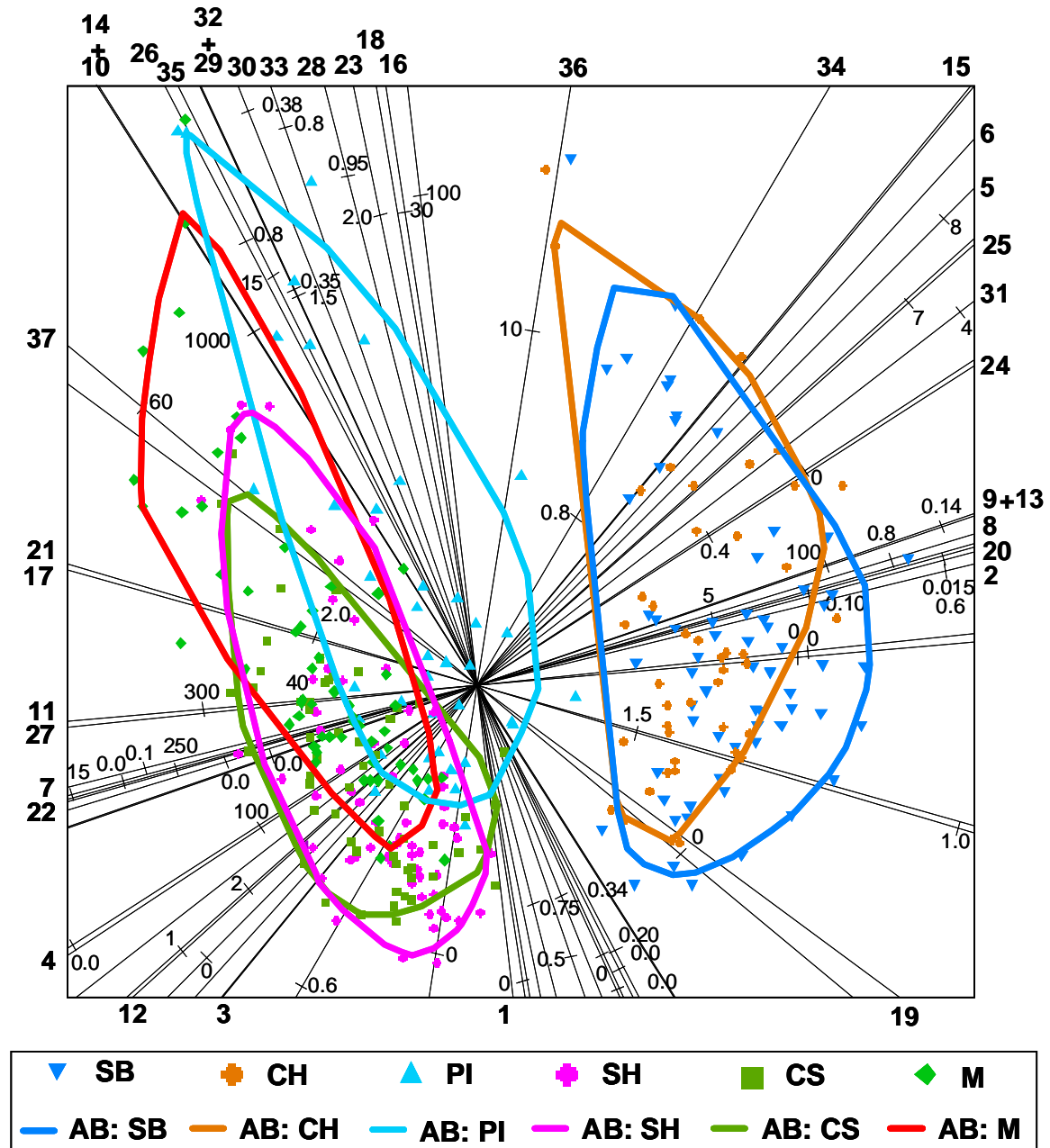


Figure 7.2. Cultivar based PCA bi-plot constructed using volatile data with interpolated 37-dimensional target of wines from different cultivars (Sauvignon Blanc (SB), Chardonnay (CH), Pinotage (PI), Shiraz (SH), Cabernet Sauvignon (CS), and Merlot (M)). α -Bags (AB) are used to characterize the probability cloud and to measure the degree of overlap among/between groups. Axes numbers 1 to 37 correspond to compound numbers listed in **Tables 7.1.** and **7.2.**

The 37 variables are represented as 37 axes in **Figure 7.2**. The calibration of these axes in the scale of the original measurements (i.e. mg/L) allows the graphical determination for any data point of values for each variable, by its projection onto the different axes. The white wine cultivars are clearly grouped on the right-hand side of the PCA bi-plot, and are separated from the red wine cultivars on the left-hand side. Slight separation between the two white cultivars is observed in this figure. A similar but more pronounced separation of Pinotage wines from the other red cultivars is also evident, which is in agreement with the ANOVA results presented in **Table 7.2**.

In a PCA bi-plot, the correlation of variables with objects (cases or groupings), and between variables themselves, depends on many factors. PCA bi-plots may be interpreted using different approaches, including trends in the magnitude of the variables, angles and distances between variables as well as the distance between the data points. In this report only the former approach is considered to elucidate patterns as the angles and distances between variables and the data points were not measured.

Variables with axes in close proximity are highly correlated. The degree of their correlation mainly depends on the size of the angle between the axes (i.e. the smaller the angle the higher the magnitude of the correlation): when two axes are perpendicular, their correlation is zero. The correlation could be negative or positive. For instance, ethyl esters of C₄, C₆, C₈, and C₁₀ acids and hexyl acetate are highly positively correlated. Isoamyl alcohol and diethyl succinate are negatively correlated with these esters and positively correlated with each other. 1-Hexanol, the content of which is independent of fermentation, but rather related to vine variety [29], was negatively correlated with hexanoic acid. The branched acids, isobutyric and isovaleric acid, showed high positive association. It is also apparent from **figure 7.2**. that ethyl esters of C₄, C₆, C₈, and C₁₀ acids, as well as isoamyl acetate, hexyl acetate, 2-phenylethyl acetate and C₆ and C₈ acids, showed positive correlation to a varying degree. These compounds were found to exist at higher concentrations in the white wines. On the contrary, higher alcohol (1-propanol, isobutanol, isoamyl alcohol, 1-hexanol and 2-phenylethyl alcohol) levels were higher in red wines. The presence of insoluble solids and suspended particles in the must are believed to increase the formation of higher alcohols [30]. Reduced concentrations of higher alcohols in white wine have therefore been associated with limited the amounts of insoluble solids in

the must [31]. The branched acids (isobutyric and isovaleric), ethyl-D-lactate, and diethyl succinate were also measured with higher levels in red wines. These compounds also presented positive correlation with each other to a varying degree. Volatile phenols, which are believed to be produced during wood maturation [32], seem to be present at slightly higher concentrations in red wines. In particular, volatile phenol levels were higher in Pinotage wines, compared to the other red cultivars studied. Similarly, n-acids of C₃ and C₅ as well as 5-methyl furfural were also measured at slightly higher concentration in Pinotage wines. These observations could be related to higher incidence of wood maturation for the Pinotage wines analysed here, as outlined previously.

It therefore seems that most of the correlation between variables in **figure 7.2**. is related to the differences between the red and white wines, as evidenced by the principle grouping of wine samples.

7.3.5. Discriminant analysis (DA)

Discriminant analysis (DA) is a statistical test technique used for prediction purposes that examines the set of variables associated with a given object and assigns the object to a group or class based on similarities and differences between variables. In DA, a linear discriminant function is formulated that describes the importance of the independent variables in differentiating objects of known group membership (i.e. Cultivar).

In the current work, discriminant analysis was used to determine if young South African wines could be classified according to cultivar using their volatile components, considering that only slight differences in volatile composition were observed as a function of geographical origin. Prediction studies were performed between white and red wines, between the two white cultivars, and among the red cultivars, by random division of the data into a training set (70% of samples) and a test set (30% of samples). A best subsets analysis approach was adopted where DA models were fitted (on the training data) for all subsets of predictors or variables. Wilk's lambda was used to select the 10 best models. The reasoning behind selecting 10 models is that those variables which tend to be included repeatedly in the models may be determined. These variables are then deemed as good predictors of cultivar.

The best models were then evaluated using the test set to see how well the models predicted test data. Hence, the results obtained in this section were used to review the discriminant model as a predictive tool, and to expand on the interpretation of the predictive analysis in order to identify the classification of each group (i.e. cultivar).

An initial prediction study between red and white wines was based on the 10 best models. Only variables that showed the highest selection rate (variables with correct prediction values $\geq 90\%$ and selected in most of the models) were used. A total of twelve compounds with high percentage prediction were observed (**Figure 7.3.A**). Only four of these variables (hexyl acetate, ethyl octanoate, ethyl-D-lactate, and diethyl succinate) were selected as predictors, since these variables were picked in most of the models. As mentioned above, the former two compounds were present in higher concentrations in white wines, while the levels of the latter two compounds were lower in white wines (also see **Figure 7.2.**). These four esters correctly predicted 100% of the wine samples in all the models, with the exception of the second model where 99% of the red wines were predicted correctly.

The high selection rate of ethyl-D-lactate and diethyl succinate is an indication that these compounds can be used as predictors for differentiation between white and red wines. The higher levels of ethyl lactate in red wines could be due to longer skin contact [33]. In addition, the higher levels of ethyl lactate can presumably be ascribed to higher incidence of malolactic fermentation [16]. The observation could also arise from the transformation of lactic and succinic acids to form ethyl lactate and diethyl succinate during fermentation and maturation, as red wine contains on average higher amounts of these acids [22].

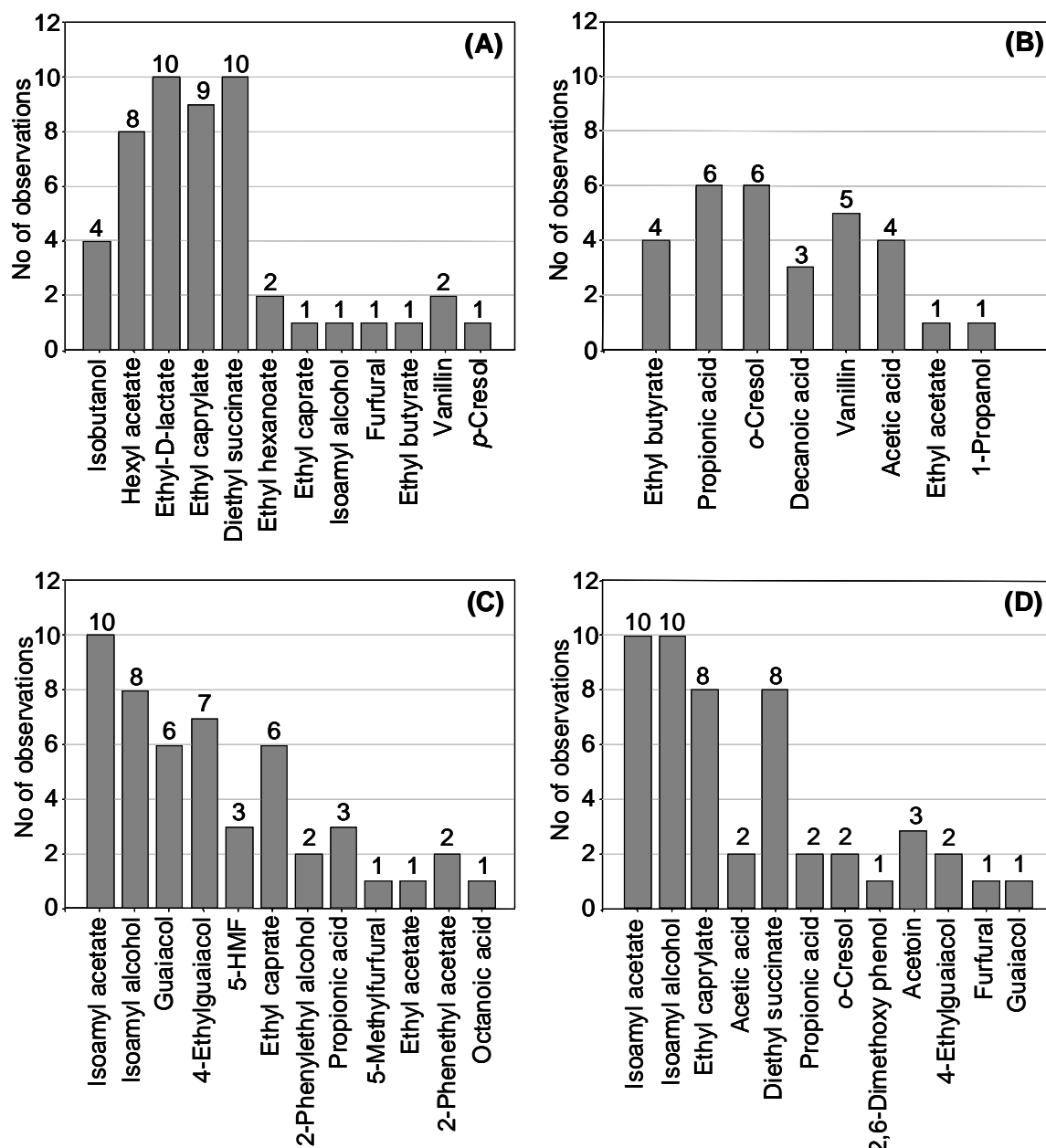


Figure 7.3. Histogram representation of variables selected as possible predictors for 10 discriminant models. Numbers indicate the number of models a variable was used in. Models were constructed to study prediction (A) between white and red wines; (B) between Sauvignon Blanc and Chardonnay cultivars; (C) among the four different red cultivars (PI, SH, CS, and M); and (D) of Pinotage wines.

While the successful classification of red and white wines was obtained using this approach, this is of limited practical relevance. Therefore, a detailed classification of the Sauvignon Blanc (SB) and Chardonnay (CH) using the same methodology was performed. A total of eight compounds showed more than 90% correct prediction for these two white cultivars (**Figure 7.3.B**), although only five (ethyl butyrate, acetic acid, propionic acid, *o*-cresol, and vanillin) were selected as they were repeatedly used in most of the models. To evaluate the classification of SB and CH, a PCA biplot was constructed using these five variables. It is clear from **Figure 7.4.A** that the

two white cultivars are relatively well separated. Especially differences in the levels of ethyl butyrate between these cultivars seem to be important in this differentiation. Ethyl butyrate has been shown to be a powerful impact odorant for CH wine, with high odor activity values measured in Chinese wines of this cultivar [34]. Also, vanillin plays an important discriminative role. The higher levels of vanillin in Chardonnay could be related to the common practice of producing wooded wines of this cultivar in South Africa [16].

Using a similar approach, discriminant analysis (DA) was performed for the four red wine cultivars Pinotage (PI), Shiraz (SH), Cabernet Sauvignon (CS), and Merlot (M). Twelve compounds appeared to be predictors for these cultivars, five of which were selected repeatedly in different models (**Figure 7.3.C**). However, with the exception of Pinotage, the correct prediction percentage for the other red cultivars was low. As a result further analysis focused on differentiation of Pinotage wines from the other three red wine cultivars.

A well-defined grouping of Pinotage wines observed in **Figure 7.2**. was an indication of the unique volatile composition in young wines of this cultivar. In the ten models developed for the DA of Pinotage, twelve variables were observed to have high prediction value (**Figure 7.3.D**). Four of these (isoamyl acetate, isoamyl alcohol, ethyl octanoate, and diethyl succinate) demonstrated a high selection rate per model, and were therefore considered as variables with high potential in predicting of this cultivar. A multidimensional extrapolation of these four variables was constructed as a PCA bi-plot (**Figure 7.4.B**) in order to identify the significance of these four compounds in differentiating Pinotage wines from the other red cultivars. It is evident that Pinotage wines are separated from the rest of the studied red cultivars using the identified variables as predictors. Levels of the isoamyl acetate in particular are higher in Pinotage, while isoamyl alcohol levels are lower for wines of this cultivar, which is in agreement with the previous reports [16,35].

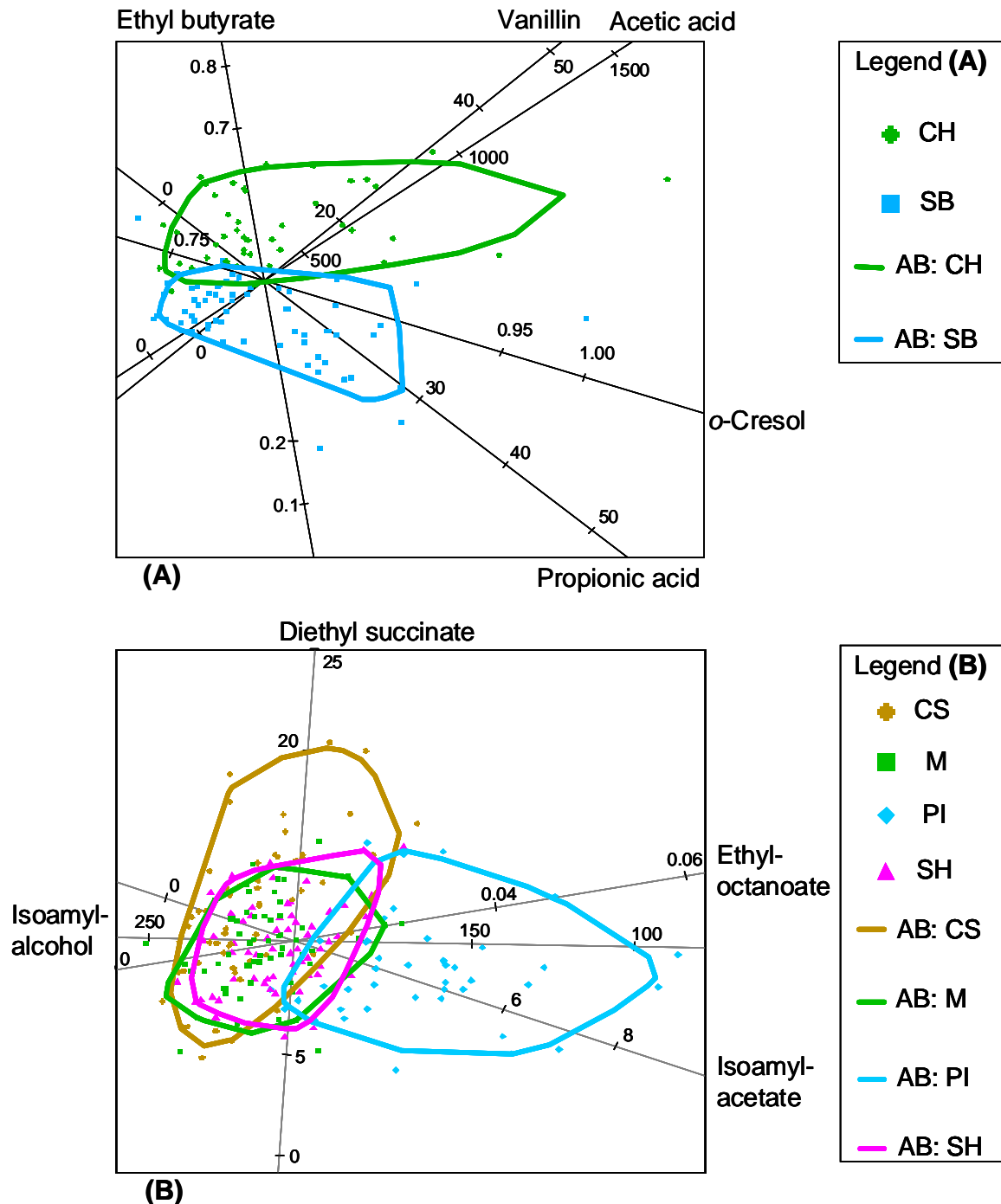


Figure 7.4. PCA bi-plots constructed using the predicting variables identified by discriminant analysis for: **(A)** the two white cultivars Sauvignon Blanc (SB) and Chardonnay (CH); **(B)** Pinotage (PI) wines. Other red cultivars included in **(B)** are Shiraz (SH), Cabernet Sauvignon (CS), and Merlot (M).

The importance of esters in the differentiation of Pinotage wines is in accordance with the fruity character of this cultivar: according to van Wyk et al. [3], young Pinotage wines are characterized by higher amounts of esters. In the same report, it was also indicated that isoamyl acetate may be an important varietal compound for Pinotage wine. Young Pinotage wines are characterized by a fruity bouquet, which is absent

from either must or grape [3]. This suggests that this particular aroma characteristic is produced during fermentation.

A comprehensive study of the factors affecting the characteristic young Pinotage flavor [36], which, to the best of our knowledge, has not been published elsewhere, implicates the higher levels of free amino content in Pinotage musts as the most influential parameter in this regard. This work included sensory evaluation of the characteristic young Pinotage flavor with quantitative analysis of the major volatiles by GC, and supported the importance of isoamyl acetate as a principle contributor to this flavor. Factors such as soil type, geographical origin, rootstock and metal content of the must were shown not to contribute to the characteristic young Pinotage aroma, or indeed affect the levels of isoamyl acetate significantly in the studied wines. In contrast, factor such as harvest date, must pH (lower pH is more favorable), skin contact and fermentation (increases in both are favorable) and yeast strain were found to be influential in the formation of the Pinotage aroma (although the effect of yeast strain was found to be of secondary importance to the must composition). The general conclusion of this study was that higher levels of free amino content in the must, which was partially due to higher levels of amino acids, led to higher yeast activity and the faster onset of anaerobic fermentation conditions. This in turn leads to the production of higher levels of saturated fatty acids in yeast cell membranes. Taken together, these factors were concluded to increase the likelihood of ester formation, presumably via catalysis of alcohol acetyltransferase [24]. Particularly in the case of isoamyl acetate, the result is the presence of this compound at levels above its odor threshold in red wines [36].

Our quantitative results are in agreement with these finding, particularly the higher levels of isoamyl acetate and ethyl octanoate, and lower levels of isoamyl alcohol, which are shown to be important distinguishing characteristics for young Pinotage wines from other red varieties (**Figure 7.4.B**). Moreover, the results of Joubert [36] are supported by evidence that certain yeast strains have the ability to promote the formation of isoamyl acetate [37]. Higher alcohols are formed by yeast, either directly from sugars or from grape amino acids, which involves the formation of keto-acidic and carbonyls as intermediates by removal of ammonia and carbon dioxide, respectively, prior to the formation of alcohols via reduction. Through this pathway

leucine produces isoamyl alcohol which is then converted to isoamyl acetate. Indeed, higher levels of leucine were measured in Pinotage musts by Joubert [36]. Moreover, higher levels of ammonia were measured in Pinotage musts compared to other South African grape varieties [38], while Ough and Lee [39] measured higher levels of isoamyl acetate in wines produced from must with higher total nitrogen content. It has also been shown that factors that increase the fermentation rate (yeast biomass, oxygenation, high temperature, and suspended particles) increase the fermentation of higher alcohols [40].

A further potential contributory factor to the higher isoamyl acetate levels in Pinotage wines might be the characteristically high concentrations of caffeic acid in these wines [18]: in the presence of N-acetyl-cystein, caffeic acid has been shown to inhibit the decrease of esters during storage [41], although this is expected to play a minor role in young wines analysed here.

It is known that the typical Pinotage fermentation bouquet decreases with ageing, and unless the wine is stored at low temperatures, this bouquet usually disappears after two or more years [3]. Wines stored in wooden containers tend to lose the typical bouquet faster than equivalent wines stored in steel tanks. The typical Pinotage fermentation bouquet would therefore be retained longer if wines are not aged in small cooperage, are bottled relatively young and stored under cool conditions. On the other hand, a slightly different style of Pinotage wine without the typical fermentation bouquet will be obtained by ageing such a wine in small cooperage or even large cooperage over a relatively long period of time.

It is clear from the precedent discussion that the impressive differentiation of Pinotage wines from other red wine varieties evident from **Figure 7.4.B** would not be as significant if older wines were included in the study, largely due to the fact that isoamyl acetate and ethyl octanoate would be expected to play less discriminatory roles for such wines. Nevertheless, the importance of especially isoamyl acetate in differentiation of young Pinotage wines is once again highlighted by our results.

It is also worth noting that a similar tendency for high isoamyl acetate levels in Pinot noir wines has been reported [42]. Pinotage was cross-bred from Pinot noir and

Cinsaut (Hermitage) varieties, which indicates that the characteristics of the former variety may be related to those observed in Pinotage wines.

7.4. Conclusions

Chemometric techniques were used to study the variation in major volatile content in a large number of young South African wines. ANOVA results indicated significant differences in volatile content between different cultivars, especially between Pinotage wines and the other varieties. Significant differences between the two white wine cultivars were also evident. Factor analysis (FA) showed that fermentation practices and wood ageing were primarily responsible for the variation in volatile content of the investigated wines. PCA bi-plots proved especially valuable in relating the different variables responsible for differentiation between wine samples. Finally, DA was used to identify prominent variables useful in the prediction of wine cultivar. The integration of DA with PCA bi-plot presentations was used to study the contribution of a small number of variables to differentiation of South African wines of different cultivar. Generally speaking, the results of the statistical approaches were complementary. They illustrated the differences between the red and white wines, between the two white cultivars, and highlighted the unique character of Pinotage wines in relation to other red wine cultivars in terms of the volatile compounds studied. Isoamyl acetate was seen as influential in this latter differentiation, and the most likely reasons for this phenomenon have been highlighted.

7.5. References

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**Characterization of volatile components
of Pinotage wines using comprehensive
two-dimensional gas chromatography
coupled to time-of-flight mass
spectrometry (GC × GC-TOFMS)[†]**

[†]Submitted to Food Chemistry as “Characterization of volatile components of Pinotage wines using comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC × GC-TOFMS)”, Berhane T. Weldegergis, André de Villiers, Christopher McNeish, Suresh Seethapathy, Tadeusz Górecki, Andrew M. Crouch.

Abstract

As part of the ongoing research into the chemical composition of the uniquely South African wine cultivar Pinotage, the volatile composition of nine young wines of this cultivar was investigated using comprehensive two-dimensional gas chromatography (GC × GC) in combination with time-of-flight mass spectrometry (TOFMS). Headspace solid phase micro extraction (HS-SPME) using a carboxen/polydimethylsiloxane (CAR/PDMS) fiber was used to extract the volatile compounds from the wine matrix. Extracts were analyzed using an in-house developed GC × GC system equipped with a single jet, liquid nitrogen-based cryogenic modulator. In the current study, 206 compounds previously reported in wine and related matrices have been detected in nine Pinotage wines. Positive identification for 48 compounds was performed using authentic standards, while tentative identification of 158 compounds was based on deconvoluted mass spectra and comparison of linear retention indices (LRI) with literature values. Identified compounds included esters, alcohols, aldehydes, ketones, acids, acetals, furans and lactones, sulfur compounds, nitrogen compounds, terpenes, hydrocarbons, volatile phenols and pyrans. Volatile compounds potentially capable of influencing wine aroma are highlighted. Many of the compounds were common to all 9 wines, although volatile components unique to specific samples were also observed. The results represent the most detailed characterization of volatile constituents of this cultivar reported to date.

Key words:

Comprehensive two-dimensional gas chromatography (GC × GC), Time-of-flight mass spectrometry (TOFMS), Wine, Pinotage, Volatiles, SPME.

8.1. Introduction

The aroma of wine is an essential characteristic in product evaluation and therefore plays an important role in consumer preference. Wine aroma is determined by the combined effects of several hundreds of chemically diverse volatile compounds [1]. Numerous odor-active compounds already exist in the grape; still, many more are produced during fermentation and maturation [2]. Their combined influence contributes to the character of wine and distinguishes one wine from another. Pinotage is a unique South African red wine cultivar cross-bred from Pinot Noir and Cinsaut varieties in the mid 1920s. It is known for its distinctive fruity character [3-5]. In order to characterize the unique qualities of Pinotage wines, elucidation of the compounds that contribute to the aroma and flavor of this variety is important.

Wine volatiles are commonly analyzed using gas chromatography (GC). Since these compounds may exist at widely varying concentrations, ranging from ng/L to per cent level, proper sample preparation prior to GC analysis is essential. Common sample preparation techniques including liquid liquid extraction (LLE) [6] and solid phase extraction (SPE) [7], solid phase microextraction (SPME) [1] and stir bar sorptive extraction (SBSE) [8,9] have been successfully applied for these analyses. Nevertheless, despite extensive research, universal sample preparation and analysis techniques suitable for the analysis of compounds with varying physicochemical properties from a complex matrix such as wine remain a challenge.

Due to the complexity of wine volatile fractions, identification and quantitation of constituents (especially minor ones) using conventional one-dimensional chromatography is hampered by frequent co-elutions, even when using high-efficiency capillary columns, selective stationary phases and programmed oven temperature conditions. Comprehensive two-dimensional gas chromatography (GC × GC) is a much more powerful technique for the analysis of complex volatile fractions. GC × GC typically involves the combination of a long non-polar column with a short polar column connected in series through a special interface (modulator). With properly selected orthogonal separation mechanisms, GC × GC allows the separation of a large number of compounds in a single chromatographic run due to the added selectivity of the second column and inherently high peak capacity. GC × GC has

successfully been applied to the analysis of flavor compounds in different food matrices such as: cheese [10], pepper [11], oil [12], sour cream [13], coffee beans [14], honey [15], fish [16], etc. Ryan et al. [17] used GC × GC in combination with nitrogen phosphorus detection (NPD) and time-of-flight mass spectrometry (TOFMS) for the identification of methoxypyrazines in Sauvignon Blanc wine. Other authors [18,19] applied GC × GC for the analysis of grape volatiles. The combination of GC × GC with TOFMS adds an extra dimension of information in terms of full mass spectral data acquisition and mass spectral continuity, which permits the deconvolution of spectra for co-eluting peaks [18,20].

To date, few literature reports have dealt with Pinotage volatiles. Limited qualitative and quantitative data pertaining mainly to the major volatiles common to most wines have been reported [8,9,21,22]. As part of our ongoing research on the chemical composition of Pinotage wines, in the present work we report the volatile constituents of nine young Pinotage wine samples of 2006 vintage determined by GC × GC-TOFMS. Initial results are limited to those compounds extracted using a generic HS-SPME method and previously identified in wine and wine-related samples. Further research into potential novel and influential volatile constituents will be reported elsewhere. To the best of our knowledge, no in-depth study on Pinotage volatile composition has been reported to date.

8.2. Experimental

8.2.1. Samples, chemicals and materials

A total of 9 young Pinotage wines from 2006 vintage were obtained from the South African Young Wine Show. Each wine was from a different producer and geographical origin in South Africa. The wines were transferred under argon to completely filled amber vials and shipped to the University of Waterloo (ON, Canada) for analysis. NaCl (ACS grade) was obtained from EMD Chemicals (Gibbstown, NJ.), while C₆ - C₁₈ *n*-alkanes (99%) used for linear retention index determination were from Sigma-Aldrich (St. Louis, MO). A carboxen/polydimethylsiloxane (CAR/PDMS, 75 μm) SPME fiber (Supelco, Bellefonte, PA) was used. Water for blank determinations was purified using Barnstead Nanopure water purification system (Thermo Scientific, Mississauga, ON).

8.2.2. Instrumentation

An in-house developed GC × GC system consisting of an Agilent 6890 GC (Agilent Technologies, Palo Alto, CA, USA) equipped with a single jet, liquid nitrogen cryogenic modulator and coupled to a Pegasus III time-of-flight mass spectrometer (LECO Corp., St. Joseph, MI) was used for all analyses [23]. The column set consisted of a 30 m × 0.25 mm i.d. × 1.00 μm d_f VF-1 (Varian, Mississauga, ON) as a primary column (1D) coupled to a 1.5 m × 0.25 mm i.d. × 0.25 μm d_f SolGel-Wax phase second dimension column (SGE, Austin, TX). A modulation period of 4 s was used with the cryogenic trap cooled to -196 °C using liquid nitrogen. The separation was performed using the following temperature program: initial temperature 40 °C, kept for 0.2 min, ramped at 3 °C/min to 225 °C and held for 10 min. The injector was operated at 275 °C in the splitless mode, with a splitless time of 2 min. Hydrogen was used as carrier gas at a constant flow of 0.8 mL/min and an initial inlet pressure of 18.2 psi. The transfer line was maintained at 250 °C. Ions in the mass range 35 - 250 amu were acquired at a rate of 50 spectra/s. The ion source temperature was 225 °C and the detector voltage was set to -1595 V.

8.2.3. Sample preparation

Sample preparation was based on a slightly modified method described by Setkova et al. [24]. Ten milliliter aliquots of the samples were transferred to 20 mL crimp-top headspace vials. Five gram aliquots of ACS grade sodium chloride, pre-baked at 250 °C and cooled to room temperature before use, were added to the vials together with PTFE-coated stir bars. The vials were then sealed immediately with PTFE-lined septa and aluminum crimp-top caps using a hand crimper. The resulting solutions were maintained at a temperature of 23 °C in a water bath before sampling. SPME in the headspace mode was performed for ten minutes with stirring at 500 rpm, followed by desorption of the fiber in the GC split/splitless injector port at 275 °C for 5 min. After the analysis, selected SPME fibers were desorbed again for 5 min at 275 °C in the injector port. No sample carry-over was observed, but fiber blanks showed the presence of petroleum hydrocarbons, most likely picked from laboratory air. System blanks were run daily prior to sample analysis to confirm cleanliness of the system. All analyses were performed in duplicate.

8.2.4. Data analysis

Data processing was performed automatically using the peak detection algorithm of the ChromaTOF software (LECO Corp. version 2.22). Compounds were identified using authentic standards (when available), while for the rest tentative identification was based on mass spectra comparison with NIST 05 and Wiley 275 libraries. A series of *n*-alkanes (C₆ -C₁₈) were also analyzed to establish first-dimension retention indices (RI₁) for each peak. Experimental retention indices (RI_{exp.}) were calculated according to the report by Marques et al. [25] and compared to literature values (RI_{lit.}) for identification purposes. A chromatographic blank run with the fiber was performed and necessary corrections were applied for the compounds observed in the samples.

8.3. Results and discussion

To date, very few studies on the volatile composition of Pinotage have been reported [8,9]. These studies exclusively used conventional capillary GC on polar (wax) phases and at most ~40 compounds have been identified and quantified. Advanced chromatographic techniques are required for the detailed investigation of the volatile composition of Pinotage wines, in order to benefit local producers. Taking this into account, GC × GC-TOFMS was used in the current investigation for the purpose of in-depth characterization of Pinotage volatiles. In addition to the significantly enhanced resolving power of this technique, it also offers improved signal to noise ratios and the power of spectral deconvolution using TOFMS.

In complex matrices such as wine, containing a large number of volatiles of wide-ranging physicochemical properties, frequent co-elutions are observed on any single stationary phase. This limitation is overcome in comprehensive two-dimensional GC by subjecting the sample to separation based on two different mechanisms, e.g. vapor pressures in 1D and polarity in 2D. **Figure 8.1.** illustrates the benefits of this approach for wine analysis. Here butyl acetate (**16**) and ethyl-S-lactate (**17**) co-elute in the first dimension due to their similar boiling points, but are separated based on differences in polarity in the second dimension. Similarly, the ethyl ester of *trans*-2-butenoic acid (**21**) and 4-methyl-1-pentanol (**92**) are separated according to differences in polarity. On the other hand, 4- (**92**) and 3-methyl pentanol (**93**), which have similar retention

times in the second dimension, are separated on the non-polar column in the first dimension. Figure 8.1. demonstrates excellent performance of the system: peak widths in the second dimension were smaller than 100 ms for many analytes. Even the somewhat tailing peak of 3-methyl-1-pentanol (**93**) was less than 200 ms wide at the base. All first dimension peaks were sampled at least three times across their profiles, which assured that 1D separation was preserved. The slight tailing seen for peak (**17**) in Figure 8.1. was also observed for other compounds of high polarity present at high levels. Tailing in the first dimension was related to the incompatibility of the polar compounds with the non-polar stationary phase used in 1D. Tailing in the second dimension was mainly related to modulator overloading with high concentration analytes. Owing to the relatively large diameter of the 2D column (0.25 mm) and the correspondingly higher amount of the stationary phase compared to a comparable 0.1 mm ID column, overloading of the 2D column was observed much less frequently than is typical for 0.1 mm ID columns [26].

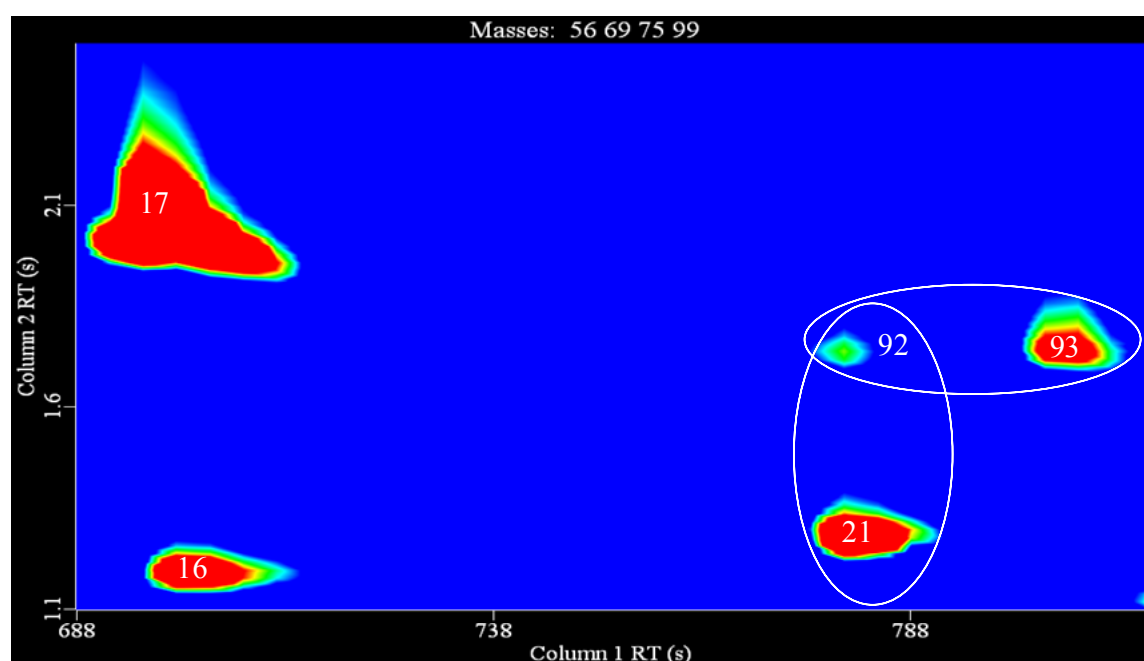


Figure 8.1. Extracted ion chromatograms illustrating the separation of butyl acetate (**16**), ethyl-S-lactate (**17**), 2-butenic acid, ethyl ester, (E)- (**21**), 4-methyl-1-pentanol (**92**) and 3-methyl-1-pentanol (**93**). For detailed compound identification, refer to **Table 8.1**.

A relatively generic HS-SPME method was used to extract the volatiles for the analysis. An SPME fiber coated with Carboxen adsorbent kept in place by polydimethylsiloxane binder (CAR/PDMS) was selected for the study as this fiber was previously reported to have good enrichment for wine volatiles [27]. The chromatographic method proved sufficiently reproducible, as evidenced by generally

negligible retention time variations for analytes detected in multiple samples. These variations are shown in **Table 8.1**. as the number of modulation period(s) (NMP) in the 1st dimension and standard deviation (SD) in the 2nd dimension. In addition, the method was shown to be suitably sensitive to allow the identification of various trace-level compounds such as methoxy pyrazines.

Considering that this investigation was the first step in comprehensive screening of Pinotage volatiles, the signal to noise ratio (S/N) used during data processing was varied to also include minor peaks. Due to a lack of authentic standards for numerous compounds, tentative identification for those compounds lacking standards was based in the first instance on a comparison of the deconvoluted mass spectra with NIST 05 and Wiley 275 spectral libraries, performed using ChromaTOF software with a match value of 70% as a minimum requirement. In addition, linear retention indices (LRI) were experimentally determined [25] in the first dimension using a homologous series of *n*-alkanes and were compared with literature values. The retention indices were calculated from the retention times of the *n*-alkanes bracketing a given analyte in the modulated chromatogram. In a properly optimized GC × GC separation, each peak eluting from the first dimension column is sampled at least three times, which leads to the same analyte showing in several consecutive 2nd dimension chromatograms (“slices”). To calculate the RIs, the averaged 1st dimension retention time was used for each compound. A maximal absolute retention index difference of 30 compared to literature values was used as the selection criterion in this study. Deviation of this magnitude was considered reasonable taking into account that the literature values were determined using one-dimensional systems.

Table 8.1. Volatile compounds identified in Pinotage wines using HS-SPME-GC × GC-TOFMS.

No.	Compounds	RT1(s) ± NMP ^a	Average RT2 (s) ± SD ^b	Similarity ^c	Reverse ^d	LRI _{cal.} ^e	LRI _{lit.} ^f	Wines ^g
Esters								
1	Formic acid, ethyl ester (Ethyl formate) ^h	168 ± 0	1.04 ± 0.03	893	893	< 600 ^j	495	1,3,6,7
2	Acetic acid, methyl ester (Methyl acetate)	188 ± 2	1.06 ± 0.02	975	975	< 600 ^j	513	1 – 9
3	Acetic acid, ethyl ester (Ethyl acetate) ^h	264 ± 1	1.18 ± 0.11	952	952	612	611	1 – 9
4	Acetic acid, 1-methylethyl ester (Isopropyl acetate)	344 ± 0	1.09 ± 0.01	756	793	652	653	2, 3
5	Formic acid, butyl ester (Butyl formate)	400	1.6	777	777	680	696	6
6	Acetic acid, 2-propenyl ester (2-Propenyl acetate)	408	1.08	749	800	684	675	2
7	Propanoic acid, ethyl ester (Ethyl propanoate)	448 ± 2	1.16 ± 0.03	957	957	688	688	1 – 9
8	Acetic acid, propyl ester (Propyl acetate)	452 ± 1	1.12 ± 0.07	943	943	705	707	2,3,5,8,9
9	Propanoic acid, 2-methyl-, ethyl ester (Ethyl isobutyrate) ^h	556 ± 0	1.16 ± 0.04	931	931	745	745	1 – 9
10	Acetic acid, 2-methylpropyl ester (Isobutyl acetate)	592 ± 1	1.20 ± 0.04	970	970	759	758	1 – 9
11	1-Butanol, 3-methyl-, formate (Isoamyl formate)	636 ± 0	1.20 ± 0.04	815	815	777	775	2,4,6
12	Pyruvic acid, ethyl ester (Ethyl pyruvate)	644 ± 1	1.76 ± 0.05	954	954	780	785	1 – 9
13	Butanoic acid, ethyl ester (Ethyl butyrate) ^h	664 ± 0	1.21 ± 0.03	954	954	788	787	1 – 9
14	Butanoic acid, 1-methylethyl ester (Isopropyl butyrate)	672	1.52	725	737	791	716	2
15	Propanoic acid, propyl ester (Propyl propanoate)	692	1.16	744	772	798	796	5
16	Acetic acid, butyl ester (Butyl acetate)	700 ± 2	1.23 ± 0.03	927	927	801	800	1 – 9
17	Propanoic acid, 2-hydroxy-, ethyl ester, (S)- (Ethyl-S-lactate)	700 ± 2	2.06 ± 0.04	988	988	799	800	1 – 9
18	Propanoic acid, 2-hydroxy-, ethyl ester (Ethyl lactate)	724 ± 3	2.13 ± 0.10	985	985	807	806	1 – 9
19	Formic acid, pentyl ester (Pentyl formate)	744 ± 2	1.46 0.04	808	858	815	810	1,4,5,6,8
20	Butanoic acid, 2-propenyl ester (Allyl butyrate)	772	1.28	806	832	825	850	7
21	2-Butenoic acid, ethyl ester, (E)- (<i>trans</i> -Ethyl 2-butenoate) ^h	780 ± 0	1.32 ± 0.06	938	938	827	827	1,2,4,6,7,9
22	Butanoic acid, 2-methyl-, ethyl ester (Ethyl 2-methylbutanoate) ^h	816 ± 1	1.17 ± 0.05	947	947	839	839	1,5,7,9
23	Acetic acid, methoxy-, ethyl ester (Ethyl methoxyacetate) ⁱ	820	1.86	727	748	840	-	6
24	Butanoic acid, 3-methyl-, ethyl ester (Ethyl isovalerate)	820 ± 1	1.17 ± 0.04	916	916	840	840	1 – 9
25	Propanoic acid, 2-hydroxy-, 1-methylethyl ester, (S)- ((S)-Isopropyl lactate) ⁱ	832	2.84	851	876	844	-	5
26	1-Butanol, 3-methyl-, acetate (Isoamyl acetate) ^h	892 ± 2	1.27 ± 0.06	942	953	862	861	1 – 9
27	4-Pentenyl acetate	916	1.26	803	869	871	861	1

Analysis of Pinotage wines using HS-SPME-GC × GC-TOFMS

28	Pentanoic acid, ethyl ester (Ethyl pentanoate) ^h	964 ± 0	1.20 ± 0.06	776	776	887	887	1,6,7,9
29	Acetic acid, pentyl ester (Pentyl acetate)	996 ± 2	1.25 ± 0.08	913	913	887	887	1,4,5,6,7,8,9
30	1-Butanol, 2-methyl-, acetate (2-Methylbutyl acetate)	1004 ± 2	1.24 ± 0.11	850	873	866	868	1,5,6,7
31	Hexanoic acid, methyl ester (Methyl hexanoate)	1040 ± 0	1.29 ± 0.09	934	934	911	913	1,2,5,6,7,8,9
32	Butanoic acid, 3-hydroxy-, ethyl ester (Ethyl 3-hydroxybutyrate)	1060	2.28	917	917	918	947	1
33	2-Butanone, 4-hydroxy-, acetate	1084	1.16	723	794	925	921	9
34	Pentanoic acid, 4-methyl-, ethyl ester (Ethyl 4-methylpentanoate)	1172	1.20	734	788	953	951	6
35	Butanoic acid, 2-hydroxy-3-methyl-, ethyl ester (Ethyl 2-hydroxyisovalerate)	1180 ± 0	1.63 ± 0.01	745	760	955	968*	6,9
36	Propanoic acid, 2-hydroxy-, 2-methylpropyl ester	1180	1.76	803	803	955	983	6
37	Propanoic acid, 2-hydroxy-, butyl ester (Butyl lactate) ⁱ	1180 ± 0	1.75 ± 0.07	907	919	955	-	1,9
38	1-Butanol, 3-methyl-, propanoate (Isoamyl propanoate)	1184 ± 0	1.24 ± 0.07	834	858	956	954	1,2,4,5,8
39	Hexanoic acid, ethyl ester (Ethyl hexanoate) ^h	1276 ± 0	1.22 ± 0.04	950	950	985	985	1 – 9
40	3-Hexen-1-ol, acetate, (E)-	1300 ± 0	1.31 ± 0.10	856	856	993	996	1,5,6
41	Acetic acid, hexyl ester (Hexyl acetate) ^h	1320 ± 0	1.24 ± 0.05	959	959	999	999	1 – 9
42	Propanoic acid, 2-methyl-, 2-methylbutyl ester (2-Methylbutyl isobutyrate)	1328 ± 0	1.16 ± 0.06	901	908	1001	1002	5,6,9
43	Ethyl 2-hexenoate	1408 ± 0	1.32 ± 0.05	832	832	1027	1026	1,6,7,9
44	Butanoic acid, 3-methylbutyl ester (Isoamyl butyrate)	1460 ± 0	1.21 ± 0.03	860	892	1043	1044	1,2,5,6,7,9
45	Pentanoic acid, 2-hydroxy-4-methyl-, ethyl ester	1468 ± 0	1.65 ± 0.05	841	852	1046	1060 [28]*	1,5
46	Propanoic acid, 2-hydroxy-, 3-methylbutyl ester (isoamyl lactate)	1504 ± 1	1.66 ± 0.04	881	881	1057	1082	1,6,9
47	Butanedioic acid, ethyl methyl ester	1576	1.82	737	864	1080	1070	6
48	Hexanoic acid, propyl ester (Propyl hexanoate)	1580 ± 0	1.24 ± 0.04	772	815	1081	1081	5,6
49	Heptanoic acid, ethyl ester (Ethyl heptanoate)	1592 ± 0	1.23 ± 0.05	924	924	1084	1084	1,2,5,7,9
50	Butanoic acid, 3-methyl-, pentyl ester (n-Amyl isovalerate)	1620 ± 0	1.15 ± 0.01	816	859	1094	1093	1,6
51	Acetic acid, heptyl ester (heptyl acetate)	1632 ± 0	1.21 ± 0.04	912	912	1098	1096	1,5,6,7
52	Octanoic acid, methyl ester (Methyl octanoate)	1672 ± 0	1.26 ± 0.04	912	912	1111	1111	1,2,5,6,7,8,9
53	Butanedioic acid, diethyl ester (Diethyl succinate) ^h	1804 ± 1	1.60 ± 0.05	970	970	1153	1149	1 – 9
54	Benzoic acid, ethyl ester (Ethyl benzoate) ^h	1812	1.60	711	748	1157	1157	5
55	Octanoic acid, ethyl ester (Ethyl octanoate) ^h	1892 ± 0	1.19 ± 0.03	927	927	1184	1184	1 – 9
56	Benzoic acid, 2-hydroxy-, methyl ester (Methyl salicylate)	1896 ± 0	1.81 ± 0.01	878	878	1185	1183	2,9
57	Propanedioic acid, oxo-, diethyl ester (Diethyl oxomalonate)	1944	1.56	806	806	1202	1188	6

Analysis of Pinotage wines using HS-SPME-GC × GC-TOFMS

58	Benzeneacetic acid, ethyl ester (Ethyl phenylacetate) ^h	2012 ± 0	1.77 ± 0.07	867	932	1225	1224	1,5
59	Hexanoic acid, 3-methylbutyl ester (Isoamyl hexanoate)	2044 ± 0	1.17 ± 0.05	940	940	1238	1238	1,2,5,6,7,9
60	Acetic acid, 2-phenylethyl ester (2-Phenylethyl acetate) ^h	2048 ± 0	1.72 ± 0.07	915	941	1239	1244	1,2,3,4,5,6,7,9
61	Nonanoic acid, ethyl ester (Ethyl nonanoate) ^h	2176 ± 0	1.21 ± 0.04	889	889	1286	1288	6,9
62	Ethyl 9-decenoate	2416 ± 0	1.22 ± 0.03	787	830	1374	1371	6,9
63	Decanoic acid, ethyl ester (Ethyl decanoate) ^h	2444 ± 0	1.16 ± 0.02	921	921	1384	1382	1 – 9
64	Octanoic acid, 3-methylbutyl ester (Isoamyl octanoate)	2576 ± 0	1.15 ± 0.06	846	846	1436	1435	1,5,6
65	Butanoic acid, 2-phenylethyl ester (2-Phenylethyl butyrate)	2744	2.44	824	824	1505	1491 [29]*	2
66	Dodecanoic acid, ethyl ester (Ethyl dodecanoate)	2936 ± 0	1.17 ± 0.02	846	846	1584	1583	1,5,6,7,9
67	Benzoic acid, 4-hydroxy-, n-heptyl ester (Heptyl 4-hydroxy benzoate)	3764	3.38	789	789	1891	1877	1
Alcohols								
68	Ethyl alcohol ^h	132 ± 0	1.55 ± 0.15	965	965	< 600 ^j	416	1 – 9
69	2-Propanol	196 ± 0	1.41 ± 0.01	881	916	< 600 ^j	500	3,6,8
70	2-Propenol	208 ± 0	1.75 ± 0.01	913	913	< 600 ^j	549	8,9
71	1-Propanol ^h	224 ± 1	1.48 ± 0.04	966	966	< 600 ^j	548	1 – 9
72	2-Butanol	252	1.36	887	887	606	581	4
73	2-Butanol (Isomer)	256 ± 0	1.39 ± 0.04	925	925	608	585	4,8,9
74	1-Propanol, 2-methyl- (Isobutanol) ^h	292 ± 0	1.56 ± 0.02	925	925	626	626	1 – 9
75	1-Butanol ^h	352 ± 0	1.66 ± 0.03	928	928	656	655	1 – 9
76	1-Penten-3-ol	384 ± 0	1.65 ± 0.01	848	906	672	672	2,6,9
77	2-Pentanol	420 ± 0	1.51 ± 0.03	903	903	690	691	2,4,5,7,9
78	3-Pentanol	424 ± 0	1.47 ± 0.04	948	948	692	693	1 – 9
79	2-Pentanol (Isomer)	424	1.5	848	848	692	681	1
80	4-Penten-2-ol	420	1.34	815	948	690	662	8
81	3-Buten-1-ol, 3-methyl-	492 ± 0	1.92 ± 0.04	949	949	720	717	1,2,5,6,7,8,9
82	1-Butanol, 3-methyl- (Isoamyl alcohol) ^h	500 ± 2	1.78 ± 0.14	946	946	723	724	1 – 9
83	1-Butanol, 2-methyl- (Active Amyl alcohol) ^h	516 ± 1	1.80 ± 0.06	928	928	730	728	1 – 8
84	1-Pentanol (Amyl alcohol)	592 ± 1	1.69 ± 0.11	912	928	759	760	1 – 9
85	2-Penten-1-ol, (E)-	596	2.18	780	839	761	760	4
86	2,3-Butanediol	600 ± 0	1.48 ± 0.02	912	912	762	743	1,2,4,5,6,7,9
87	2-Buten-1-ol, 2-methyl-	600 ± 0	2.23 ± 0.01	820	840	763	762	5,9

Analysis of Pinotage wines using HS-SPME-GC × GC-TOFMS

88	2,3-Butanediol (Isomer)	628 ± 1	2.33 ± 0.07	909	909	773	768	1,2,3,4,5,6,7,9
89	2-Pentanol, 3-methyl-	692	2.10	807	807	798	797	6
90	2-Hexanol	736 ± 0	2.03 ± 0.10	823	859	813	795	2,6
91	1-Propanol, 3-ethoxy-	772	2.04	919	919	825	837	1
92	4-Methyl-1-pentanol	780 ± 0	1.84 ± 0.06	915	915	826	821	1 – 9
93	3-Methyl-1-pentanol	808 ± 0	1.84 ± 0.06	903	903	835	829	1 – 9
94	3-Hexen-1-ol, (E)-	820 ± 0	1.98 ± 0.04	916	916	840	840	1,3,6,9
95	3-Hexen-1-ol, (Z)- ^h	832 ± 0	2.14 ± 0.07	934	934	846	846	1,2,5,6,9
96	2-Hexen-1-ol, (E)-	864	2.24	793	812	855	854	9
97	1-Hexanol ^h	876 ± 0	1.81 ± 0.04	919	919	858	858	1 – 9
98	2-Heptanol	972 ± 0	1.64 ± 0.07	916	916	890	889	1,2,4,5,9
99	1-Heptanol	1196 ± 0	1.78 ± 0.07	904	904	960	960	1,2,3,4,5,6,7,9
100	2-Hepten-1-ol, (E)-	1228	1.68	731	778	970	968	6
101	5-Hepten-2-ol, 6-methyl-	1268	1.64	775	794	983	974	1
102	2-Octanol ^h	1296	1.54	848	848	991	992	1
103	Isooctanol	1368 ± 0	1.68 ± 0.00	845	845	1014	995	2,9
104	2,6-Dimethyl-4-heptanol ⁱ	1468 ± 0	1.62 ± 0.03	842	869	1046	-	6,9
105	1-Octanol	1516 ± 0	1.68 ± 0.07	888	888	1061	1061	1,2,5,6,7
106	2-Phenylethyl alcohol ^h	1636 ± 0	3.28 ± 0.07	947	947	1098	1098	1 – 9
107	1-Nonanol	1828	1.56	877	894	1163	1163	1
Aldehydes								
108	Acetaldehyde	120 ± 1	1.00 ± 0.02	992	992	< 600 ^j	372	1 – 9
109	Propanal	152 ± 2	1.04 ± 0.03	941	983	< 600 ^j	461	1 – 9
110	2-Propenal (Acrolein)	156 ± 1	1.05 ± 0.07	971	971	< 600 ^j	463	2,4,5,8,9
111	2-Methyl-propanal (Isobutanal)	208 ± 0	1.06 ± 0.02	804	804	< 600 ^j	538	2,3,7
112	2-Methyl-2-propenal (Isobutenal)	216 ± 1	1.12 ± 0.02	824	872	< 600 ^j	553	3,5,7
113	Butanal (Butyraldehyde) ^h	240 ± 0	1.08 ± 0.03	784	784	600	600	3,5
114	Methylglyoxal (Pyruvaldehyde)	308	1.44	915	945	634	644	8
115	3-Methyl-butanal (Isovaleraldehyde)	332 ± 0	1.17 ± 0.02	955	955	646	645	1 – 9
116	2-Methyl-butanal	384 ± 0	1.08 ± 0.03	752	752	668	665	3,4
117	Pentanal (Valeraldehyde)	416 ± 1	1.09 ± 0.03	830	866	688	687	1 – 8

Analysis of Pinotage wines using HS-SPME-GC × GC-TOFMS

118	2-Methyl-2-butenal (E)-	512 ± 2	1.38 ± 0.07	887	938	727	724	1,2,5
119	2-Methyl-2-butenal (2,3-Dimethylacrolein)	520 ± 0	1.38 ± 0.02	918	918	731	730	3,4,8
120	2-Pentenal, (E)-	544	1.32	712	723	741	743	1
121	Hexanal ^h	652 ± 1	1.26 ± 0.05	892	892	783	784	1 – 9
122	3-Hexenal, (Z)-	780 ± 1	1.45 ± 0.01	755	804	829	834	2,3,4
123	Heptanal	960	1.24	799	799	886	885	1
124	Benzaldehyde ^h	1140 ± 0	2.20 ± 0.12	956	956	943	942	1 – 7
125	Octanal	1284 ± 0	1.28 ± 0.06	853	871	988	988	1,2,3,8,9
126	Benzeneacetaldehyde (Phenylacetaldehyde)	1392 ± 0	2.21 ± 0.08	952	952	1022	1022	1 – 9
127	Nonanal ^h	1604	1.34	849	849	1089	1088	5
128	2-Nonenal, (Z)-	1628	1.02	752	760	1096	1098	5
129	Decanal ^h	1916	1.26	799	816	1192	1192	9
Ketones								
130	2,3-Butanedione	228	1.10	959	959	< 600 ^j	586	6
131	2-Propanone, 1-hydroxy- (Acetol)	292	1.34	829	829	626	625	2
132	2-Butanone, 3-methyl-	384 ± 0	1.22 ± 0.04	924	949	672	677	1,5,6,7
133	2,3-Pentanedione	392 ± 0	1.34 ± 0.00	867	885	676	676	3,5,6
134	2-Pentanone ^h	396 ± 1	1.27 0.03	860	860	677	680	1,3,6,7
135	3-Pentanone	412 ± 2	1.19 ± 0.03	920	954	684	683	1,2,5,6,8
136	2-Butanone, 3-hydroxy- (Acetoin)	416 ± 1	2.33 ± 0.02	884	884	688	687	2,3,6,7
137	3-Penten-2-one	488 ± 1	1.49 ± 0.04	961	961	719	719	1,3,4,5,6,7,8,9
138	Cyclopentanone	540	1.34	790	829	739	747	4
139	3-Octanone	1236 ± 0	1.28 ± 0.05	924	924	972	973	1,5,6,7,9
140	2-Octanone	1244	1.24	922	922	975	976	1
141	4-Heptanone, 2,6-dimethyl-	1196	1.16	772	806	960	962	4
142	Cyclohexanone, 2,2,6-trimethyl-	1404 ± 0	1.23 ± 0.01	842	842	1025	1022	1,6,9
143	Acetophenone ^h	1476 ± 0	2.15 ± 0.05	830	830	1048	1048	1,2,5,6
144	2-Nonen-4-one	1504	1.14	797	797	1057	1065	8
145	2-Nonanone	1568 ± 0	1.34 ± 0.00	894	894	1077	1078	6,9
Acids								
146	Formic acid	132 ± 0	2.11 ± 0.04	905	905	< 600 ^j	512	1,7

Analysis of Pinotage wines using HS-SPME-GC × GC-TOFMS

147	Acetic acid ^h	260 ± 0	3.36 ± 0.10	965	965	610	610	1 – 9
148	Propanoic acid, 2-methyl- (Isobutyric acid) ^h	580 ± 0	3.06 ± 0.07	892	892	755	753	1,5,6,7,9
149	Acetic acid, methoxy- (Methoxyacetic acid)	628 ± 1	3.76 ± 0.00	993	993	772	752	2,5
150	Acetic acid, hydroxy- (Glycolic acid)	816 ± 2	1.96 ± 0.08	980	980	841	819	3,8,9
151	Propanoic acid, 2-hydroxy- (Lactic acid)	816	2.04	959	997	839	838	5
152	Butanoic acid, 3-methyl- (Isovaleric acid) ^h	816 ± 0	2.85 ± 0.09	900	900	839	840	1,4,5,6,7,9
153	Hexanoic acid ^h	1236 ± 1	1.93 ± 0.17	921	921	973	971	1,2,5,6,7,9
Acetals								
154	1,1-Diethoxyethane (Acetal)	492 ± 0	1.07 ± 0.04	811	830	720	718	1,3,4,5
155	2,4,5-Trimethyl-1,3-dioxolane	540 ± 2	1.17 ± 0.04	914	914	741	761	1 – 9
156	1,1-Diethoxy-2-methylpropane (Propane, 1,1-diethoxy-2-methyl-)	860 ± 0	1.12 ± 0.03	891	891	853	859 [30]*	1,2,3,5,6
157	1,1-Diethoxy-2-methylbutane (Butane, 1,1-diethoxy-3-methyl-)	1152 ± 0	1.10 ± 0.05	736	761	948	952*	1,5,6
158	1-(1-Ethoxyethoxy)pentane (Pentane, 1-(1-ethoxyethoxy)-)	1212	1.16	745	762	965	970 [30]*	5
159	1,1-Diethoxypentane (Pentane, 1,1-diethoxy-)	1316	1.30	740	740	998	995 [30]*	1
Furans and Lactones								
160	Furan	164	1.02	726	935	< 600 ⁱ	492	5
161	Furan, 2,5-dimethyl-	444 ± 0	1.13 ± 0.01	810	877	702	700	1,9
162	2-Furancarboxaldehyde (Furfural) ^h	728 ± 0	3.17 ± 0.07	901	915	810	810	1,2,3,5,6
163	2-Acetylfuran (Acetylfuran)	896 ± 1	1.10 ± 0.03	807	938	866	870	2,8
164	2(3H)-Furanone, dihydro- (γ -Butyrolactone)	920 ± 2	0.19 ± 0.09	941	941	873	871	1 – 9
165	2(3H)-Furanone, dihydro-5-methyl- (γ -Pentalactone)	1056	3.26	874	874	916	914	5
166	2(3H)-Furanone, dihydro-3,5-dimethyl-	1144 ± 2	1.26 ± 0.03	779	813	946	947	5,7
167	Ethyl 2-furoate	1424 ± 0	2.02 ± 0.00	828	877	1032	1009 [31]	1,6
Sulfur containing compounds								
168	Sulfur dioxide ⁱ	100 ± 1	1.10 ± 0.05	967	967	< 600 ⁱ	-	1 – 9
169	Dimethyl sulfide	176 ± 0	1.00 ± 0.02	864	914	< 600 ⁱ	493	1,2,3,5
170	Methyl thiolacetate	408 ± 0	1.26 ± 0.04	923	923	684	683	4,5,6
171	Ethyl thiolacetate	580	1.30	782	803	755	749	6
172	Thiophene, 2-methyl-	620 ± 0	1.33 ± 0.05	818	834	770	770	1,6,7,8,9

Analysis of Pinotage wines using HS-SPME-GC × GC-TOFMS

Nitrogen containing compounds								
173	2-Methylpropylamine (Isobutylamine)	216 ± 0	1.06 ± 0.00	779	882	< 600 ⁱ	588	8
174	2-Butanamine, 2-methyl- ⁱ	300	1.02	761	785	630	-	8
175	Pyrrolidine	936	1.04	791	888	706	695	1
176	2-Methoxy-3-(1-methylethyl)- Pyrazine [2-Methoxy-3-isopropylpyrazine (IPMP)] ^h	1564	1.18	902	902	1082	1081	9
177	2-Methoxy-3-(1-methylpropyl)- Pyrazine [2-Methoxy-3-sec-butylpyrazine (SBMP)] ^h	1892	2.58	735	735	1184	1159	9
178	2-Methoxy-3-(2-methylpropyl)- Pyrazine [2-Methoxy-3-isobutylpyrazine (IBMP)] ^h	1972	1.25	671	671	1210	1211	9
Terpenes								
179	Cumene	1068 ± 0	1.18 ± 0.04	962	962	920	916	1 – 9
180	Isocumene	1164 ± 0	1.21 ± 0.06	964	964	950	949	1,2,4,5,6,7,8,9
181	1-Methyl-4-(1-methylethylidene)-cyclohexane [4(8)-p-Menthene]	1288	1.04	705	861	989	998	9
182	2-Methyl-5-(1-methylethyl)-1,3-Cyclohexadiene (α-Phellandrene)	1312	1.08	708	708	996	996	2
183	1,4-Epoxy-p-Menthane (Isocineole)	1364 ± 0	1.11 ± 0.01	755	887	1013	1011	6,9
184	<i>o</i> -Cymene	1392 ± 0	1.18 ± 0.05	963	963	1022	1022	1 – 9
185	1-Methyl-4-(1-methylethenyl)-cyclohexene (Limonene) ^h	1420 ± 0	1.13 ± 0.04	911	915	1030	1028	1,2,4,5,7,9
186	1,8-Epoxy-p-Menthane (Eucalyptol)	1424 ± 0	1.09 ± 0.03	814	814	1030	1030	4,6,9
187	3,5,5-Trimethyl-2-cyclohexen-1-one (Isophoron)	1480	1.32	734	758	1049	1074	6
188	1-Methyl-4-(1-methylethyl)- 1,3-cyclohexadiene (α-Terpinene)	1508	1.12	745	789	1058	1040	7
189	5-Ethenyltetrahydro-α,α,5-trimethyl- <i>cis</i> -2-Furanmethanol [Linalool oxide, (Z)-] ^h	1536	1.36	866	866	1067	1067	9
190	1,3,3-Trimethylbicyclo[2.2.1]heptan-2-one (Fenchon) ^h	1580	1.30	876	876	1081	1080	3
191	5-Ethenyltetrahydro-α,α,5-trimethyl- <i>trans</i> -2-Furanmethanol (Linalool oxide, (E)-)	1580	1.38	839	839	1081	1081	9
192	1-Methyl-4-(1-methylethylidene)-1-cyclohexene (Terpinolen)	1608	1.12	797	806	1090	1089	9
193	3,7-Dimethyl-1,6-octadien-3-ol (Linalool) ^h	1612 ± 0	1.51 ± 0.06	783	796	1091	1091	1,6,7
194	2,6,6-Trimethylbicyclo[3.1.1]heptan-3-ol (Isopinocampheol)	1612	1.52	728	728	1091	1120	5
195	α,α,4-Trimethyl- cyclohexanemethanol, (p-Menthan-8-ol)	1768	1.48	755	780	1143	1162 ^{**}	9
196	1,6-Octadien-3-ol, 3,7-dimethyl-, formate (Linalool formate)	1844 ± 0	1.09 ± 0.04	747	747	1168	1170	6,9

Analysis of Pinotage wines using HS-SPME-GC × GC-TOFMS

197	4-Methyl-1-(1-methylethyl)-3-Cyclohexen-1-ol (4-Terpineol)	1868	1.44	762	762	1176	1175	9
198	$\alpha,\alpha,4$ -Trimethyl-3-cyclohexene-1-methanol (α -Terpineol) ^h	1900 ± 0	1.60 ± 0.04	907	909	1187	1185	1,2,5,6,7,8,9
199	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl) acetate (4-Terpineol acetate)	2116 ± 1	1.19 ± 0.03	911	911	1264	1270	1,2,4,5,6,7,8,9
200	1,1,6-Trimethyl-1,2-dihydro-naphthalene (TDN)	2372 ± 0	1.30 ± 0.04	808	827	1358	1336	1,6,9
201	1,1,6-Trimethyl-1,2,3,4-tetrahydro-naphthalene (TTN)	2384 ± 0	1.22 ± 0.05	811	834	1362	1340	1,6,9
202	2-Buten-1-one, 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-, (E)- (β -Damascenone)	2432 ± 0	1.38 ± 0.02	790	844	1380	1373	1,7,9
Volatile phenols								
203	2,4-Bis(1,1-dimethylethyl)-phenol	2748	2.04	779	779	1506	1502	6
204	Butylated hydroxytoluene (BHT) ^h	2756 ± 0	1.26 ± 0.02	861	869	1510	1505	1,5,6,7,9
Pyrans								
205	2H-Pyran-2-one, tetrahydro-	992	1.34	761	774	896	910	8
206	2H-Pyran, 2-ethenyltetrahydro-2,6,6-trimethyl-	1232 ± 0	1.09 ± 0.04	803	803	971	960	6,7,8,9

^a RT1(s) ± NMP: 1st dimension retention times with the variation in modulation period (MP) among the samples where a compound was detected. ^b SD: Standard deviation of 2nd dimension retention times among the samples where a compound was detected. ^c Forward similarity (value out of 1000). ^d Reverse similarity (value out of 1000). ^e LRI_{cal}: linear retention indices experimentally determined. ^f LRI_{lit}: linear retention indices (taken from NIST 05 library unless otherwise specified). ^g Wines: the wine samples in which the compound was identified (numbers 1,2,3,...9 are codes given to the nine Pinotage wine samples, consecutively). ^h Compound identity confirmed using authentic standards. ⁱ Identification was based only on mass spectra from the NIST 05 library. ^j LRI_{cal} < 600 estimated since C₆ was the lowest *n*-alkane analysed. ^{*} LRI_{lit}: LRI obtained from a column with (5%-Phenyl)-methylpolysiloxane stationary phase. ^{**} LRI_{lit}: LRI obtained under isothermal conditions.

The results presented in **Table 8.1**. demonstrate that the position of a compound in the two-dimensional separation space was reproducible. Hence, the presence of certain compounds could be established based on positive identification of the same compounds in other samples, through the correspondence of the retention times in both dimensions. However, often these tentative identifications were rejected due to low mass spectral match quality (see for example Figure 8.3. further). In addition, it should be noted that many peaks detected with good spectral matches were excluded from the results presented here because there were no other means to confirm their identity. More than 200 volatile compounds presented in **Table 8.1**. were identified (positively or tentatively) in the nine samples using authentic standards (when available), mass spectra and linear retention indices as outlined previously. A detailed rationalization of the different classes of compounds identified, focusing on those that may contribute to wine aroma, is given below.

Esters. Esters are abundant wine volatiles produced during fermentation and through esterification occurring during wine ageing. Young Pinotage wines are characterized by relatively high concentrations of esters [5]. Among the nine samples analyzed, 67 esters were detected. Of these, 17 were positively identified using authentic standards (indicated by superscript “h” in Table 8.1.). Most ethyl and acetate esters were mainly separated in the 1st dimension (1D) and displayed very similar and rather low 2nd dimension (2D) retention times (RTs). In contrast, hydroxyl substituted esters such as ethyl lactate (see **Figure 8.1**.) tended to be more retained in the second dimension. Similarly, ethyl esters of di-acids and aromatic esters had longer second dimension RTs due to their higher polarity.

Esters previously reported in Pinotage wines included ethyl-acetate (**3**), -butyrate (**13**), -lactate (**18**), -isovalerate (**24**), -hexanoate (**39**), -octanoate (**55**), -phenylacetate (**58**), -9-decenoate (**62**), -decanoate (**63**) and dodecanoate (**66**), as well as isoamyl acetate (**26**), hexyl acetate (**41**), diethyl succinate (**53**) and 2-phenylethyl acetate (**60**) [8,9,21]. These esters were common to most of the wines analyzed. Isoamyl acetate was reported to be an impact odorant characteristic of the Pinotage varietal [5]. This compound was detected at relatively high levels in all wines. According to Ferreira et al. [32], ethyl esters of hexanoic (**39**) and octanoic acids (**55**), which have low odor thresholds of 5 and 14 µg/L, respectively, are important aroma constituents.

Minor concentrations of other carboxylic acids and alcohols produced during fermentation may lead to the production of esters capable of contributing to wine aroma. For instance, ethyl esters of 2-methyl- (**22**) and 3-methylbutyric acids (**24**) were reported to play a role in the aroma of a wine [33]. Other naturally rare ethyl esters which may have some impact on the wine aroma include ethyl 2-, 3- and 4-methylpentanoate and ethyl cyclohexanoate [34], which reveal pleasant strawberry/licuorice-like odors. The concentrations of these esters tend to increase with wine age due to slow esterification of their corresponding acids formed during fermentation. However, only one of the four esters, ethyl 4-methylpentanoate, can be found in young wines at low levels [34]. Indeed, only ethyl-4-methylpentanoate (**34**) was detected in one of the nine wines analyzed here.

Allyl butyrate (**20**) and ethyl methoxyacetate (**23**), both identified tentatively, have previously been reported in Moutai Chinese liquor [35], and pentyl formate (also identified tentatively) (**19**) in grape brandy [36]. We could not find literature reports demonstrating the occurrence of these compounds in wine.

Alcohols. Alcohols were the second largest group of identified volatiles, amounting to a total of 40 compounds in all 9 wines. The identity of 10 of these was confirmed using authentic standards. Unlike most esters, the alcohols reported here showed varying retention times in both dimensions (**Table 8.1**). Alcohols such as isoamyl-alcohol (**82**) showed noticeable tailing and even wraparound, which could be attributed to their high concentrations and polarities. Ethanol masked a number of minor compounds due to its high concentration. **Figure 8.2** depicts some of the aliphatic alcohols identified in Pinotage wines.

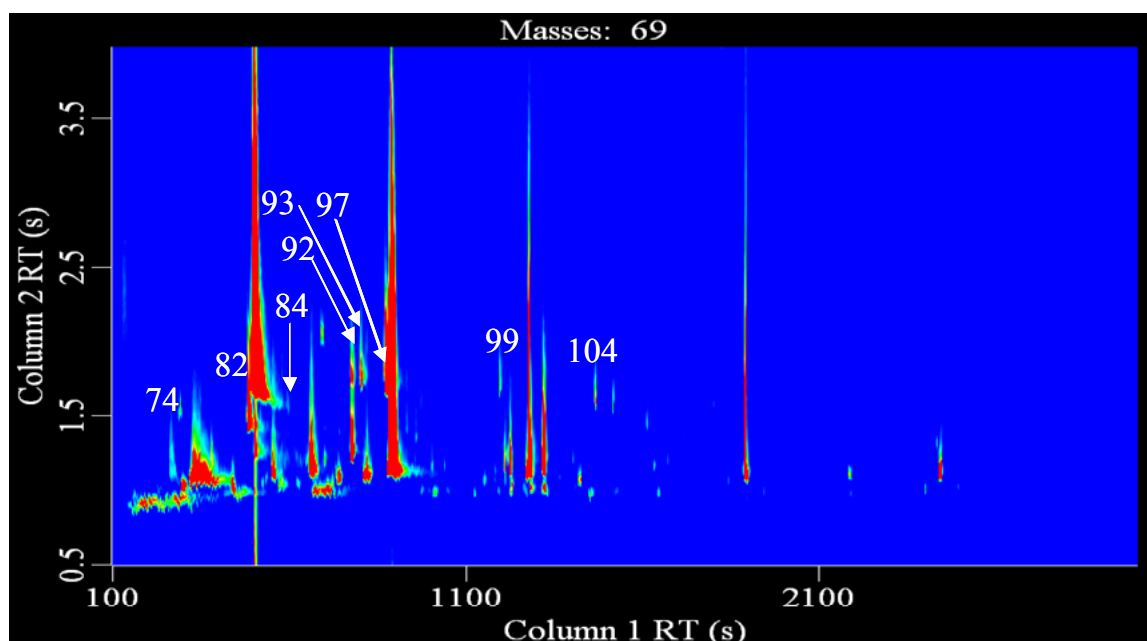


Figure 8.2. Extracted ion contour plot depicting selected alcohols in a Pinotage wine. Peak numbers correspond to **Table 8.1**.

Alcohols are produced as secondary metabolic products of yeast [37]. Di-alcohols such as butanediol (**86** & **88**) are produced from carbohydrates [38]. Isoamyl alcohol (**82**) and 2-phenylethyl alcohol (**106**) have odor thresholds of 30 and 14 mg/L, respectively, in red wine [32]. As these compounds usually occur at levels above their odor thresholds, they are important odorants in wine, and have previously been identified in Pinotage wines [8,9]. Isobutanol (**74**), 1-butanol (**75**), 1-pentanol (**84**), 1-hexanol (**97**) and 1-octanol (**105**) have also been reported in Pinotage [8,9,21]. With the exception of 1-octanol, which was identified in only 5 wines, all these alcohols were detected in each of the 9 samples. 2-Hepten-1-ol, (E) (**100**; identified tentatively) has been previously reported in grape brandy [36], but to the best of our knowledge, this is the first evidence of its occurrence in wine.

Carbonyls. Aldehydes and ketones are highly volatile constituents of alcoholic beverages. In the present study 22 aldehydes and 16 ketones are reported. Most aldehydes were retained somewhat stronger than ketones in the second dimension. Hydroxy- substituted (acetoin, **136**) and aromatic carbonyls (benzaldehyde **124**, benzeneacetaldehyde, **126** and acetophenone, **143**) showed longer retention times in the second dimension, as expected. Aldehydes and ketones are believed to result from the direct oxidation of their corresponding alcohols and fatty acids, respectively [39]. Other authors suggested that carbonyls result from the degradation of amino acids and sugars [40].

Among the carbonyls, acetaldehyde (**108**, odor threshold of 500 mg/L) [41] is a major component and generally represents more than 90% of the total aldehyde content in wine. Benzeneacetaldehyde (**126**) has been described as contributing a honey odor above its odor threshold level of 1 µg/L [39]. This compound was identified in each of the 9 wine samples. In addition, a significant number of unsaturated aldehydes were identified as well. For instance, acrolein (2-propenal, **110**), which is known for its pungent odor and peppery smell, was identified in five of the nine wine samples. This compound may be produced by bacteria from glycerol [42]. In addition to the mono-keto group, C₄ and C₅ di-ketones were identified in a few samples. These di-ketones are formed in wine by oxidative decarboxylation of 2-acetolactate [42]. Acetoin and 2-octanone have been reported previously in Pinotage wines [8,9]. Isobutenal (2-methyl-2-propenal, **112**) and 3-octanone (**139**), both identified tentatively, have only been reported before in grape brandy [36] and grape juice [43], respectively.

Acids. Generally speaking few acids were identified using the current analytical method. This was most likely related to the low sample/headspace and headspace/fiber partition coefficients of these ionizable species [8]. Typically, acids showed high retention in the second dimension, and in fact some of these highly polar compounds showed tailing and wraparound due to their high polarity (for example acetic acid). Acetic acid is known to contribute a vinegar odor [44], and was the dominant acid (based on peak area), in agreement with a previous report [8]. Of the 8 acids identified in the current study, five: formic acid (**146**), acetic acid (**147**), isobutyric acid (**148**), isovaleric acid (**152**) and hexanoic acid (**153**) have been reported previously in Pinotage wines [8,9,21]. Isovaleric acid is known as a very powerful contributor to wine flavor [32]. Further optimization of the method and the sample preparation procedure in particular, is required for the detailed study of the acid content of Pinotage wines.

Acetals. Acetals comprise both cyclic and acyclic di-oxo-compounds, and are produced in wine as secondary products during maturation. Câmara et al. [45], reported the formation of different heterocyclic acetals from acetaldehyde and glycerol via acetalization. Of the 6 acetals reported, 2,4,5-trimethyl-1,3-dioxolane (**155**) was identified in all wines with a good match factor (**Table 8.1**).

Furans and lactones. The furan-related compounds identified here are unsaturated heterocyclic compounds with a 5-membered ring as a basic structure and have been identified in a wide range of foodstuffs (including wine) in both desirable and undesirable circumstances. In the present report, a total of 8 different compounds categorized under this group and including esters, aldehydes, ketones and lactones were detected (**Table 8.1**). Some furan derivatives are believed to be sourced from wood cooperage [46]. Furfural (**162**) is one of the many aldehydes that is released to the wine from wood and has previously been reported in Pinotage wine [8,9]. The level of furfural can increase after drying or seasoning of the wood, mainly when high temperatures are used [47]. It has also been suggested that the release of furfural into wine increases significantly with toasting levels [48]. This increase may have important sensory impact. According to Spillman et al. [49], the amount of furfural and other aldehydes sourced from wood decreases during ageing due to biological reduction in the course of both alcoholic and malolactic fermentation to form the corresponding alcohols. γ -Butyrolactone (**164**) was identified in all wines with greater than 90% match factor. For the rest of the furans identified, some discrepancies among the samples were observed, which could be due to differences in maturation practices as outlined above.

Sulfur compounds. Volatile sulfur compounds play a remarkable role in the aroma of food and beverages, even when present at low concentrations. These compounds can be produced through either enzymatic processes in yeast, or non-enzymatic processes through different chemical, photochemical or thermal reactions during winemaking and storage [50]. Five low molecular weight sulfur compounds have been detected in Pinotage. Among the identified compounds, sulfur dioxide (SO₂, **168**), commonly used to prevent undesired microbiological growth [51], was detected in all samples.

Sulfides and thiols that have a negative impact on wine odor are divided into light (boiling point < 90 °C) and heavy (boiling point > 90 °C) compounds and can diverse olfactory contribution to wine aroma. Dimethyl sulfide (DMS, **169**) is reported to contribute positively to wine bouquet. DMS is characterized by quince or/and truffle odor and has a perception threshold of 5 µg/L. This compound is synthesized by yeast from cysteine. The concentration of DMS depends on grape varieties and can vary during ageing [50,52].

Nitrogen containing compounds. Nitrogen in wine is sourced from the degradation of amino acids and is used for the synthesis of other nitrogen compounds by yeast cells [53]. The best known volatile nitrogen compounds in wine are 3-alkyl-2-methoxypyrazines, which are commonly present in Sauvignon Blanc and Cabernet Sauvignon grape varieties. Methoxypyrazines are nitrogenated heterocycles produced by the metabolism of amino acids [54]. In the current study three methoxypyrazines were detected: 2-methoxy-3-isopropylpyrazine (IPMP, **176**), 2-methoxy-3-sec-butylpyrazine (SBMP, **177**) and 2-methoxy-3-isobutylpyrazine (IBMP, **178**). Note that IBMP is included in **Table 8.1**. despite the fact that the match factor for this compound was less than the requisite 70% as its identity was confirmed using an authentic standard. The levels of IBMP in red wine exceeded those of the other two compounds by a factor 10, and since similar extraction efficiencies are expected for all three methoxypyrazines, it is likely that the poor match factor for IBMP may be ascribed to co-elution.

Methoxypyrazines are characterized by very low perception thresholds of 1 – 2 ng/L and 15 ng/L in white and red wines, respectively. These compounds are well-known for their contribution to vegetative, herbaceous, green bell and pepper character of wines [17,54]. To the best of our knowledge this is the first time methoxypyrazines are being reported in Pinotage wines. These results are in agreement with unpublished results obtained using LC-MS/MS [55]. Although no quantitation was performed in the current study, it is known that the levels of IBMP commonly vary between 0.4 and 10 ng/L in red wines, while SBMP and IPMP are typically below 10% of these levels. This indicates that the analytical method used here was capable of ultra-trace level determination of some compounds.

Terpenes. Terpenes are important varietal aroma compounds that are biosynthesised from acetyl-coenzyme A (CoA). Various types of monoterpene compounds have been reported in grapes including hydrocarbons and oxygen-containing compounds, such as monoterpenols, monoterpendiols and monoterpenes [56]. A large proportion of terpenes (~90%) are present as non-volatile glycosides in the grape, which can be hydrolyzed (enzymatically or chemically) to the corresponding free forms during fermentation and ageing [57]. Moreover, during wine processing and ageing changes

in concentration and the formation of new compounds can take place due to acid-catalyzed rearrangements [57,58].

In the current study 24 terpenes have been identified (positively or tentatively), most of them being present in one to three samples. Exceptions are terpene hydrocarbons including cumene (**179**), isocumene (**180**), *o*-cymene (**184**) and limonene (**185**) as well as an alcohol, α -terpineol (**198**) and an ester, 4-terpineol acetate (**199**), which were positively identified in more than 6 wines. A very similar terpene profile to that reported for grapes using GC × GC-TOFMS [18] was obtained in the present study. Moreover, terpene profiles and levels were found to vary significantly between different samples. **Figure 8.3.** presents a comparison between terpene profiles for two different Pinotage wines. Two compounds – isocineole (**183**) and linalool formate (**196**) were not detected at all in sample W4. Note that only eucalyptol (**186**) and 4-terpineol acetate (**199**) were tentatively identified in this sample using our criteria, since the match factors for linalool (**193**) and α -terpineol (**198**) were below 70%.

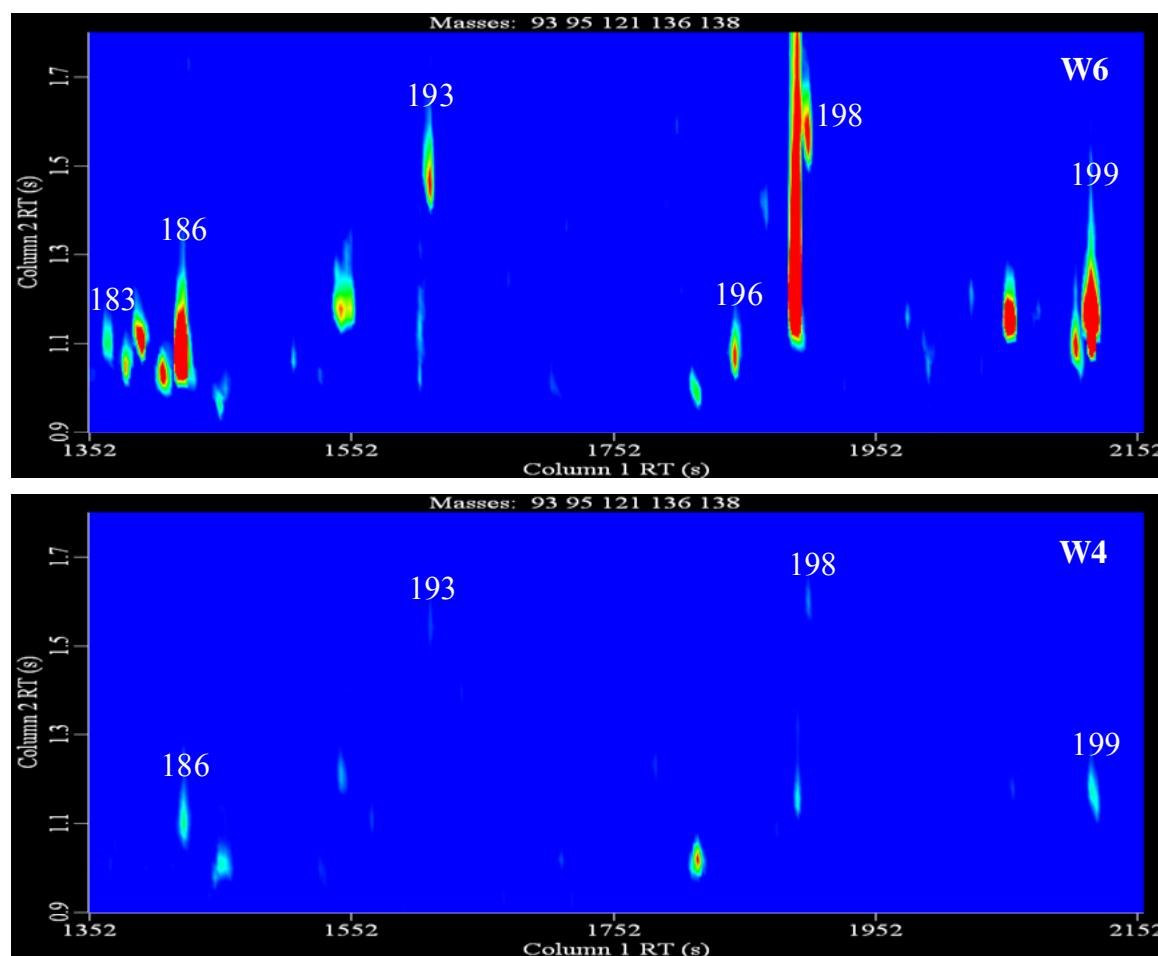


Figure 8.3. Comparison of terpene profiles between two different Pinotage wines. Peak numbers correspond to **Table 8.1**.

The characteristic varietal terpene composition may be influenced to some extent by geographical origin. For instance, a comparison of Riesling wines in cooler (Germany) and in warmer (South Africa) climates have shown very similar monoterpene profiles, but the levels were lower in the warmer climate wines [59]. However, variations due to climatic differences are expected to be less significant for the samples analyzed here, and the differences in terpene content between these samples can more likely be ascribed to variations in fermentation conditions [57,58].

The monoterpene alcohols linalool (**193**) and α -terpineol (**198**) are known for floral aroma properties and are important impact odorants. The odor perception thresholds of these compounds range from 50 to 400 $\mu\text{g/L}$. Monoterpene hydrocarbons such as α -phellandrene (**182**), limonene (**185**), α -terpinene (**188**) and terpinolen (**192**), as well as several monoterpene ketones and esters were also detected. Three norisoprenoid derivatives, 1,1,6-trimethyl-1,2-dihydro-naphthalene (TDN, **200**), 1,1,6-trimethyl-1,2,3,4-tetrahydro-naphthalene (TTN, **201**) and β -damascenone (**202**) were also

tentatively identified. These compounds are characterized by high RTs in the first dimension, due to relatively low vapor pressures. These C₁₃ norisoprenoid derivatives can be formed in grapes or/and wine due to degradation of C₄₀ terpenes like carotenoids. TDN may be responsible for a kerosene or petrol odor in some wines and has a sensory threshold of 20 µg/L. Concentration of this compound increases significantly during bottle ageing [54,59,60]. β-Damascenone is an influential contributor to wine aroma and is believed to be present in all grape varieties [32,41,54]. This compound is characterized by a complex tropical fruit aroma and has an odor threshold of 50 ng/L.

Fenchon (**190**) and *p*-menthan-8-ol (**195**) were previously reported in grape [36], but this is the first report of these compounds in wine. Fenchon was positively identified by an authentic standard, while *p*-menthan-8-ol was identified tentatively.

Hydrocarbons. In this study, over 20 hydrocarbons (not listed in table 8.1.) were detected. The sources of these hydrocarbons were most probably from the laboratory air, as most of them were also detected in blank analyses. It is also worth noting that the sample preparation procedure used favored the extraction of these molecules, even if they were present at very low concentrations. Most of these hydrocarbons have been identified previously in wine and in cork stoppers [61]. Aromatic hydrocarbons have previously been detected in wine, where their presence is chiefly associated with contamination arising from petroleum-derived products [62]. Jordão et al. [63] also reported the presence of aromatic hydrocarbons in oak-wood.

Volatile phenols. Although the sample preparation method employed here hardly revealed high-boiling compounds like volatile phenols, 2,4-Bis(1,1-dimethylethyl)-phenol (**203**) and butylated hydroxytoluene (BHT, **204**) were detected in 1 and 5 samples, respectively. The source of these two compounds in wine is unclear, although plastic containers (plastic cap inserts were used for transport of the wine samples) have been reported as possible sources of BHT in wine [64].

Pyrans. Pyran-related compounds have a six membered ring as a basic chemical structure. In the current study two pyrans: 2H-pyran-2-one, tetrahydro- (**205**) and 2H-pyran, 2-ethenyltetrahydro-2,6,6-trimethyl- (**206**), were detected.

8.4. Conclusions

The methodology applied proved successful for the most detailed screening of volatile compounds in Pinotage wines reported to date. This is largely due to the intrinsically high resolving power and sensitivity of comprehensive 2D GC coupled to TOFMS. The proposed method was also found to be reproducible, but the time- and labor intensive nature of data interpretation seems to preclude its usage in routine analysis for now. In total, 206 volatile compounds belonging to various chemical classes were identified (positively or tentatively). Many of the compounds were common to all samples, while others were uniquely identified in only a few, possibly reflecting differences in viticultural and wine-making practices. Differences may also be ascribed to the presence of co-eluting compounds and the low levels of occurrence, both of which make accurate identification difficult.

Several limitations were encountered in the methodology applied. First, the high level of ethanol, acetaldehyde, acetic acid and certain esters and alcohols masked a potentially large number of minor compounds and hampered their identification, even with the deconvolution software. Secondly, less volatile, highly polar and large molecular weight compounds such as acids, volatile phenols, lactones, etc., which could contribute significantly to wine flavor, were not detected. This was likely related to the sample preparation technique used, which favored the extraction of non-polar and highly volatile compounds. While less volatile compounds might also be effectively extracted by the Carboxen coating used, they are notoriously difficult to desorb from the fiber. Despite these drawbacks, the methodology proved suitable for the screening of a large number of wine volatiles. Future work will focus on the development of more selective sample preparation procedures to allow the detection of particular classes of minor wine volatiles.

It should be pointed out that all the compounds reported in this paper have previously been identified in wine or related products, although most of them are identified for the first time in Pinotage wine. Many compounds reported here may potentially contribute to the unique aroma of wine of this cultivar, notably sulfur compounds, terpenes and methoxypyrazines. These results therefore represent a valuable contribution to the knowledge of this uniquely South African cultivar and might eventually be used to improve wine-making practices for the production of Pinotage

wines. While the goal of the current report was the identification of volatile constituents, future work will focus on the detailed evaluation of the effect of various wine-making practices on the volatile content. For such work, well-defined samples will be sourced from the local industry. Further work should also include quantitative measurements, which, in combination with odor threshold values, may contribute to better understanding of Pinotage wine aroma.

8.5. References

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Solid phase extraction (SPE) in combination with comprehensive two-dimensional gas chromatography (GC × GC) coupled to time-of-flight mass spectrometry (TOFMS) for the detailed investigation of volatiles in South African wines[†]

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Abstract

Comprehensive two-dimensional gas chromatography in combination with time-of-flight mass spectrometry (GC × GC-TOFMS) has been applied for the analysis of volatile compounds in three young South African red wines. In spite of the significant benefits offered by GC × GC-TOFMS for the separation and identification of volatiles in such a complex matrix, previous results utilizing headspace solid phase micro-extraction (HS-SPME) demonstrated certain limitations. These were primarily associated with the choice of sample preparation technique, which failed to extract some of the influential semi-volatile wine constituents. Therefore, in the current report, we utilized solid phase extraction (SPE) in combination with GC × GC-TOFMS for the detailed investigation of particularly low-level semi-volatiles in South African wine. Most polar major volatiles were removed during a rinsing step employing 50% methanol, thereby improving identification of trace-level compounds. 214 Compounds previously reported in grapes and related beverages were tentatively identified based on mass spectral data and retention indices, while 62 compounds were identified using authentic standards. The developed method proved particularly beneficial for the analysis of terpenes, lactones and volatile phenols and allows us to report the presence of numerous volatile compounds for the first time in Pinotage wines.

Key Word:

Solid Phase Extraction (SPE), Comprehensive Two-Dimensional Gas Chromatography (GC × GC), Time-of-Flight Mass Spectrometry (TOFMS), Volatiles, Wine.

9.1. Introduction

In excess of 800 volatile compounds have been reported in wine [1]. The levels of these compounds depend on many enological and viticultural factors, and their combined effect determines wine aroma, which is an influential factor in quality assessment. Wine volatiles can broadly be sub-divided into three groups: impact odorants, major volatiles and off-flavors. The former class includes compounds with characteristic odor properties such as varietal compounds [2], which are linked to a specific grape cultivar. Impact odorant may also be imparted to the wine medium from external factors like wood [3] or evolve in wine via chemical reactions during wine-making, from crushing of the grape berries through fermentation, maturation and ageing [3,4]. Major volatiles commonly exist at higher concentrations and do not contribute to the wine aroma individually, but rather collectively. These compounds are responsible for the so-called base aroma of wines. Most esters and alcohols are categorized as major volatiles, and are mainly produced during fermentation [4,5]. Off-flavors are volatile compounds associated with deterioration of wine aroma. These compounds could originate in various ways including bacterial spoilage and oxidation [4]. The aroma contribution for specific compounds may vary between positive and negative, depending on their concentrations [6]. Considering the diverse factors that determine the quality and perception of wine aroma, analytical methods suitable for the assessment of the volatile compounds associated with wine aroma are essential.

The study of wine volatiles is normally performed using gas chromatography (GC). Conventional capillary GC (cGC) methods are characterized by high separation power, relatively wide applicability, simplicity and durability. Nevertheless, wine analysis represents a severe analytical challenge due to the large number of volatile compounds present. Even the high peak capacity of cGC is insufficient for the separation of all of these compounds existing at different levels in a single sample [7].

Almost two decades since its first appearance in chromatographic literature [8], comprehensive two-dimensional gas chromatography (GC × GC) is receiving increasing attention for the analysis of complex samples. GC × GC is theoretically capable of producing improved separation of volatile compounds in complex mixtures

due to the distribution of analytes over a retention plane created by two independent columns. The enhanced resolution of peaks is a result of unrelated (orthogonal) separation mechanisms provided by the two columns, resulting in much higher peak capacity. In addition, GC × GC is expected to provide more reliable identification of analytes, and highly structured patterns are often obtained due to the presence of two retention mechanisms [7,9].

Sample pretreatment is an essential step in the analysis of wine volatiles, and several techniques have been described in the literature. Liquid liquid extraction (LLE) has been extensively applied [1,3,10-12]. Yet, due to limited sensitivity, labor intensiveness, high cost and environmental concerns, LLE is gradually being replaced by alternative methods. Solid phase extraction (SPE) is increasingly being used for the extraction of wine volatiles [3,10,13-16]. Advantages include higher selectivity and sensitivity, ease of automation, and reduced environmental risk. Sorptive-based sample preparation techniques such as solid phase micro extraction (SPME) [17] and stir bar sorptive extraction (SBSE) [18] have also been extensively applied for the investigation of wine volatiles [5,19-22].

Despite significant advances, there is no universal sample preparation technique for wine volatiles, and methods are selected based on the analytical goals. We recently used headspace (HS) SPME in combination with GC × GC-TOFMS to investigate Pinotage volatiles [23]. While this method provided significantly improved resolution and sensitivity, and allowed identification of more than 200 compounds, HS-SPME showed limited application for some influential high-boiling aroma compounds. For example, lactones and volatile phenols were not detected in the previous study. In addition, the extraction of some compounds present at high levels in wine, such as highly volatile polar compounds, resulted in tailing peaks which obscured minor compounds. In order to overcome some of these shortcomings, we report a modified experimental procedure in the current work. A different column set and instrumental configuration was used, while the utility of SPE as complementary sample pretreatment technique in combination with GC × GC was investigated specifically for the analysis of high-boiling potentially influential wine volatiles.

9.2. Materials and methods

9.2.1. Wine samples

Two Pinotage and one Cabernet Sauvignon sample of vintage 2006, supplied by three South African producers, were analysed. The two Pinotage wines were the same wines studied before using the HS-SPME-GC × GC-TOFMS [23].

9.2.2. Chemicals and reagents

Methanol, ethanol and dichloromethane were obtained from Sigma-Aldrich (St. Louis, MO). NaHCO₃ was obtained from UNIVAR[®] (Helsinki, Finland). Water was purified by Milli-Q water purification system (Millipore, Bedford, MA). Volatile standards (see **Table 9.1.**) were purchased from Sigma-Aldrich, Fluka (Zwijndrecht, Netherlands), Riedel-de Haën (Steinheim, Germany), and Merck (Darmstadt, Germany). Series of *n*-alkanes were obtained from Supelco (Bellefonte, PA).

9.2.3. Chromatographic conditions

Experiments were performed on a LECO Pegasus[®] 4D GC × GC-TOFMS system (LECO Corp., St. Joseph, MI). This instrument consisted of an Agilent 7890A GC (Agilent Technologies, Palo Alto, CA, USA) incorporating LECO's thermal modulator (dual-stage quad-jet) and a secondary oven mounted inside the primary GC oven. The column set consisted of a 30 m × 0.25 mm i.d. × 0.25 μm d_f Rxi[®] – 5Sil MS (Restek, Penn Eagle Park, CA, USA) as primary column coupled via a press-tight column connector (Restek) to a 0.8 m × 0.18 mm i.d. × 0.18 μm d_f Rtx – PCB secondary column (Restek). The separation was performed using the following temperature program: 35 °C kept for 2 min, ramped at 3 °C/min to 250 °C and held for 10 min. The secondary (2D) oven was operated at 20 °C higher than the primary (1D) oven throughout. A modulation period of 4 s (hot pulse of 1.00 s) was used. 1 μL sample extract was injected in splitless mode, with a splitless time of 2 min at an injector temperature of 280 °C. Helium (99.999% purity; Afrox, Johannesburg, South Africa) was used as carrier gas at a constant flow of 1.00 mL/min.

A Pegasus® IV time-of-flight mass spectrometer (LECO Corp.) was used for analyte detection. The transfer line and the ion source were maintained at 250 and 200 °C, respectively. The detector voltage was set to -1650 V and the MS was operated in electron impact ionization mode (70 eV). Ions were collected in the mass range of 35 - 350 amu at an acquisition rate of 100 spectra/s. ChromaTOF v 4.13 software (LECO Corp.) was used for instrument control, data acquisition and data processing. Identification was based on retention times of authentic standards (when available) and comparison of mass spectra with NIST 2005 library. A series of *n*-alkanes were analyzed under the same conditions to determine first dimension linear retention indices for each compound.

Tentative identification was based on the comparison of mass spectra with the NIST 2005 library using a minimum similarity value of 70% as the criterion, as well as experimentally determined linear retention indices (LRI) [24] compared to literature values. A maximum deviation of 30 between the experimental and literature LRI values was used as a criterion. In addition, positive identification of 62 (~ 23%) compounds was based on comparison of retention times with authentic standards. Note that for some compounds (hexanoic acid, **157**; phenol, **258**; 2,6-dimethyl phenol, **262** and 4-ethylguaiacol, **266**), identification was confirmed using authentic standards, despite mass spectral similarities being less than 70% (presumably due to their low concentrations and/or co-elution).

9.2.4. Solid phase extraction (SPE) procedure

The solid phase extraction (SPE) procedure reported by Campo et al. [14] was adapted with slight modification. Strata™ SDB-L (Styrene-Divinylbenzene, 100 µm, 260A, 50 mg/3 mL; Phenomenex, Torrance, USA) SPE cartridges were consecutively conditioned using 10 mL each of dichloromethane, methanol and a 15% (v/v) solution of ethanol in water. 40 mL wine was loaded and rinsed using 20 mL of an aqueous solution containing 50% methanol (v/v) and 1% NaHCO₃ in order to remove major volatiles and other interfering compounds. The cartridge was then dried for 10 min under vacuum. Analytes were eluted using 1.5 mL dichloromethane, and stored at 4 °C until analysis.

9.3. Results and discussion

The combination of comprehensive two-dimensional gas chromatography with high speed time-of-flight mass spectrometry has enabled us to identify (positively or tentatively) 276 compounds in the three young South African wines. Wine samples for the current study were selected in the first instance to allow comparison with previous results obtained by HS-SPME. The Cabernet Sauvignon sample was selected to study potential differences in volatile content between these two cultivars.

Figure 9.1. illustrates an analytical ion chromatogram (AIC) of the Cabernet Sauvignon wine highlighting a portion of the two-dimensional contour plot. It is clear that the number of compounds displayed in this figure could not have been separated using conventional 1D GC methods. In fact, only about a quarter of the compounds detected here could be completely resolved in one dimension. An Rtx-PCB column was used in the second dimension because it offered unique selectivity, particularly for aromatic compounds. This explains why non-polar compounds such as aliphatic esters were poorly retained in the 2nd dimension, while cyclic compounds such as aromatics, lactones and pyrans were more retained.

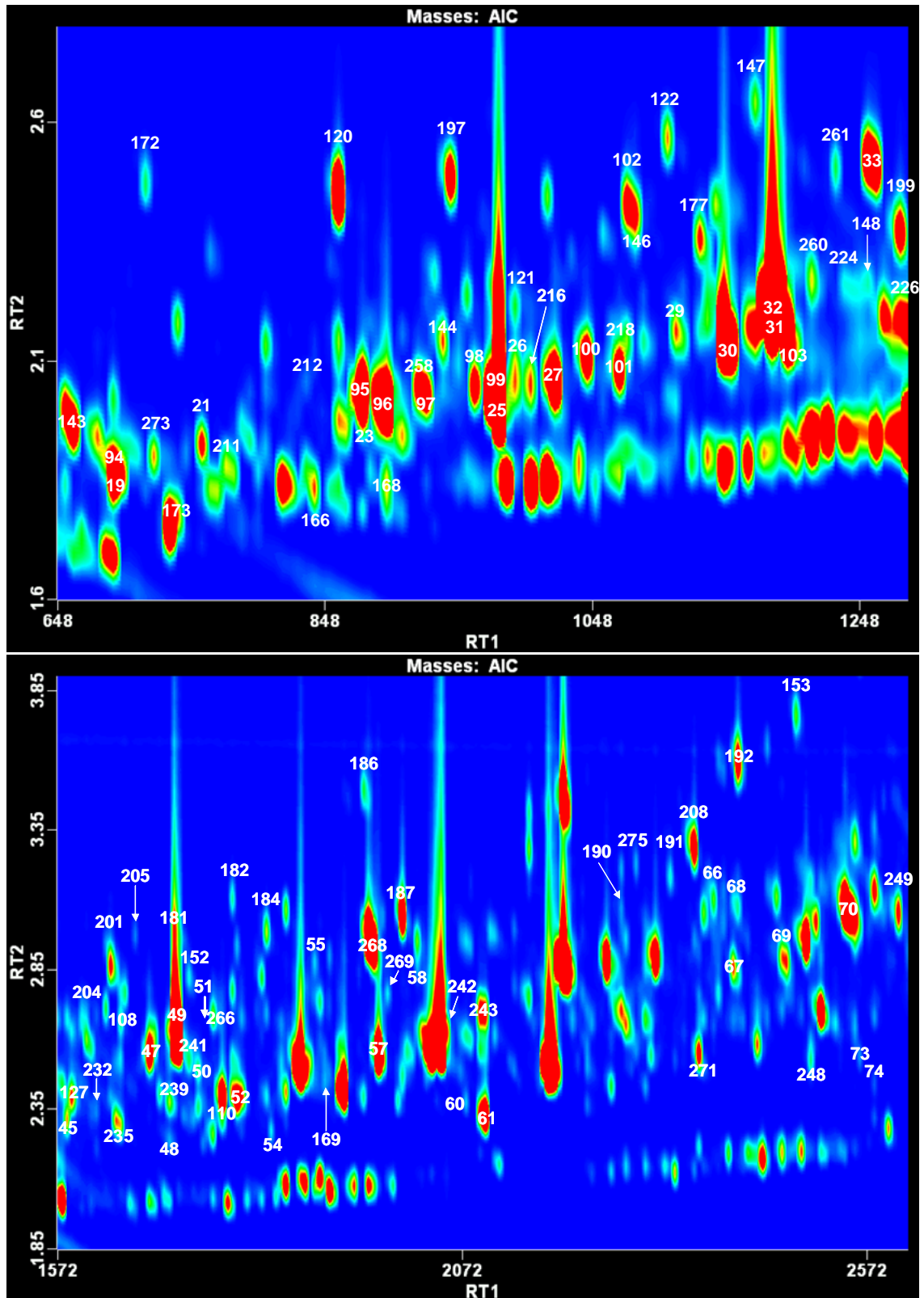


Figure 9.1. Analytical ion chromatogram (AIC) contour plot obtained for the SPE-GC×GC-TOFMS analysis of a Cabernet Sauvignon wine. Compound numbers correspond to **Table 9.1**.

Table 9.1. Volatile compounds identified in three South African wines by SPE-GC × GC-TOFMS.

No.	Compound	RT1 ^a	RT2 ^b	Similarity ^c	Reverse ^d	P1 ^e	P2 ^e	CSI ^e	^j LRI _{cal.}	^g LRI _{lit.}	Identification ^h
Esters											
1	Acetic acid, ethyl ester (Ethyl acetate)*	196	1.00	959	959	+	+	-	581	588	RI, MS, Tent.
2	Propanoic acid, ethyl ester (Ethyl propanoate)*	288	1.26	936	936	+	+	+	686	684	RI, MS, Tent.
3	Acetic acid, propyl ester (Propyl acetate)*	296	1.28	959	959	+	+	+	691	703	RI, MS, Tent.
4	Butanoic acid, methyl ester (Methyl butanoate)	304	1.31	952	952	+	+	+	703	714	RI, MS, Tent.
5	Propanoic acid, 2-hydroxy-, methyl ester (Methyl lactate)	324	1.41	799	799	+	-	-	715		MS, Tent.
6	Propanoic acid, 2-methyl-, ethyl ester (Ethyl isobutyrate)*	360	1.40	871	871	+	+	+	737	729	RI, MS, Tent.
7	Acetic acid, chloro-, methyl ester ⁱ	364	1.55	929	929	+	+	-	740	731	RI, MS, Tent.
8	Acetic acid, 2-methylpropyl ester (Isobutyl acetate)*	388	1.47	935	935	+	+	+	754	758	RI, MS, STD
9	1-Butanol, 3-methyl-, formate (Isoamyl formate)*	428	1.53	773	784	-	-	+	779	792	RI, MS, Tent.
10	Butanoic acid, ethyl ester (Ethyl butyrate)*	448	1.55	925	937	+	+	+	791	787	RI, MS, STD
11	Pyruvic acid, ethyl ester (Ethyl pyruvate)*	464	1.64	850	894	-	-	+	801	802 [10]	RI, MS, Tent.
12	Acetic acid, butyl ester (Butyl acetate)*	476	1.61	919	919	+	+	+	806	807	RI, MS, STD
13	Acetic acid, dimethoxy-, methyl ester	536	1.63	897	990	-	+	-	832		MS, Tent.
14	2-Butenoic acid, ethyl ester, (E)- (<i>trans</i> -Ethyl 2-butenate)*	548	1.77	924	924	+	+	+	837	835	RI, MS, STD
15	Butanoic acid, 2-methyl-, ethyl ester (Ethyl 2-methylbutanoate)*	556	1.67	891	891	+	+	+	840	842	RI, MS, Tent.
16	Butanoic acid, 3-methyl-, ethyl ester (Ethyl isovalerate)*	568	1.70	908	908	+	+	+	845	846	RI, MS, STD
17	1-Butanol, 3-methyl-, acetate (Isoamyl acetate)*	628	1.85	944	947	+	+	+	871	872	RI, MS, STD
18	1-Butanol, 2-methyl-, acetate (2-Methylbutyl acetate)*	632	1.91	814	817	+	+	+	873	877	RI, MS, Tent.
19	Pentanoic acid, ethyl ester (Ethyl pentanoate)*	692	1.83	908	908	+	+	+	898	898	RI, MS, STD
20	Acetic acid, pentyl ester (Pentyl acetate)*	732	1.88	829	829	-	+	-	912	912	RI, MS, Tent.
21	Hexanoic acid, methyl ester (Methyl hexanoate)*	760	1.91	866	866	+	+	+	922	922	RI, MS, Tent.
22	Butanoic acid, 2-hydroxy-3-methyl-, ethyl ester (Ethyl 2-hydroxyisovalerate)*	876	2.02	739	760	+	-	+	961	968	RI, MS, Tent.
23	Pentanoic acid, 4-methyl-, ethyl ester (Ethyl 4-methylpentanoate)*	880	1.94	811	811	-	+	-	963	969 [10]	RI, MS, Tent.
24	Propanoic acid, 2-hydroxy-, butyl ester (Butyl lactate)*	880	2.04	951	951	+	+	+	963		MS, Tent.
25	Hexanoic acid, ethyl ester (Ethyl hexanoate)*	980	2.02	924	933	+	+	+	997	998	RI, MS, STD
26	3-Hexen-1-ol, acetate, (E)-*	988	2.04	835	835	-	+	-	999	998	RI, MS, Tent.
27	Acetic acid, hexyl ester (Hexyl acetate)*	1020	2.05	949	949	+	+	+	1010	1010	RI, MS, STD
28	Butanedioic acid, dimethyl ester (Dimethyl succinate)	1076	2.27	909	909	+	-	-	1029	1034	RI, MS, Tent.
29	Ethyl 2-hexenoate*	1112	2.15	719	719	-	-	+	1042	1046	RI, MS, Tent.
30	Pentanoic acid, 2-hydroxy-4-methyl-, ethyl ester*	1148	2.16	933	933	+	+	+	1054	1078	RI, MS, Tent.
31	Propanoic acid, 2-hydroxy-, 3-methylbutyl ester (Isoamyl	1184	2.21	862	862	+	+	+	1066	1082	RI, MS, Tent.

Analysis of South African wines using SPE-GC × GC-TOFMS

	lactate)*										
32	Propanedioic acid, diethyl ester (Diethyl malonate)	1184	2.25	952	952	+	+	+	1066	1069	RI, MS, Tent.
33	Benzoic acid, methyl ester (Methyl benzoate)	1256	2.51	928	928	+	+	+	1091	1092	RI, MS, STD
34	Hexanoic acid, propyl ester (Propyl hexanoate)*	1264	2.05	768	776	+	-	-	1093	1094	RI, MS, Tent.
35	Heptanoic acid, ethyl ester (Ethyl heptanoate)*	1276	2.11	902	902	+	+	+	1097	1097	RI, MS, Tent.
36	Ethyl 2,2-diethoxypropionate	1288	2.06	776	800	-	+	-	1101	1106 [25]	RI, MS, Tent.
37	Butanedioic acid, ethyl methyl ester ^{i,*}	1308	2.30	935	935	+	+	+	1108	1116	RI, MS, Tent.
38	Octanoic acid, methyl ester (Methyl octanoate)*	1352	2.18	917	917	+	+	+	1123	1125	RI, MS, Tent.
39	Acetic acid, phenylmethyl ester (Phenylmethyl acetate)	1460	2.51	894	894	+	+	-	1160	1161	RI, MS, Tent.
40	Benzoic acid, ethyl ester (Ethyl benzoate)*	1480	2.53	854	873	+	+	+	1167	1170	RI, MS, Tent.
41	Formic acid, 2-phenylethyl ester (2-Phenylethyl formate)	1496	2.61	821	846	-	+	+	1172	1174 [26]	RI, MS, Tent.
42	Butanedioic acid, diethyl ester (Diethyl succinate)*	1508	2.36	957	957	+	+	+	1176	1179	RI, MS, STD
43	Benzoic acid, 2-hydroxy-, methyl ester (Methyl salicylate)*	1544	2.69	813	823	+	+	+	1188	1189	RI, MS, Tent.
44	Octanoic acid, ethyl ester (Ethyl octanoate)*	1564	2.25	913	914	+	+	+	1195	1196	RI, MS, STD
45	Butanedioic acid, methyl-, diethyl ester (Diethyl methylsuccinate)	1584	2.30	898	898	+	+	+	1202	1205 [25]	RI, MS, Tent.
46	1,4-Butanediol, diacetate	1604	2.43	986	986	-	+	-	1209	1210 [27]	RI, MS, Tent.
47	Benzeneacetic acid, ethyl ester (Ethyl phenylacetate)*	1684	2.55	966	966	+	+	+	1238	1243	RI, MS, STD
48	Hexanoic acid, 3-methylbutyl ester (Isoamyl hexanoate)*	1712	2.24	804	804	-	+	+	1249	1250	RI, MS, Tent.
49	Acetic acid, 2-phenylethyl ester (2-Phenylethyl acetate)*	1720	2.63	947	963	+	+	+	1252	1252	RI, MS, STD
50	Butanedioic acid, hydroxy-, diethyl ester (Ethyl malate) ⁱ	1744	2.50	898	898	-	+	+	1260	1244	RI, MS, Tent.
51	Benzoic acid, 2-hydroxy-, ethyl ester (Ethyl salicylate)	1756	2.68	697	830	+	+	+	1265	1267	RI, MS, STD
52	Pentanedioic acid, diethyl ester (Diethyl glutarate)	1792	2.40	914	914	+	+	+	1278	1281	RI, MS, Tent.
53	Octanoic acid, propyl ester (Propyl octanoate)	1828	2.27	703	708	+	+	-	1291	1300 [28]	RI, MS, Tent.
54	Nonanoic acid, ethyl ester (Ethyl nonanoate)*	1836	2.27	912	912	+	+	+	1294	1294	RI, MS, STD
55	Benzoic acid, 2,3-dihydroxy-, methyl ester	1892	2.86	918	924	+	+	+	1314		MS, Tent.
56	Decanoic acid, methyl ester (Methyl decanoate)	1912	2.33	700	703	+	+	-	1322	1324	RI, MS, Tent.
57	Benzenepropanoic acid, ethyl ester (Ethyl hydrocinnamate)	1968	2.69	817	826	+	-	+	1343	1347	RI, MS, Tent.
58	Benzenepropanoic acid, α -hydroxy-, methyl ester ⁱ	2016	2.79	778	803	+	-	-	1361	1336	RI, MS, Tent.
59	Succinoic acid, 2-hydroxy-3-methyl-, diethyl ester	2044	2.65	764	764	+	+	+	1334	1349	RI, MS, Tent.
60	Ethyl 9-decenoate*	2076	2.38	900	930	+	+	+	1384	1387	RI, MS, Tent.
61	Decanoic acid, ethyl ester (Ethyl decanoate)*	2096	2.34	909	909	+	+	+	1392	1390	RI, MS, STD
62	Octanoic acid, 3-methylbutyl ester (Isoamyl octanoate)*	2228	2.37	718	801	+	+	-	1445	1442	RI, MS, Tent.
63	Benzoic acid, 4-hydroxy-, methyl ester (Methylparaben)	2236	2.84	866	883	-	+	-	1448	1459	RI, MS, STD
64	2-Propenoic acid, 3-phenyl-, ethyl ester (Ethyl cinnamate)	2268	2.94	759	872	+	+	+	1461	1463	RI, MS, Tent.
65	Heptanedioic acid, diethyl ester (Diethyl pimelate)	2316	2.58	726	733	+	+	+	1481	1480 [25]	RI, MS, Tent.

Analysis of South African wines using SPE-GC × GC-TOFMS

66	Benzoic acid, 4-hydroxy-3-methoxy-, methyl ester (Methyl vanillate)	2380	3.11	971	971	+	+	+	1507	1496	RI, MS, Tent.
67	Benzoic acid, 4-hydroxy-, ethyl ester (Ethylparaben)	2408	2.85	916	916	+	+	+	1519		MS, Tent.
68	Benzoic acid, 2,5-dihydroxy-, methyl ester (Methyl gentisate)	2408	3.09	870	881	+	+	+	1519	1519	RI, MS, Tent.
69	Benzeneacetic acid, 4-hydroxy-, ethyl ester (Ethyl 4-hydroxyphenylacetate)	2468	2.89	865	865						MS, Tent.
70	Benzoic acid, 4-hydroxy-3-methoxy-, ethyl ester (Ethyl vanillate)	2544	3.09	867	867	+	+	+	1577	1579	RI, MS, Tent.
71	Octanedioic acid, diethyl ester (Ethyl suberate)	2556	2.62	754	766	-	+	+	1582	1583 [25]	RI, MS, Tent.
72	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate ⁱ	2560	2.30	779	784	-	+	-	1584	1591	RI, MS, Tent.
73	Propanoic acid, 2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester	2560	2.62	800	835	+	+	+	1584	1578 [29]	RI, MS, Tent.
74	Dodecanoic acid, ethyl ester (Ethyl dodecanoate)*	2576	2.46	783	801	-	-	+	1591	1591	RI, MS, Tent.
75	Dodecanoic acid, 1-methylethyl ester (Isopropyl dodecanoate)	2652	2.42	734	741	+	+	+	1624	1617	RI, MS, Tent.
76	Benzeneacetic acid, 4-hydroxy-3-methoxy-, ethyl ester (Ethyl homovanillate)	2664	3.04	743	811	-	-	+	1630		MS, Tent.
77	Ethyl citrate ⁱ	2708	2.84	823	903	-	+	-	1650	1655	RI, MS, Tent.
78	Dodecanoic acid, 3-hydroxy-, ethyl ester	2896	2.70	772	774	-	+	-	1735	1742	RI, MS, Tent.
79	Hexadecanoic acid, ethyl ester (Ethyl palmitate) ^k	3408	2.69	793	800	+	-	+	1950	1975	RI, MS, STD
80	Octadecanoic acid, methyl ester (Methyl octadecanoate) ^k	3652	2.83	757	761	+	-	+	2054	2065	RI, MS, Tent.
Alcohols											
81	1-Butanol*	268	1.23	808	827	+	-	-	663	668	RI, MS, STD
82	2-Pentanol*	308	1.27	768	782	-	+	-	705	705	RI, MS, Tent.
83	1-Butanol, 3-methyl- (Isoamyl alcohol)*	324	1.37	965	965	+	+	+	715	713	RI, MS, STD
84	1-Butanol, 2-methyl-, (Active amyl alcohol)*	332	1.36	808	808	+	+	+	720	697	RI, MS, STD
85	2-Hexanol*	464	1.65	720	748	+	+	-	801	801	RI, MS, Tent.
86	2,3-Butanediol*	468	1.65	794	794	-	-	+	803	806	RI, MS, STD
87	2-Ethyl-1-butanol	528	1.57	793	823	+	-	-	828	828 [30]	RI, MS, Tent.
88	4-Methyl-1-pentanol*	528	1.68	909	909	+	+	+	828	833	RI, MS, Tent.
89	3-Methyl-1-pentanol*	548	1.72	936	936	+	+	+	837	840 [30]	RI, MS, Tent.
90	3-Hexen-1-ol, (E)-*	560	1.74	953	953	-	+	+	842	845	RI, MS, Tent.
91	3-Hexen-1-ol, (Z)-*	576	1.81	706	852	-	-	+	849	850	RI, MS, STD
92	2-Hexen-1-ol, (E)-*	596	1.80	806	815	+	-	+	857	865	RI, MS, Tent.
93	1-Hexanol*	608	1.82	939	939	+	+	+	862	860	RI, MS, Tent.
94	2-Heptanol*	696	1.86	850	870	+	+	+	900	900	RI, MS, Tent.
95	3-Hepten-1-ol ⁱ	876	2.02	723	732	-	+	+	961	941	RI, MS, Tent.

Analysis of South African wines using SPE-GC × GC-TOFMS

96	1-Heptanol*	892	2.01	926	926	+	+	+	967	961	RI, MS, Tent.
97	1-Octen-3-ol	920	2.12	704	742	+	+	+	976	978	RI, MS, STD
98	5-Hepten-2-ol, 6-methyl-	960	2.05	886	887	+	-	+	990	997	RI, MS, Tent.
99	3-Octanol	972	2.05	747	785	-	-	+	994	994	RI, MS, STD
100	1-Heptanol, 6-methyl- (Isooctanol) ^{i, *}	1044	2.10	805	814	+	+	+	1018	1055	RI, MS, Tent.
101	2-Ethyl-1-hexanol	1068	2.08	940	940	+	+	+	1027	1025	RI, MS, Tent.
102	Benzyl alcohol	1076	2.41	880	880	+	+	+	1029	1029	RI, MS, STD
103	1-Octanol*	1196	2.14	919	919	+	+	+	1070	1071	RI, MS, Tent.
104	2-Nonanol	1292	2.14	957	957	+	+	-	1103	1100	RI, MS, Tent.
105	2-Phenylethyl alcohol*	1304	2.58	940	949	+	+	+	1107	1108	RI, MS, STD
106	1-Nonanol*	1496	2.17	773	825	+	+	+	1172	1172	RI, MS, Tent.
107	2-Phenoxy ethanol	1616	2.73	728	742	-	+	-	1214	1226	RI, MS, Tent.
108	Benzenepropanol (Phenylpropyl alcohol)	1652	2.69	831	841	+	+	+	1227	1225	RI, MS, Tent.
109	2-Ethylphenethyl alcohol (2-Phenyl-1-butanol)	1704	2.65	753	771	+	+	+	1246	1270	RI, MS, Tent.
110	1-Decanol	1772	2.32	739	739	+	+	+	1270	1270	RI, MS, STD
111	1-Undecanol	2092	2.34	801	804	-	+	-	1390	1387	RI, MS, Tent.
112	1,9-Nonanediol ⁱ	2224	2.63	722	745	-	-	+	1443	1414	RI, MS, Tent.
113	1-Dodecanol	2296	2.45	711	750	+	+	-	1472	1473	RI, MS, STD
114	1-Hexadecanol ^k	3156	2.60	785	802	-	+	-	1848	1875 [28]	RI, MS, Tent.
Aldehydes											
115	2-Methyl propanal (Isobutanal)*	180	1.30	727	732	+	+	+	562	560	RI, MS, Tent.
116	2-Methyl butanal*	240	1.17	797	797	-	+	-	631	635	RI, MS, Tent.
117	2-Methyl pentanal	348	1.46	864	864	-	+	+	730	746	RI, MS, Tent.
118	Hexanal*	440	1.69	898	898	+	+	+	786	792	RI, MS, STD
119	2-Heptenal, (Z)-	852	2.31	853	859	+	+	+	953	952	RI, MS, Tent.
120	Benzaldehyde*	860	2.44	951	951	+	+	+	956	955	RI, MS, STD
121	Octanal*	988	2.20	744	828	+	+	+	999	1002	RI, MS, Tent.
122	Benzeneacetaldehyde (Phenylacetaldehyde)*	1104	2.56	952	952	+	+	+	1039	1037	RI, MS, Tent.
123	4-Methyl benzaldehyde	1220	2.78	824	890	-	+	+	1078	1079	RI, MS, Tent.
124	4-Ethyl benzaldehyde	1460	2.77	868	868	+	+	+	1160	1164	RI, MS, Tent.
125	3,5-Dimethyl benzaldehyde ⁱ	1508	2.89	814	816	+	+	+	1176	1149	RI, MS, Tent.
126	3-Phenyl-2-propenal (Cinnamaldehyde)	1576	2.99	792	792	+	+	+	1199	1193 [31]	RI, MS, Tent.
127	Decanal*	1592	2.39	721	784	-	+	-	1205	1203	RI, MS, STD
128	2,4-Dimethyl benzaldehyde	1612	3.00	781	784	+	+	+	1212	1185	RI, MS, Tent.
129	Benzeneacetaldehyde, α -ethylidene-	1756	2.87	761	766	+	+	+	1265	1273	RI, MS, Tent.
130	4-Hydroxy benzaldehyde	1996	2.88	850	880	+	-	-	1354	1368 [32]	RI, MS, Tent.

Analysis of South African wines using SPE-GC × GC-TOFMS

131	Vanillin	2088	3.26	879	890	+	+	+	1389	1389	RI, MS, Tent.
132	Dodecanal	2132	2.52	769	804	-	+	+	1406	1408	RI, MS, Tent.
133	Methylvanillin	2296	3.29	745	754	-	-	+	1472		MS, Tent.
134	Syringaldehyde	2700	3.58	754	761	-	+	+	1646	1670	RI, MS, Tent.
Ketones											
135	2-Butanone	188	0.99	905	905	+	+	+	567	575	RI, MS, Tent.
136	2-Pentanone*	260	1.24	934	934	+	+	+	654	653	RI, MS, STD
137	3-Pentanone*	272	1.27	921	921	+	+	+	668	685	RI, MS, Tent.
138	3-Penten-2-one*	332	1.52	709	891	-	-	+	712	721	RI, MS, Tent.
139	2-Methyl-3-pentanone	348	1.44	935	935	+	+	+	730	748	RI, MS, Tent.
140	3-Hydroxy-3-methyl-2-butanone ⁱ	384	1.35	729	859	-	-	+	752	778	RI, MS, Tent.
141	4-Methyl-3-penten-2-one	436	1.68	702	761	-	+	-	784	792	RI, MS, Tent.
142	3-Heptanone	652	1.92	784	834	-	+	+	881	884	RI, MS, Tent.
143	2-Heptanone	664	1.94	921	921	+	+	+	886	884	RI, MS, Tent.
144	6-Methyl-5-hepten-2-one	936	2.14	793	793	+	-	+	982	984	RI, MS, Tent.
145	2-Octanone*	948	2.14	755	789	+	-	-	986	985	RI, MS, Tent.
146	2,2,6-Trimethyl cyclohexanone*	1080	2.34	717	764	-	+	+	1031	1036	RI, MS, Tent.
147	Acetophenone*	1168	2.64	967	967	+	+	+	1061	1060	RI, MS, STD
148	2-Nonanone*	1252	2.27	903	903	+	-	+	1089	1090	RI, MS, Tent.
149	4-Acetyl-1-methylcyclohexene	1368	2.60	722	726	+	+	+	1129	1158 [33]	RI, MS, Tent.
150	2-Hydroxyacetophenone	1448	2.83	773	813	+	-	-	1156	1152 [34]	RI, MS, Tent.
151	4-Methylacetophenone	1484	2.79	802	816	+	+	-	1168	1179	RI, MS, Tent.
152	2,4-Dimethylacetophenone ⁱ	1736	2.81	850	865	+	+	+	1257	1230	RI, MS, Tent.
153	2,6-Dimethoxybenzoquinone	2484	3.76	922	922	+	+	+	1551		MS, Tent.
154	Benzophenone	2648	3.32	724	834	-	+	-	1623	1621	RI, MS, Tent.
155	2,3-Dimethyl-1,4-naphthalenedione	2652	3.76	852	856	+	+	+	1624		MS, Tent.
156	Acetosyringone	2860	3.51	711	758	-	+	-	1719	1741	RI, MS, STD
Acids											
157	Hexanoic acid*	916	1.89	602	639	+	-	-	971	970	RI, MS, STD
158	n-Hexadecanoic acid ^k	3340	2.75	842	867	+	+	+	1922	1925	RI, MS, Tent.
159	Octadecanoic acid ^k	3712	2.91	856	857	+	+	-	2080	2075	RI, MS, Tent.
Acetals											
160	2-Methyl-1,3-Dioxolane ^j	244	1.12	748	789	+	-	-	636	627	RI, MS, Tent.
161	1,1-Diethoxy ethane*	316	1.27	945	945	+	+	+	710	715	RI, MS, Tent.
162	2,4,5-Trimethyl-1,3-dioxolane*	352	1.41	910	910	+	+	+	732	745 [35]	RI, MS, Tent.

Analysis of South African wines using SPE-GC × GC-TOFMS

163	Propane, 1-(1-ethoxyethoxy)-	484	1.53	882	882	+	-	-	809	805	RI, MS, Tent.
164	1,1-Diethoxy-2-methylpropane (Propane, 1,1-diethoxy-2-methyl-)*	576	1.64	802	802	+	-	+	849	865	RI, MS, Tent.
165	Butane, 1-(1-ethoxyethoxy)-	612	1.64	762	762	+	-	-	864		MS, Tent.
166	1,1-Diethoxypentane (Pentane, 1,1-diethoxy-)*	836	1.83	725	804	+	+	+	948		MS, Tent.
167	1,1-Diethoxy-2-methylbutane (Butane, 1,1-diethoxy-3-methyl-)*	840	1.82	796	809	-	+	-	949	952	RI, MS, Tent.
168	1-(1-Ethoxyethoxy)pentane (Pentane, 1-(1-ethoxyethoxy)-)*	896	1.85	838	852	+	-	+	968	970 [36]	RI, MS, Tent.
169	Benzene, (2,2-diethoxyethyl)-	1896	2.50	818	819	+	+	+	1316		MS, Tent.
Furans and Lactones											
170	Furan-2-carbaldehyd (Furfural)*	512	1.96	851	863	+	+	+	821	818	RI, MS, STD
171	2-Acetyl furan (Acetylfuran)*	712	2.21	847	853	-	+	-	905	907	RI, MS, Tent.
172	2(3H)-Furanone, dihydro- (γ -Butyrolactone)*	716	2.46	951	959	+	+	+	907	908	RI, MS, Tent.
173	2-tert-Butyl-4-methylfuran [†]	736	1.78	785	897	-	-	+	914	915	RI, MS, Tent.
174	Methyl 2-furoate	900	2.27	800	854	+	+	+	969	980	RI, MS, Tent.
175	2-Pentylfuran	948	2.02	879	879	+	-	-	986	987	RI, MS, Tent.
176	1-(2-Furanyl)-1-propanone	996	2.39	805	827	+	+	-	1002	1005	RI, MS, Tent.
177	Ethyl 2-furoate*	1132	2.34	925	925	+	+	+	1048	1047	RI, MS, Tent.
178	6-Hexanolactone	1392	2.99	894	900	+	+	+	1137		MS, Tent.
179	γ -Heptanolactone	1424	2.92	789	801	+	+	+	1148	1159	RI, MS, Tent.
180	2,3-Dihydrobenzofuran (Coumaran)	1616	2.52	822	822	+	-	-	1214	1224	RI, MS, Tent.
181	γ -Octalactone	1716	3.02	858	859	+	+	+	1250	1255	RI, MS, Tent.
182	β -Octalactone	1788	3.10	890	890	+	+	+	1276	1283	RI, MS, Tent.
183	<i>trans</i> -Methyl- γ -octalactone (<i>trans</i> -Oak lactone)	1808	2.97	879	880	+	+	-	1283	1292 [32]	RI, MS, STD
184	Ethyl 5-oxotetrahydro-2-furancarboxylate	1828	3.03	884	884	+	+	+	1291		MS, Tent.
185	<i>cis</i> -Methyl- γ -octalactone (<i>cis</i> -Oak lactone)	1896	3.01	906	907	+	+	-	1316	1325 [32]	RI, MS, STD
186	Phthalolactone	1952	3.48	936	936	+	+	+	1337		MS, Tent.
187	γ -Nonalactone	1996	3.05	895	895	+	+	+	1354	1354	RI, MS, STD
188	5-Methylphthalide	2124	3.49	890	891	+	+	+	1402	1385	RI, MS, Tent.
189	1,2-Benzopyrone (Cumarin)	2184	3.68	838	844	+	+	+	1427	1428	RI, MS, Tent.
190	γ -Decalactone	2264	3.09	730	823	+	+	+	1459	1467	RI, MS, STD
191	δ -Decalactone	2328	3.18	940	940	+	+	+	1485	1490	RI, MS, Tent.
192	Dihydroactinidiolide	2412	3.59	889	911	+	+	+	1521	1532 [25]	RI, MS, Tent.
Sulfur compounds											
193	Methyl thiolacetate*	276	1.29	877	877	-	+	-	672	701	RI, MS, Tent.
194	Ethyl thiolacetate*	384	1.55	862	862	-	+	-	752	756	RI, MS, Tent.

Analysis of South African wines using SPE-GC × GC-TOFMS

195	3-(Methylthio) propanal (Methional)	704	2.26	759	761	+	+	+	903	905	RI, MS, Tent.
196	3-(Methylthio)-1-propanol (Methionol)	912	2.26	713	732	-	-	+	974	977	RI, MS, Tent.
197	2-Methyltetrahydrothiophen-3-one	944	2.47	882	882	+	+	+	984	990	RI, MS, Tent.
198	2-Thiophenaldehyde	972	2.61	916	917	-	+	+	994	1003	RI, MS, Tent.
199	Ethyl methylthiopropionate	1276	2.37	856	860	+	+	+	1097	1098	RI, MS, Tent.
200	5-Methyl-2-thiophenecarboxaldehyde	1332	2.83	826	836	+	-	-	1116	1116	RI, MS, Tent.
201	Benzothiazole	1636	2.92	935	935	+	+	+	1221	1220	RI, MS, Tent.
Nitrogen-containing compounds											
202	Pyrrole-2-carboxaldehyde, 1-methyl-	984	2.53	721	843	+	-	-	998	1022	RI, MS, Tent.
203	2-Phenylethylamine ⁱ	1328	3.02	800	879	-	+	-	1115	1111	RI, MS, Tent.
204	3-Pyridinecarboxylic acid, ethyl ester (Ethyl nicotine)	1608	2.69	872	883	+	+	+	1211	1218	RI, MS, Tent.
205	Quinoline	1668	2.98	843	868	+	+	+	1233	1233	RI, MS, STD
206	Isoquinoline	1728	3.21	828	863	+	-	-	1254	1255	RI, MS, Tent.
207	Indole	1816	2.92	927	947	+	+	+	1289	1290	RI, MS, Tent.
208	Acetamide, N-(2-phenylethyl)-	2356	3.29	907	907	+	+	+	1497	1492	RI, MS, Tent.
209	Indole-3-ethanol (Tryptophol)	2912	3.72	924	924	+	+	+	1743		MS, Tent.
Terpens											
210	α-Thujene	776	1.94	834	866	-	+	+	927	928	RI, MS, Tent.
211	1S-α-Pinene	780	1.86	919	930	+	+	+	929	937	RI, MS, Tent.
212	Isocumene*	836	2.04	881	902	+	+	+	948	958	RI, MS, Tent.
213	β-Pinene	908	1.96	771	775	-	+	+	972	971	RI, MS, Tent.
214	Sabinene	948	1.87	776	780	+	-	-	986	986	RI, MS, Tent.
215	β-Myrcene	948	1.95	736	756	+	+	-	986	987	RI, MS, Tent.
216	3-Carene	1000	2.07	819	833	+	+	+	1003	1004	RI, MS, Tent.
217	<i>o</i> -Cymene*	1048	2.20	799	861	+	-	+	1020	1021	RI, MS, Tent.
218	Limonene*	1064	2.18	866	874	+	+	+	1025	1026	RI, MS, STD
219	β-Phellandrene	1068	2.17	745	797	+	-	+	1027	1026	RI, MS, Tent.
220	Eucalyptol*	1072	2.15	875	875	-	+	+	1028	1029	RI, MS, Tent.
221	β-Ocimene, (E)-	1120	2.07	738	772	+	+	-	1044	1044	RI, MS, Tent.
222	3,7-Dimethyl-2,6-octadien-1-ol	1120	2.08	729	739	-	-	+	1044	1044	RI, MS, Tent.
223	Dehydro-p-cymene	1228	2.36	726	820	+	+	+	1081	1080	RI, MS, Tent.
224	5-Ethenyltetrahydro-α,α,5-trimethyl- <i>cis</i> -2-furanmethanol (Linalool oxide, (Z)-)*	1240	2.23	752	760	+	+	+	1085	1087	RI, MS, STD
225	2,6,6-Trimethyl-bicyclo[3.1.1]heptan-3-one	1244	2.14	736	765	+	-	+	1086	1109	RI, MS, Tent.
226	Linalool*	1280	2.18	881	889	+	+	+	1099	1100	RI, MS, STD

Analysis of South African wines using SPE-GC × GC-TOFMS

227	3,5,5-Trimethyl-2-cyclohexen-1-one (Isophoron)*	1344	2.71	934	934	+	+	+	1120	1118	RI, MS, Tent.
228	α-Campholenal	1356	2.53	718	789	+	-	-	1125	1126 [37]	RI, MS, Tent.
229	4-Oxoisophorone	1404	2.80	904	904	+	+	+	1141	1143	RI, MS, Tent.
230	p-Cymen-8-ol	1548	2.51	902	902	-	+	-	1190	1188	RI, MS, Tent.
231	α,α,4-Trimethyl-3-cyclohexene-1-methanol, (α-Terpineol)*	1564	2.54	884	892	+	+	+	1195	1197	RI, MS, STD
232	7-Methyl-3-methylene-6-octen-1-ol	1620	2.34	825	844	+	+	+	1215		MS, Tent.
233	cis-Carveol	1624	2.58	808	813	+	+	-	1217	1215	RI, MS, Tent.
234	β-Cyclocitral	1624	2.65	769	786	+	-	+	1217	1219	RI, MS, Tent.
235	β-Citronellol	1648	2.30	872	872	+	+	+	1225	1226	RI, MS, STD
236	Isogeraniol	1656	2.36	762	769	+	-	-	1228	1254 [33]	RI, MS, Tent.
237	Citronellyl formate	1668	2.39	700	749	+	+	-	1233	1249	RI, MS, Tent.
238	2,6-Dimethyl-1,7-octadiene-3,6-diol	1700	2.32	747	767	+	-	-	1244	1265	RI, MS, Tent.
239	Nerol	1712	2.37	897	897	+	+	+	1249	1249	RI, MS, STD
240	Cuminol	1724	2.64	709	724	-	-	+	1253	1270	RI, MS, Tent.
241	4-Terpineol acetate*	1732	2.52	871	871	+	+	+	1256	1282	RI, MS, STD
242	2-Buten-1-one, 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-, (E)- (β-Damascenone)*	2052	2.63	790	790	+	+	+	1375	1376	RI, MS, Tent.
243	2-Propenal, 3-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	2096	2.70	738	742	+	+	+	1392		MS, Tent.
244	1,1,6-Trimethyl-1,2-dihydronaphthalene, (TDN)*	2120	2.78	715	776	+	-	-	1401	1354	RI, MS, Tent.
245	trans-β-Ionone	2228	2.50	717	719	-	-	+	1445	1442	RI, MS, Tent.
246	β-Farnesene	2244	2.33	732	783	+	+	-	1451	1450	RI, MS, Tent.
247	(Z,E)-α-Farnesene	2336	2.38	805	837	-	+	-	1489	1491	RI, MS, Tent.
248	cis,trans-Nerolidol	2500	2.53	937	937	+	+	+	1558	1561	RI, MS, Tent.
249	3-Hydroxy-β-damascone [†]	2608	3.06	789	821	+	+	+	1605	1591	RI, MS, Tent.
250	3-Oxo-α-ionol	2672	3.29	829	829	+	+	+	1633	1632	RI, MS, Tent.
251	3-Oxo-7,8-dihydro-α-ionone	2732	3.54	740	768	+	-	+	1660		MS, Tent.
252	α-Bisabolol	2784	2.88	778	812	-	+	-	1684	1662 [33]	RI, MS, Tent.
253	4-Oxo-2,3-dehydro-β-ionol	2796	3.33	751	762	-	-	+	1689		MS, Tent.
254	3-Oxo-7,8-dihydro-α-ionol (Blumenol C)	2800	3.41	843	849	+	+	+	1691	1713	RI, MS, Tent.
255	Nerolidyl acetate	2804	2.76	759	769	-	+	+	1693	1715	RI, MS, Tent.
256	4-Oxo-7,8-dihydro-β-ionol	2840	3.33	841	841	+	-	+	1709		MS, Tent.
257	(2E,6E)-Farnesol	2848	2.71	801	803	+	+	+	1713	1717	RI, MS, STD
Volatile Phenols											
258	Phenol	916	2.06	669	768	-	+	+	975	977	RI, MS, STD
259	o-Cresol	1160	2.17	956	956	+	+	+	1058	1055	RI, MS, STD

Analysis of South African wines using SPE-GC × GC-TOFMS

260	<i>p</i> -Cresol	1224	2.21	926	926	+	+	+	1080	1080	RI, MS, STD
261	Guaiacol	1228	2.51	926	926	+	+	+	1081	1085	RI, MS, STD
262	2,6-Dimethyl phenol	1296	2.48	579	654	+	+	+	1105	1105	RI, MS, STD
263	2,3-Dimethyl phenol	1444	2.34	833	838	+	+	+	1154	1149	RI, MS, STD
264	4-Ethyl phenol	1464	2.40	946	946	+	-	-	1161	1161	RI, MS, STD
265	4-Methyl guaiacol	1540	2.61	741	777	+	+	+	1187	1185	RI, MS, STD
266	4-Ethyl guaiacol	1768	2.70	675	752	+	-	+	1270	1270	RI, MS, STD
267	4-Vinyl guaiacol	1868	2.78	795	829	+	-	-	1305	1308	RI, MS, Tent.
268	2,6-Dimethoxy phenol	1964	2.88	970	970	+	+	+	1342	1345	RI, MS, STD
269	Eugenol	1980	2.77	863	871	+	+	+	1348	1348	RI, MS, STD
270	3,5-dimethoxy phenol	2272	2.97	771	793	-	-	+	1463		MS, Tent.
271	2,4-Bis(1,1-dimethylethyl) phenol*	2372	2.49	912	912	+	+	+	1503	1519	RI, MS, Tent.
272	Methoxy eugenol	2576	3.00	817	827	+	+	+	1591	1609	RI, MS, Tent.
Pyrans											
273	2H-Pyran, 2-ethoxytetrahydro-*	724	1.88	816	851	+	+	+	910	920 [25]	RI, MS, Tent.
274	2H-Pyran, 3,6-dihydro-4-methyl-2-(2-methyl-1-propenyl)-	1432	2.30	703	706	-	+	-	1150	1153	RI, MS, Tent.
275	2H-Pyran-2-one, 5,6-dihydro-6-pentyl- ⁱ	2284	3.29	718	823	+	+	+	1468	1499	RI, MS, Tent.
276	3,4-Dihydro-8-hydroxy-3-methyl-iso-coumarin (Mellein)	2448	3.66	909	934	+	+	+	1536	1514	RI, MS, Tent.

^a RT1: Retention time on the primary column, ^b RT2: retention time on the secondary column, ^c Similarity: spectra match value on forward reading, ^d Reverse: spectra match value on backward reading, ^e P1: Pinotage sample 1, P2: Pinotage sample 2, ^e CS1: Cabernet Sauvignon wine sample 1, ^f LRT_{cal.}: Calculated linear retention indices, ^g LRI_{lit.}: Linear retention indices from literature obtained from the NIST 2005 library unless specified otherwise, ^h Identification criteria (RI, MS, STD = identification based on retention indices, mass spectra and retention time of authentic standards; MS, Tent. = tentative identification based on MS; RI, MS, Tent. = tentative identification based on RI and MS). ⁱ Literature LRI corresponds to a 100% polydimethylsiloxane (PDMS) or equivalent stationary phase. ^j Literature value of LRI was obtained from analysis under isothermal conditions. ^k Extrapolated LRI. *Compounds also reported in our previous study [23]. +: Detected and -: Not detected.

Table 9.1. lists the compounds identified grouped according to different chemical classes. In general, the compounds identified in the current study showed higher match factors compared to the previous work [23], which can be ascribed to removal of co-eluting compounds using the SPE procedure. In particular, the number of polar major wine volatiles (e.g. acids, alcohols and esters) identified in this work was dramatically reduced by their removal during the rinsing step. In addition, polar compounds were often characterized by tailing peaks and even resulted in wraparound in our previous work. This was not observed in the current study (**Figure 9.1.**) in part due to the choice of a less polar column in the second dimension and a secondary oven. This resulted in less co-elution due to tailing phenomena and improved peak identification. The SPE method employed therefore proved to be particularly suitable for the analysis of the more apolar, high-boiling wine volatiles.

Table 9.2. compares the number of compounds identified in each class using HS-SPME and SPE methods, respectively. More compounds than previously reported in wine and related matrices were detected in the current study: in particular significantly more terpenes, volatile phenols as well as furans and lactones. It is apparent that the SPE method generally revealed more apolar and high-boiling (based on RT1) compounds. This emphasizes the complementary nature of the extraction techniques, with HS-SPME better suited for more volatile analytes, whereas the selective nature of SPE is best exploited for trace-level analysis of semi-volatiles. This indicates the lack of a universal sample preparation technique for wine volatile analysis. A more detailed discussion of each chemical class, with the emphasis on influential flavor compounds, is outlined below.

Table 9.2. Comparison of volatile compounds detected in South African wines using HS-SPME-GC × GC-TOFMS [23] and SPE-GC × GC-TOFMS.

Class	Number of compounds		
	HS-SPME ^a	SPE ^b	Common ^c
Esters	67	80	42
Alcohols	40	34	19
Aldehydes	22	20	7
Ketones	16	22	7
Acids	8	3	1
Acetals	6	10	6
Furans and lactones	8	23	4
Sulfur compounds	5	9	2
Nitrogen-containing compounds	6	8	0
Terpenes	24	48	11
Volatile phenols	2	15	1
Pyrans	2	4	1

^aData from [23]. ^bCurrent method. ^cCompounds common to both methods.

Esters. Numerically, the largest group of flavor compounds in wine consists of esters. Esters are usually present in especially young wines at concentrations above their sensory thresholds and are therefore considered as important contributors to young wine flavor. Their levels in wine depend on sugar content and fermentation conditions (yeast, temperature, pH, aeration, etc.) [4,6]. Generally esters contribute to the desirable fermentation bouquet of young wines. As the wine ages, the fruity and fresh note is likely to disappear as the levels of both ethyl and acetate esters decrease. On the other hand, the concentrations of di-protic ethyl esters increase with time due to esterification of their corresponding acids [1].

Usually, the pleasant smell associated with esters is due to the collective effect of many esters. However, a specific aroma property can also be associated with a specific ester. For instance, ethyl butyrate (**10**), isoamyl acetate (**17**), ethyl hexanoate (**25**), hexyl acetate (**27**), ethyl octanoate (**44**) and 2-phenylethyl acetate (**49**) are reported as important individual contributors to the fruity note of wine [11,38,39]. Isoamyl acetate in particular has been shown to be an influential compound determining the fermentation aroma of Pinotage wines [38].

Among the 80 esters identified, 42 were also reported in the previous work [23]. These are mainly the low-boiling esters. Of the 38 esters only identified in the current work, most were relatively high-boiling aromatic and higher aliphatic esters. Other

esters only identified in this work include low-boiling (**4**, **5**, **28**, **33** and **39**) and medium to high-boiling (**55**, **56**, **63**, **66**, **68** and **80**) methyl esters. These methyl esters may be formed in wine from their corresponding acids via methylation [40]. Among the aromatic esters identified in this work, ethyl cinnamate (**64**) was reported as key aroma compound in red wines [41]. Additionally, compounds which are regarded as wood extractable such as methyl- and ethyl-vanillate (**66** and **70**) were tentatively identified. Jarauta et al. [3] reported the presence of these compounds in wine and the likely increase of their levels when stored in stainless steel, possibly due to the hydrolysis of glycosidic precursors.

Alcohols. Alcohols are produced by yeast from carbohydrates or amino acids during fermentation and are important contributors to the wine flavor. The alcohol content of wines might increase slightly during ageing due to hydrolysis of esters. A total of 34 alcohols were identified, less than reported previously using HS-SPME [23]. Especially the polar low molecular weight alcohols, often present at high levels, were not detected here. These compounds were removed during the rinsing step of the SPE procedure.

Isoamyl alcohol (**83**) and 2-phenylethyl alcohol (**105**) are important contributors to the floral/rose aroma of young wines, as they mostly exist at levels above their odor thresholds [1]. The C₆- alcohols 1-hexanol (herbaceous odor, **93**), 3-hexen-1-ol, (*E*)- (green-floral odor, **90**) and 3-hexen-1-ol, (*Z*)- (green odor, **91**) are essential contributors to wine flavor and are formed from lipoxygenases acting on linoleic or linolenic acid in crushed berry tissue [4]. In fact according to Oliveira et al. [42], the ratio between the latter two isomers may be used to trace the origin of wine. Other important odorous alcohols identified in the current study are 3-methyl-1-pentanol (roasty odor, **89**) and 1-octene-3-ol (noble rot odor, **97**) [4]. Some alcohols (**85**, **104** and **113**) were only identified in the Pinotage samples, whereas, **91**, **99** and **112** were detected only in the Cabernet Sauvignon sample.

Carbonyls. Although a similar number (38 and 42 for HS-SPME and SPE, respectively) of carbonyl compounds were detected using both methods (**Table 9.2.**), only 14 were common to both. Most of these were relatively low-boiling compounds. Carbonyls include both aldehydes and ketones, and the current study reveals a total of

20 and 22 from each class. Aldehydes can be classified as either primary aroma compounds due to their existence in the grape, or secondary aroma compounds formed as by-products during fermentation. C₆- aldehydes like hexanal (**118**) are formed from fatty acid precursors during grape crushing [2,4,43]. Aldehydes can contribute different flavors to wine, including nutty, bruised apples, herbaceous, grassy, green, fatty, fruity and pungent aromas [2,4,43]. Wine aldehyde composition may fluctuate over time as a result of oxidation/reduction reactions. For instance, higher aldehydes such as 2-methyl propanal (**115**) and 2-methyl butanal (**116**) serve as intermediates for the formation of fusel alcohols. Aldehydes also indirectly affect the color of wine due to their reaction with anthocyanins [2,43]. The presence of cinnamaldehyde (**126**) is ascribed to ageing in oak barrels [4]. Benzeneacetaldehyde (**122**), which is characterized by a low odor threshold of 2 µg/L, contributes rose odor to wine aroma [4]. This compound is present neither in grape nor in a freshly fermented wine and is produced during ageing. It can also be formed in the wine via oxidative degradation of phenylalanine as well as by direct oxidation of 2-phenylethyl alcohol (**105**) [3]. In addition, some aromatic aldehydes including vanillin (**131**), methyl vanillin (**133**) and syringaldehyde (**134**) were also detected. The former is known to be an important contributor of distinctive vanilla odor to wine [6].

Ketones are formed during fermentation. Ferreira and de Pinho [12] indicated 2,2,6-trimethyl-cyclohexanone (**146**) as being responsible for the rock-rose-like aroma of port wines. This compound is believed to be formed in wine from thermal degradation of β-carotene, and its level depends on the pH of the wine (lower pH favors its formation) and increases during ageing. The rest of the ketones listed in **Table 9.1**. are not considered to contribute significantly to wine flavor [4].

Acids. In the previous study, volatile acids such as acetic acid showed tailing and wraparound. In this work, most polar acids were removed by rinsing with a basic aqueous solution. Nevertheless, the three major acids hexanoic (**157**), hexadecanoic (**158**) and octadecanoic acid (**159**) were detected. These are commonly present in all wines. Neither **158** nor **159** were detected in the previous work as these are high boiling compounds. Hexanoic acid (**157**) is reported to exist from trace levels to mg/L quantities and can contribute a sweet aroma to wine [4,6].

Acetals. In the current study 10 acetals were detected, six of which (**161**, **162**, **164**, **166**, **167** and **168**) were also observed in the previous report [23]. Acetals are formed from the reaction of aldehydes with ethanol during fermentation and ageing [6,44]. Their levels depend on the ethanol concentration and pH of the wine [2,6]. Acetals generally contribute less to wine aroma than their corresponding alcohols and aldehydes [2], but are important flavor compounds in sherry [4,6]. The levels of acetals are expected to be higher in fortified wines compared to table wines due to higher alcohol content of the former. 1,1-Diethoxy ethane (**161**) is one of the important acetals that has been reported to contribute to wine aroma [4,6].

Furans and lactones. The profiles of selected lactones for a Pinotage and Cabernet Sauvignon wine are presented in the extracted ion chromatograms in **Figure 9.2**. In the current study a total of 23 compounds classified under this group (**Table 9.1**) were detected. This represents a three-fold increase compared to the HS-SPME method [23], mainly with respect to lactones. Only γ -butyrolactone (**172**) was detected in both studies. From the sensory point of view, the oak lactones (whiskey lactones): *trans*- (**183**) and *cis*-methyl- γ -octalactone (**185**), are responsible for oak flavor of wine. These two isomers were identified only in the two Pinotage wines. They are released to wine from the wood and their concentration increases with storage time. Increased toasting of the barrel can diminish the fresh oak aromas generally attributed to these compounds. The *cis*- isomer is always present at higher levels (**Figure 9.2**.) and is the most important isomer as far as wine aroma is concerned, since it has a lower perception threshold (92 $\mu\text{g/L}$) compared to the *trans*- isomer (460 $\mu\text{g/L}$) [45]. The ratio of these two isomers can be used to distinguish wines aged in different wood barrels. Excess in concentration of oak lactones can also lead to undesired aroma (resinous, varnish and coconut-like flavors) [45,46].

Another group of lactones of relevance to the sensory properties, particularly of wines aged in oak wood, are the γ -lactones (**Figure 9.2**): octalactone (**181**, odor threshold 7 $\mu\text{g/L}$ in water), nonalactone (**187**, odor threshold 30 $\mu\text{g/L}$ in white wine) and decalactone (**190**, odor threshold 10 $\mu\text{g/L}$ in model wine) [47]. These compounds contribute coconut and peach-like flavor and their levels also increase with storage time. They are formed via cyclisation of the corresponding γ -hydroxycarboxylic acids

[1]. γ -Nonalactone (**187**) was also indicated as degradation product of lignin [45]. Although γ -lactones are more potent than the corresponding δ -lactones, both are believed to contribute significantly to the wine flavor. For instance, δ -decalactone (**191**) contributes peachy flavor [47]. γ -Butyrolactone (**172**), described as contributing a sweet roasted character, is also released from wood [3]. This compound might also be formed during fermentation from diprotic acids like glutamic and succinic acid [45].

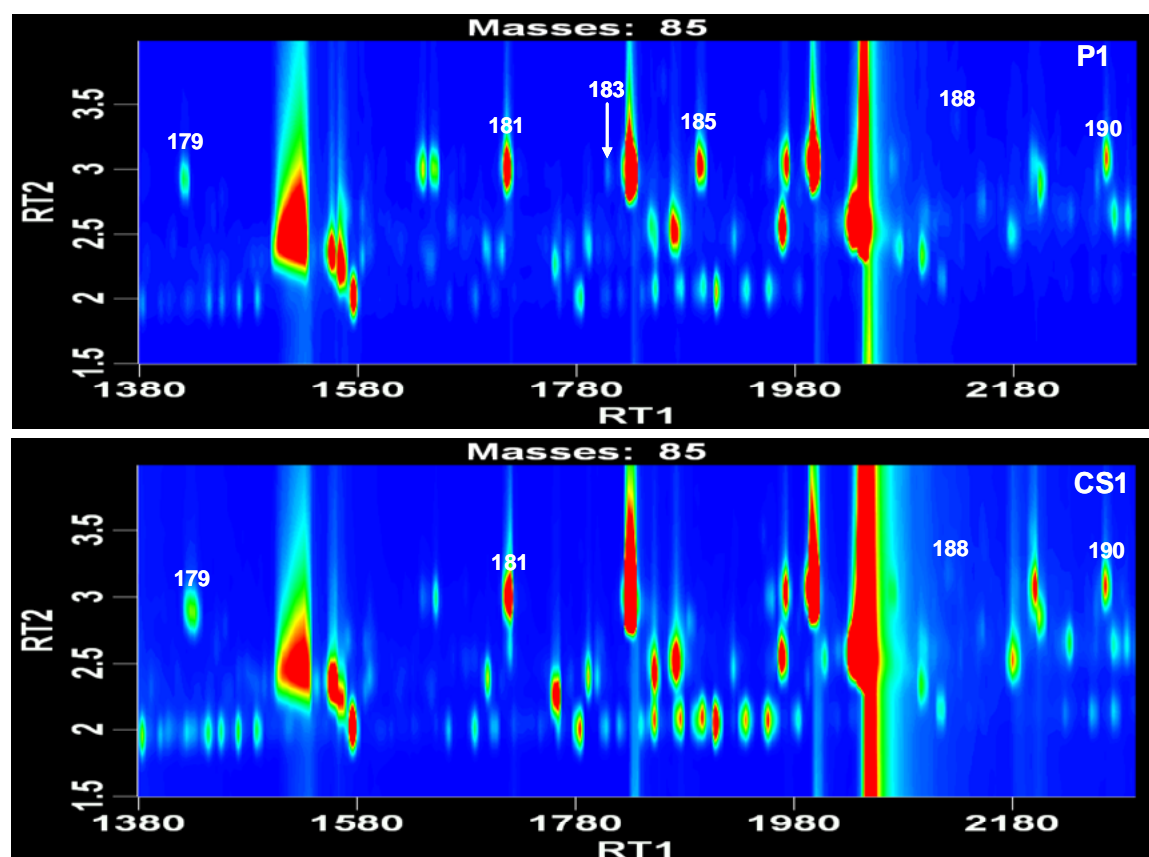


Figure 9.2. Comparison of extracted ion contour plots for Pinotage (P1) and Cabernet Sauvignon (CS1) wines, illustrating the profiles of selected lactones. Compound numbers correspond to **Table 9.1**.

Only three (**170**, **171** and **177**) of the furans identified here were reported in the previous work. The major furan compounds were furfural (**170**), 1-(2-furanyl)-1-propanone (**176**), methyl- (**174**) and ethyl- (**177**) 2-furate, as well as ethyl 5-oxotetrahydro-2-furancarboxylate (**184**). Furfural is formed through degradation of carbohydrates from wood via the Maillard reaction. The amount of this compound in wine also depends on the age of the barrel and the degree of wood toasting. The level of furfural is expected to increase during ageing and can be used as an indication for the age of the wine [1]. Acetylfuran (**171**), which has a sweet balsamic-cinnamic note,

was also identified in one of the Pinotage wines. This compound can be produced in a similar fashion as that outlined for furfural [48].

Sulfur compounds. Sulfur containing compounds can be grouped as ‘light’ and ‘heavy’ depending on their size. The compounds reported here are mainly classified under the latter category, as opposed to the previous study [23], where mostly light sulfur compounds were identified (only methyl thiolacetate (**193**) and ethyl thiolacetate (**194**) were detected in the previous work). The contribution of sulfur compounds to wine aroma may be negative or positive.

Sulfur compounds in wine include a wide range of functionalities. Thiophene aldehydes such as 2-thiophenaldehyde (thiofurfural, **198**) and 5-methyl-2-thiophenecarboxaldehyde (**200**) are formed in wine by the reaction of short chain aldehydes or furfural with hydrogen sulfide from amino acids [2]. Thiofurfural (**198**) contributes roasted, coffee-like notes to wine flavor. The oxidation of 5-methyl-2-thiophenecarboxaldehyde (**200**) can lead to the formation of 2-methyltetrahydrothiophen-3-one (**197**). This compound has an odor threshold of 90 µg/L and contributes a gas aroma to wine. Benzothiazole (**201**) has an odor threshold of 50 µg/L and is characterized by a rubber aroma. This compound has been reported to cause reductive defects in wine at 11 µg/L (below its threshold [6]).

Methionol (**196**), which was identified only in the Cabernet Sauvignon sample, has a perception threshold of 1.20 mg/L [6]. This compound is expected to exist at higher levels if the wine is fermented in oak barrels compared to stainless steel tanks [16]. Methionol is a yeast fermentation product produced from methionine via the Ehrlich reaction. The aldehydic form methional (**195**) is formed as an intermediate in this process [6]. Methional is a key odorant present in oxidized white wines, and is responsible for a cooked vegetable off-flavor [49]. The olfactory threshold of this compound in model wine is estimated to be about 0.5 µg/L; in oxidized wines methional can be present at significantly higher levels.

Nitrogen-containing compounds. Volatile nitrogen compounds encompass amines, acetamides (**208**), and heterocyclic compounds (**205**, **206**, **207**, and **209**). The latter group can be considered as important contributors to wine aroma. Other compounds

that can be classified under this group are pyrroles (**202**) and pyrazines. Due to their very low (ng/L) odor thresholds [6], methoxypyrazines are considered important odorants, especially for Sauvignon Blanc and Cabernet Sauvignon wines. The three principal methoxypyrazines ((2-Methoxy-3-isopropylpyrazine (IPMP), 2-Methoxy-3-sec-butylpyrazine (SBMP) and 2-Methoxy-3-isobutylpyrazine (IBMP)) were identified in a Pinotage wine in our previous study [23]. The same wine was not analyzed in the current study, so we were unable to confirm their presence. Methoxypyrazines were not detected in any of the three wines analysed here. N-(2-phenylethyl)-acetamide (**208**) is sourced from the corresponding amine (**203**) [50]. The two heterocyclic compounds indole (**207**) and tryptophol (**209**) are associated with off-flavors [51]. They are believed to be formed in wine as degradation products of L-tryptophan during the fermentation process. The former possesses a jasmine odor, and can have strong odor intensity in wine [51]. The concentration of tryptophol (**209**) was reported to increase in wine fermented in oak barrels [16].

Terpenes. In the current study a total of 48 terpene compounds were detected, including mono- and poly-terpene hydrocarbons, alcohols, carbonyls and esters, as well as C₁₃ norisoprenoids and C₁₅ sesquiterpenes. This is double the number of terpenoids detected in our previous work [23], with only 11 compounds common to both methods. Once again, this is largely due to the SPE method employed here, which is more suitable for the analysis of low-level apolar and high-boiling compounds such as terpenes. Terpenes are well-known varietal compounds of *Vitis vinifera* grapes [19,52], and their contribution to the wine aroma is significant. Most terpenes exist in grapes and are categorized as primary aroma compounds. Nevertheless, substantial evidence exists to show the formation of terpene-related compounds during fermentation, maturation and ageing. Carrau et al. [52] reported increasing concentrations of terpenes in wine during fermentation. Other authors [53,54] have also reported the release of terpenes from non-volatile glycosidic precursors during fermentation and ageing, as well as transformation of free terpenes through acid-catalyzed rearrangements as in the conversion of linalool (**226**) to α -terpeneol (**231**) and nerol (**239**). These three monoterpenols together with β -citronellol (**235**) are responsible for the characteristic floral aroma of wines and have low olfactory thresholds in the range of 50 – 400 μ g/L. Eucalyptol (**220**), a terpene

ether, is associated with an eucalyptus odor. This compound has an odor threshold of 1.3 µg/L and its concentration increases in the grape with ripening. Fariña et al. [55] recently proposed the formation of eucalyptol in red wine from limonene (**218**).

The C₁₃ norisoprenoids are thought to be responsible for complex wine flavors variously described as grassy, tea, lime, honey and pineapple. These compounds, similar to the monoterpenes, occur in grapes largely as bound glycosides and non-bound carotenoid precursors. Their aroma contribution is mainly determined by *trans*-β-damascenone (**242**), 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN, **244**) and *trans*-β-ionone (**245**). The latter has a very low perception threshold of 1.5 µg/L. Whereas damascenone and TDN were identified in our previous report [23], *trans*-β-ionone (**245**) was only identified in the current work in the Cabernet Sauvignon wine. This compound was absent in the Pinotage wines analysed in both studies. Other C₁₃ norisoprenoids such as 3-hydroxy-β-damascone (**249**), 3-oxo-α-ionol (**250**), 3-oxo-7,8-dihydro-α-ionone (**251**), 4-oxo-2,3-dehydro-β-ionol (**253**), 3-oxo-7,8-dihydro-α-ionol (Blumenol C, **254**), and 4-oxo-7,8-dihydro-β-ionol (**256**), as well as the C₁₅ sesquiterpene compounds (*Z,E*)-α-farnesene (**247**), *cis,trans*-nerolidol (**248**), α-bisabolol (**252**) and (2E,6E)-farnesol (**257**) are reported here for the first time in South African wines, and Pinotage wines in particular. A number of hydrocarbon monoterpenes detected here such as limonene (**218**), cymene (**217**), myrcene (**215**) as well as the C₁₅ sesquiterpenol farnesol (**257**), are reported to contribute a resin-like odor to wine [6,53].

It is noted that some of the terpenes were identified in only one of the cultivars. For example, β-myrcene (**215**), β-ocimene, (E)- (**221**), *cis*-carveol (**233**), citronellyl-formate (**237**), and β-farnesene (**246**) were detected only in the two Pinotage wines. On the other hand, 3,7-dimethyl-2,6-octadien-1-ol (**222**), *trans*-β-ionone (**245**), and 4-oxo-2,3-dehydro-β-ionol (**253**) were identified only in the Cabernet Sauvignon wine. Different terpene profiles between the samples analysed are highlighted in **Figure 9.3**. *p*-Cymen-8-ol (**230**), (*Z,E*)-α-farnesene (**247**), and α-bisabolol (**252**) were detected only in Pinotage P2. On the other hand, nerolidyl acetate (**255**) and 4-oxo-7,8-dihydro-β-ionol (**256**) were not detected in either of the Pinotage wines. Although terpenes are known varietal compounds [53], the observed variation could also be

related to diverse fermentation processes [52]. In the absence of the detailed history of the wines analysed here no clear conclusions can be drawn using these data. However, it is clear that SPE sample preparation in combination with GC × GC-TOFMS analysis represents a useful analytical methodology to study terpene profiles in different wines.

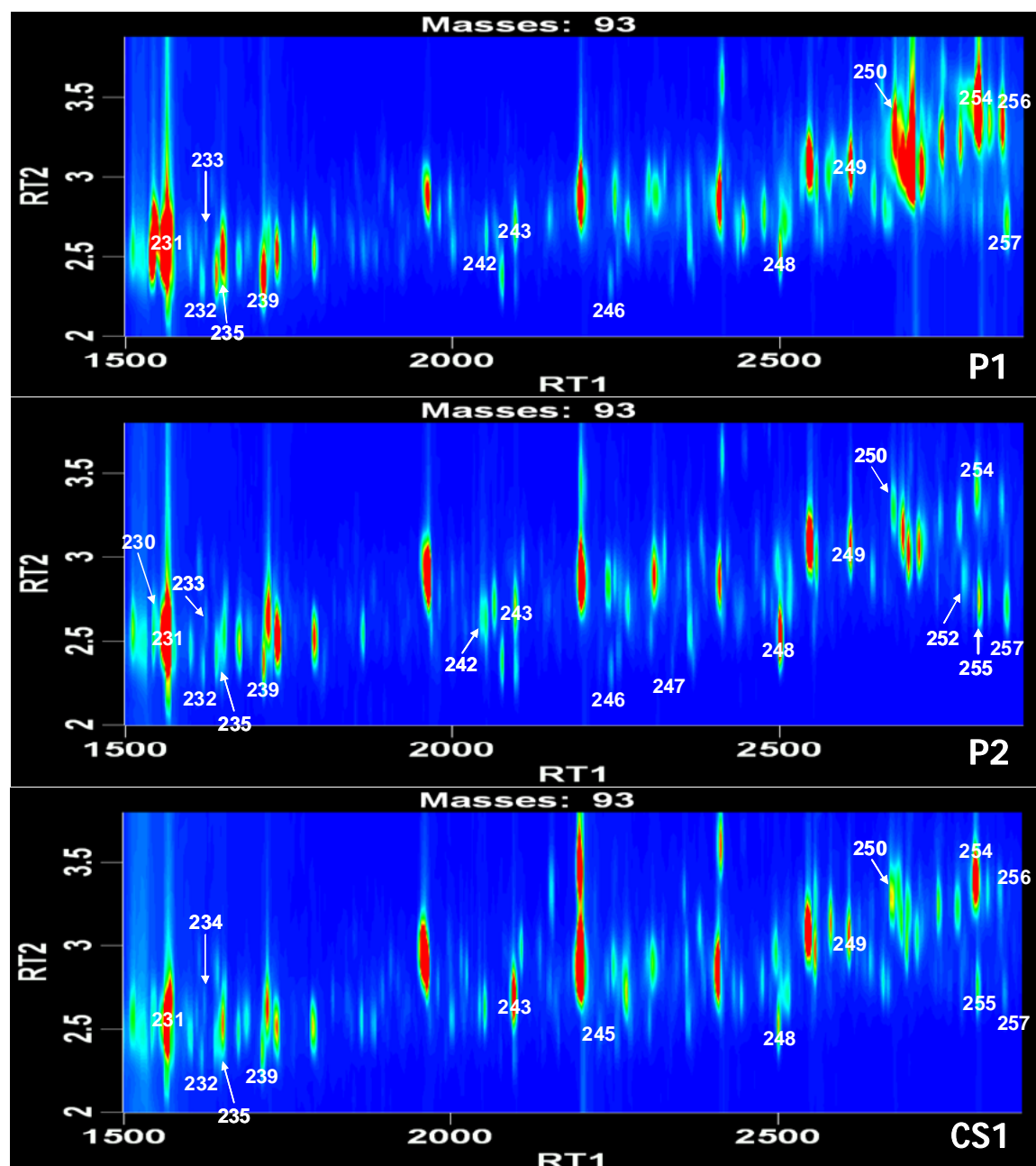


Figure 9.3. Extracted ion contour plots comparing terpene profiles for the three wines analysed (Pinotage 1 and 2 (P1 and P2) and Cabernet Sauvignon (CS1)).

Hydrocarbons. Saturated and unsaturated straight chain hydrocarbons are commonly present in the surface wax layer of grapes [50]. 26 Aliphatic, cyclic and aromatic

hydrocarbons have been identified. These compounds are not listed in **Table 9.1.** as their contribution to wine aroma is insignificant. Aromatic hydrocarbons have previously been detected in wine, where their presence was mainly associated with contamination arising from petroleum-derived products [56].

Volatile phenols. Of the 15 volatile phenols identified in the current study, 14 were not detected in the previous study [23]. This is presumably related to the limited volatility of these relatively polar compounds. The SPE method used here is clearly better suited for the determination of volatile phenols than HS-SPME [23]. The volatile phenols reported here (**Table 9.1.**) comprised different functional groups including alcohols, aldehydes, and ketones. The former group includes, phenol (**258**), *o*-cresol (**259**), *p*-cresol (**260**), guaiacol (**261**), 2,6-dimethyl phenol (**262**), 2,3-dimethyl phenol (**263**), 4-ethyl phenol (**264**), 4-methyl guaiacol (**265**), 4-ethyl guaiacol (**266**), 4-vinyl guaiacol (**267**), 2,6-dimethoxy phenol, (**268**), eugenol (**269**), 3,5-dimethoxy phenol (**270**), and methoxy eugenol (**272**). Most of these volatile phenols are believed to be sourced from wood lignins via different pathways including thermal degradation during wood toasting, extraction of lignin monomers, and ethanolysis of lignin. They are responsible for smoky, toasty, and burnt aroma in wine. The levels of volatile phenols can vary between white and red wines – for example, ethyl phenols exist at higher- and vinyl phenols at lower levels in red wines [6,45].

Ethyl and vinyl phenols are also related to aroma defects in wine. 4-Ethyl phenol (**264**, stable and sweaty saddles odor) and 4-vinyl phenol (medicinal and paint odor) are associated with *Brettanomyces* spoilage [6,45]. 4-Vinyl guaiacol (**267**, carnations odor) and 4-ethyl guaiacol (**266**, smoky and spicy odor) contribute to wine flavor at normal levels, but may also be associated with *Brettanomyces* spoilage. Below certain levels (140 and 620 µg/L for 4-ethyl guaiacol and 4-ethyl phenol, respectively), these two compounds can contribute positively to the aroma of red wines [6,45]. 4-Methyl guaiacol (**265**, smoky, toasted and ash) and eugenol (**269**, clove-like) are also important contributors to wine aroma. These compounds are characterized by spicy flavors, and together with compounds characterized by smoky flavor like guaiacol and substituted guaiacols (**261**, **265**, and **266**), their levels are expected to increase with toasting of wood. The present work serves to demonstrate that GC × GC is a powerful

separation method for the analysis of volatile phenols, although the choice of sample preparation technique is critical.

9.4. Conclusions

While GC × GC-TOFMS as analytical technique has previously been shown to provide significant benefits in terms of the high-resolution and sensitive analysis of wine volatiles, the usefulness of the technique does depend on the sample preparation technique for specific target analytes. In the present report, we have demonstrated that the use of SPE on reversed phase material offers several advantages. Primarily, polar volatiles may easily be removed from the sample matrix. More importantly, since many polar wine volatiles are present at relatively high concentrations, their removal prior to analysis allows the accurate determination of a wide variety of apolar higher-boiling impact odorants. In particular, this approach proved beneficial for the analysis of terpenes, volatile phenols, lactones, and sulfur containing compounds. In addition, considering that many of the reported compounds are typically present at very low levels (in the region of $\mu\text{g/L}$), this technique is sufficiently sensitive to allow their determination in wine. It is clear that despite the inherent advantages associated with GC × GC-TOFMS, the selection of sample preparation method plays an essential role in determining the types of compounds that may be analysed. Future work will focus on the application of both the developed HS-SPME- and SPE-GC × GC-TOFMS methods for the detailed characterization of volatile compounds in a small number of well-defined wines. Through careful selection of wine samples, it is hoped that the effects of different viticultural and enological factors on the volatile composition of South African wines may be ascertained.

9.5. References

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General conclusions

The aroma of wine is mainly determined by the content of volatile compounds. The purpose of this study was to shed lights on the chemical constituents associated with the flavor and aroma of South African wines.

Initial work focused on the use of gas chromatography for the analysis of wine volatiles. An analytical method which is reliable and easy to implement was developed and validated. This method was based on stir bar sorptive extraction (SBSE) in the headspace mode, and was useful for the extraction of both polar and non-polar compounds. Although SBSE utilizes polydimethylsiloxane (PDMS) extracting phase, which is non-polar, the extraction ability of the method for polar compounds, mainly acids, relies heavily on pH adjustment of the aqueous sample. The method was very sensitive as it proved capable of detecting compounds that are normally present at low levels (ng/L to $\mu\text{g/L}$) in wine. The method also demonstrated good reliability including linearity, reproducibility, recovery as well as limits of detection and quantitation. The detailed report is highlighted in chapter 5.

The developed SBSE method above was further improved by making important modifications (chapter 6). The main changes included reduction of the sample volume by half; reduction of extraction time from 4 hours to 1 hour and improving the model blank wine used for dilution of the sample prior to extraction. The first two changes impact on the improvement of the method from a cost benefit point of view. Following validation, this modified method showed good results in terms of recovery, reproducibility, linearity as well as limits of detection and quantitation. The method was extensively applied for the analysis of a large number of young Pinotage wines of vintages 2005 and 2006. Subjection of the quantitative measurements of these wines to chemometrical data analysis techniques revealed relevant information that can be used to characterize Pinotage wines. A clear distinction between Pinotage wines of the two vintages was achieved. Principal component analysis (PCA) indicated that the 2005 vintage wines were distinguished by their higher levels of wood-related compounds, whereas the 2006 vintage wines were characterized by higher levels of fermentation compounds. These two characteristics are highly related to the wine-making technologies used during production.

Next (chapter 7), a large number of young South African wines from various cultivars including white wines (Sauvignon Blanc and Chardonnay) and red wines (Shiraz, Cabernet Sauvignon and Merlot), all from vintage 2005, were analyzed using the modified SBSE method. The Pinotage wines of 2005 vintage were also included in this part of the study. ANOVA showed significant differences among various grape varieties and few regions of origin. In addition, significant differences were observed between the two white wine cultivars and among the four red wine cultivars. Once again, it was shown that the principal variations in the volatile composition were due to fermentation practices and wood ageing. Clear separation was observed between white and red wines using a PCA bi-plot. However, the striking part was the distinguishing of Pinotage wines from the other red cultivars, which led to further investigation using discriminant analysis (DA). During DA analysis, five compounds (ethyl butyrate, acetic acid, propanoic acid, *o*-cresol, and vanillin) were found to be potential predictors for the two white cultivars, with ethyl butyrate playing a very prominent role for the Chardonnay cultivar. DA also revealed the unique character of Pinotage wines in relation to the other red cultivars. The distinctive nature of Pinotage wines was mainly due to higher levels of isoamyl acetate and lower levels of isoamyl alcohol; the former being classified as a varietal compound for Pinotage expressed by a fruity (banana) aroma.

In order to further explore the volatile content of South African wines, comprehensive gas chromatographic analysis using two columns with opposite polarity was investigated (Chapters 8 and 9). This approach was used to improve the separation of large number of volatiles. The first approach was to apply solid phase micro-extraction (SPME) in the headspace mode in combination with comprehensive two-dimensional gas chromatography (GC \times GC) coupled to time-of-flight mass spectrometry (TOFMS). 206 Volatile compounds previously reported as components of wine and related matrices were identified (see chapter 8) in 9 Pinotage wines of vintage 2006. The large number of identified compounds could mainly be attributed to the high resolving power and large peak capacity of GC \times GC. The use of TOFMS also adds another dimension due to its identification power. Despite the remarkable potential of this method for fingerprinting, it was clear from the type of compounds identified that HS-SPME extracts mainly highly volatile compounds. As a result of the high levels of ethanol, acetic acid and acetaldehyde, these compounds were

dominant in the chromatograms, masking quite a large number of minor compounds. In addition, the method failed to present the high boiling compounds such as volatile phenols, lactones and other influential flavor compounds.

In order to overcome some of these problems, solid phase extraction (SPE) was used instead for the extraction of volatile and semi-volatile compounds from the wine matrix. SPE was chosen as it allows the elimination of lower boiling and highly polar compounds that exist in wine in large concentrations, such as ethanol and acetic acid, which were shown to hinder the proper identification of many compounds using SMPE. Due to its selective nature, SPE also provided cleaner extracts. The SPE-GC × GC-TOFMS combination allowed identification of 276 compounds previously reported as components of grape and grape-related beverages. These compounds include potential contributors to the wine flavor such as terpenes, volatile phenols, lactones, and sulfur containing compounds. Taking into account the level of most of the compounds reported here (mostly $\mu\text{g/L}$ levels and lower), the technique can be regarded as sensitive in terms of its detection ability. Many of the 276 compounds were only identified in this work, in particular the volatile phenols and lactones as well as most of the aromatic esters and the sesquiterpenes. This indicates the advantage of the SPE extraction method employed. This work is detailed in chapter 9.

The principal achievements of the research presented here include:

- New analytical methods based on SBSE in combination with GC suitable for the routine analysis of wine volatiles.
- Detailed investigation of volatile content in young South African wines using GC × GC-TOFMS.
- Quantitative results represent a significant contribution to our current knowledge of the volatile composition of young South African wines.
- Distinctive features of the volatile composition of the uniquely South African cultivar Pinotage have been highlighted.

Future work should extend the research of the current study in an inclusive manner for both young and old wines in order to understand the effect of ageing on wine aroma. It is also vital to share the information obtained in this manner with wine producers in order to improve wine-making procedures.

Selected tables

Selected tables

Table 1. Information of the wines analysed in this study: origin, source, year of production and code for each wine.

No.	Wine Code ^a	Region Code ^b	Producer Code ^c	No.	Wine Code ^a	Region Code ^b	Producer Code ^c	No.	Wine Code ^a	Region Code ^b	Producer Code ^c	No.	Wine Code ^a	Region Code ^b	Producer Code ^c
Young Red Wines from Vintage 2005															
PINOTAGE															
1	PI1	W	C33	34	PI34	S	C100	66	SH17	W	C46	99	SH50	RO	C56
2	PI2	W	C34	35	PI35	SW	C71	67	SH18	P	C1	100	SH51	RO	C59
3	PI3	W	C38	36	PI36	SW	C103	68	SH19	P	C105	101	SH52	RO	C84
4	PI4	W	C39	37	PI37	SW	C104	69	SH20	P	C2	102	SH53	RO	C61
5	PI5	W	C40	38	PI38	SW	C90	70	SH21	P	C3	103	SH54	RO	C62
6	PI6	W	C77	39	PI39	SW	C91	71	SH22	P	C4	104	SH55	RO	C109
7	PI7	W	C41	40	PI40	RO	C53	72	SH23	P	C5	105	SH56	RO	C110
8	PI8	W	C43	41	PI41	RO	C56	73	SH24	P	C7	106	SH57	OR	C86
9	PI9	W	C78	42	PI42	RO	C57	74	SH25	P	C73	107	SH58	OR	C101
10	PI10	W	C44	43	PI43	RO	C62	75	SH26	P	C8	108	SH59	OR	C69
11	PI11	W	C80	44	PI44	OR	C86	76	SH27	P	C106	109	SH60	OR	C70
12	PI12	W	C45	45	PI45	OR	C101	77	SH28	P	C10	110	SH61	OR	C111
13	PI13	W	C46	46	PI46	OR	C69	78	SH29	P	C74	111	SH62	SW	C71
14	PI14	W	C47	47	PI47	OR	C70	79	SH30	P	C92	112	SH63	SW	C103
15	PI15	P	C2	48	PI48	KK	C88	80	SH31	P	C13	113	SH64	SW	C90
16	PI16	P	C3	49	PI49	UP	C102	81	SH32	P	C14	114	SH65	SW	C112
17	PI17	P	C4	SHIRAZ				82	SH33	S	C94	115	SH66	SW	C99
18	PI18	P	C5	50	SH1	W	C33	83	SH34	S	C20	116	SH67	KK	C88
19	PI19	P	C7	51	SH2	W	C76	84	SH35	S	C96	117	SH68	UP	C102
20	PI20	P	C10	52	SH3	W	C34	85	SH36	S	C22	CABERNET SAUVIGNON			
21	PI21	P	C74	53	SH4	W	C36	86	SH37	S	C23	118	CS1	P	C105
22	PI22	P	C92	54	SH5	W	C37	87	SH38	S	C24	119	CS2	P	C2
23	PI23	P	C13	55	SH6	W	C38	88	SH39	S	C25	120	CS3	P	C3
24	PI24	P	C14	56	SH7	W	C40	89	SH40	S	C17	121	CS4	P	C4
25	PI25	S	C93	57	SH8	W	C77	90	SH41	S	C97	122	CS5	P	C5
26	PI26	S	C94	58	SH9	W	C41	91	SH42	S	C31	123	CS6	P	C113
27	PI27	S	C95	59	SH10	W	C43	92	SH43	S	C99	124	CS7	P	C7
28	PI28	S	C20	60	SH11	W	C44	93	SH44	S	C100	125	CS8	P	C73
29	PI29	S	C96	61	SH12	W	C79	94	SH45	RO	C51	126	CS9	P	C9
30	PI30	S	C97	62	SH13	W	C80	95	SH46	RO	C52	127	CS10	P	C106
31	PI31	S	C98	63	SH14	W	C107	96	SH47	RO	C53	128	CS11	P	C10
32	PI32	S	C31	64	SH15	W	C108	97	SH48	RO	C55	129	CS12	P	C74
33	PI33	S	C99	65	SH16	W	C45	98	SH49	RO	C83	130	CS13	P	C75

Cabernet Sauvignon continue >>>

Selected tables

131	CS14	P	C92	170	CS53	W	C37	208	M22	S	C96	247	M61	KK	C87
132	CS15	P	C13	171	CS54	W	C38	209	M23	S	C21	248	M62	KK	C88
133	CS16	P	C114	172	CS55	W	C39	210	M24	S	C23	Young White Wines from Vintage 2005			
134	CS17	P	C14	173	CS56	W	C40	211	M25	S	C24	SAUVIGNON BLANC			
135	CS18	P	C115	174	CS57	W	C41	212	M26	S	C17	1	SB1	RO	C48
136	CS19	S	C15	175	CS58	W	C43	213	M27	S	C29	2	SB2	RO	C49
137	CS20	S	C93	176	CS59	W	C79	214	M28	S	C31	3	SB3	RO	C50
138	CS21	S	C94	177	CS60	W	C45	215	M29	S	C99	4	SB4	RO	C51
139	CS22	S	C20	178	CS61	W	C46	216	M30	S	C100	5	SB5	RO	C52
140	CS23	S	C96	179	CS62	OR	C86	217	M31	RO	C53	6	SB6	RO	C53
141	CS24	S	C22	180	CS63	OR	C101	218	M32	RO	C55	7	SB7	RO	C54
142	CS25	S	C116	181	CS64	OR	C69	219	M33	RO	C83	8	SB8	RO	C55
143	CS26	S	C24	182	CS65	OR	C70	220	M34	RO	C56	9	SB9	RO	C56
144	CS27	S	C25	183	CS66	SW	C71	221	M35	RO	C57	10	SB10	RO	C57
145	CS28	S	C17	184	CS67	SW	C112	222	M36	RO	C59	11	SB11	RO	C58
146	CS29	S	C98	185	CS68	KK	C88	223	M37	RO	C60	12	SB12	RO	C59
147	CS30	S	C117	186	CS69	UP	C102	224	M38	RO	C62	13	SB13	RO	C60
148	CS31	S	C68	MERLOT				225	M39	RO	C109	14	SB14	RO	C61
149	CS32	S	C99	187	M1	W	C33	226	M40	RO	C118	15	SB15	RO	C62
150	CS33	S	C100	188	M2	W	C76	227	M41	RO	C110	16	SB16	RO	C63
151	CS34	RO	C49	189	M3	W	C34	228	M42	RO	C66	17	SB17	RO	C64
152	CS35	RO	C51	190	M4	W	C36	229	M43	P	C105	18	SB18	RO	C65
153	CS36	RO	C55	191	M5	W	C37	230	M44	P	C2	19	SB19	RO	C66
154	CS37	RO	C52	192	M6	W	C38	231	M45	P	C3	20	SB20	RO	C67
155	CS38	RO	C53	193	M7	W	C39	232	M46	P	C5	21	SB21	S	C15
156	CS39	RO	C83	194	M8	W	C40	233	M47	P	C7	22	SB22	S	C16
157	CS40	RO	C56	195	M9	W	C77	234	M48	P	C73	23	SB23	S	C17
158	CS41	RO	C59	196	M10	W	C41	235	M49	P	C10	24	SB24	S	C18
159	CS42	RO	C60	197	M11	W	C42	236	M50	P	C74	25	SB25	S	C19
160	CS43	RO	C84	198	M12	W	C43	237	M51	P	C75	26	SB26	S	C20
161	CS44	RO	C61	199	M13	W	C44	238	M52	P	C92	27	SB27	S	C21
162	CS45	RO	C62	200	M14	W	C80	239	M53	P	C13	28	SB28	S	C22
163	CS46	RO	C109	201	M15	W	C108	240	M54	OR	C86	29	SB29	S	C23
164	CS47	RO	C110	202	M16	W	C46	241	M55	OR	C101	30	SB30	S	C24
165	CS48	RO	C66	203	M17	W	C47	242	M56	OR	C69	31	SB31	S	C25
166	CS49	W	C33	204	M18	S	C15	243	M57	OR	C70	32	SB32	S	C26
167	CS50	W	C76	205	M19	S	C93	244	M58	SW	C71	33	SB33	S	C27
168	CS51	W	C34	206	M20	S	C94	245	M59	SW	C104	34	SB34	S	C28
169	CS52	W	C36	207	M21	S	C20	246	M60	SW	C90	35	SB35	S	C29

Sauvignon Blanc continued >>>

Selected tables

36	SB36	S	C30	76	CH4	RO	C52	113	CH41	P	C7	23	PI72	RO	C109
37	SB37	S	C31	77	CH5	RO	C53	114	CH42	P	C73	24	PI73	RO	C52
38	SB38	S	C32	78	CH6	RO	C54	115	CH43	P	C74	25	PI74	RO	C62
40	SB40	W	C34	79	CH7	RO	C55	116	CH44	P	C75	26	PI75	RO	C61
41	SB41	W	C35	80	CH8	RO	C83	117	CH45	P	C12	27	PI76	RO	C124
43	SB43	W	C37	81	CH9	RO	C56	118	CH46	P	C14	28	PI77	SW	C104
44	SB44	W	C38	82	CH10	RO	C57	119	CH47	OR	C86	29	PI78	SW	C90
45	SB45	W	C39	83	CH11	RO	C58	120	CH48	OR	C68	30	PI79	SW	C112
46	SB46	W	C40	84	CH12	RO	C59	121	CH49	OR	C69	31	PI80	SW	C71
47	SB47	W	C41	85	CH13	RO	C84	122	CH50	OR	C70	32	PI81	SW	C125
48	SB48	W	C42	86	CH14	RO	C61	123	CH51	KK	C87	33	PI82	KK	C126
49	SB49	W	C43	87	CH15	RO	C62	124	CH52	KK	C88	34	PI83	KK	C89
50	SB50	W	C44	88	CH16	RO	C63	125	CH53	KK	C89	35	PI84	KK	C127
51	SB51	W	C45	89	CH17	RO	C64	126	CH54	SW	C90	36	PI85	KK	C88
52	SB52	W	C46	90	CH18	RO	C65	Young Pinotage Wines from Vintage 2006				37	PI86	S	C100
53	SB53	W	C47	91	CH19	RO	C66	1	PI50	W	C33	38	PI87	S	C19
54	SB54	P	C1	92	CH20	RO	C85	2	PI51	W	C39	39	PI88	S	C96
55	SB55	P	C2	93	CH21	W	C33	3	PI52	W	C108	40	PI89	S	C95
56	SB56	P	C3	94	CH22	W	C76	4	PI53	W	C45	41	PI90	OR	C128
59	SB59	P	C6	95	CH23	W	C34	5	PI54	W	C38	42	PI91	OR	C86
60	SB60	P	C7	96	CH24	W	C36	6	PI55	W	C46				
61	SB61	P	C8	97	CH25	W	C38	7	PI56	W	C77				
62	SB62	P	C9	98	CH26	W	C40	8	PI57	W	C119				
63	SB63	P	C10	99	CH27	W	C77	9	PI58	W	C34				
64	SB64	P	C11	100	CH28	W	C41	10	PI59	W	C36				
65	SB65	P	C12	101	CH29	W	C42	11	PI60	W	C120				
66	SB66	P	C13	102	CH30	W	C43	12	PI61	P	C5				
67	SB67	P	C14	103	CH31	W	C78	13	PI62	P	C3				
68	SB68	OR	C68	104	CH32	W	C44	14	PI63	P	C121				
69	SB69	OR	C69	105	CH33	W	C79	15	PI64	P	C122				
70	SB70	OR	C70	106	CH34	W	C80	16	PI65	P	C7				
71	SB71	SW	C71	107	CH35	W	C81	17	PI66	P	C13				
72	SB72	SW	C72	108	CH36	W	C45	18	PI67	P	C123				
	CHARDONNAY			109	CH37	W	C82	19	PI68	P	C74				
73	CH1	RO	C48	110	CH38	W	C46	20	PI69	P	C14				
74	CH2	RO	C49	111	CH39	P	C1	21	PI70	RO	C55				
75	CH3	RO	C51	112	CH40	P	C2	22	PI71	RO	C53				

^a Identification code for each wine, ^b Region codes: Worcester (W), Paarl (P), Stellenbosch (S), Swartland (SW), Robertson (RO), Olifants River (OR), Klein Karoo (KK), Upington (UP) ^c Code for each wine producer (cellar or estate).

Selected tables

Table 2. Quantitative (mg/l) data of 49 young Pinotage wines (PI1 – PI49) from vintage 2005.

COMPOUND	PI1	PI2	PI3	PI4	PI5	PI6	PI7	PI8	PI9	PI10	PI11	PI12	PI13	PI14	PI15	PI16	PI17
Ethyl acetate	98.6	89.2	142	139	180	172	71.7	98.6	134	177	167	95.3	89.9	223	131	204	170
Ethyl butyrate	0.217	0.526	0.328	0.329	0.370	0.259	0.181	0.268	0.533	0.373	0.469	0.281	0.312	0.340	0.252	0.362	0.301
1-Propanol	77.5	56.3	34.9	38.5	33.7	116	65.8	91.8	65.7	81.3	40.4	45.2	63.9	62.4	46.5	49.9	59.7
Isobutanol	42.4	32.3	60.2	59.5	61.8	56.5	3.97	29.5	93.5	60.5	75.4	78.9	54.2	40.4	56.1	66.0	67.2
Isoamyl acetate	4.23	6.23	2.10	5.04	2.92	2.72	6.42	4.52	3.57	8.74	7.07	3.81	3.01	4.59	3.11	4.77	3.71
n-Butanol	9.41	1.23	8.32	11.8	11.5	11.3	5.42	2.53	9.73	10.3	9.49	7.68	7.41	11.0	10.1	8.07	11.4
Isoamyl alcohol	167	167	163	146	160	145	164	168	189	193	138	192	159	142	164	152	147
Ethyl hexanoate	0.148	0.554	0.234	0.230	0.247	0.137	0.114	0.182	0.145	0.280	0.455	0.240	0.369	0.167	0.195	0.232	0.250
Hexyl acetate	19.0 ^a	28.1 ^a	10.1 ^a	17.4 ^a	4.56 ^a	14.5 ^a	9.27 ^a	12.2 ^a	8.72 ^a	27.5 ^a	46.7 ^a	14.3 ^a	16.5 ^a	8.71 ^a	11.1 ^a	12.0 ^a	13.1 ^a
Acetoin	120	34	19.6	34.7	21.8	19.2	144	111	56.6	42.3	46.2	30.0	51.3	19.8	17.5	20.8	21.1
Ethyl-D-lactate	345	243	263	191	354	153	280	243	177	142	344	465	234	192	297	446	277
1-Hexanol	0.527	0.731	1.03	0.985	0.912	0.425	0.669	1.01	0.163	50.1 ^a	0.252	0.362	0.326	0.529	94.0 ^a	0.974	0.894
Ethyl octanoate	19.6 ^a	86.9 ^a	28.1 ^a	36.7 ^a	35.4 ^a	16.8 ^a	17.9 ^a	25.9 ^a	22.3 ^a	34.3 ^a	71.6 ^a	29.0 ^a	41.7 ^a	24.5 ^a	28.3 ^a	35.0 ^a	37.3 ^a
Acetic acid	1518	385	316	314	731	825	2626	1702	648	433	403	979	710	657	411	588	572
Furfural	18.1	3.10	17.6	13.9	17.9	19.7	34.9	26.7	5.20	19.6	1.41	1.19	9.07	21.4	17.4	15.5	0.994
Propionic acid	34.1	11.3	10.1	11.1	16.4	19.1	47.6	41.4	11.9	6.80	8.74	24.0	12.6	11.9	11.9	11.2	14.4
Isobutyric acid	1.31	0.857	1.77	0.796	2.01	0.402	1.62	1.54	1.61	0.944	1.84	1.40	0.713	1.53	1.13	0.950	0.999
5-Methylfurfural	0.486	0.302	67.9 ^a	0.632	0.568	0.621	0.709	0.835	47.2 ^a	9.16 ^a	46.0	0.517	0.207	0.145	92.1 ^a	0.441	0.664
n-Butyric acid	3.37	2.27	4.97	3.99	4.97	4.50	4.02	4.32	1.97	5.03	65.3 ^a	4.96	3.70	0.210	4.01	45.8 ^a	4.48
Ethyl decanoate	4.84 ^a	5.55 ^a	4.95 ^a	8.45 ^a	7.89 ^a	3.72 ^a	4.58 ^a	3.96 ^a	5.52 ^a	6.25 ^a	11.2 ^a	7.69 ^a	5.07 ^a	4.68 ^a	5.19 ^a	4.64 ^a	6.35 ^a
Isovaleric acid	1.83	1.38	1.71	1.64	1.72	1.34	1.85	1.50	1.88	1.49	1.36	1.78	1.52	1.45	1.56	1.56	1.59
Diethyl succinate	7.78	9.09	15.4	9.01	9.05	7.28	7.63	12.2	6.91	7.55	8.26	9.39	14.8	8.36	11.8	9.66	7.53
n-Valeric acid	1.79	1.48	1.67	1.38	1.53	1.52	1.80	1.72	1.57	1.46	1.54	1.67	1.60	1.45	1.44	1.51	1.39
2-Phenethyl acetate	0.231	0.360	91.8 ^a	0.217	62.6 ^a	86.6 ^a	0.206	0.142	85.1 ^a	0.222	0.218	0.268	0.112	0.104	0.169	0.185	87.2 ^a
Hexanoic acid	3.24	4.34	4.39	3.71	3.55	2.91	3.46	3.29	3.13	3.14	4.22	3.21	3.85	2.86	3.16	3.66	3.41
Guaiacol	0.802	0.771	0.294	0.175	0.363	0.281	1.14	0.750	0.516	0.258	0.216	0.487	40.9 ^a	0.251	0.249	0.283	0.504
trans-oak-lactone	1.03	1.05	1.04	1.03	1.04	1.03	nd ^b	1.03	1.04	1.04	nd ^b	1.04	1.04	1.04	1.04	1.04	1.04
2-Phenylethyl alcohol	15.5	12.5	19.0	12.6	11.2	10.1	15.5	24.6	11.0	10.8	8.75	23.1	15.9	8.74	13.3	13.2	8.47
cis-oak-lactone	0.991	1.02	1.01	0.981	1.04	1.01	1.01	1.02	0.987	0.997	0.986	0.991	nd ^b	1.01	0.993	1.04	0.992
o-Cresol	0.932	0.837	0.81	0.777	0.812	0.817	1.00	0.968	0.840	0.802	0.826	0.850	0.780	0.822	0.808	0.795	0.825
Phenol	2.08	0.896	0.500	0.37	0.618	0.641	2.29	3.27	1.55	0.702	0.486	1.13	0.351	0.378	0.794	0.369	0.730
4-Ethylguaiacol	0.376	0.370	0.353	0.347	0.354	0.349	0.391	0.373	0.359	0.352	0.348	0.359	0.340	0.350	0.353	0.348	0.363
Octanoic acid	1.60	2.299	1.66	1.76	1.44	1.18	1.62	1.59	1.33	1.31	1.69	1.73	1.52	1.06	1.46	1.46	1.63
p-Cresol	0.431	0.322	0.275	0.338	0.251	0.248	0.363	0.344	0.328	0.248	0.249	0.307	0.239	0.244	0.257	0.254	0.271
Eugenol	0.807	0.716	0.589	0.560	0.592	0.583	0.951	0.781	0.696	0.602	0.569	0.690	0.510	0.555	0.574	0.561	0.651
Decanoic acid	0.72	0.725	0.766	0.711	0.655	0.629	0.685	0.718	0.664	0.637	0.694	0.801	0.684	0.610	0.705	0.675	0.685
2,6-Dimethoxy phenol	33.2	6.19	4.59	5.00	9.71	8.75	53.7	45.2	7.41	4.68	3.66	10.7	6.44	7.99	4.67	5.26	7.18
5-Hydroxymethylfurfural	13.0	7.86	5.19	5.05	6.41	5.79	23.2	19.7	3.16	5.81	6.10	6.46	2.78	3.08	0.903	0.588	0.840
Vanillin	81.7	39.2	29.2	24.6	28.7	30.9	141	52.7	33.3	25.7	20.2	51.0	17.4	18.7	22.0	18.0	35.8

Table 2 Continue >>>

Selected tables

COMPOUND	P18	P19	P20	P21	P22	P23	P24	PI25	PI26	PI27	PI28	PI29	PI30	PI31	PI32	PI33	PI34
Ethyl acetate	193	99.9	96.5	168	150	198	196	153	119	190	144	77.9	175	124	93.2	77.3	185
Ethyl butyrate	0.305	0.230	0.249	0.293	0.152	0.422	0.245	0.239	0.266	0.326	0.248	0.221	0.260	0.237	0.256	0.162	0.251
1-Propanol	24.8	37.1	52.3	112	41.4	163	48.9	55.0	89.7	18.2	83.1	41.4	103	39.6	40.8	48.1	130
Isobutanol	57.3	45.9	40.7	37.5	66.0	36.3	41.9	30.7	28.4	28.1	45.3	62.1	99.6	103	66.6	59.3	56.4
Isoamyl acetate	4.78	6.26	6.57	4.08	0.919	7.78	2.53	6.21	9.59	7.43	3.59	4.53	3.24	6.43	8.09	2.55	3.90
n-Butanol	1.85	8.78	5.50	8.42	7.55	8.70	8.04	8.15	11.3	9.14	1.02	1.96	8.59	7.72	10.7	8.79	11.1
Isoamyl alcohol	160	142	158	123	122	147	133	136	138	128	153	163	146	200	201	145	173
Ethyl hexanoate	0.229	0.149	0.147	0.202	63.3 ^a	0.282	0.123	0.161	0.129	0.205	0.141	0.141	0.131	0.152	0.176	0.180	81.2 ^a
Hexyl acetate	29.7 ^a	6.47 ^a	12.0 ^a	13.8 ^a	5.34 ^a	26.3 ^a	11.2 ^a	52.9 ^a	19.7 ^a	22.6 ^a	18.5 ^a	11.5 ^a	11.6 ^a	13.6 ^a	21.7 ^a	6.39	9.39 ^a
Acetoin	59.3	65.3	140	1.84	51.6	176	21.4	35.1	66.2	17.3	61.5	109	2.44	37.6	45.4	86.1	98.7
Ethyl-D-lactate	260	315	283	288	208	258	3.94	435	308	343	366	235	257	313	399	474	239
1-Hexanol	0.770	0.168	0.681	0.927	0.553	0.671	0.820	0.190	0.525	0.597	0.141	0.967	0.782	0.726	0.778	0.116	0.876
Ethyl octanoate	34.6 ^a	21.3 ^a	32.7 ^a	25.5 ^a	11.3 ^a	35.9 ^a	16.9 ^a	25.9 ^a	27.1 ^a	42.9 ^a	22.5 ^a	21.3 ^a	22.2 ^a	22.4 ^a	23.9 ^a	29.9 ^a	9.38 ^a
Acetic acid	675	1079	1004	404	596	550	460	652	1427	615	698	1537	621	828	1146	1467	736
Furfural	7.37	14.7	21.6	24.2	20.7	6.15	17.8	17.3	20.5	16.7	17.5	23.4	18.2	18.6	16.9	18.1	20.0
Propionic acid	19.8	26.9	32.2	11.5	12.5	16.6	12.5	15.2	33.0	16.2	15.6	34.0	13.4	13.6	34.4	40.2	16.7
Isobutyric acid	1.21	0.732	1.76	0.867	2.64	1.57	1.32	0.995	1.62	1.60	1.05	2.25	1.13	2.30	3.01	5.36	1.74
5-Methylfurfural	0.677	0.480	0.666	4.54 ^a	0.717	0.288	0.715	0.153	0.810	64.0 ^a	0.689	0.820	0.671	0.497	0.406	0.130	0.685
n-Butyric acid	1.15	2.52	2.96	4.28	4.65	1.84	89.8 ^a	4.60	4.00	65.5 ^a	4.37	4.33	3.39	4.16	2.15	3.63	5.06
Ethyl decanoate	6.68 ^a	3.29 ^a	8.09 ^a	3.57 ^a	3.17 ^a	6.55 ^a	5.82 ^a	6.88 ^a	8.32 ^a	0.0735 ^a	9.98 ^a	3.30	4.68 ^a	4.73 ^a	6.61 ^a	5.54	0.976 ^a
Isovaleric acid	1.53	1.36	1.48	1.27	1.52	1.79	1.41	1.50	1.63	1.55	1.59	1.59	1.37	2.01	1.85	4.46	1.66
Diethyl succinate	8.86	11.0	11.4	11.2	7.28	9.84	8.25	9.35	11.7	6.10	10.1	11.0	8.56	5.30	10.1	12.2	11.6
n-Valeric acid	1.54	1.53	1.67	1.40	1.44	1.51	1.41	1.52	1.63	1.62	1.46	1.72	1.30	1.41	1.77	3.80	1.39
2-Phenethyl acetate	0.212	49.2 ^a	0.275	0.106	74.8 ^a	0.238	99.4 ^a	0.431	0.407	0.341	0.218	0.219	0.136	0.560	0.549	0.175	0.201
Hexanoic acid	3.36	3.38	3.35	2.94	2.50	3.73	2.46	3.54	3.50	4.01	3.54	3.62	2.43	3.09	3.56	5.66	2.70
Guaiacol	0.440	0.853	0.567	0.25	0.324	0.478	0.205	0.410	0.923	0.332	0.333	0.899	0.214	0.204	0.520	0.793	0.379
trans-oak-lactone	1.04	1.05	nd ^b	nd ^b	nd ^b	nd ^b	1.03	1.04	nd ^b	1.04	1.03	1.04	1.03	nd ^b	1.04	1.04	nd ^b
2-Phenylethyl alcohol	17.6	14.6	21.7	9.01	10.5	14.0	10.3	13.6	18.8	11.1	16.6	21.6	9.72	36.8	32.0	29.5	21.9
cis-oak-lactone	1.04	1.01	0.996	nd ^b	nd ^b	nd ^b	0.983	0.992	1.00	0.996	0.990	1.00	0.978	0.982	0.991	0.996	0.982
o-Cresol	0.871	0.892	0.950	0.805	0.805	0.855	0.807	0.804	0.921	0.815	0.816	0.954	0.793	0.789	0.900	0.929	0.813
Phenol	1.08	1.44	1.82	0.830	0.586	1.04	0.449	0.337	1.56	0.429	0.394	1.84	0.318	0.187	1.28	1.42	0.498
4-Ethylguaiacol	0.355	0.377	0.369	0.351	0.355	0.359	0.348	0.359	0.382	0.355	0.358	0.381	0.348	0.350	0.363	0.375	0.352
Octanoic acid	1.48	1.83	1.73	1.10	1.04	1.452	1.12	1.56	2.01	1.88	1.94	1.81	1.23	1.45	2.29	3.33	1.08
p-Cresol	0.260	0.386	0.307	0.241	0.265	0.300	0.229	0.252	0.343	0.267	0.244	0.373	0.227	0.221	0.333	0.312	0.259
Eugenol	0.641	0.802	0.695	0.569	0.594	0.612	0.539	0.689	0.776	0.592	0.610	0.853	0.590	0.550	0.659	0.779	0.616
Decanoic acid	0.680	0.739	0.714	0.612	0.606	0.641	0.609	0.716	0.727	0.781	0.752	0.704	0.625	0.628	0.977	1.92	0.603
2,6-Dimethoxy phenol	7.40	13.1	24.6	6.71	5.50	7.01	4.85	4.70	8.62	3.46	5.08	13.8	9.60	3.60	6.31	8.27	3.40
5-Hydroxymethylfurfural	1.22	3.08	2.68	0.917	0.711	0.895	0.713	8.44	23.9	6.84	6.90	27.8	7.10	6.63	20.1	16.4	5.94
Vanillin	37.4	66.4	41.2	24.3	27.1	28.8	20.8	27.3	86.9	30.4	25.0	49.6	22.4	14.9	29.6	71.1	21.8

Table 2 Continue >>>

Selected tables

COMPOUND	PI35	PI36	PI37	PI38	PI39	PI40	PI41	PI42	PI43	PI44	PI45	PI46	PI47	PI48	PI49
Ethyl acetate	102	220	192	201	146	180	112	156	99.2	142	84.9	146	89.4	99.0	146
Ethyl butyrate	0.254	0.351	0.409	0.452	0.284	0.222	0.127	0.257	0.199	0.318	0.328	0.455	0.290	0.322	0.203
1-Propanol	30.1	211	16.5	118	15.7	32.1	58.5	36.9	44.8	16.2	52.5	62.2	37.0	12.2	77.3
Isobutanol	51.0	48.8	0.232	42.5	38.2	84.6	83.4	118	41.9	57.5	41.8	60.5	50.2	61.8	51.0
Isoamyl acetate	3.12	0.929	0.421	7.77	3.38	1.70	1.02	2.00	0.696	3.73	6.83	8.24	6.27	3.03	3.04
n-Butanol	5.53	9.67	1.71	8.35	10.5	1.95	8.13	7.32	1.45	9.57	1.24	11.6	25.7 ^a	76.5 ^a	9.26
Isoamyl alcohol	177	175	149	142	166	198	159	190	152	169	198	157	168	170	160
Ethyl hexanoate	0.195	0.228	0.318	0.247	0.155	0.165	0.100	0.168	0.151	0.204	0.248	0.333	0.310	0.264	0.239
Hexyl acetate	10.0 ^a	5.54 ^a	5.59 ^a	16.4 ^a	10.1 ^a	5.90 ^a	6.99 ^a	4.58 ^a	8.98 ^a	12.8 ^a	13.9 ^a	18.9 ^a	22.6 ^a	19.2 ^a	7.51 ^a
Acetoin	104	65.9	52.9	66.8	25.5	83.9	2.75	91.1	149	10.1	31.5	67.1	62.3	49.6	56.1
Ethyl-D-lactate	289	915	274	320	145	259	183	128	393	345	326	191	402	93.9	313
1-Hexanol	0.251	0.802	0.983	0.566	0.207	0.127	0.925	0.234	56.4 ^a	0.286	0.916	0.515	0.864	0.571	73.6 ^a
Ethyl octanoate	26.5 ^a	28.9 ^a	44.3 ^a	31.7 ^a	23.0 ^a	19.4 ^a	11.2 ^a	25.7 ^a	23.5 ^a	27.9 ^a	30.6 ^a	34.7 ^a	54.3 ^a	39.0 ^a	34.0 ^a
Acetic acid	1359	910	708	681	584	974	679	468	1245	734	1027	1051	1081	852	677
Furfural	20.0	17.8	15.6	21.1	17.7	12.0	13.6	3.03	24.6	12.5	0.692	16.0	19.0	5.08	11.3
Propionic acid	35.2	15.4	11.8	14.5	16.5	27.1	10.2	7.01	38.8	9.95	20.7	20.9	16.7	8.90	10.3
Isobutyric acid	2.43	2.96	0.851	1.64	1.92	2.26	1.75	2.89	1.64	2.93	3.58	2.41	2.95	2.64	
5-Methylfurfural	0.581	35.0 ^a	0.654	0.641	62.6 ^a	52.3 ^a	0.499	0.144	0.789	0.537	0.503	0.393	0.542	0.727	0.456
n-Butyric acid	3.13	5.01	36.3 ^a	5.14	1.25	1.56	4.07	0.862	3.76	4.96	0.768	1.63	4.76	0.820	3.11
Ethyl decanoate	4.09 ^a	4.19 ^a	7.46 ^a	6.41 ^a	5.56 ^a	2.90 ^a	1.93 ^a	5.16 ^a	4.83 ^a	6.72 ^a	3.90 ^a	3.39 ^a	9.37 ^a	6.59 ^a	4.45 ^a
Isovaleric acid	1.78	1.68	1.58	1.48	1.53	1.49	1.52	1.62	1.53	1.92	1.61	1.54	1.80	1.82	1.45
Diethyl succinate	10.6	11.4	13.6	6.39	10.9	6.78	7.91	7.89	5.72	12.6	10.6	9.24	13.3	4.74	4.56
n-Valeric acid	1.77	1.57	1.40	1.45	1.48	1.47	1.43	1.61	1.68	1.49	1.60	1.60	1.74	1.54	1.44
2-Phenethyl acetate	0.126	41.9 ^a	65.9 ^a	0.241	0.31	65.9 ^a	73.7 ^a	70.3 ^a	0.223	0.161	0.190	0.455	0.386	0.203	68.3 ^a
Hexanoic acid	4.03	4.38	4.05	3.81	3.15	2.63	2.66	3.20	3.72	3.67	3.96	4.16	4.42	3.52	4.63
Guaiacol	0.975	0.352	0.254	0.412	0.298	0.614	0.153	0.325	0.754	0.188	0.268	0.592	0.436	0.511	0.112
trans-oak-lactone	nd ^b	1.03	1.04	nd ^b	1.04	1.04	1.04	1.04	nd ^b	nd ^b	1.04	1.05	1.03	1.04	1.04
2-Phenylethyl alcohol	25.1	11.5	15.3	9.44	19.4	19.1	13.7	16.6	24.5	20.7	14.0	13.9	22.1	14.4	8.58
cis-oak-lactone	0.991	0.993	0.977	nd ^b	0.980	0.998	0.985	0.988	1.01	0.983	0.986	0.992	0.993	0.989	0.992
o-Cresol	0.935	0.805	0.801	0.825	0.817	0.903	0.768	0.843	0.963	0.775	0.824	0.884	0.835	0.851	0.765
Phenol	1.97	0.735	0.401	0.956	0.554	1.69	0.821	0.968	1.97	0.431	0.796	3.14	0.876	0.963	0.296
4-Ethylguaiacol	0.381	0.357	0.355	0.351	0.347	0.359	0.346	0.354	0.371	0.346	0.355	0.362	0.355	0.355	0.344
Octanoic acid	2.02	1.39	1.92	1.73	1.87	1.20	1.06	1.32	1.88	1.59	1.87	1.40	2.22	1.68	1.52
p-Cresol	0.357	0.270	0.286	0.295	0.269	0.296	0.221	0.305	0.302	0.317	0.265	0.305	0.269	0.288	0.266
Eugenol	0.867	0.604	0.561	0.669	0.530	0.711	0.558	0.610	0.702	0.533	0.580	0.697	0.666	0.663	0.539
Decanoic acid	0.790	0.678	0.826	0.645	0.693	0.647	0.642	0.646	0.755	0.695	0.693	0.716	0.832	0.726	0.658
2,6-Dimethoxy phenol	26.9	12.2	9.88	9.94	10.1	17.1	11.4	8.44	8.42	15.7	34.0	38.9	24.1	7.00	15.1
5-Hydroxymethylfurfural	10.1	2.92	3.96	2.97	2.46	3.62	4.44	1.04	16.3	2.79	12.2	7.79	4.64	0.198	2.74
Vanillin	113	30.8	37.5	52.9	25.8	60.9	49.0	31.9	41.7	54.9	36.9	45.9	45.7	47.3	3.05

^a Measured in µg/L. ^b nd: not detected. For details of the wines refer to **Table 1**. For analytical conditions refer to **chapter 6, section 6.2**.

Selected tables

Table 3. Quantitative (mg/l) data of 68 young Shiraz wines (SH1 – SH68) from vintage 2005.

COMPOUND	SH1	SH2	SH3	SH4	SH5	SH6	SH7	SH8	SH9	SH10	SH11	SH12	SH13	SH14	SH15	SH16	SH17
Ethyl acetate	90.9	132	82.6	151	179	149	166	109	61.1	104	132	152	161	124	186	99.8	87.8
Ethyl butyrate	0.161	0.271	0.252	0.201	0.198	0.291	0.300	0.256	0.122	0.185	0.318	0.237	0.268	0.196	0.282	0.278	0.242
1-Propanol	27.0	62.6	35.7	46.1	35.7	41.7	33.1	78.8	24.9	40.9	31.6	21.0	31.5	21.4	29.5	54.3	79.9
Isobutanol	96.3	92.6	78.1	98.9	144	123	65.8	50.9	26.8	47.4	67.2	80.4	86.8	79.3	47.4	86.4	73.4
Isoamyl acetate	2.39	3.28	2.68	2.218	0.891	2.09	1.27	1.87	13.5 ^a	2.43	1.70	0.938	2.92	2.14	2.79	2.49	2.27
n-Butanol	7.22	0.358	9.56	1.41	1.76	0.784	10.9	11.9	10.4	10.9	0.244	9.47	11.5	11.2	2.71	9.32	8.65
Isoamyl alcohol	203	225	201	177	201	215	163	194	160	202	146	239	197	203	163	201	196
Ethyl hexanoate	0.114	0.164	0.199	0.148	91.0 ^a	0.175	0.171	0.196	71.4 ^a	0.106	0.191	0.158	0.236	0.113	0.232	0.191	0.151
Hexyl acetate	11.0 ^a	9.94 ^a	17.3 ^a	14.6 ^a	5.36 ^a	11.0 ^a	4.36 ^a	13.9 ^a	7.10 ^a	8.16 ^a	9.52 ^a	5.90 ^a	17.9 ^a	6.90 ^a	20.9 ^a	16.5 ^a	9.15 ^a
Acetoin	55.2	14.7	24.3	19.4	50.8	11.1	53.5	55.1	138	69.6	3.38	28.0	8.01	11.5	21.3	100	26.9
Ethyl-D-lactate	255	121	262	247	135	192	314	282	198	127	154	361	223	132	234	393	211
1-Hexanol	0.942	0.632	0.806	1.90	1.09	1.27	1.21	1.65	0.844	0.647	0.758	1.84	1.31	0.677	1.69	0.398	1.15
Ethyl octanoate	12.4 ^a	17.5 ^a	22.9 ^a	12.6 ^a	7.76 ^a	16.0 ^a	20.2 ^a	17.3 ^a	6.65 ^a	10.8 ^a	21.9 ^a	13.1 ^a	31.6 ^a	14.8 ^a	26.3 ^a	23.5 ^a	13.0 ^a
Acetic acid	254	311	389	301	292	240	374	322	934	573	374	445	406	346	334	487	467
Furfural	15.3	9.22	13.6	10.6	8.58	10.6	10.9	10.9	17.9	13.5	14.4	16.1	12.6	12.1	17.2	13.1	2.10
Propionic acid	12.2	5.72	12.8	6.84	5.50	5.84	6.84	5.72	28.0	23.9	8.76	10.8	9.35	7.19	8.01	16.0	14.0
Isobutyric acid	1.06	1.24	1.52	0.946	1.59	1.15	1.88	0.357	2.04	1.94	1.24	4.70	2.03	2.19	3.41	1.25	2.54
5-Methylfurfural	44.0 ^a	0.403	17.1 ^a	0.478	0.404	0.393	0.490	0.463	0.627	0.627	0.632	0.681	0.584	0.473	0.722	0.482	79.5 ^a
n-Butyric acid	0.515	3.35	0.367	3.05	2.58	3.00	2.98	2.90	3.46	3.13	4.44	3.39	3.17	3.51	3.69	2.65	0.316
Ethyl decanoate	3.70 ^a	4.21 ^a	7.99 ^a	2.35 ^a	1.76 ^a	2.61 ^a	3.23 ^a	3.36 ^a	1.99 ^a	3.27 ^a	3.13 ^a	3.07	7.78 ^a	2.68 ^a	3.69 ^a	6.26 ^a	5.13 ^a
Isovaleric acid	1.52	1.78	1.56	1.53	1.51	1.46	1.43	1.45	1.70	1.78	1.67	2.25	1.70	2.13	1.81	1.64	2.61
Diethyl succinate	8.30	9.78	10.2	5.81	7.31	8.61	10.9	12.6	9.57	14.2	12.2	10.5	11.6	14.5	12.9	8.72	8.05
n-Valeric acid	1.55	1.36	1.46	1.44	1.33	1.26	1.38	1.35	1.74	1.69	1.46	1.63	1.60	1.44	1.71	1.83	2.51
2-Phenethyl acetate	0.238	0.238	0.183	0.104	83.2 ^a	0.112	50.5 ^a	0.223	0.112	0.175	0.135	79.4 ^a	0.233	0.269	0.207	0.239	95.3 ^a
Hexanoic acid	2.72	2.56	3.13	2.67	2.16	2.10	2.44	2.38	3.24	3.18	2.81	4.09	3.60	2.70	4.47	3.67	3.92
Guaiacol	0.276	62.2 ^a	0.161	92.7 ^a	39.6 ^a	0.101	0.186	87.3 ^a	0.607	0.447	0.370	0.310	0.136	0.218	0.300	0.457	0.355
trans-oak-lactone	nd ^b	1.03	nd ^b	1.04	1.03	nd ^b	nd ^b	nd ^b	1.03	nd ^b	nd ^b	1.03	1.03	1.03	1.03	nd ^b	nd ^b
2-Phenylethyl alcohol	23.7	28.6	15.6	9.54	18.2	16.2	12.5	16.4	23.5	43.9	15.3	41.7	23.5	41.8	21.0	23.1	15.4
cis-oak-lactone	0.977	0.967	0.984	0.975	0.972	nd ^b	nd ^b	nd ^b	0.998	0.991	nd ^b	nd ^b	nd ^b	0.971	nd ^b	0.987	0.979
o-Cresol	0.791	0.749	0.776	0.755	0.734	0.749	0.750	0.756	0.908	0.894	0.788	0.795	0.779	0.778	0.816	0.861	0.831
Phenol	0.539	0.831	0.302	80.6 ^a	0.739	14.9 ^a	0.324	0.176	1.24	1.43	0.740	0.608	0.698	0.517	0.811	0.871	0.683
4-Ethylguaiacol	0.353	0.337	0.348	0.338	0.337	0.336	0.343	0.341	0.370	0.358	0.348	0.345	0.341	0.344	0.346	0.362	0.355
Octanoic acid	1.18	1.13	1.40	1.11	0.977	0.939	1.02	1.05	1.24	1.37	1.07	2.01	1.63	1.35	1.85	1.84	2.19
p-Cresol	0.297	0.213	0.248	0.236	0.204	0.205	0.231	0.235	0.288	0.307	0.257	0.268	0.272	0.244	0.272	0.295	0.282
Eugenol	0.571	0.498	0.536	0.518	0.496	0.519	0.574	0.533	0.697	0.651	0.662	0.619	0.534	0.527	0.581	0.652	0.619
Decanoic acid	0.680	0.621	0.632	0.640	0.641	0.642	0.607	0.604	0.655	0.677	0.626	0.853	0.767	0.715	0.739	0.785	1.09
2,6-Dimethoxy phenol	5.09	2.41	4.58	2.95	2.86	3.16	3.66	3.52	9.16	10.6	6.54	8.33	8.33	7.63	6.54	14.0	7.05
5-Hydroxymethylfurfural	5.49	2.35	7.16	2.79	2.39	2.70	2.32	2.70	16.5	11.1	3.23	3.17	2.79	2.84	2.99	14.7	6.59
Vanillin	20.1	14.8	18.8	18.2	14.5	19.4	20.1	19.4	56.4	12.2	47.0	37.6	28.2	20.9	25.6	8.91	28.2

Table 3 Continue >>>

Selected tables

COMPOUND	SH18	SH19	SH20	SH21	SH22	SH23	SH24	SH25	SH26	SH27	SH28	SH29	SH30	SH31	SH32	SH33	SH34
Ethyl acetate	187	158	145	116	154	128	82.3	147	152	150	116	168	165	158	167	91.0	149
Ethyl butyrate	0.286	0.253	0.288	0.312	0.248	0.232	0.151	0.217	0.222	0.286	0.181	0.216	0.196	0.267	0.221	0.202	0.242
1-Propanol	28.2	25.8	138	31.7	38.3	21.2	39.4	34.7	34.8	35.4	24.0	13.3	31.4	154	33.0	32.1	74.1
Isobutanol	67.5	98.5	57.7	92.0	93.4	64.3	83.5	79.2	59.8	81.4	60.6	70.0	55.5	50.0	85.6	75.4	63.4
Isoamyl acetate	1.59	1.03	1.18	3.81	2.34	0.849	1.27	3.13	1.12	2.14	1.95	3.24	1.28	1.77	4.26	2.49	2.72
n-Butanol	10.5	6.50	1.98	1.77	2.01	11.8	7.80	9.98	1.12	11.2	8.84	10.4	1.87	1.72	6.03	9.62	2.93
Isoamyl alcohol	170	209	176	258	176	222	186	181	150	200	192	230	123	168	235	180	168
Ethyl hexanoate	0.199	0.124	0.121	0.220	0.125	0.162	97.2 ^a	0.119	0.204	0.147	0.107	0.131	82.7 ^a	0.153	98.7 ^a	0.115	0.102
Hexyl acetate	5.98 ^a	4.19 ^a	5.38 ^a	13.2 ^a	10.0 ^a	6.72 ^a	6.29 ^a	12.7 ^a	4.51 ^a	7.87 ^a	7.94 ^a	10.1 ^a	6.72 ^a	8.02 ^a	8.89 ^a	18.3 ^a	12.1 ^a
Acetoin	8.93	21.4	19.8	86.5	70.6	31.7	92.8	70.2	52.6	69.4	123	13.9	0.344	57.1	81.7	144	12.0
Ethyl-D-lactate	71.4	180	113	431	217	254	267	236	211	150	233	253	235	236	174	243	174
1-Hexanol	87.2 ^a	0.333	0.186	0.855	0.263	2.96	0.522	39.2 ^a	0.116	0.456	0.784	0.284	0.638	0.504	0.211	0.850	0.487
Ethyl octanoate	17.6 ^a	8.10 ^a	12.2 ^a	24.5 ^a	14.6 ^a	14.3 ^a	9.29 ^a	17.8 ^a	30.3 ^a	13.7 ^a	12.7 ^a	11.0 ^a	14.5 ^a	16.2 ^a	13.9 ^a	12.5 ^a	12.3 ^a
Acetic acid	334	377	1196	397	566	641	755	341	264	257	1510	287	272	392	242	362	193
Furfural	10.1	14.0	13.3	13.7	12.9	19.1	11.3	10.7	10.4	17.2	29.1	14.8	11.9	13.1	11.2	1.85	10.4
Propionic acid	5.61	6.53	6.53	6.32	5.16	3.72	19.6	4.90	4.70	5.29	44.2	6.41	5.29	6.86	5.49	8.21	3.08
Isobutyric acid	0.944	1.31	0.731	1.07	1.88	2.16	3.51	1.57	1.13	3.85	2.23	2.44	1.67	0.731	2.06	3.77	2.13
5-Methylfurfural	0.411	0.513	0.468	0.503	0.531	0.65	0.728	0.467	0.481	0.720	0.767	0.703	0.599	0.592	0.519	0.674	0.461
n-Butyric acid	3.57	3.40	3.45	3.50	2.61	5.08	2.18	2.99	3.80	4.14	4.44	4.03	3.34	3.90	3.35	2.62	2.88
Ethyl decanoate	2.99 ^a	1.53 ^a	2.73 ^a	5.40 ^a	3.21 ^a	2.17 ^a	3.54 ^a	3.03 ^a	7.12 ^a	3.28 ^a	4.29 ^a	2.96 ^a	5.06 ^a	4.12 ^a	1.18 ^a	4.35 ^a	2.37 ^a
Isovaleric acid	1.75	1.85	1.60	1.83	1.51	1.89	1.55	1.53	1.68	1.92	1.89	2.38	1.67	1.61	1.72	1.76	1.54
Diethyl succinate	9.33	6.43	10.6	11.2	8.11	11.3	12.3	10.9	14.6	11.3	15.6	15.2	9.04	13.6	9.84	11.9	10.1
n-Valeric acid	1.34	1.41	1.44	1.31	1.33	1.39	1.69	1.49	1.52	1.71	1.69	1.59	1.51	1.41	1.43	1.81	1.51
2-Phenethyl acetate	65.1 ^a	42.5 ^a	73.3 ^a	0.274	0.106	75.1 ^a	0.175	0.255	57.1 ^a	0.140	0.288	0.554	0.171	84.6 ^a	0.372	0.527	0.191
Hexanoic acid	2.73	1.43	2.51	2.91	2.58	2.72	3.28	2.30	3.39	3.52	3.49	3.45	2.54	2.42	2.66	3.66	2.62
Guaiacol	0.163	0.226	0.194	0.282	0.257	0.237	0.676	65.7 ^a	0.157	0.335	0.910	0.521	0.186	0.168	0.182	0.334	30.1 ^a
trans-oak-lactone	1.03	1.04	1.03	nd ^b	1.03	nd ^b	1.03	1.03	1.10	1.03	nd ^b	nd ^b	nd ^b	1.03	1.04	nd ^b	1.04
2-Phenylethyl alcohol	18.9	14.6	15.2	61.0	19.8	40.9	31.0	15.7	22.5	33.7	54.8	81.4	15.44	17.8	56.1	32.3	19.1
cis-oak-lactone	nd ^b	0.970	0.964	0.971	0.969	nd ^b	1.01	nd ^b	1.13	nd ^b	1.00	nd ^b	nd ^b	0.969	0.985	0.990	0.968
o-Cresol	0.751	0.773	0.768	0.769	0.761	0.807	0.879	0.756	0.775	0.814	0.977	0.804	0.789	0.785	0.771	0.818	0.755
Phenol	38.5 ^a	0.661	0.274	0.387	0.247	0.565	1.13	0.359	0.448	1.30	1.83	0.661	0.367	0.657	0.474	0.854	0.863
4-Ethylguaiacol	0.338	0.340	0.337	0.347	0.346	0.345	0.372	0.341	0.345	0.351	0.375	0.354	0.344	0.344	0.342	0.357	0.335
Octanoic acid	1.08	1.18	1.08	1.39	1.32	1.35	1.43	1.03	1.59	1.41	1.76	1.77	1.30	1.04	1.26	1.57	1.11
p-Cresol	0.216	0.246	0.221	0.227	0.220	0.237	0.335	0.239	0.236	0.281	0.332	0.276	0.262	0.236	0.238	0.297	0.216
Eugenol	0.542	0.558	0.545	0.587	0.603	0.550	0.740	0.522	0.517	0.616	0.793	0.661	0.537	0.537	0.532	0.584	0.497
Decanoic acid	0.607	0.658	0.627	0.714	0.666	0.701	0.680	0.638	0.716	0.671	0.743	0.754	0.769	0.686	0.725	0.713	0.624
2,6-Dimethoxy phenol	2.86	6.17	5.35	5.73	5.54	7.22	16.1	4.01	4.82	12.8	25.5	6.26	4.82	5.62	4.33	3.20	1.28
5-Hydroxymethylfurfural	2.39	2.54	2.54	2.60	2.31	8.90	12.7	2.12	2.05	2.45	21.6	3.18	2.54	2.60	1.96	8.60	1.86
Vanillin	18.8	21.0	21.0	22.2	29.5	21.0	42.1	12.6	13.0	29.9	123	37.0	16.8	14.7	12.2	38.7	14.4

Table 3 Continue >>>

Selected tables

COMPOUND	SH35	SH36	SH37	SH38	SH39	SH40	SH41	SH42	SH43	SH44	SH45	SH46	SH47	SH48	SH49	SH50	SH51
Ethyl acetate	65.4	121	133	156	108	156	125	82.9	66.9	138	147	140	112	164	125	114	115
Ethyl butyrate	0.179	0.149	0.178	0.304	0.168	0.269	0.255	0.171	0.162	0.244	0.178	0.207	0.200	0.274	0.229	0.144	0.204
1-Propanol	21.7	24.0	38.5	30.3	31.3	33.0	12.7	14.4	20.7	68.5	39.7	32.6	30.0	19.8	88.3	30.1	40.0
Isobutanol	113	85.9	59.5	79.0	109	100	35.8	79.8	94.0	53.5	120	144	127	69.5	60.0	109	94.6
Isoamyl acetate	0.506	1.63	1.62	2.58	0.871	2.80	1.52	1.35	1.80	3.59	1.34	2.48	2.17	5.72	1.75	3.06	3.25
n-Butanol	8.19	1.07	10.8	10.9	10.7	2.15	11.0	1.09	10.1	11.2	0.454	3.09	0.611	1.21	8.85	0.135	10.8
Isoamyl alcohol	226	206	157	211	187	226	163	235	196	171	232	249	305	262	186	249	192
Ethyl hexanoate	90.6 ^a	81.1 ^a	0.121	0.152	72.0 ^a	0.201	0.173	94.3 ^a	0.115	0.161	87.7 ^a	0.113	0.143	0.191	0.169	92.3 ^a	0.153
Hexyl acetate	4.72 ^a	7.62 ^a	12.2 ^a	9.05 ^a	5.12 ^a	10.7 ^a	13.3 ^a	8.90 ^a	16.1 ^a	12.5 ^a	5.15 ^a	11.5 ^a	5.50 ^a	15.4 ^a	13.9 ^a	13.3 ^a	12.9 ^a
Acetoin	96.6	143	33.7	58.7	66.5	28.3	67.2	117	79.8	24.2	29.3	26.5	15.5	41.5	17.0	29.2	53.0
Ethyl-D-lactate	153	115	189	258	209	300	216	312	159	275	200	349	231	258	243	179	136
1-Hexanol	0.496	0.451	0.779	0.335	0.451	0.753	1.90	0.877	0.383	0.694	1.06	2.10	1.11	0.746	3.67	1.92	1.59
Ethyl octanoate	11.3 ^a	11.1 ^a	19.1 ^a	14.0 ^a	8.38 ^a	28.7 ^a	15.3 ^a	7.21 ^a	14.9 ^a	19.4 ^a	7.13 ^a	10.3 ^a	11.5 ^a	22.2 ^a	12.4 ^a	8.27 ^a	14.8 ^a
Acetic acid	580	362	435	290	1430	130	383	743	464	427	311	374	445	322	283	246	389
Furfural	8.06	11.9	16.2	11.8	14.9	10.8	13.0	17.9	4.43	16.9	16.9	19.7	19.0	15.6	14.7	9.69	11.8
Propionic acid	12.3	3.33	6.16	3.08	6.16	2.46	4.10	27.1	9.85	7.39	8.01	9.35	10.0	7.58	8.25	5.84	7.01
Isobutyric acid	1.75	2.67	1.21	1.07	2.17	1.07	1.38	2.32	1.19	1.75	2.42	3.49	7.23	4.35	0.762	5.66	2.84
5-Methylfurfural	0.323	0.440	0.476	0.463	0.555	0.443	0.554	0.508	0.328	0.713	0.708	29.0 ^a	0.138	22.4 ^a	0.531	0.417	0.565
n-Butyric acid	1.55	2.91	3.53	3.60	3.60	2.77	5.11	1.98	1.60	3.56	3.13	3.44	2.93	3.53	3.28	2.46	3.87
Ethyl decanoate	2.46 ^a	2.20 ^a	4.76 ^a	2.96 ^a	1.87 ^a	4.80 ^a	5.93 ^a	3.75 ^a	5.29 ^a	2.31 ^a	1.74 ^a	3.57 ^a	2.23 ^a	4.13 ^a	2.43 ^a	2.16 ^a	5.47 ^a
Isovaleric acid	1.76	2.04	1.53	1.57	1.73	1.60	1.67	2.18	1.63	1.74	1.72	2.10	2.50	2.16	1.43	2.55	2.05
Diethyl succinate	12.7	9.98	5.80	12.1	8.43	12.0	10.0	10.6	8.65	9.26	7.51	12.4	8.57	12.0	10.2	11.1	9.30
n-Valeric acid	1.57	1.39	1.36	1.35	1.42	1.37	1.52	1.77	1.57	1.59	1.48	1.40	1.41	1.46	1.34	1.35	1.51
2-Phenethyl acetate	0.237	0.276	0.140	0.214	69.0 ^a	0.231	0.107	0.603	0.283	0.195	91.3 ^a	0.375	0.159	0.626	0.150	0.471	0.182
Hexanoic acid	3.04	2.67	2.29	2.18	2.10	2.73	2.86	3.77	2.87	3.13	2.65	2.80	2.75	3.13	2.26	2.70	2.43
Guaiacol	0.760	0.196	0.253	0.150	0.179	0.107	0.266	0.525	0.449	0.303	0.268	0.294	0.332	0.208	0.201	28.9 ^a	0.117
trans-oak-lactone	1.03	nd ^b	1.05	nd ^b	nd ^b	nd ^b	1.03	nd ^b	nd ^b	1.04	nd ^b	nd ^b	1.03	1.03	nd ^b	1.03	1.04
2-Phenylethyl alcohol	52.9	49.3	17.5	25.5	24.6	27.6	19.3	57.9	28.8	18.4	21.9	39.6	51.4	53.0	14.4	73.7	16.4
cis-oak-lactone	0.987	nd ^b	1.00	nd ^b	nd ^b	nd ^b	nd ^b	0.985	0.986	nd ^b	nd ^b	nd ^b	0.973	0.964	nd ^b	nd ^b	0.976
o-Cresol	0.855	0.768	0.786	0.759	0.775	0.764	0.789	0.925	0.844	0.817	0.783	0.797	0.794	0.782	0.769	0.754	0.768
Phenol	0.930	0.159	0.312	0.248	0.536	0.316	0.709	1.43	1.28	1.02	0.278	0.995	0.710	0.810	0.518	0.191	0.702
4-Ethylguaiacol	0.373	0.346	0.346	0.342	0.345	0.342	0.343	0.356	0.358	0.343	0.341	0.346	0.347	0.344	0.345	0.337	0.345
Octanoic acid	1.28	1.38	1.15	1.08	0.980	1.22	1.37	1.89	1.57	1.43	1.11	1.40	1.40	1.66	0.937	1.24	0.945
p-Cresol	0.305	0.217	0.240	0.219	0.251	0.229	0.230	0.407	0.356	0.304	0.226	0.238	0.247	0.235	0.226	0.223	0.234
Eugenol	0.773	0.542	0.571	0.543	0.561	0.523	0.546	0.678	0.634	0.584	0.585	0.624	0.634	0.566	0.567	0.485	0.541
Decanoic acid	0.631	0.672	0.662	0.635	0.628	0.647	0.694	0.822	0.846	0.737	0.652	0.713	0.707	0.704	0.623	0.717	0.642
2,6-Dimethoxy phenol	5.12	1.89	2.56	1.71	1.71	1.71	2.56	10.4	4.60	6.58	3.05	6.54	7.63	3.98	3.27	2.78	3.27
5-Hydroxymethylfurfural	11.2	1.75	2.24	1.86	2.24	1.60	1.86	8.64	8.94	3.84	3.29	2.99	3.29	2.84	3.36	2.35	2.42
Vanillin	85.1	21.3	28.4	21.3	42.5	17.0	23.0	31.2	51.0	21.2	28.2	37.6	43.4	23.5	43.4	11.8	20.1

Table 3 Continue >>>

Selected tables

COMPOUND	SH52	SH53	SH54	SH55	SH56	SH57	SH58	SH59	SH60	SH61	SH62	SH63	SH64	SH65	SH66	SH67	SH68
Ethyl acetate	157	194	145	149	107	159	83.3	120	100	162	87.8	157	146	175	165	188	173
Ethyl butyrate	0.133	0.184	0.168	0.213	0.138	0.235	0.294	0.188	0.268	0.164	0.194	0.287	0.313	0.334	0.216	0.536	0.247
1-Propanol	24.5	13.8	19.2	23.3	25.4	40.0	20.5	20.3	40.1	20.9	26.6	129	42.1	35.4	27.8	25.6	54.6
Isobutanol	115	59.7	67.2	73.5	91.3	82.1	106	156	108	94.1	98.9	56.4	59.2	68.9	88.6	88.8	82.9
Isoamyl acetate	1.69	0.992	1.63	1.08	1.09	2.00	3.37	3.47	2.40	0.902	2.29	2.12	1.76	3.10	1.60	3.18	1.31
n-Butanol	0.457	9.37	0.600	11.1	11.7	81.1 ^a	11.4	3.08	9.55	11.4	7.73	2.16	10.1	8.05	3.44	2.51	6.06
Isoamyl alcohol	237	165	189	203	174	210	249	282	208	208	196	191	158	171	224	208	193
Ethyl hexanoate	84.2 ^a	84.2 ^a	0.102	0.112	0.102	0.130	0.197	0.103	0.140	85.4 ^a	0.117	0.140	0.172	0.228	0.153	0.228	0.153
Hexyl acetate	7.09 ^a	5.07 ^a	8.56 ^a	5.44 ^a	6.58 ^a	8.83 ^a	10.6 ^a	10.0 ^a	12.2 ^a	5.06 ^a	11.2 ^a	7.36 ^a	10.2 ^a	14.1 ^a	10.6 ^a	14.6 ^a	6.19 ^a
Acetoin	11.6	37.9	84.8	34.3	62.8	21.1	31.9	61.1	18.4	55.4	18.5	61.8	37.9	38.2	66.7	67.1	12.8
Ethyl-D-lactate	183	339	171	101	161	305	188	173	246	170	204	320	233	250	498	269	300
1-Hexanol	3.04	0.357	0.927	1.42	1.54	0.914	0.632	0.114	0.365	1.75	0.448	0.798	0.537	0.458	3.57	1.20	1.48
Ethyl octanoate	4.68 ^a	10.8 ^a	9.77 ^a	11.8 ^a	9.63 ^a	10.1 ^a	20.1 ^a	13.1 ^a	18.0 ^a	7.47 ^a	14.4 ^a	15.7 ^a	15.3 ^a	33.5 ^a	9.68 ^a	27.1 ^a	14.5 ^a
Acetic acid	275	584	322	240	246	445	667	301	534	492	779	467	701	334	346	311	252
Furfural	13.6	15.4	9.15	6.93	10.6	5.60	8.55	12.8	4.62	11.7	20.6	16.5	9.23	10.5	9.71	10.7	12.3
Propionic acid	5.72	9.35	5.61	5.39	5.97	10.40	20.0	6.52	15.6	7.19	14.0	9.35	5.72	6.23	6.37	5.72	4.90
Isobutyric acid	4.31	3.78	2.30	1.11	1.54	2.45	2.60	3.51	1.46	3.75	2.31	1.13	0.268	0.438	2.47	1.58	1.99
5-Methylfurfural	0.595	0.126	0.388	0.315	0.430	72.7 ^a	0.528	0.604	0.202	0.514	91.5 ^a	0.650	0.388	0.514	0.430	0.420	0.426
n-Butyric acid	3.61	0.253	2.91	2.61	2.93	3.82	1.80	3.29	1.04	3.35	1.38	3.87	3.37	4.11	3.05	3.47	2.77
Ethyl decanoate	1.65 ^a	2.27 ^a	3.64 ^a	4.13 ^a	2.75 ^a	2.49 ^a	2.34 ^a	6.26 ^a	5.68 ^a	0.545 ^a	2.99 ^a	2.01 ^a	5.43 ^a	7.85 ^a	2.30 ^a	4.79 ^a	2.07 ^a
Isovaleric acid	1.91	2.16	2.13	1.74	1.58	1.74	1.77	2.24	1.83	2.20	1.90	1.63	1.43	1.58	2.20	1.85	1.47
Diethyl succinate	7.64	8.21	8.67	11.4	9.74	12.1	11.5	7.02	10.0	7.52	12.7	12.0	10.9	15.4	14.0	12.5	5.50
n-Valeric acid	1.56	1.58	1.37	1.413	1.40	1.48	1.60	1.52	1.99	1.46	1.61	1.58	1.48	1.39	1.51	1.28	1.30
2-Phenethyl acetate	0.407	92.0 ^a	0.318	0.114	0.120	0.137	0.228	1.56	0.163	83.8 ^a	0.220	96.6 ^a	0.145	0.345	0.241	0.156	57.2 ^a
Hexanoic acid	3.07	2.75	2.91	2.69	2.46	2.71	3.21	2.93	3.30	2.99	3.21	2.97	2.85	3.05	3.59	2.84	2.76
Guaiacol	0.162	0.333	72.8 ^a	56.7 ^a	84.7 ^a	0.333	0.306	0.135	0.290	0.158	0.344	0.123	60.4 ^a	0.145	0.127	68.2 ^a	0.152
trans-oak-lactone	nd ^b	nd ^b	1.03	nd ^b	nd ^b	1.03	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	1.03	1.03	nd ^b	nd ^b	nd ^b	1.03
2-Phenylethyl alcohol	79.8	31.0	53.8	42.0	19.6	18.4	33.1	69.8	15.8	42.8	34.4	20.7	15.1	29.9	57.5	25.2	15.4
cis-oak-lactone	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	0.970	0.996	nd ^b	0.992	nd ^b	0.985	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	0.968
o-Cresol	0.790	0.804	0.755	0.748	0.769	0.803	0.866	0.792	0.838	0.770	0.821	0.794	0.770	0.765	0.762	0.753	0.764
Phenol	0.805	0.668	0.255	0.213	0.387	0.614	0.947	0.801	0.792	0.366	0.599	0.222	0.211	0.656	0.383	0.874	0.501
4-Ethylguaiacol	0.344	0.349	0.338	0.338	0.339	0.341	0.356	0.342	0.353	0.341	0.359	0.335	0.338	0.340	0.341	0.340	0.341
Octanoic acid	1.25	1.27	1.61	1.25	1.10	1.28	1.42	1.73	1.49	1.19	1.32	1.14	1.58	1.31	1.82	1.45	1.30
p-Cresol	0.257	0.260	0.225	0.223	0.231	0.264	0.293	0.260	0.268	0.228	0.277	0.236	0.231	0.222	0.230	0.207	0.215
Eugenol	0.517	0.558	0.496	0.483	0.509	0.608	0.619	0.512	0.581	0.535	0.606	0.524	0.497	0.501	0.504	0.515	0.553
Decanoic acid	0.670	0.691	0.744	0.718	0.716	0.705	0.687	0.813	0.763	0.711	0.668	0.707	0.791	0.721	0.685	0.713	0.673
2,6-Dimethoxy phenol	6.11	8.33	3.65	3.26	5.72	7.05	6.11	6.11	2.24	4.16	7.63	3.52	4.58	5.09	3.74	2.84	8.92
5-Hydroxymethylfurfural	2.39	4.22	2.39	2.32	2.70	3.74	11.8	3.58	8.31	2.42	7.16	3.43	2.53	2.99	2.53	2.70	2.12
Vanillin	17.6	43.4	12.0	11.5	18.2	43.4	29.7	21.7	22.6	16.1	24.5	17.6	14.8	12.5	13.1	12.5	8.42

^a Measured in µg/L. ^b nd: not detected. For details of the wines refer to **Table 1**. For analytical conditions please refer to **chapter 6, section 6.2**.

Selected tables

Table 4. Quantitative (mg/l) data of 69 young Cabernet Sauvignon wines (CS1 – CS69) from vintage 2005.

COMPOUND	CS1	CS2	CS3	CS4	CS5	CS6	CS7	CS8	CS9	CS10	CS11	CS12	CS13	CS14	CS15	CS16	CS17
Ethyl acetate	149	105	132	123	113	112	107	86.2	101	125	72.9	143	137	103	118	85.3	142
Ethyl butyrate	0.205	0.205	0.292	0.201	0.220	0.265	0.142	0.207	0.208	0.266	0.124	0.280	0.154	0.137	0.300	0.177	0.153
1-Propanol	17.2	49.6	33.4	38.1	32.1	16.7	40.3	25.7	24.7	26.4	25.8	8.36	63.0	22.2	31.8	27.4	15.2
Isobutanol	85.2	149	75.7	115	112	69.8	78.2	107	202	113	90.7	64.7	129	88.9	61.8	84.4	54.2
Isoamyl acetate	1.72	2.72	0.615	1.75	1.27	0.495	1.01	3.18	0.890	2.06	0.868	3.16	0.895	1.09	0.923	1.47	0.777
n-Butanol	5.62	7.34	6.05	6.68	6.72	8.47	1.14	9.99	10.2	9.16	4.25	11.1	9.83	2.40	2.72	4.25	3.39
Isoamyl alcohol	198	225	215	241	254	257	250	233	314	275	240	200	199	162	214	247	195
Ethyl hexanoate	1.17	0.111	0.228	0.129	0.145	0.277	90.5 ^a	0.146	0.103	0.176	64.2 ^a	0.179	94.2 ^a	41.2 ^a	1.26	0.117	71.1 ^a
Hexyl acetate	6.82 ^a	6.53 ^a	5.68 ^a	7.23 ^a	5.28 ^a	4.09 ^a	0.005	13.2 ^a	4.32 ^a	5.25 ^a	4.41 ^a	10.5 ^a	4.69 ^a	4.83 ^a	4.38 ^a	5.51 ^a	6.90 ^a
Acetoin	7.47	45.1	35.3	54.6	20.5	10.7	62.7	61.0	12.3	75.0	69.6	36.9	6.16	50.2	15.7	67.8	7.13
Ethyl-D-lactate	392	203	411	283	249	353	253.5	288	144	257	231	230	179	161	207	227	206
1-Hexanol	0.225	14.3 ^a	1.02	1.18	14.2	1.82	0.307	0.361	0.757	74.1 ^a	0.316	0.482	0.274	0.878	0.335	12.9	0.154
Ethyl octanoate	16.2 ^a	13.6 ^a	25.6 ^a	9.82 ^a	17.1 ^a	25.7 ^a	0.008	18.5 ^a	8.44 ^a	18.3 ^a	5.22 ^a	24.9 ^a	12.5 ^a	6.56 ^a	19.1 ^a	16.2 ^a	7.63 ^a
Acetic acid	404	367	807	448	538	425	405.6	1854	145	734	475	423	367	336	384	425	538
Furfural	14.6	14.0	4.30	21.0	19.1	19.4	9.717	8.26	2.47	18.7	16.2	15.2	12.0	11.5	11.7	19.9	16.5
Propionic acid	5.27	3.96	8.79	6.59	4.40	6.59	4.307	16.1	5.49	6.59	5.17	4.84	3.82	3.66	4.19	4.88	5.86
Isobutyric acid	1.04	2.83	4.51	5.19	2.77	1.04	2.035	3.32	2.97	3.03	3.86	0.707	1.02	0.725	1.17	1.86	3.14
5-Methylfurfural	0.588	0.618	0.459	0.270	58.6 ^a	35.5 ^a	0.417	0.455	0.248	0.169	24.9 ^a	29.7 ^a	0.568	0.362	0.594	83.6 ^a	27.6 ^a
n-Butyric acid	4.73	4.02	2.12	0.275	4.26	4.61	2.749	1.25	0.207	4.95	5.06	4.57	3.12	2.10	4.29	4.19	3.09
Ethyl decanoate	9.16 ^a	5.29 ^a	6.74 ^a	1.72 ^a	7.80 ^a	5.78 ^a	0.002	7.26 ^a	3.10 ^a	2.19 ^c	1.54 ^a	5.88 ^a	3.94 ^a	1.60 ^a	6.65 ^a	3.34 ^a	4.33 ^a
Isovaleric acid	1.75	2.16	2.07	2.17	2.11	2.44	2.26	2.21	2.28	2.46	1.87	1.90	1.73	1.54	2.00	1.51	2.11
Diethyl succinate	16.2	10.2	16.4	10.5	10.8	24.1	11.22	14.6	8.21	10.2	10.8	17.4	8.24	10.9	12.0	10.2	9.78
n-Valeric acid	1.41	1.70	1.83	1.73	1.51	1.62	1.269	1.93	1.54	1.54	1.46	1.51	1.37	1.24	1.38	1.77	1.56
2-Phenethyl acetate	0.223	0.215	0.122	36.8 ^a	0.134	0.115	0.131	0.505	96.9 ^a	0.193	0.162	0.308	0.130	0.297	71.6 ^a	0.162	82.9 ^a
Hexanoic acid	3.12	2.60	3.96	1.65	3.08	2.73	2.464	3.58	2.95	3.51	2.32	3.17	2.33	1.98	2.80	3.65	3.02
Guaiacol	0.182	0.183	0.517	0.554	0.400	0.388	0.048	0.782	0.462	0.415	0.176	0.197	0.151	67.4 ^a	0.191	0.504	0.447
trans-oak-lactone	nd ^b	nd ^b	1.03	1.04	nd ^b	nd ^b	nd ^b	nd ^b	1.04	nd ^b	nd ^b	nd ^b	nd ^b	1.03	1.04	1.04	1.04
2-Phenylethyl alcohol	35.6	29.2	31.9	24.7	24.3	37.1	73.03	52.5	90.1	72.2	67.0	61.8	26.3	30.5	54.9	58.9	58.1
cis-oak-lactone	0.971	0.970	0.995	0.997	0.974	0.970	0.971	0.976	0.972	0.969	nd ^b	0.973	0.967	0.967	0.997	0.973	0.982
o-Cresol	0.780	0.776	0.830	0.807	0.799	6.24	0.75	0.873	0.822	0.810	0.783	0.787	0.759	0.757	0.765	0.813	0.790
Phenol	61.2 ^a	0.754	1.37	0.608	0.599	0.890	0.897	3.26	0.635	0.759	0.405	0.323	15.6 ^a	0.843	0.094	0.552	0.687
4-Ethylguaiacol	0.346	0.345	0.357	0.364	0.359	0.358	0.339	0.370	0.359	0.358	0.345	0.347	0.345	0.340	0.348	0.359	0.355
Octanoic acid	1.76	1.15	1.36	1.64	1.36	1.59	1.244	1.47	1.36	1.85	1.10	1.32	1.14	1.21	1.25	1.70	1.52
p-Cresol	0.234	0.231	0.267	1.35	0.274	0.262	0.212	0.323	0.278	0.290	0.253	0.232	0.231	0.217	0.224	0.251	0.236
Eugenol	0.545	0.552	0.691	0.730	0.700	0.609	0.525	0.793	0.654	0.620	0.536	0.527	0.557	0.515	0.545	0.667	0.679
Decanoic acid	0.682	0.668	0.634	0.653	0.665	0.679	0.731	0.753	0.655	0.722	0.634	0.648	0.648	0.749	0.705	0.851	0.838
2,6-Dimethoxy phenol	2.54	3.27	3.63	3.23	6.41	2.31	2.035	29.1	3.24	14.4	2.42	3.53	2.54	3.27	2.00	6.89	3.45
5-Hydroxymethylfurfural	0.289	0.267	0.444	0.422	0.29	0.333	0.142	3.29	0.273	0.289	0.222	0.333	0.222	0.178	0.222	0.311	0.267
Vanillin	13.8	17.12	32.9	32.9	23.1	19.8	10.9	94.8	39.9	21.4	11.0	21.7	17.1	14.8	15.1	36.1	25.0

Table 4 Continue >>>

Selected tables

COMPOUND	CS18	CS19	CS20	CS21	CS22	CS23	CS24	CS25	CS26	CS27	CS28	CS29	CS30	CS31	CS32	CS33	CS34
Ethyl acetate	124	101	72.9	168	93.5	96.1	62.6	179	120	102	129	57.5	91.6	78.6	68.2	90.8	140
Ethyl butyrate	0.197	43.6 ^a	0.187	0.249	0.198	0.133	0.142	0.143	0.218	0.124	0.293	0.155	0.225	0.189	0.155	0.183	0.213
1-Propanol	23.1	0.686	8.46	7.39	9.50	11.9	8.96	22.0	29.0	46.1	34.7	7.12	35.4	18.5	10.3	42.3	21.4
Isobutanol	111	104	92.5	60.3	53.7	58.0	12.3	113	135	95.7	132	32.9	101	69.6	98.3	0.175	58.5
Isoamyl acetate	0.719	3.36	41.1 ^a	2.19	3.53	40.8 ^a	1.69	0.692	0.598	1.24	2.35	3.33	0.943	35.1 ^a	24.7 ^a	2.84	2.69
n-Butanol	2.52	6.19	7.80	2.21	2.72	8.30	6.37	6.04	9.08	11.1	8.52	10.4	4.55	8.17	9.24	7.04	9.69
Isoamyl alcohol	241	282	254	249	243	204	255	193	263	587	249	279	250	269	235	235	213
Ethyl hexanoate	0.104	0.116	0.111	0.161	0.170	91.9 ^a	77.3 ^a	94.5 ^a	0.102	0.114	0.180	0.125	94.4 ^a	0.102	76.9 ^a	99.5 ^a	0.194
Hexyl acetate	3.61 ^a	10.3 ^a	6.47 ^a	7.53 ^a	14.6 ^a	13.6 ^a	6.51 ^a	3.82 ^a	3.48 ^a	4.76 ^a	7.80 ^a	8.19 ^a	4.34 ^a	5.25 ^a	6.28 ^a	9.27 ^a	17.5 ^a
Acetoin	15.3	42.7	110	3.99	80.7	15.5	159	98.7	49.0	55.0	61.8	42.3	56.4	32.2	0.724	14.4	115
Ethyl-D-lactate	210	232	154	173	173	249	199	6.14	235	5.19	270	253	259	296	124	280	78.4
1-Hexanol	0.341	0.468	1.05	0.791	0.888	0.117	1.01	0.460	0.603	0.545	1.11	0.569	0.839	0.261	0.304	0.436	1.75
Ethyl octanoate	6.43 ^a	7.15 ^a	12.6 ^a	17.6 ^a	20.4 ^a	9.98 ^a	7.52 ^a	15.8 ^a	8.04 ^a	7.44 ^a	17.2 ^a	16.2 ^a	9.45 ^a	7.66 ^a	10.4 ^a	11.5 ^a	24.5 ^a
Acetic acid	605	450	601	402	442	225	902	458	392	361	451	309	301	475	301	601	506
Furfural	15.7	9.70	1.31	16.0	1.91	11.2	6.54	15.7	10.7	16.2	18.9	1.23	18.3	21.3	17.2	21.2	1.67
Propionic acid	6.59	6.64	13.0	5.64	17.0	18.7	16.9	7.36	5.64	7.36	8.90	3.67	5.64	10.6	8.46	11.3	6.51
Isobutyric acid	4.28	0.940	5.71	0.440	4.09	2.77	3.21	1.95	1.30	0.604	1.63	2.36	2.13	2.98	1.17	1.70	5.45
5-Methylfurfural	0.115	0.434	0.628	0.662	0.490	0.691	0.191	62.0 ^a	0.403	0.700	0.649	0.210	0.167	0.189	0.597	0.684	0.397
n-Butyric acid	4.85	3.08	4.32	3.81	1.83	0.656	0.661	4.28	2.88	3.33	23.8 ^a	0.196	3.49	1.40	4.31	3.64	4.02
Ethyl decanoate	1.45 ^a	4.53 ^a	5.57 ^a	8.80 ^a	8.22 ^a	3.68 ^a	2.95 ^a	9.88 ^a	5.26 ^a	5.00 ^a	4.59 ^a	4.42 ^a	2.23 ^a	4.63 ^a	5.68 ^a	3.38 ^a	7.83 ^a
Isovaleric acid	2.28	1.96	2.03	1.61	2.25	2.13	3.15	1.91	2.13	1.77	2.25	2.93	2.262	2.76	1.66	2.29	2.24
Diethyl succinate	8.75	5.23	9.87	9.53	15.4	9.98	12.3	11.9	7.12	19.3	13.4	15.4	11.6	13.0	8.44	14.2	13.6
n-Valeric acid	1.58	1.31	1.72	1.30	1.65	1.60	1.66	1.59	1.31	1.38	1.73	1.49	1.51	1.83	1.39	1.41	1.72
2-Phenethyl acetate	89.1 ^a	0.387	0.337	0.546	0.842	0.390	0.342	0.104	63.5 ^a	0.218	0.351	0.962	0.121	0.415	0.307	0.597	0.143
Hexanoic acid	2.85	2.32	3.49	2.87	3.61	2.96	3.34	2.58	2.58	2.76	3.02	3.06	3.02	4.02	2.39	2.65	4.16
Guaiacol	0.330	69.9 ^a	0.222	0.269	0.435	0.241	0.579	0.204	70.7 ^a	0.275	0.281	0.453	0.205	0.218	78.7 ^a	0.472	0.610
trans-oak-lactone	1.04	1.03	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	1.03	1.04	1.03	nd ^b	nd ^b	1.04	nd ^b	nd ^b	1.04	1.04
2-Phenylethyl alcohol	59.7	25.4	66.5	26.1	85.1	57.9	44.8	22.7	53.9	51.7	49.4	155	56.7	132	42.0	78.8	52.2
cis-oak-lactone	0.970	0.974	nd ^b	0.971	0.982	nd ^b	0.975	0.971	0.970	0.980	nd ^b	0.973	0.974	nd ^b	nd ^b	0.970	1.01
o-Cresol	0.797	0.745	0.852	0.770	0.859	0.904	0.857	0.789	0.758	0.785	0.819	0.824	0.800	0.827	0.802	0.822	0.830
Phenol	0.426	41.0 ^a	0.702	0.160	0.714	1.24	0.956	0.325	0.083	0.448	0.804	0.648	0.978	0.785	0.334	1.04	1.10
4-Ethylguaiacol	0.349	0.339	0.347	0.349	0.358	0.350	0.359	0.349	0.340	0.351	0.352	0.362	0.347	0.349	0.341	0.363	0.364
Octanoic acid	1.71	1.03	1.54	1.46	2.33	1.41	1.58	1.43	1.40	1.54	1.56	1.24	1.81	2.68	1.18	1.40	2.46
p-Cresol	1.44	0.218	0.246	0.244	0.303	0.323	0.275	0.267	0.233	0.267	0.264	0.241	0.285	0.291	0.227	0.269	0.305
Eugenol	0.597	0.530	0.577	0.610	0.648	0.585	0.677	0.558	0.503	0.589	0.575	0.606	0.571	0.546	0.531	0.678	0.738
Decanoic acid	0.829	0.611	0.714	0.695	0.675	0.649	0.721	0.671	0.732	0.762	0.743	0.633	0.882	0.955	0.655	0.769	0.677
2,6-Dimethoxy phenol	3.49	6.46	8.08	1.95	4.25	5.07	12.9	6.46	5.46	5.61	15.5	5.93	5.75	11.7	6.46	13.5	9.76
5-Hydroxymethylfurfural	0.222	1.29	2.57	2.61	3.43	2.26	5.15	6.46	1.29	2.57	2.86	96.2 ^a	2.57	3.43	2.57	3.43	3.04
Vanillin	16.5	14.9	11.2	18.7	14.9	14.9	44.7	17.9	11.2	22.3	21.4	25.5	15.4	8.93	11.2	57.8	70.2

Table 4 Continue >>>

Selected tables

COMPOUND	CS35	CS36	CS37	CS38	CS39	CS40	CS41	CS42	CS43	CS44	CS45	CS46	CS47	CS48	CS49	CS50	CS51
Ethyl acetate	106	100	94.9	4.81	89.1	112	117	93.3	123	134	61.6	115	112	128	89.3	99.8	125
Ethyl butyrate	0.141	0.233	0.165	40.0 ^a	0.225	0.120	0.216	5.98	7.77	0.183	0.217	0.187	0.149	0.271	0.165	0.207	0.267
1-Propanol	22.6	27.2	23.5	14.4	40.4	47.4	48.4	27.9	27.9	18.6	24.7	18.6	28.3	83.7	36.4	11.3	52.3
Isobutanol	94.0	89.8	78.3	38.1	71.7	159	103	90.3	125	102	67.5	83.9	98.1	76.0	86.9	33.1	76.1
Isoamyl acetate	0.633	3.50	1.52	41.4 ^a	1.60	2.33	0.964	1.23	2.50	1.36	2.64	0.832	1.29	2.72	2.24	1.96	35.7 ^a
n-Butanol	7.06	8.20	7.95	4.85	2.31	0.333	10.0	7.36	7.94	9.04	7.74	9.25	2.21	2.41	10.5	1.52	8.34
Isoamyl alcohol	195	208	250	162	209	257	206	175	284	224	253	278	236	168	226	170	196
Ethyl hexanoate	91.1 ^a	0.191	0.123	8.76 ^a	0.251	63.8 ^a	0.207	0.154	0.127	0.115	0.163	0.186	73.0 ^a	91.8 ^a	97.2 ^a	0.218	0.142
Hexyl acetate	4.33 ^a	13.9 ^a	6.03 ^a	3.35 ^a	0.140	6.16 ^a	4.18 ^a	7.19 ^a	8.77 ^a	4.88 ^a	8.76 ^a	4.16 ^a	4.97 ^a	10.2 ^a	9.59 ^a	6.45 ^a	7.28 ^a
Acetoin	60.5	80.5	20.4	58.5	62.2	17.0	32.3	40.6	61.2	55.7	18.8	9.69	35.2	44.7	38.9	28.3	21.4
Ethyl-D-lactate	130	185	299	187	266	72.7	200	166	374	179	281	318	194	197	252	183	259
1-Hexanol	7.18	1.26	1.78	0.645	2.59	1.41	1.63	0.862	2.37	0.334	0.522	1.26	1.46	73.4 ^a	0.534	2.08	0.178
Ethyl octanoate	10.8 ^a	27.6 ^a	14.1 ^a	5.24 ^a	24.6 ^a	3.48 ^a	20.3 ^a	24.1 ^a	11.0 ^a	17.3 ^a	23.8 ^a	20.4 ^a	6.95 ^a	13.7 ^a	8.91 ^a	31.9 ^a	15.0 ^a
Acetic acid	376	1502	251	96.8	676	721	706	434	1051	736	751	509	601	631	230	148	190
Furfural	10.8	5.45	2.71	15.6	14.5	15.8	12.6	1.16	17.1	13.1	20.0	19.0	15.8	10.1	16.3	16.6	1.23
Propionic acid	2.50	7.51	4.13	0.345	3.76	3.42	3.58	2.93	4.70	3.95	5.37	5.64	3.76	2.56	27.1	6.02	7.72
Isobutyric acid	0.715	1.62	1.39	4.06	1.07	6.09	0.885	1.94	4.08	2.45	5.17	3.81	5.93	0.470	1.84	2.93	5.55
5-Methylfurfural	0.524	0.251	40.2 ^a	33.5 ^a	0.600	0.116	0.574	26.1 ^a	52.3 ^a	0.713	0.247	0.225	0.107	0.312	0.217	0.153	11.0 ^a
n-Butyric acid	3.46	1.69	4.54	5.11	3.81	4.14	3.37	5.03	3.58	0.216	0.477	4.92	3.25	2.44	1.76	3.86	0.753
Ethyl decanoate	5.71 ^a	8.10 ^a	5.37 ^a	0.899 ^a	8.87 ^a	1.32 ^a	5.66 ^a	8.45 ^a	5.79 ^a	5.96 ^a	5.90 ^a	9.14 ^a	3.18 ^a	3.36 ^a	2.82 ^a	12.3 ^a	2.90 ^a
Isovaleric acid	1.79	2.12	2.42	2.21	1.90	2.16	1.82	1.95	2.88	2.28	1.95	2.80	2.09	1.51	1.71	1.83	2.65
Diethyl succinate	8.83	20.7	5.74	12.6	12.8	6.61	14.5	20.4	12.8	14.4	6.85	21.7	9.44	10.3	9.25	14.6	12.7
n-Valeric acid	1.37	1.63	1.56	1.48	1.47	1.57	1.41	1.67	1.45	1.51	1.74	1.64	1.43	1.35	1.68	1.53	2.66
2-Phenethyl acetate	0.129	0.371	0.246	70.4 ^a	24.1 ^a	0.397	0.108	0.142	0.960	0.178	0.297	0.209	0.517	0.177	0.247	0.120	97.1 ^a
Hexanoic acid	2.66	3.75	3.68	2.71	3.48	2.52	2.98	3.35	3.55	2.82	4.25	4.56	2.45	2.19	2.81	4.90	4.19
Guaiacol	0.115	0.680	0.526	0.391	0.262	0.380	0.165	0.388	0.309	0.163	0.158	0.326	0.174	51.4 ^a	0.248	0.162	0.151
trans-oak-lactone	nd ^b	1.03	1.04	nd ^b	1.03	nd ^b	1.04	nd ^b	nd ^b	1.03	nd ^b	nd ^b	1.04	1.04	nd ^b	nd ^b	nd ^b
2-Phenylethyl alcohol	29.6	40.6	42.3	19.5	41.8	62.6	27.3	41.7	126	61.4	52.3	168	73.7	17.1	30.4	21.0	26.7
cis-oak-lactone	0.969	0.972	1.01	0.973	0.979	0.969	1.03	0.968	0.975	0.968	nd ^b	0.974	0.981	nd ^b	nd ^b	0.973	nd ^b
o-Cresol	0.744	0.833	0.821	0.785	0.775	0.778	0.760	0.832	0.796	0.777	0.809	0.814	0.793	0.749	0.900	0.785	0.826
Phenol	0.812	1.13	0.312	0.458	0.183	0.505	34.3 ^a	0.907	0.506	0.297	0.422	1.19	0.250	0.881	0.795	0.352	0.581
4-Ethylguaiacol	0.343	0.370	0.359	0.345	0.352	0.355	0.346	0.358	0.353	0.346	0.345	0.355	0.348	0.340	0.349	0.345	0.348
Octanoic acid	1.38	1.73	1.61	1.05	1.40	1.06	1.40	1.55	0.683	1.43	2.29	1.78	1.46	1.03	1.30	2.33	1.53
p-Cresol	0.226	0.316	0.248	0.234	0.245	0.261	0.234	0.289	0.260	0.237	0.252	0.268	0.305	0.217	0.272	0.242	0.234
Eugenol	0.528	0.761	0.723	0.551	0.594	0.644	0.575	0.611	0.550	0.501	0.554	0.613	0.547	0.529	0.576	0.540	0.560
Decanoic acid	0.661	0.791	0.672	0.637	0.783	0.663	0.799	0.781	0.810	0.693	0.755	0.738	0.874	0.661	0.746	0.700	0.818
2,6-Dimethoxy phenol	5.70	8.14	6.18	6.92	6.92	6.92	6.92	12.8	5.56	7.32	5.42	7.32	3.52	4.88	4.34	4.14	3.86
5-Hydroxymethylfurfural	2.79	6.20	0.120	2.91	3.04	5.39	2.97	5.45	3.10	3.04	3.87	3.16	4.40	1.86	6.52	1.15	1.15
Vanillin	37.3	83.0	61.3	29.0	41.5	39.0	34.9	10.6	41.5	40.7	23.7	37.3	24.9	20.8	9.02	9.02	5.41

Table 4 Continue >>>

Selected tables

COMPOUND	CS52	CS53	CS54	CS55	CS56	CS57	CS58	CS59	CS60	CS61	CS62	CS63	CS64	CS65	CS66	CS67	CS68	CS69
Ethyl acetate	103	123	128	110	116	71.4	58.5	114	82.9	79.0	136	85.8	90.1	83.9	88.6	123	120	93.8
Ethyl butyrate	0.157	0.173	0.198	0.229	7.42	0.261	0.163	0.196	0.217	0.250	0.304	0.240	0.163	0.258	0.283	0.231	0.401	0.173
1-Propanol	51.2	30.7	37.4	62.0	23.8	18.3	8.86	27.9	38.3	58.8	20.3	22.2	33.6	35.1	28.3	25.4	35.5	34.3
Isobutanol	94.2	106	97.6	64.2	102	29.4	16.7	108	79.6	57.3	80.0	82.6	90.3	37.3	80.8	66.3	73.1	35.2
Isoamyl acetate	1.78	0.858	1.27	4.57	1.74	4.44	45.8 ^a	1.79	27.4 ^a	33.4 ^a	3.84	29.5 ^a	2.07	24.2 ^a	33.1 ^a	1.99	2.04	0.873
n-Butanol	5.78	8.22	6.67	8.78	9.45	7.29	7.95	8.66	10.3	0.851	7.43	7.49	4.37	10.5	7.14	1.39	1.46	11.4
Isoamyl alcohol	207	257	222	195	216	269	216	277	233	191	186	241	232	224	208	162	182	199
Ethyl hexanoate	0.197	0.124	0.129	1.15	1.27	0.190	0.135	0.132	0.135	0.155	0.267	0.175	0.165	0.155	0.202	0.194	0.244	0.140
Hexyl acetate	11.3 ^a	5.60 ^a	5.18 ^a	17.4 ^a	6.54 ^a	16.5 ^a	8.08 ^a	6.46 ^a	6.75 ^a	5.74 ^a	12.0 ^a	7.89 ^a	7.20 ^a	6.94 ^a	9.99 ^a	9.24 ^a	7.72 ^a	5.12 ^a
Acetoin	44.2	20.3	56.2	11.4	41.2	8.64	15.7	21.6	60.6	61.0	49.9	35.9	39.7	17.4	25.2	63.4	67.5	38.8
Ethyl-D-lactate	328	157	271	245	268	284	169	201	264	166	240	209	215	238	252	141	161	370
1-Hexanol	2.30	1.25	0.955	0.702	0.815	0.701	1.45	1.34	0.844	0.328	0.520	0.412	1.14	0.417	0.666	88.9 ^a	0.794	1.35
Ethyl octanoate	21.0 ^a	10.9 ^a	14.0 ^a	26.3 ^a	23.5 ^a	20.5 ^a	15.0 ^a	13.7 ^a	15.0 ^a	18.6 ^a	35.5 ^a	21.2 ^a	19.6 ^a	17.9 ^a	26.6 ^a	26.1 ^a	29.3 ^a	14.1 ^a
Acetic acid	148	138	98.7	217	94.7	199	1067	197	527	452	406	430	361	219	165	442	352	430
Furfural	14.7	14.5	14.2	20.1	11.0	18.0	17.7	4.34	8.55	1.66	10.7	1.41	11.7	17.5	1.68	12.0	16.7	16.4
Propionic acid	4.52	4.02	4.02	6.02	4.12	9.56	4.19	10.0	18.1	8.44	3.76	8.40	6.60	5.58	9.94	5.08	6.77	7.69
Isobutyric acid	1.31	1.45	1.67	1.35	0.570	5.35	3.45	1.71	0.772	0.960	0.930	1.68	2.25	0.908	6.10	14.8 ^a	0.713	1.08
5-Methylfurfural	0.702	75.6 ^a	11.8 ^a	0.101	0.609	0.462	0.444	0.250	0.239	0.184	0.543	0.107	0.603	0.632	0.64	0.434	0.519	0.674
n-Butyric acid	4.72	4.03	2.35	4.56	3.52	58.7 ^a	4.82	0.686	1.62	1.31	3.86	0.974	3.39	4.81 ^a	0.429	2.85	4.33	5.08
Ethyl decanoate	5.94 ^a	7.27 ^a	3.63 ^a	8.38 ^a	8.01 ^a	3.66 ^a	3.79 ^a	5.99 ^a	3.12 ^a	3.55 ^a	8.70 ^a	3.80 ^a	7.25 ^a	7.49 ^a	6.99 ^a	6.11 ^a	5.84 ^a	3.48 ^a
Isovaleric acid	1.86	1.98	1.88	1.89	1.70	2.15	2.23	2.29	1.71	1.68	1.56	2.00	2.08	1.71	1.69	1.38	1.64	1.67
Diethyl succinate	12.5	5.81	5.14	14.3	14.5	15.8	17.2	12.1	13.5	9.83	16.8	12.4	20.1	10.2	13.9	10.7	7.62	14.3
n-Valeric acid	1.43	1.43	1.31	1.61	1.47	1.90	1.94	1.52	1.49	1.46	1.41	1.60	1.36	1.49	1.84	1.48	1.40	1.46
2-Phenethyl acetate	0.250	0.144	0.192	0.442	0.144	0.543	0.210	0.208	0.160	91.8 ^a	0.178	0.307	0.301	0.152	0.167	0.187	0.119	0.148
Hexanoic acid	3.56	2.87	2.46	3.17	2.76	4.37	3.98	3.42	2.49	2.53	2.92	3.36	2.89	2.66	4.16	2.67	2.84	3.35
Guaiacol	0.171	0.180	0.136	0.247	0.104	0.128	13.4 ^a	0.581	0.303	0.196	30.4 ^a	1.36	0.176	0.134	0.181	57.2 ^a	0.276	0.232
trans-oak-lactone	1.03	1.04	nd ^b	1.04	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	1.03	nd ^b	1.04	nd ^b	nd ^b	1.04	nd ^b	nd ^b
2-Phenylethyl alcohol	29.9	46.0	25.0	32.9	19.7	51.6	62.6	88.7	48.0	28.4	18.3	57.5	48.4	34.2	43.5	26.2	18.1	21.3
cis-oak-lactone	0.994	0.987	0.968	0.991	0.969	nd ^b	nd ^b	0.976	nd ^b	nd ^b	0.981	nd ^b	0.975	nd ^b	nd ^b	0.969	0.969	0.972
o-Cresol	0.773	0.768	0.763	0.805	0.754	0.823	0.832	0.851	0.864	0.826	0.748	0.825	0.762	0.804	0.850	0.758	0.795	0.775
Phenol	0.152	56.0 ^a	82.2 ^a	0.362	69.9 ^a	1.05	0.859	0.731	0.657	0.471	0.897	0.488	20.7 ^a	0.498	0.859	0.808	0.379	0.296
4-Ethylguaiacol	0.347	0.347	0.347	0.350	0.344	0.341	0.337	0.366	0.353	0.346	0.342	0.396	0.348	0.344	0.347	0.342	0.350	0.348
Octanoic acid	1.51	1.29	1.18	1.43	1.17	2.44	2.59	1.44	1.15	1.17	1.36	1.75	1.31	1.27	1.58	1.20	1.28	1.60
p-Cresol	0.277	0.259	0.833	0.284	0.241	0.240	0.275	0.274	0.291	0.254	0.224	0.261	0.239	0.233	0.246	0.214	0.241	0.224
Eugenol	0.539	0.538	0.546	0.593	0.522	0.538	0.503	0.630	0.581	0.561	0.491	0.542	0.540	0.534	0.548	0.509	0.564	0.566
Decanoic acid	0.636	0.660	0.626	0.684	0.648	0.982	1.18	0.682	0.638	0.641	0.639	0.695	0.802	0.663	0.687	0.747	0.701	0.684
2,6-Dimethoxy phenol	2.48	2.76	2.76	2.76	4.42	3.64	3.07	5.52	3.57	2.67	6.51	8.61	5.17	2.98	3.86	4.31	5.17	6.80
5-Hydroxymethylfurfural	1.05	1.53	1.92	2.00	1.07	1.15	1.28	2.30	6.54	2.23	3.10	2.71	1.72	1.18	1.28	1.61	1.72	2.45
Vanillin	8.46	10.8	10.4	11.8	10.8	9.02	8.73	27.1	12.9	6.29	21.6	13.1	14.9	5.41	6.76	14.0	14.9	16.0

^a Measured in µg/L. ^b nd: not detected. For details of the wines refer to **Table 1**. For analytical conditions refer to **chapter 6, section 6.2**.

Selected tables

Table 5. Quantitative (mg/l) data of 62 young Merlot wines (M1 – M62) from vintage 2005.

COMPOUND	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12	M13	M14	M15	M16	M17
Ethyl acetate	65.6	154	88.3	176	159	143	107	172	143	88.2	184	86.2	131	86.6	177	147	139
Ethyl butyrate	0.174	0.324	0.257	0.267	0.282	0.339	0.320	0.348	0.226	0.201	0.257	0.222	0.202	0.211	0.207	0.266	0.243
1-Propanol	32.9	84.5	69.3	27.5	15.3	18.1	38.3	46.2	43.0	25.0	34.3	32.6	38.9	17.5	21.8	27.8	24.1
Isobutanol	77.8	101	80.2	61.6	57.5	78.2	52.4	104	97.5	70.2	79.9	65.3	99.1	84.6	74.3	84.5	65.5
Isoamyl acetate	2.52	2.36	2.38	1.80	4.37	1.04	3.37	1.07	2.14	2.41	0.589	2.59	1.45	2.41	1.45	1.22	2.27
n-Butanol	0.744	0.459	10.3	0.209	3.26	1.48	11.2	9.63	11.5	10.8	8.50	11.5	10.9	10.3	9.73	9.49	8.75
Isoamyl alcohol	312	277	187	212	245	225	168	234	248	213	198	242	224	224	225	230	175
Ethyl hexanoate	0.101	0.250	0.139	0.168	0.197	0.264	0.213	0.150	0.144	85.0 ^a	0.199	95.0 ^a	0.153	0.180	0.139	0.216	0.174
Hexyl acetate	7.47 ^a	4.77 ^a	6.91 ^a	9.52 ^a	11.8 ^a	5.58 ^a	8.33 ^a	4.26 ^a	7.40 ^a	4.50 ^a	3.62 ^a	5.93 ^a	6.17 ^a	5.64 ^a	7.39 ^a	5.32 ^a	11.7 ^a
Acetoin	13.4	51.0	5.81	58.06	92.7	42.0	147	68.5	19.6	33.5	55.5	41.3	18.7	65.5	26.3	0.443	105
Ethyl-D-lactate	230	239	214	432.6	294	236	147	240	239	166	274	238	161	120	251	197	189
1-Hexanol	0.329	0.278	0.566	0.93	1.06	0.191	0.895	0.713	0.865	0.798	20.5 ^a	0.217	0.784	0.952	1.54	0.976	0.593
Ethyl octanoate	8.45 ^a	27.1 ^a	14.2 ^a	0.021	23.3 ^a	30.2 ^a	27.7 ^a	20.0 ^a	16.1 ^a	9.50 ^a	22.7 ^a	8.34 ^a	17.2 ^a	26.6 ^a	15.2 ^a	22.1 ^a	29.6 ^a
Acetic acid	453	210	252	246.4	883	846	1426	199	363	152	314	378	189	272	204	197	350
Furfural	0.766	5.26	17.1	2.741	10.6	4.20	26.0	19.2	20.1	20.3	17.2	9.81	0.835	17.8	0.144	17.4	88.8 ^a
Propionic acid	17.2	13.8	7.55	12.07	22.7	12.2	44.4	6.83	7.73	0.900	2.49	14.4	6.05	6.83	12.1	3.66	11.5
Isobutyric acid	2.08	3.56	0.959	2.505	2.56	3.12	1.56	4.38	2.68	1.47	2.43	1.77	2.23	0.882	2.34	1.62	2.92
5-Methylfurfural	0.188	0.304	0.698	0.437	0.387	0.195	0.711	0.153	0.697	66.9 ^a	61.2 ^a	0.221	0.234	0.487	36.0 ^a	91.0 ^a	0.184
n-Butyric acid	0.723	1.42	4.74	2.375	2.03	2.10	4.62	0.373	0.497	4.97	5.03	1.31	4.00	3.34	0.708	4.66	4.89
Ethyl decanoate	3.61 ^a	6.77 ^a	4.11 ^a	0.005	8.11 ^a	2.77 ^a	4.80 ^a	5.39 ^a	5.10 ^a	3.55 ^a	3.74 ^a	3.30 ^a	3.26 ^a	5.15 ^a	2.63 ^a	5.97 ^a	6.85 ^a
Isovaleric acid	1.95	2.30	1.60	1.843	2.32	2.41	1.88	2.12	2.43	1.69	2.10	1.91	1.73	1.64	2.07	1.90	1.95
Diethyl succinate	8.77	10.3	8.59	6.229	5.49	27.5	11.6	14.1	13.2	8.78	11.9	10.4	11.6	9.20	11.9	11.4	10.4
n-Valeric acid	1.56	1.50	1.57	1.648	1.57	1.64	1.69	1.65	1.53	1.53	1.78	1.75	1.51	1.42	1.58	1.62	1.67
2-Phenethyl acetate	0.306	0.149	83.6 ^a	0.115	0.338	0.104	0.201	70.4 ^a	0.245	81.2 ^a	54.1 ^a	0.114	0.164	0.141	0.165	96.5 ^a	0.173
Hexanoic acid	2.55	3.08	2.71	2.979	3.78	3.44	3.52	3.68	3.35	2.49	4.30	2.78	3.42	2.56	3.71	3.78	3.86
Guaiacol	0.385	0.494	0.164	0.375	0.678	0.530	1.08	0.406	0.449	0.444	0.345	0.470	0.471	0.181	0.428	0.309	0.638
trans-oak-lactone	1.03	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	1.03	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b
2-Phenylethyl alcohol	46.4	39.3	19.4	33.05	61.9	63.7	18.4	40.1	57.3	33.9	22.6	46.9	4.88	32.0	44.2	33.0	23.1
cis-oak-lactone	0.973	nd ^b	0.981	nd ^b	nd ^b	nd ^b	0.964	nd ^b	nd ^b	0.981	0.970	0.982	0.979	0.971	nd ^b	nd ^b	nd ^b
o-Cresol	0.818	0.832	0.781	0.84	0.876	0.830	0.937	0.796	0.812	0.797	0.799	0.858	0.813	0.794	0.832	0.807	0.832
Phenol	0.632	0.739	0.121	0.675	2.19	1.82	1.88	0.624	0.744	0.301	0.545	0.687	0.618	0.416	0.711	0.494	1.41
4-Ethylguaiacol	0.353	0.360	0.346	0.354	0.366	0.357	0.384	0.354	0.352	0.361	0.351	0.361	0.354	0.346	0.351	0.348	0.356
Octanoic acid	1.24	1.21	1.19	1.181	1.59	1.13	1.35	1.34	1.40	1.27	1.34	1.54	1.15	1.32	1.45	1.75	1.69
p-Cresol	0.253	0.288	0.229	0.291	0.327	0.293	0.383	0.281	0.285	0.242	0.298	0.285	0.283	0.258	0.274	0.293	0.287
Eugenol	0.626	0.666	0.558	0.607	0.713	0.643	0.790	0.617	0.624	0.673	0.602	0.667	0.662	0.575	0.629	0.580	0.638
Decanoic acid	0.635	0.608	0.590	0.626	0.684	0.682	0.670	0.645	0.656	0.644	0.679	0.769	0.634	0.737	0.657	0.803	0.816
2,6-Dimethoxy phenol	8.85	2.68	3.22	2.874	9.72	3.22	27.0	3.35	3.45	0.712	1.83	8.05	3.22	4.83	4.20	3.29	8.02
5-Hydroxymethylfurfural	6.62	0.902	3.68	3.36	1.70	3.34	12.0	2.45	2.30	2.76	5.63	7.36	1.03	3.50	2.45	2.23	1.94
Vanillin	21.3	24.8	9.34	11.2	86.9	48.3	103	14.9	33.0	22.2	6.71	22.4	60.1	10.7	15.7	10.2	21.1

Table 5 Continue >>>

Selected tables

COMPOUND	M18	M19	M20	M21	M22	M23	M24	M25	M26	M27	M28	M29	M30	M31	M32	M33	M34
Ethyl acetate	169	141	68.7	90.0	77.5	130	163	132	189	155	90.9	74.2	139	84.9	113	131	103
Ethyl butyrate	0.219	0.174	0.189	0.239	0.152	0.333	0.282	0.234	0.356	0.221	0.214	0.165	0.212	0.140	0.240	0.194	0.179
1-Propanol	16.8	18.7	17.8	24.9	19.6	25.2	78.2	20.3	43.9	21.0	38.6	17.1	25.7	22.3	23.2	16.7	24.4
Isobutanol	112	72.6	54.6	64.3	80.8	142	64.2	82.4	85.3	104	75.1	112	53.2	104	89.9	66.8	69.0
Isoamyl acetate	1.48	1.90	1.44	1.96	1.38	1.01	2.36	2.46	2.38	0.960	1.92	1.92	2.29	0.415	2.86	0.910	3.11
n-Butanol	8.64	0.204	11.2	11.5	2.33	0.811	0.750	9.91	0.470	9.20	0.506	1.20	10.3	0.775	0.946	11.9	10.8
Isoamyl alcohol	272	196	215	229	265	202	226	235	221	214	245	224	204	301	238	220	310
Ethyl hexanoate	0.210	98.6 ^a	94.7 ^a	0.167	80.7 ^a	0.196	0.159	0.148	0.188	0.121	93.3 ^a	81.4 ^a	0.100	0.108	0.174	0.153	63.3 ^a
Hexyl acetate	4.48 ^a	7.48 ^a	7.01 ^a	6.68 ^a	4.70 ^a	3.93 ^a	7.67 ^a	6.61 ^a	5.54 ^a	4.51 ^a	4.67 ^a	4.78 ^a	6.46 ^a	3.79 ^a	8.88 ^a	5.51 ^a	7.69 ^a
Acetoin	27.1	18.0	59.8	45.2	115	14.5	86.4	19.7	20.4	3.68	41.5	17.0	156	8.44	23.2	58.7	125
Ethyl-D-lactate	251	142	183	249	137	182	300	261	154	314	422	110	250	153	161	235	209
1-Hexanol	0.911	79.1 ^a	0.138	0.993	70.0 ^a	0.871	0.320	10.2 ^a	0.669	0.996	0.734	0.976	0.750	0.779	0.630	0.821	1.28
Ethyl octanoate	24.9 ^a	13.3 ^a	12.5 ^a	21.9 ^a	9.24 ^a	27.9 ^a	23.3 ^a	20.4 ^a	18.9 ^a	17.1 ^a	8.38 ^a	8.25 ^a	8.71 ^a	11.9 ^a	23.0 ^a	16.8 ^a	4.47 ^a
Acetic acid	631	371	526	461	625	322	673	316	325	379	807	442	1226	1665	156	367	891
Furfural	8.20	21.3	17.8	18.3	1.19	5.04	17.4	18.5	1.13	18.5	7.38	15.1	14.8	2.65	1.19	4.45	13.4
Propionic acid	16.9	16.8	11.8	12.7	14.4	14.7	33.0	8.62	13.3	8.45	15.2	8.62	33.0	10.9	10.0	16.5	34.1
Isobutyric acid	4.93	1.67	2.14	1.13	4.08	1.19	2.66	3.79	1.23	1.77	5.81	4.05	3.13	2.81	1.97	2.81	7.79
5-Methylfurfural	0.471	85.2 ^a	0.154	36.1 ^a	0.201	0.133	0.500	0.151	0.166	8.81 ^a	0.360	69.1 ^a	0.557	15.6 ^a	0.145	0.318	0.595
n-Butyric acid	1.75	4.90	4.80	5.17	5.16	57.0 ^a	2.17	0.325	1.02	0.504	4.53	3.83	2.09	0.209	0.798	1.26	3.21
Ethyl decanoate	6.23	4.58 ^a	2.56 ^a	5.50 ^a	3.12 ^a	7.97 ^a	5.47 ^a	3.57 ^a	6.22 ^a	5.04 ^a	3.46 ^a	4.10 ^a	5.01 ^a	6.92 ^a	7.72 ^a	4.13 ^a	1.48 ^a
Isovaleric acid	2.46	2.08	1.86	2.14	2.23	1.48	2.17	2.10	1.86	2.25	2.08	1.64	2.40	2.62	1.96	1.92	3.79
Diethyl succinate	7.00	9.75	13.5	10.7	9.33	9.09	7.81	13.8	10.2	10.1	12.9	8.02	9.87	9.92	10.7	5.80	8.44
n-Valeric acid	1.64	1.58	1.46	1.50	1.55	1.46	1.62	1.53	1.63	1.71	1.81	1.57	1.88	1.54	1.55	1.57	1.75
2-Phenethyl acetate	0.142	0.310	0.263	0.268	0.202	51.6 ^a	0.188	0.302	0.170	0.109	0.234	0.153	0.237	0.109	0.255	0.122	0.365
Hexanoic acid	3.68	4.10	2.39	2.96	2.54	2.77	3.59	3.12	3.61	3.28	3.72	2.86	4.89	3.26	2.95	3.33	3.43
Guaiacol	0.635	0.360	0.318	0.317	0.303	0.478	0.687	0.326	0.397	0.469	0.650	0.114	0.761	0.440	0.429	0.370	0.606
trans-oak-lactone	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	1.07	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b
2-Phenylethyl alcohol	46.1	52.6	44.0	34.8	70.1	17.5	44.9	51.9	24.9	46.8	71.3	36.4	54.0	87.9	36.8	51.0	75.9
cis-oak-lactone	nd ^b	nd ^b	0.976	nd ^b	0.975	nd ^b	1.01	nd ^b	nd ^b	nd ^b	0.986	0.976	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b
o-Cresol	0.845	0.818	0.786	0.803	0.817	0.843	0.901	0.802	0.836	0.813	0.865	0.776	0.911	0.835	0.824	0.853	0.891
Phenol	1.68	0.640	0.611	0.767	0.605	1.55	1.40	0.519	0.942	0.737	1.48	0.242	1.61	0.821	0.820	1.14	1.46
4-Ethylguaiacol	0.363	0.353	0.355	0.350	0.351	0.356	0.365	0.351	0.356	0.354	0.364	0.344	0.367	0.356	0.356	0.352	0.360
Octanoic acid	1.76	1.74	1.18	1.38	1.32	1.05	1.36	1.25	1.64	1.41	1.82	1.25	2.27	1.09	1.10	1.26	1.24
p-Cresol	0.337	0.270	0.255	0.286	0.321	0.282	0.296	0.261	0.303	0.294	0.324	0.230	0.321	0.288	0.748	0.274	0.300
Eugenol	0.741	0.642	0.636	0.564	0.609	0.646	0.685	0.593	0.575	0.639	0.734	0.537	0.776	0.635	0.618	0.616	0.642
Decanoic acid	0.680	0.829	0.618	0.647	0.649	0.637	0.667	0.612	0.674	0.720	0.711	0.635	0.951	0.651	0.665	0.627	0.678
2,6-Dimethoxy phenol	11.4	8.56	10.8	11.0	13.2	10.8	15.5	5.70	4.53	5.13	4.28	6.27	32.4	13.2	3.30	8.80	8.97
5-Hydroxymethylfurfural	2.22	1.67	3.89	1.67	2.11	2.18	7.51	1.13	1.11	0.712	4.78	1.60	6.98	1.35	2.41	0.193	3.87
Vanillin	76.1	26.3	53.3	38.1	37.3	60.9	71.5	38.8	19.0	34.3	49.9	15.2	85.4	30.3	29.1	2.78	16.7

Table 5 Continue >>>

Selected tables

COMPOUND	M35	M36	M37	M38	M39	M40	M41	M42	M43	M44	M45	M46	M47	M48	M49	M50	M51
Ethyl acetate	189	171	203	86.7	103	165	124	191	100	189	195	122	117	176	95.5	147	175
Ethyl butyrate	0.241	0.209	0.234	0.266	0.199	0.140	0.199	0.310	0.200	0.275	0.295	0.232	0.181	0.238	0.142	0.238	0.191
1-Propanol	21.5	38.2	26.3	14.9	21.2	22.3	13.5	60.1	3.53	34.5	20.4	30.0	7.42	24.2	19.9	37.0	12.3
Isobutanol	128	93.4	79.3	90.5	105	120	47.6	67.5	25.6	72.4	59.4	95.0	36.4	100	114	36.6	77.0
Isoamyl acetate	1.53	0.507	1.40	2.43	2.17	0.723	1.33	2.20	0.945	1.49	1.90	0.922	0.986	2.84	1.85	1.58	0.831
n-Butanol	10.4	9.39	0.389	10.9	0.725	8.85	11.7	9.07	0.251	11.8	10.34	8.38	3.23	11.7	9.19	8.69	10.9
Isoamyl alcohol	273	237	217	244	296	207	267	168	222	231	175	241	204	240	197	154	247
Ethyl hexanoate	99.1 ^a	0.164	0.185	0.198	0.116	62.4 ^a	0.192	0.140	0.107	0.179	0.153	0.192	78.6 ^a	0.144	76.4 ^a	0.154	0.135
Hexyl acetate	4.18 ^a	3.57 ^a	8.46 ^a	7.83 ^a	5.71 ^a	3.79 ^a	6.19 ^a	5.48 ^a	3.89 ^a	4.22 ^a	6.08 ^a	5.18 ^a	3.61 ^a	9.54 ^a	4.96 ^a	7.27 ^a	4.63 ^a
Acetoin	13.0	83.5	73.4	54.1	121	65.0	57.5	13.0	43.4	56.2	5.81	31.4	32.2	35.2	6.53	51.6	31.0
Ethyl-D-lactate	17.3	236	269	252	146	197	292	268	271	195	215	201	212	222	164	257	158
1-Hexanol	0.247	0.310	0.997	0.283	0.268	0.791	1.46	0.655	0.208 ^a	33.9 ^a	0.239	1.46	1.02	0.319	0.480	0.166	1.71
Ethyl octanoate	10.3 ^a	20.7 ^a	31.1 ^a	27.9 ^a	12.8 ^a	9.27 ^a	21.0 ^a	22.3 ^a	6.52 ^a	23.0 ^a	19.4 ^a	20.0 ^a	9.15 ^a	18.8 ^a	11.2 ^a	24.9 ^a	13.6 ^a
Acetic acid	305	697	566	349	1507	967	675	449	1425	445	291	828	427	460	713	428	755
Furfural	0.472	3.69	3.95	2.01	7.25	4.80	16.2	17.4	14.5	12.6	0.342	8.27	3.54	15.3	21.0	20.4	16.2
Propionic acid	10.7	12.3	14.3	3.10	19.3	10.6	32.8	9.75	32.5	9.84	7.10	17.8	9.52	11.6	24.4	6.69	16.2
Isobutyric acid	3.48	3.24	2.44	2.55	2.31	4.19	3.63	2.53	4.36	1.11	0.782	2.25	1.85	1.79	3.16	0.608	2.33
5-Methylfurfural	67.7 ^a	0.310	0.481	12.5 ^a	0.119	0.419	0.522	0.110	0.609	0.520	0.112	0.374	0.320	0.635	0.104	0.715	0.612
n-Butyric acid	0.715	0.969	1.83	0.102	0.860	0.850	2.00	4.14	3.48	3.96	0.519	1.53	0.985	3.95	27.3 ^a	4.99	4.48
Ethyl decanoate	5.26 ^a	5.53 ^a	68.4 ^c	5.43 ^a	6.13 ^a	5.47 ^a	6.90 ^a	4.54 ^a	1.60 ^a	5.49 ^a	4.03 ^a	4.32 ^a	3.42 ^a	4.36 ^a	4.59 ^a	5.10 ^a	3.96 ^a
Isovaleric acid	2.53	2.25	1.99	2.41	2.85	2.24	3.04	1.84	2.38	1.96	1.57	2.05	1.77	1.95	2.14	1.61	2.34
Diethyl succinate	4.77	10.9	15.0	6.16	9.09	9.14	11.3	13.7	6.48	12.7	9.93	10.4	7.96	12.1	9.88	13.5	8.61
n-Valeric acid	1.57	1.74	2.01	1.87	1.59	1.92	1.75	1.58	1.62	1.42	1.51	1.85	1.58	1.58	1.92	1.55	1.61
2-Phenethyl acetate	0.109	45.0 ^a	0.123	0.198	0.249	98.0 ^a	0.207	0.110	77.6 ^a	0.209	0.135	0.108	65.1 ^a	0.300	0.126	0.112	0.103
Hexanoic acid	3.52	4.17	5.37	3.37	3.36	4.29	4.84	3.32	2.95	2.788	2.68	3.63	2.38	3.62	3.01	3.40	3.68
Guaiacol	0.421	0.621	0.504	0.257	0.574	0.604	0.701	0.385	0.683	0.274	0.482	0.434	0.632	0.29	0.334	0.258	0.329
trans-oak-lactone	nd ^b	1.03	nd ^b	nd ^b	1.03	nd ^b	nd ^b	1.03	1.03	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	1.03
2-Phenylethyl alcohol	60.3	36.4	41.1	44.0	66.3	50.2	95.8	13.4	43.7	41.6	21.0	40.7	32.5	36.6	41.6	23.2	73.4
cis-oak-lactone	nd ^b	0.970	nd ^b	0.980	0.964	nd ^b	nd ^b	0.968	0.964	nd ^b	nd ^b	nd ^b	0.986	nd ^b	0.975	nd ^b	0.965
o-Cresol	0.826	0.843	0.856	0.813	0.871	0.845	0.908	0.798	0.872	0.763	0.809	0.859	0.832	0.790	0.807	0.814	0.793
Phenol	0.726	0.853	1.31	0.706	1.33	0.868	1.50	0.486	1.19	1.49	0.464	1.03	0.844	0.830	0.452	0.418	0.541
4-Ethylguaiacol	0.357	0.368	0.363	0.351	0.361	0.359	0.362	0.353	0.362	0.347	0.357	0.356	0.366	0.347	0.356	0.350	0.348
Octanoic acid	1.27	1.17	1.68	1.43	1.24	1.26	1.74	1.40	1.39	1.32	1.05	1.28	1.17	1.51	1.50	1.31	1.60
p-Cresol	0.279	0.295	0.325	0.253	0.301	0.312	0.343	0.266	0.292	0.245	0.278	0.298	0.302	0.251	0.265	0.262	0.254
Eugenol	0.605	0.722	0.640	0.603	0.697	0.692	0.740	0.636	0.708	0.544	0.666	0.608	0.724	0.577	0.617	0.560	0.584
Decanoic acid	0.660	0.653	0.709	0.712	0.649	0.672	0.702	0.750	0.757	0.671	0.637	0.701	0.639	0.707	0.775	0.644	0.671
2,6-Dimethoxy phenol	5.91	4.48	10.4	3.45	9.89	6.52	24.2	0.212	7.91	6.32	3.79	5.85	1.62	2.63	3.82	4.26	3.95
5-Hydroxymethylfurfural	2.58	1.57	6.19	0.658	3.79	4.12	6.79	0.967	3.70	1.06	1.69	3.39	5.10	1.61	3.51	0.203	1.85
Vanillin	8.00	43.9	29.1	12.6	51.9	55.4	81.0	0.675	69.0	23.0	46.6	23.9	44.0	30.0	34.5	21.6	34.5

Table 5 Continue >>>

Selected tables

COMPOUND	M52	M53	M54	M55	M56	M57	M58	M59	M60	M61	M62
Ethyl acetate	172	134	176	89.6	163	100	86.4	159	188	368	120
Ethyl butyrate	0.174	0.265	0.364	0.322	0.203	0.270	0.232	0.234	0.348	0.462	0.230
1-Propanol	18.35	22.2	23.8	22.9	14.8	31.2	31.6	19.7	57.5	38.2	23.2
Isobutanol	65.41	49.4	77.1	73.0	48.8	125	123	66.5	45.4	132	92.9
Isoamyl acetate	1.487	0.647	2.06	1.10	1.08	1.16	1.39	0.701	3.52	3.48	1.74
n-Butanol	9.403	9.48	10.1	11.9	9.49	0.779	9.92	1.38	6.95	7.79	10.5
Isoamyl alcohol	152.6	172	221	216	203	228	231	196	157	194	226
Ethyl hexanoate	0.078	0.172	0.216	0.171	0.171	0.171	0.133	0.125	0.257	0.234	0.166
Hexyl acetate	0.007	4.48 ^a	6.88 ^a	6.45 ^a	4.56 ^a	6.93 ^a	6.50 ^a	3.36 ^a	12.9 ^a	9.26 ^a	5.68 ^a
Acetoin	154.4	13.1	35.2	19.7	35.1	9.80	9.78	49.4	73.8	48.6	132
Ethyl-D-lactate	219.7	162	335	213	180	242	221	214	298	265	198
1-Hexanol	0.565	0.292	0.398	0.399	0.348	78.9 ^a	0.617	0.589	0.887	0.834	63.3 ^a
Ethyl octanoate	0.014	14.8 ^a	19.5 ^a	19.4 ^a	20.0 ^a	27.0 ^a	18.2 ^a	15.5 ^a	29.1 ^a	37.2 ^a	31.7 ^a
Acetic acid	1522	428	436	410	358	427	747	356	842	313	626
Furfural	16.71	18.1	5.19	19.5	20.1	14.2	99.4 ^a	16.8	4.03	16.3	9.04
Propionic acid	38.1	9.74	15.9	11.9	5.71	5.09	17.0	10.8	21.6	7.05	19.1
Isobutyric acid	3.466	0.211	1.47	1.08	4.94	4.08	0.740	1.39	1.67	1.77	2.93
5-Methylfurfural	0.54	0.610	0.394	0.473	0.127	71.8 ^a	0.702	71.0 ^a	0.176	0.612	0.165
n-Butyric acid	2.369	3.39	2.51	4.02	4.98	3.60	4.60	5.13	3.01	4.22	2.00
Ethyl decanoate	0.003	4.83	9.29 ^a	3.45 ^a	5.68 ^a	0.527 ^a	5.44 ^a	3.62 ^a	9.60 ^a	7.95 ^a	0.936 ^a
Isovaleric acid	2.323	1.54	1.72	1.64	2.29	1.79	1.61	1.82	2.00	1.74	2.38
Diethyl succinate	11.15	11.2	10.8	9.02	13.4	9.14	8.97	10.2	11.7	10.3	20.9
n-Valeric acid	1.719	1.44	1.54	1.39	1.66	1.58	1.48	1.44	1.78	1.35	1.59
2-Phenethyl acetate	0.187	67.7 ^a	90.4 ^a	0.137	82.3 ^a	0.107	84.7 ^a	0.100	0.153	0.120	0.149
Hexanoic acid	3.377	3.00	3.19	3.14	4.31	3.04	2.71	2.56	4.71	1.39	3.61
Guaiacol	0.819	0.264	0.666	0.320	0.437	0.171	0.229	0.335	0.431	0.287	0.608
trans-oak-lactone	nd ^b	1.03	1.03	nd ^b	1.04	1.03	nd ^b	nd ^b	nd ^b	nd ^b	1.03
2-Phenylethyl alcohol	37.91	24.4	24.9	33.4	49.7	21.3	29.8	43.5	19.0	13.3	62.4
cis-oak-lactone	nd ^b	0.971	0.964	0.976	0.976	0.986	0.979	nd ^b	nd ^b	nd ^b	0.964
o-Cresol	0.906	0.810	0.836	0.796	0.817	0.769	0.803	0.786	0.862	0.765	0.855
Phenol	1.633	0.465	1.06	0.485	0.739	0.218	0.322	0.268	0.821	0.177	0.921
4-Ethylguaiacol	0.363	0.344	0.366	0.354	0.352	0.348	0.348	0.350	0.352	0.351	0.362
Octanoic acid	1.504	1.37	1.37	1.29	1.65	1.53	1.32	1.18	1.80	1.16	1.50
p-Cresol	0.321	0.262	0.284	0.238	0.297	0.238	0.238	0.254	0.305	0.236	0.281
Eugenol	0.713	0.581	0.727	0.627	0.630	0.578	0.591	0.558	0.618	0.582	0.701
Decanoic acid	0.757	0.785	0.746	0.612	0.691	0.679	0.669	0.643	0.708	0.602	0.668
2,6-Dimethoxy phenol	23.86	5.93	1.27	0.506	1.18	0.603	4.14	2.56	8.83	4.25	8.51
5-Hydroxymethylfurfural	6.702	1.11	4.74	3.32	2.33	3.27	2.68	1.94	3.40	1.11	2.22
Vanillin	61.91	20.7	58.4	35.1	21.4	14.6	34.5	20.7	33.5	25.9	51.8

^a Measured in µg/L. ^b nd: not detected. For details of the wines refer to **Table 1**. For analytical conditions refer to **chapter 6, section 6.2**.

Selected tables

Table 6. Quantitative (mg/l) data of 72 young Sauvignon Blanc wines (SB1 – SB72) from vintage 2005.

COMPOUND	SB1	SB2	SB3	SB4	SB5	SB6	SB7	SB8	SB9	SB10	SB11	SB12	SB13	SB14	SB15	SB16	SB17	SB18
Ethyl acetate	68.2	93.2	117	83.3	145	73.1	125	98.6	85.0	98.0	137	190	102	189	151	110	104	143
Ethyl butyrate	0.363	0.300	0.330	0.321	0.399	0.336	0.381	0.379	0.350	0.419	0.541	0.471	0.456	0.482	0.487	0.552	0.425	0.392
1-Propanol	20.7	12.4	18.4	17.8	47.4	18.8	24.8	23.1	16.9	11.6	17.0	9.33	21.0	12.7	4.36	1.68	10.4	12.9
Isobutanol	25.1	15.4	10.6	12.9	31.7	10.9	1.01	41.3	15.3	16.1	21.8	39.8	27.8	47.7	36.2	17.3	23.2	18.8
Isoamyl acetate	2.46	7.40	7.40	6.32	24.7 ^a	6.43	10.7	20.2 ^a	4.31	24.9 ^a	30.2 ^a	26.3 ^a	26.8 ^a	4.04	6.03	5.25	4.32	4.79
n-Butanol	1.74	8.95	1.50	3.33	0.396	3.76	6.21	7.21	1.38	1.87	11.3	9.57	8.69	1.24	9.82	6.83	9.70	8.00
Isoamyl alcohol	130	147	137	132	125	141	153	110	140	113	130	136	120	139	137	138	114	132
Ethyl hexanoate	0.521	0.534	0.407	0.444	0.435	0.426	0.401	0.446	0.429	0.678	0.778	0.772	0.678	0.710	0.816	0.888	0.781	0.616
Hexyl acetate	53.7 ^a	0.121	89.8 ^a	80.9 ^a	83.5 ^a	81.9 ^a	60.5 ^a	0.109	0.142	57.4 ^a	0.110	58.1 ^a	59.1 ^a	63.1 ^a	89.9 ^a	80.6 ^a	59.8 ^a	97.4 ^a
Acetoin	16.6	22.9	38.2	13.6	54.9	39.4	33.5	39.9	30.4	65.1	4.97	55.9	47.3	55.3	61.8	65.5	52.8	64.5
Ethyl-D-lactate	40.3	13.0	19.0	19.2	9.95	5.86	1.34	6.50	10.6	17.6	17.7	12.5	10.9	19.8	7.56	26.2	15.3	17.4
1-Hexanol	0.664	0.95	0.470	0.722	0.447	0.210	1.01	1.28	0.798	0.162	0.541	0.511	1.16	0.612	54.7 ^a	0.939	0.601	0.928
Ethyl octanoate	87.8 ^a	86.3 ^a	72.4 ^a	60.8 ^a	63.0 ^a	67.9 ^a	79.2 ^a	64.7 ^a	83.5 ^a	0.121	0.115	0.132	0.126	0.121	0.117	0.143	0.128	62.6 ^a
Acetic acid	320	356	178	309	142	249	88.9	103	313	174	171	237	142	187	236	296	239	323
Furfural	10.2	13.7	13.4	12.8	16.8	15.0	10.9	12.2	18.1	19.2	0.100	18.0	17.3	17.8	19.7	0.353	16.3	21.2
Propionic acid	18.4	20.5	8.19	18.2	6.83	22.5	5.54	6.40	22.8	11.4	10.8	12.1	10.8	10.2	13.6	14.1	9.31	13.5
Isobutyric acid	1.33	6.27	6.14	1.11	1.05	1.17	1.17	1.31	2.30	1.16	0.407	1.42	0.882	0.275	0.201	1.01	2.34	0.777
5-Methylfurfural	0.369	0.951	94.9 ^a	0.424	0.602	0.172	0.399	0.335	0.552	0.666	0.704	0.122	14.5 ^a	0.603	32.9 ^a	0.318	20.8 ^a	0.114
n-Butyric acid	2.78	2.05	2.99	0.370	3.52	3.15	2.67	2.43	4.60	4.36	0.542	4.14	4.57	3.44	36.0 ^a	1.91	3.95	1.63
Ethyl decanoate	9.58 ^a	4.78 ^a	7.53 ^a	8.33 ^a	9.30 ^a	1.94 ^a	6.29 ^a	2.60 ^a	4.61 ^a	14.3 ^a	7.58 ^a	22.1 ^a	11.1 ^a	13.9 ^a	13.0 ^a	14.6 ^a	14.5 ^a	10.5 ^a
Isovaleric acid	1.47	1.42	1.42	2.18	1.13	2.16	1.19	1.15	3.38	1.21	1.39	1.27	1.42	1.34	1.27	1.50	1.28	1.57
Diethyl succinate	4.78	4.34	4.25	4.32	4.23	4.16	4.06	4.38	4.26	4.36	4.36	4.22	4.47	4.25	4.25	4.45	4.53	4.31
n-Valeric acid	1.49	1.75	1.47	2.63	1.25	2.65	1.24	1.28	3.36	1.34	1.46	1.48	1.56	1.44	1.45	1.62	1.50	1.45
2-Phenethyl acetate	0.256	0.322	0.246	0.255	0.333	0.422	0.232	0.210	0.628	0.126	0.311	0.239	0.181	0.264	0.564	0.304	0.156	0.344
Hexanoic acid	5.32	4.81	4.43	6.04	2.99	6.91	4.14	3.10	5.97	4.26	5.34	4.90	6.00	5.79	6.46	7.68	5.82	7.78
Guaiacol	0.380	0.532	0.252	0.282	0.134	0.214	0.139	11.3 ^a	0.283	73.8 ^a	65.5 ^a	0.126	89.4 ^a	0.178	0.275	0.214	0.160	0.311
trans-oak-lactone	nd ^b	1.03	nd ^b	1.03	nd ^b	1.03	1.03	nd ^b	1.04	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b
2-Phenylethyl alcohol	12.4	10.3	8.13	9.61	4.28	8.54	6.36	4.12	10.4	5.71	7.56	7.49	7.10	10.3	12.3	13.6	7.21	11.7
cis-oak-lactone	0.996	1.01	0.979	0.999	0.967	0.993	0.967	0.968	1.01	0.969	0.969	0.971	0.970	0.970	0.970	0.971	0.971	0.971
o-Cresol	0.893	0.908	0.761	0.895	0.772	0.933	0.745	0.752	0.924	0.798	0.817	0.792	0.777	0.789	0.794	0.804	0.771	0.801
Phenol	1.20	1.15	0.140	0.969	0.159	0.947	0.220	0.769	0.935	0.131	0.295	0.179	0.893	0.181	0.187	1.07	0.423	0.740
4-Ethylguaiacol	0.356	0.365	0.350	0.354	0.345	0.347	0.337	0.338	0.352	0.341	0.343	0.343	0.345	0.345	0.350	0.349	0.348	0.352
Octanoic acid	3.87	2.92	3.15	3.91	1.46	5.74	2.10	1.52	3.72	2.47	2.87	2.60	2.72	3.71	3.68	4.36	2.83	7.45
p-Cresol	0.336	0.354	0.229	0.280	0.215	0.328	0.189	0.200	0.298	0.222	0.237	0.234	0.217	0.220	0.227	0.254	0.240	0.271
Eugenol	0.595	0.659	0.606	0.588	0.564	0.563	0.481	0.507	0.599	0.531	0.538	0.548	0.534	0.576	0.612	0.572	0.585	0.639
Decanoic acid	0.942	0.822	0.930	1.08	0.660	3.79	0.630	0.671	1.32	0.742	0.795	0.770	0.762	0.796	0.741	0.776	0.761	1.12
2,6-Dimethoxy phenol	6.03	7.09	4.82	5.95	2.84	5.10	2.13	1.77	6.24	5.32	3.54	3.62	3.69	4.61	4.11	5.53	5.53	4.11
5-Hydroxymethylfurfural	11.1	13.9	4.64	11.4	4.35	9.04	3.48	2.32	14.1	5.80	4.80	4.64	5.56	4.22	6.62	6.32	3.48	5.56
Vanillin	3.46	4.94	2.47	3.21	2.52	2.47s	1.24	1.38	3.95	2.06	1.70	2.47	2.60	3.29	4.49	2.75	3.29	4.49s

Table 6 Continue >>>

Selected tables

COMPOUND	SB19	SB20	SB21	SB22	SB23	SB24	SB25	SB26	SB27	SB28	SB29	SB30	SB31	SB32	SB33	SB34	SB35	SB36
Ethyl acetate	89.9	109	76.8	101	149	160	125	131	126	124	134	97.8	124	162	168	123	142	114
Ethyl butyrate	0.328	0.350	0.559	0.450	0.364	0.525	0.469	0.472	0.449	0.401	0.534	0.418	0.538	0.699	0.481	0.459	0.277	0.326
1-Propanol	22.3	15.1	24.0	31.5	15.5	9.19	27.0	21.7	12.9	40.8	23.0	23.6	27.7	31.7	28.4	14.6	21.9	33.9
Isobutanol	36.0	13.9	23.3	33.6	23.6	35.2	42.0	35.0	28.8	19.4	27.8	9.53	18.8	37.6	43.0	29.1	30.3	18.8
Isoamyl acetate	7.81	7.25	5.36	5.12	26.0 ^a	25.4 ^a	27.2 ^a	23.1 ^a	22.6 ^a	33.4 ^a	28.1 ^a	20.0 ^a	8.03	8.90	3.83	6.21	6.20	8.33
n-Butanol	8.34	8.56	2.70	2.89	8.09	0.582	9.10	8.27	7.92	11.2	11.0	2.58	9.55	2.46	11.2	7.60	9.91	6.13
Isoamyl alcohol	140	125	141	132	123	134	144	123	107	133	127	150	126	140	130	149	136	131
Ethyl hexanoate	0.445	0.747	0.513	0.635	0.389	0.727	0.722	0.648	0.615	0.483	0.745	0.421	0.524	0.737	0.596	0.690	0.357	0.429
Hexyl acetate	78.2 ^a	0.110	0.119	73.3 ^a	0.153	0.109	92.9 ^a	81.0 ^a	93.1 ^a	62.1 ^a	0.183	85.5 ^a	0.144	88.3 ^a	85.6 ^a	85.8 ^a	86.5 ^a	0.119
Acetoin	45.5	39.1	65.5	31.6	68.9	41.0	62.2	46.7	52.5	25.2	19.6	47.7	50.2	34.2	57.6	68.2	19.1	41.5
Ethyl-D-lactate	18.8	7.63	12.2	36.5	11.7	11.8	16.2	13.3	7.18	13.5	13.4	18.7	9.43	22.2	20.3	61.5	2.02	12.5
1-Hexanol	0.560	1.41	0.377	0.462	0.322	57.6 ^a	0.767	0.165	0.989	0.909	0.758	62.9 ^a	0.478	58.7 ^a	0.714	0.493	0.364	0.394
Ethyl octanoate	83.5 ^a	0.123	88.8 ^a	99.1 ^a	65.8 ^a	0.110	0.108	90.6 ^a	92.9 ^a	77.6 ^a	0.126	48.4 ^a	73.2 ^a	0.114	88.0 ^a	0.107	71.5 ^a	17.3 ^a
Acetic acid	339	245	428	285	306	267	214	186	329	214	225	238	143	115	204	389	354	194
Furfural	16.9	12.6	31.36	11.7	20.9	12.8	18.3	16.0	19.2	11.5	4.34	4.74	18.5	7.99	20.1	97.7 ^a	20.5	13.6
Propionic acid	9.31	7.31	41.7	13.9	20.8	10.2	9.92	16.0	17.4	19.8	21.9	32.1	13.9	6.40	10.4	18.9	20.8	9.26
Isobutyric acid	1.28	0.165	0.737	6.71	0.442	0.200	0.963	1.57	1.33	4.16	2.88	0.416	1.25	1.33	1.87	0.453	1.30	4.70
5-Methylfurfural	0.480	0.369	0.678	0.620	0.170	0.610	0.138	86.3 ^a	0.672	0.298	0.107	0.114	0.103	0.417	0.157	66.0 ^a	59.7 ^a	0.676
n-Butyric acid	3.52	3.28	6.67	3.24	4.57	4.28	4.27	4.10	4.77	3.91	4.52	1.38	4.32	3.55	4.64	84.0 ^a	1.24	4.31
Ethyl decanoate	7.42 ^a	11.7 ^a	3.81 ^a	17.0 ^a	24.5 ^a	27.1 ^a	21.2 ^a	4.45 ^a	10.6 ^a	3.57 ^a	28.3 ^a	4.63 ^a	17.2 ^a	13.9 ^a	4.96 ^a	6.01 ^a	14.4 ^a	2.40 ^a
Isovaleric acid	1.31	1.52	1.46	1.74	1.29	1.30	1.22	1.25	1.35	1.26	1.35	1.24	1.21	1.29	1.29	1.31	1.18	1.29
Diethyl succinate	4.41	4.62	4.35	4.77	4.22	4.17	4.54	4.27	4.32	4.47	4.26	4.20	4.13	4.54	4.32	4.69	4.25	4.17
n-Valeric acid	1.46	1.43	1.63	1.58	1.36	1.38	1.52	1.50	1.27	1.38	1.41	1.34	1.47	1.35	1.46	1.46	1.46	1.45
2-Phenethyl acetate	0.296	0.272	1.73	0.309	0.808	0.772	0.469	0.443	0.522	0.323	0.645	0.596	0.456	0.432	0.288	0.503	0.312	0.466
Hexanoic acid	5.07	6.27	4.66	6.75	4.51	5.64	4.67	4.12	3.72	3.80	6.25	4.69	4.67	4.44	5.20	5.74	3.60	4.90
Guaiacol	0.166	0.112	0.529	0.153	0.227	0.143	0.164	0.255	0.296	0.238	0.174	0.246	0.138	0.110	0.161	0.316	0.472	0.117
trans-oak-lactone	nd ^b	nd ^b	1.05	1.03	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b
2-Phenylethyl alcohol	7.46	9.48	47.4	9.39	10.6	13.0	6.22	6.03	5.49	8.95	11.4	9.48	6.52	7.24	7.81	12.8	8.97	8.97
cis-oak-lactone	0.967	nd ^b	1.03	0.988	0.975	0.973	0.973	0.972	0.971	0.977	0.970	0.972	0.971	nd ^b	0.967	0.970	0.972	0.968
o-Cresol	0.767	0.753	0.999	0.752	0.797	0.777	0.787	0.776	0.785	0.863	0.843	0.876	0.788	0.731	0.788	0.795	0.819	0.755
Phenol	0.857	0.124	1.36	0.253	0.345	0.264	0.679	0.200	0.191	0.533	0.908	0.470	0.312	0.637	0.128	0.157	0.645	62.5 ^a
4-Ethylguaiacol	0.345	0.343	0.362	0.346	0.349	0.347	0.346	0.350	0.353	0.349	0.344	0.343	0.344	0.337	0.346	0.352	0.354	0.342
Octanoic acid	2.70	3.20	2.83	3.57	3.70	3.32	2.66	1.90	1.81	2.24	4.80	4.59	2.96	2.08	2.34	3.40	2.22	4.58
p-Cresol	0.206	0.218	0.322	0.229	0.239	0.235	0.256	0.235	0.260	0.232	0.233	0.227	0.239	0.197	0.228	0.220	0.230	0.227
Eugenol	0.598	0.582	0.675	0.567	0.595	0.544	0.556	0.621	0.649	0.595	0.561	0.564	0.555	0.492	0.585	0.600	0.640	0.555
Decanoic acid	0.766	0.759	0.735	1.09	1.02	0.907	0.846	0.673	0.700	0.839	0.930	0.711	0.844	0.728	0.687	0.753	0.748	0.894
2,6-Dimethoxy phenol	3.54	2.13	2.78	3.09	4.64	3.48	3.57	1.99	1.99	1.74	1.64	1.99	1.86	2.36	1.39	2.53	2.53	1.39
5-Hydroxymethylfurfural	3.48	2.78	21.3	5.34	7.12	4.74	4.96	5.21	7.62	6.67	5.34	4.27	4.74	4.64	4.03	5.47	5.34	3.56
Vanillin	3.29	2.91	16.5	11.0	11.8	8.25	9.16	13.8	13.5	8.25	5.50	4.12	10.3	0.988	6.60	11.0	8.25	5.50

Table 6 Continue >>>

Selected tables

COMPOUND	SB37	SB38	SB39	SB40	SB41	SB42	SB43	SB44	SB45	SB46	SB47	SB48	SB49	SB50	SB51	SB52	SB53	SB54
Ethyl acetate	76.6	131	66.0	66.0	129	91.1	119	107	96.4	104	81.2	104	130	134	113	73.7	94.0	109
Ethyl butyrate	0.353	0.346	0.323	0.407	0.583	0.486	0.304	0.425	0.289	0.381	0.287	0.321	0.511	0.367	0.400	0.383	0.352	0.406
1-Propanol	41.8	21.0	24.9	14.8	31.5	17.3	13.1	33.5	16.8	28.4	21.4	17.9	14.4	33.2	17.8	27.2	41.7	4.97
Isobutanol	27.2	17.7	8.46	8.22	45.6	0.791	29.2	44.5	21.1	25.8	10.6	22.3	24.3	34.3	20.6	14.6	35.2	3.97
Isoamyl acetate	3.05	3.43	5.61	3.83	9.47	10.2	25.9 ^a	25.1 ^a	23.4 ^a	25.4 ^a	5.70	30.5 ^a	37.0 ^a	5.58	10.2	6.39	8.09	2.94
n-Butanol	7.95	10.4	8.75	9.40	10.5	11.1	10.9	0.132	11.8	5.73	1.74	11.6	8.25	2.43	6.12	11.6	10.3	3.85
Isoamyl alcohol	120	96.1	131	107	139	139	129	132	126	151	115	125	124	122	130	128	158	105
Ethyl hexanoate	0.551	0.636	0.357	0.605	0.713	0.518	0.409	0.464	0.396	0.393	0.463	0.416	0.718	0.430	0.623	0.612	0.425	0.574
Hexyl acetate	38.7 ^a	34.6 ^a	60.8 ^a	63.6 ^a	0.185	0.117	66.5 ^a	0.102	0.136	0.110	0.129	94.4 ^a	0.138	64.5 ^a	0.152	0.156	71.8 ^a	67.0 ^a
Acetoin	30.3	49.5	11.4	69.3	61.6	5.31	37.3	38.8	0.501	20.3	6.01	6.65	7.13	68.7	37.1	64.0	2.96	42.4
Ethyl-D-lactate	19.2	12.9	15.6	12.1	29.5	2.96	10.3	12.0	12.9	11.8	2.52	7.20	12.4	6.59	19.7	6.62	13.1	18.6
1-Hexanol	14.3 ^a	0.828	28.3 ^a	0.333	1.06	61.1 ^a	0.189	0.338	0.644	0.380	0.445	0.436	0.639	0.410	1.00	1.24	0.703	0.325
Ethyl octanoate	85.6 ^a	89.9 ^a	53.3 ^a	82.2 ^a	0.126	88.4 ^a	61.0 ^a	80.5 ^a	65.7 ^a	87.9 ^a	71.0 ^a	84.9 ^a	0.134	60.4 ^a	0.110	84.8 ^a	72.0 ^a	91.9 ^a
Acetic acid	143	214	518	1036	363	777	292	207	545	305	674	531	856	384	207	1347	576	343
Furfural	10.8	14.8	9.91	26.2	17.2	4.09	11.2	13.8	19.8	5.32	8.90	4.05	7.38	14.8	11.7	24.7	0.986	23.0
Propionic acid	9.06	10.4	20.3	40.7	10.2	26.4	8.14	7.68	16.3	27.1	18.3	19.4	14.5	9.46	8.14	38.5	16.3	32.3
Isobutyric acid	1.26	0.935	0.160	1.37	5.67	1.18	0.197	1.36	3.81	0.423	1.52	0.340	3.04	1.36	0.323	5.34	0.305	0.850
5-Methylfurfural	0.345	0.672	0.212	0.548	17.2 ^a	0.191	0.404	0.575	0.144	94.3 ^a	0.434	0.165	0.474	0.590	0.396	0.218	0.683	0.751
n-Butyric acid	3.08	4.59	1.06	4.38	3.60	1.75	3.23	2.93	2.89	4.40	6.57	4.74	2.09	3.54	3.71	8.31	3.59	7.60
Ethyl decanoate	7.82 ^a	2.11 ^a	7.11 ^a	9.50 ^a	36.3 ^a	10.4 ^a	15.4 ^a	7.07 ^a	7.57 ^a	27.9 ^a	10.3 ^a	22.7 ^a	38.2 ^a	10.2 ^a	15.2 ^a	1.80 ^a	6.51 ^a	9.80 ^a
Isovaleric acid	1.31	1.39	1.28	2.11	1.44	1.72	1.46	1.22	1.30	1.32	2.58	1.31	1.86	1.31	1.41	6.92	1.29	5.17
Diethyl succinate	5.30	4.56	4.17	4.37	4.53	4.33	4.21	4.20	4.14	4.09	4.15	4.20	4.25	4.33	4.65	4.48	4.31	4.64
n-Valeric acid	1.36	1.48	1.38	2.35	1.50	1.83	1.35	1.24	1.41	1.32	2.76	1.40	2.01	1.36	1.45	6.74	1.32	1.57
2-Phenethyl acetate	0.310	0.493	0.263	0.212	0.647	0.553	0.290	0.350	0.406	0.407	0.464	0.272	0.573	0.195	0.686	0.407	0.263	0.164
Hexanoic acid	3.62	5.26	4.17	5.24	7.00	4.79	5.23	3.35	4.30	3.94	5.09	4.16	8.88	3.55	5.74	9.50	4.52	4.93
Guaiacol	0.147	0.194	0.373	0.655	0.272	0.269	0.110	80.5 ^a	0.228	0.140	0.240	0.200	0.258	0.135	66.1 ^a	0.469	0.374	0.360
trans-oak-lactone	nd ^b	nd ^b	1.03	1.03	nd ^b	1.06	1.04	nd ^b	nd ^b	nd ^b	1.03	nd ^b	nd ^b	nd ^b	nd ^b	1.10	nd ^b	1.03
2-Phenylethyl alcohol	7.89	9.28	9.09	8.21	13.2	10.8	10.3	7.39	8.97	7.75	7.08	6.60	12.0	7.36	10.7	8.29	8.86	9.27
cis-oak-lactone	0.967	0.971	0.993	1.01	0.978	1.01	1.04	0.972	0.982	0.972	0.992	0.972	0.975	0.968	nd ^b	1.05	0.969	1.01
o-Cresol	0.740	0.759	0.876	0.988	0.785	0.887	0.756	0.748	0.800	0.842	0.876	0.823	0.850	0.766	0.750	0.993	0.795	0.953
Phenol	0.741	0.894	0.902	1.17	0.179	0.902	58.4 ^a	0.755	0.452	1.13	0.772	0.509	0.514	0.800	92.7 ^a	1.52	0.426	1.49
4-Ethylguaiacol	0.338	0.345	0.359	0.373	0.349	0.353	0.342	0.344	0.346	0.341	0.351	0.347	0.350	0.343	0.342	0.362	0.350	0.358
Octanoic acid	1.80	1.84	3.11	2.71	4.44	2.49	4.37	1.99	2.91	2.82	2.61	2.57	4.81	1.63	2.81	4.23	2.83	3.12
p-Cresol	0.196	0.222	0.297	0.354	0.227	0.303	0.215	0.196	0.241	0.229	0.320	0.278	0.273	0.198	0.213	0.371	0.244	0.339
Eugenol	0.513	0.588	0.609	0.695	0.599	0.578	0.555	0.544	0.594	0.544	0.599	0.558	0.569	0.563	0.514	0.685	0.686	0.610
Decanoic acid	0.685	0.694	0.805	1.02	1.03	0.876	1.05	0.686	0.906	0.863	1.10	0.852	1.23	0.651	0.727	2.54	0.766	0.864
2,6-Dimethoxy phenol	0.928	1.39	5.89	7.37	3.68	7.17	3.68	2.95	7.29	5.67	4.05	6.14	3.88	1.94	2.38	5.71	8.10	6.95
5-Hydroxymethylfurfural	3.05	3.50	12.8	18.3	3.04	10.0	3.04	3.32	7.31	4.57	11.9	6.09	9.61	2.95	2.69	9.98	3.65	16.0
Vanillin	3.67	4.71	17.9	23.8	11.9s	13.1	10.8	6.44	19.9	6.11	10.2	9.92	12.5	6.11	5.81	3.69	21.7	3.80

Table 6 Continue >>>

Selected tables

COMPOUND	SB55	SB56	SB57	SB58	SB59	SB60	SB61	SB62	SB63	SB64	SB65	SB66	SB67	SB68	SB69	SB70	SB71	SB72
Ethyl acetate	74.1	110	143	100	117	86.5	132	92.8	113	117	77.4	163	72.8	75.3	79.4	134	99.5	73.2
Ethyl butyrate	50.7 ^a	0.368	0.487	0.444	0.416	0.442	0.508	0.358	0.369	0.485	0.323	0.518	0.469	0.277	0.352	0.363	0.442	0.390
1-Propanol	15.4	30.5	15.9	42.5	36.6	8.58	18.9	18.6	37.3	28.1	10.2	17.2	52.8	20.2	21.2	28.5	5.79	15.2
Isobutanol	26.5	36.7	15.4	48.9	24.6	14.9	47.5	22.3	29.0	3.74	15.1	47.2	14.1	20.1	17.5	22.1	6.56	44.6
Isoamyl acetate	6.16	24.8 ^a	21.0 ^a	20.2 ^a	28.9 ^a	24.5 ^a	23.2 ^a	27.2 ^a	30.8 ^a	7.07	5.84	3.96	4.72	4.88	7.45	2.74	22.4 ^a	24.4 ^a
n-Butanol	11.7	9.55	8.43	8.39	4.83	10.5	11.9	41.3 ^a	7.55	8.08	6.28	9.80	3.05	2.46	6.27	1.35	2.15	11.7
Isoamyl alcohol	142	139	118	122	111	121	132	128	129	112	150	126	112	146	137	127	129	168
Ethyl hexanoate	0.507	0.400	0.553	0.436	0.755	0.723	0.775	0.407	0.534	0.732	0.520	0.613	0.774	0.384	0.575	0.286	0.480	0.440
Hexyl acetate	64.7 ^a	72.4 ^a	0.104	88.4 ^a	50.0 ^a	0.102	79.3 ^a	0.106	0.103	0.130	54.0 ^a	53.6 ^a	60.3 ^a	59.5 ^a	0.118	36.3 ^a	97.4 ^a	28.0 ^a
Acetoin	19.4	51.3	51.5	28.6	11.2	21.8	47.2	41.1	60.1	67.4	37.2	39.2	26.4	42.2	51.5	36.0	22.9	45.9
Ethyl-D-lactate	22.2	8.66	4.02	5.10	18.5	22.0	13.6	7.39	23.3	19.9	7.95	4.95	21.7	11.6	34.2	9.88	2.55	36.9
1-Hexanol	0.196	0.780	0.374	0.182	0.888	0.728	0.524	0.964	0.997	0.375	92.7 ^a	0.129	0.897	0.474	0.771	0.135	26.6 ^a	1.25
Ethyl octanoate	82.6 ^a	65.7 ^a	68.5 ^a	70.0 ^a	0.139	0.130	0.128	81.9 ^a	99.9 ^a	0.127	86.9 ^a	79.1 ^a	0.104	60.2 ^a	0.102	41.8 ^a	46.3 ^a	69.5 ^a
Acetic acid	296	178	187	162	203	294	119	123	237	309	155	187	309	392	188	121	299	170
Furfural	8.07	17.2	17.2	9.16	4.40	9.05	13.5	12.1	20.5	0.734	12.5	12.8	16.7	22.4	18.1	7.32	16.4	14.9
Propionic acid	17.1	10.2	9.31	6.83	20.7	21.6	8.19	8.90	15.6	14.6	7.88	8.90	20.5	31.4	13.5	6.83	10.2	9.31
Isobutyric acid	1.94	1.36	0.648	0.850	0.175	2.39	1.15	0.254	0.573	2.43	0.947	1.66	1.41	1.30	1.55	1.68	0.844	0.460
5-Methylfurfural	0.305	0.635	19.9 ^a	0.204	0.126	0.424	0.549	0.484	0.642	0.159	0.461	0.57	0.406	0.898	65.6 ^a	0.406	35.0 ^a	0.520
n-Butyric acid	3.09	4.12	3.66	2.07	0.158	0.964	2.79	4.91	4.98	0.880	3.64	4.07	2.75	2.91	0.321	3.41	4.82	3.40
Ethyl decanoate	0.691 ^a	13.0 ^a	6.26 ^a	7.56 ^a	24.4 ^a	19.7 ^a	16.6 ^a	20.6 ^a	15.4 ^a	14.2 ^a	18.1 ^a	2.19 ^a	0.775 ^a	4.70 ^a	21.8 ^a	4.82 ^a	81.5 ^a	0.508 ^a
Isovaleric acid	2.79	1.21	1.17	1.12	1.22	1.42	1.17	1.52	1.82	1.61	1.40	1.30	2.46	1.58	1.43	1.24	1.54	1.39
Diethyl succinate	4.95	4.10	4.17	4.16	4.76	5.35	4.57	4.18	4.48	4.67	5.33	4.31	5.14	4.25	4.41	4.31	4.09	5.35
n-Valeric acid	3.12	1.29	1.32	1.25	1.35	1.50	1.31	1.69	1.79	1.39	1.52	1.37	2.80	1.78	1.52	1.34	1.47	1.34
2-Phenethyl acetate	0.506	0.328	1.14	0.312	0.276	0.339	0.387	0.207	0.442	0.472	0.454	0.253	0.552	0.132	0.375	99.0 ^a	0.644	83.5 ^a
Hexanoic acid	6.92	3.18	3.82	2.98	4.84	5.38	4.00	3.64	5.41	5.59	4.35	5.29	6.02	3.95	6.10	3.94	5.35	3.55
Guaiacol	0.359	0.150	0.227	0.118	0.146	0.274	18.0 ^a	32.0 ^a	0.117	0.298	0.130	77.0 ^a	0.333	0.330	0.175	0.133	0.174	19.7 ^a
trans-oak-lactone	1.03	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	1.04	1.03	nd ^b	nd ^b	nd ^b	nd ^b
2-Phenylethyl alcohol	17.7	4.71	6.01	3.62	6.20	11.7	7.27	6.27	8.11	8.47	19.9	6.01	7.82	8.78	11.1	4.96	11.7	8.96
cis-oak-lactone	0.993	0.971	0.968	nd ^b	0.970	0.974	0.969	0.967	nd ^b	0.970	0.967	0.969	0.998	0.996	0.971	0.969	0.972	0.969
o-Cresol	0.879	0.780	0.774	0.741	0.836	0.854	0.754	0.756	0.799	0.800	0.756	0.747	0.925	0.942	0.784	0.723	0.773	0.770
Phenol	0.612	10.6 ^a	1.13	0.584	0.424	0.682	30.3 ^a	0.863	0.162	0.634	0.161	0.763	0.939	1.12	63.4 ^a	0.761	46.0 ^a	0.856
4-Ethylguaiacol	0.359	0.343	0.350	0.335	0.344	0.350	0.339	0.340	0.344	0.351	0.343	0.340	0.355	0.354	0.348	0.335	0.345	0.339
Octanoic acid	4.18	1.35	3.02	1.40	2.46	2.84	1.91	1.69	3.00	3.08	2.57	2.38	2.60	2.19	4.69	1.16	3.21	1.89
p-Cresol	0.289	0.219	0.247	0.185	0.228	0.236	0.210	0.197	0.233	0.340	0.213	0.205	0.297	0.313	0.240	0.182	0.212	0.211
Eugenol	0.649	0.575	0.611	0.496	0.554	0.596	0.506	0.510	0.556	0.639	0.540	0.533	0.630	0.595	0.604	0.498	0.515	0.516
Decanoic acid	1.21	0.678	0.981	0.626	0.804	0.793	0.742	0.750	0.852	0.750	0.704	0.679	0.994	0.875	0.925	0.615	0.806	0.709
2,6-Dimethoxy phenol	5.67	3.73	5.90	1.58	6.44	5.91	3.38	3.08	3.54	5.67	5.67	3.73	5.91	1.73	3.54	2.22	5.91	3.73
5-Hydroxymethylfurfural	12.1	4.64	4.80	2.05	11.6	9.94	4.35	3.48	5.56	3.48	3.48	3.97	11.6	18.3	5.56	2.78	6.96	3.09
Vanillin	3.95	2.47	3.29	1.24	4.12	4.84	1.54	2.06	2.47	4.12	3.53	2.47	4.12	2.60	2.47	1.24	2.91	1.65

^a Measured in $\mu\text{g/L}$. ^b nd: not detected. For details of the wines refer to **Table 1**. For analytical conditions refer to **chapter 6, section 6.2**.

Selected tables

Table 7. Quantitative (mg/l) data of 56 young Chardonnay wines (CH1 – CH56) from vintage 2005.

COMPOUND	CH1	CH2	CH3	CH4	CH5	CH6	CH7	CH8	CH9	CH10	CH11	CH12	CH13	CH14	CH15	CH16	CH17
Ethyl acetate	112	64.6	71.9	129	137	211	149	124	28.6	145	147	133	196	137	132	45.3	184
Ethyl butyrate	0.567	0.398	0.599	0.470	0.502	0.493	0.650	0.619	0.361	0.590	0.431	0.491	0.722	0.586	0.563	0.447	0.568
1-Propanol	27.0	7.78	9.13	21.6	54.4	5.89	1.92	23.9	39.4	22.8	27.0	20.3	34.7	61.7	40.7	12.8	43.8
Isobutanol	16.7	8.36	12.8	17.2	30.6	17.9	20.8	37.0	15.0	11.9	22.5	33.7	80.5	58.5	26.3	0.998	28.7
Isoamyl acetate	7.50	8.01	8.91	5.22	5.33	5.29	5.03	5.16	6.08	6.26	5.07	2.44	5.65	1.87	6.08	7.18	7.41
n-Butanol	11.5	6.41	0.193	11.8	7.01	11.7	11.2	0.352	11.3	12.0	9.53	10.9	9.58	10.5	9.81	3.62	1.59
Isoamyl alcohol	139	121	134	110	143	137	123	115	128	109	105	110	140	122	124	128	131
Ethyl hexanoate	0.697	0.623	0.886	0.615	0.687	0.565	0.635	0.571	0.758	0.618	0.460	0.570	0.740	0.409	0.638	0.450	0.678
Hexyl acetate	63.5 ^a	38.1 ^a	42.5 ^a	74.3 ^a	32.8 ^a	38.8 ^a	71.8 ^a	56.9 ^a	45.2 ^a	27.7 ^a	0.0715	0.313	37.9 ^a	22.1 ^a	0.106	78.5 ^a	63.0 ^a
Acetoin	31.7	42.9	15.9	64.0	65.1	58.8	58.5	68.3	28.9	30.5	45.7	57.7	5.40	41.6	1.02	68.2	67.6
Ethyl-D-lactate	36.4	11.8	18.3	1.71	7.15	3.06	9.57	5.23	22.6	9.52	2.42	75.5	6.43	157	3.03	6.71	15.6
1-Hexanol	73.3 ^a	0.448	0.964	0.238	0.847	0.552	0.416	0.482	0.981	0.700	0.917	0.269	0.705	1.69	0.616	0.793	0.901
Ethyl octanoate	0.129	90.1 ^a	0.135	0.112	87.7 ^a	89.7 ^a	88.5 ^a	86.3 ^a	0.115	98.1 ^a	53.7 ^a	94.4 ^a	0.113	55.2 ^a	0.113	77.1 ^a	0.123
Acetic acid	498	1574	1093	273	364	353	363	405	962	219	267	254	364	273	342	547	456
Furfural	16.8	22.5	9.57	18.9	15.8	18.9	19.2	1.78	23.1	13.1	15.2	13.8	6.79	12.3	20.5	19.8	1.72
Propionic acid	25.8	31.6	10.2	5.66	4.63	5.66	4.43	6.79	26.8	2.55	2.49	3.09	29.2 ^a	2.49	7.84	5.99	5.10
Isobutyric acid	0.941	1.43	0.528	1.69	0.494	0.656	0.825	0.235	0.566	1.20	0.126	1.14	0.650	0.738	0.550	1.57	1.57
5-Methylfurfural	0.417	0.796	0.438	0.122	0.599	0.717	32.2 ^a	79.1 ^a	0.773	0.578	0.573	0.448	0.619	0.523	3.21 ^a	20.0 ^a	0.353
n-Butyric acid	4.48	4.67	3.50	4.52	4.27	0.118	0.170	5.14	3.18	3.76	2.57	3.11	4.67	3.70	0.973	5.15	1.09
Ethyl decanoate	10.4 ^a	4.91 ^a	7.13 ^a	13.6 ^a	0.912 ^a	7.50 ^a	15.6 ^a	8.83 ^a	5.15 ^a	6.12 ^a	1.13 ^a	10.3 ^a	11.1 ^a	8.47 ^a	5.02 ^a	8.17 ^a	19.4 ^a
Isovaleric acid	1.62	1.87	1.68	1.35	1.45	1.54	1.58	1.31	1.69	1.30	1.21	1.18	1.40	1.52	1.37	2.06	1.53
Diethyl succinate	6.11	4.50	5.25	4.34	4.89	4.95	4.29	4.26	6.03	4.55	4.30	5.29	4.37	5.59	4.32	4.50	4.74
n-Valeric acid	1.80	2.04	1.93	1.55	1.31	1.46	1.60	1.42	1.83	1.33	1.35	1.40	1.37	1.46	1.40	1.52	1.78
2-Phenethyl acetate	0.483	0.168	0.291	0.332	0.148	0.234	0.450	0.268	0.557	0.410	0.448	73.6 ^a	0.309	64.3 ^a	0.316	0.295	0.289
Hexanoic acid	9.32	6.29	7.64	5.02	5.97	4.39	6.52	3.56	5.23	4.26	3.21	3.64	4.89	4.56	4.80	4.72	6.70
Guaiacol	0.588	0.617	0.499	0.285	0.324	0.233	0.342	0.502	0.685	0.145	0.187	78.9 ^a	0.185	0.161	0.292	0.215	0.326
trans-oak-lactone	1.03	1.05	1.03	nd ^b	nd ^b	0	nd ^b	nd ^b	nd ^b	nd ^b	1.03	1.04	nd ^b	1.03	nd ^b	1.04	1.04
2-Phenylethyl alcohol	15.3	10.2	14.2	8.53	11.9	10.9	11.8	6.01	9.93	8.71	5.33	6.33	9.92	8.80	10.3	8.79	9.35
cis-oak-lactone	1.00	1.02	1.00	0.970	nd ^b	0	nd ^b	nd ^b	nd ^b	nd ^b	0.968	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	0.976
o-Cresol	0.914	0.945	0.851	0.800	0.774	0.802	0.807	0.820	0.905	0.747	0.764	0.769	0.812	0.772	0.833	0.786	0.842
Phenol	1.16	1.58	0.812	0.445	0.164	0.484	0.546	0.680	1.63	0.844	0.614	45.1 ^a	0.428	0.196	0.475	0.669	1.16
4-Ethylguaiacol	0.360	0.366	0.365	0.350	0.352	0.347	0.351	0.358	0.369	0.346	0.345	0.341	0.344	0.346	0.349	0.347	0.358
Octanoic acid	5.05	3.07	3.72	2.31	2.23	1.78	4.11	1.49	2.85	1.94	1.42	1.44	1.87	2.00	2.47	1.94	2.65
p-Cresol	0.330	0.347	0.288	0.244	0.231	0.268	0.239	0.274	0.434	0.212	0.219	0.208	0.227	0.222	0.257	0.362	0.308
Eugenol	0.712	0.729	0.687	0.617	0.660	0.571	0.621	0.700	0.768	0.591	0.616	0.537	0.581	0.537	0.574	0.562	0.571
Decanoic acid	1.11	0.903	0.942	0.675	0.633	0.687	0.849	0.751	0.969	0.692	0.687	0.632	0.687	0.713	0.711	0.752	0.796
2,6-Dimethoxy phenol	4.02	10.7	7.83	3.56	3.92	9.75	7.58	3.41	18.0	2.37	3.41	2.62	2.80	3.26	3.73	6.53	4.12
5-Hydroxymethylfurfural	13.8	14.5	12.3	4.10	3.07	4.10	3.51	4.39	20.9	3.07	2.46	2.24	3.07	2.05	3.24	3.84	3.62
Vanillin	28.9	55.8	11.0	10.0	8.46	5.79	15.8	33.2	35.6	5.50	7.34	2.75	5.50	2.97	5.00	7.86	3.44

Table 7 Continue >>>

Selected tables

COMPOUND	CH18	CH19	CH20	CH21	CH22	CH23	CH24	CH25	CH26	CH27	CH28	CH29	CH30	CH31	CH32	CH33	CH34
Ethyl acetate	128	155	147	94.1	151	82.3	118	156	197	104	61.3	114	147	184	163	148	129
Ethyl butyrate	0.467	0.530	0.563	0.409	0.658	0.512	0.424	0.554	0.597	0.512	0.739	0.407	0.623	0.491	0.569	0.681	0.667
1-Propanol	95.4	186	9.98	29.5	76.4	36.3	7.11	3.37	24.8	39.7	38.7	33.3	71.6	43.2	21.6	14.2	32.9
Isobutanol	32.0	46.9	4.93	14.7	44.5	22.2	5.76	43.7	18.3	36.9	9.67	39.3	24.7	39.6	19.7	46.2	54.3
Isoamyl acetate	2.84	5.23	8.17	8.34	3.91	5.55	2.52	6.44	14.1	4.78	9.04	3.68	8.00	5.44	3.60	6.72	5.47
n-Butanol	8.72	10.6	7.89	7.39	1.38	5.60	8.77	12.9	0.162	9.79	9.08	10.5	0.141	9.74	9.89	9.61	7.37
Isoamyl alcohol	121	107	109	106	122	126	92.1	120	126	128	131	118	114	126	101	130	126
Ethyl hexanoate	0.601	0.549	0.841	0.814	0.691	0.494	0.779	0.855	0.603	0.653	0.789	0.505	0.659	0.579	0.725	0.648	0.689
Hexyl acetate	25.8 ^a	30.3 ^a	0.112	0.114	31.3 ^a	48.3 ^a	63.8 ^a	49.2 ^a	0.229	83.0 ^a	0.107	36.4 ^a	0.118	54.7 ^a	0.114	70.9 ^a	54.2 ^a
Acetoin	25.2	54.4	1.99	39.3	42.8	44.5	40.1	53.2	59.8	48.0	21.1	51.2	54.4	51.2	57.4	50.0	55.0
Ethyl-D-lactate	6.78	11.6	10.5	0.533	2.55	182	171	13.0	5.77	5.85	8.71	102	5.85	7.67	33.0	10.8	88.5
1-Hexanol	0.369	0.224	0.354	0.821	48.6 ^a	0.849	0.586	1.59	0.586	0.367	1.01	99.5 ^a	0.244	0.994	1.45	94.7 ^a	0.702
Ethyl octanoate	95.6 ^a	79.8 ^a	0.115	0.124	5.58 ^a	84.2 ^a	0.121	81.7 ^a	93.4 ^a	0.107	0.109	80.5 ^a	0.103	87.8 ^a	95.6 ^a	98.0 ^a	95.8 ^a
Acetic acid	219	364	421	733	489	733	497	419	586	533	2932	497	533	586	506	543	637
Furfural	8.98	18.6	4.74	15.6	12.5	6.23	2.88	17.7	19.4	16.3	18.8	15.5	16.1	15.2	17.0	15.1	16.9
Propionic acid	2.37	3.09	7.84	13.1	3.77	12.3	4.90	4.78	4.67	4.45	19.6	4.67	4.56	4.90	5.03	5.16	5.03
Isobutyric acid	1.34	0.295	2.18	3.27	0.408	0.430	0.830	2.13	1.30	0.788	0.446	34.0 ^a	1.22	2.47	0.661	2.08	0.784
5-Methylfurfural	0.477	0.685	0.315	0.729	27.0 ^a	0.294	0.345	0.236	0.678	58.8 ^a	0.394	0.473	0.396	0.571	0.565	40.6 ^a	0.659
n-Butyric acid	3.43	4.78	4.80	3.38	3.79	1.40	1.42	5.78	4.38	4.13	3.38	3.25	3.09	3.82	5.09	4.80	0.868 ^a
Ethyl decanoate	7.07 ^a	4.64 ^a	18.9 ^a	6.61 ^a	5.08 ^a	8.18 ^a	8.06 ^a	25.8 ^a	10.3 ^a	13.1 ^a	6.36 ^a	8.25 ^a	12.0 ^a	11.5 ^a	7.93 ^a	8.53 ^a	3.07 ^a
Isovaleric acid	1.24	1.37	1.44	1.24	1.34	1.35	1.48	4.46	1.41	1.29	1.77	1.25	1.31	1.43	1.54	1.43	1.48
Diethyl succinate	4.40	4.78	5.09	4.47	4.58	4.26	5.31	1.96	4.21	4.47	4.40	6.41	4.54	4.81	4.95	4.98	5.32
n-Valeric acid	1.26	1.39	1.69	1.77	1.39	1.64	1.72	0.617	1.48	1.44	2.08	1.36	1.37	1.35	1.51	1.56	1.41
2-Phenethyl acetate	79.7 ^a	0.271	0.706	0.629	0.106	0.266	0.140	0.435	0.814	0.379	0.697	0.281	0.954	0.243	0.149	0.344	0.267
Hexanoic acid	3.38	4.52	7.98	6.805	4.53	5.53	6.10	4.63	5.07	3.96	6.90	3.40	5.31	3.82	5.17	5.42	4.53
Guaiacol	91.5 ^a	1.18	0.402	0.695	0.185	0.335	0.427	1.20	0.227	0.311	0.590	0.116	0.233	0.121	0.290	0.220	0.417
trans-oak-lactone	nd ^b	nd ^b	nd ^b	nd ^b	1.03	1.03	nd ^b	nd ^b	1.03	1.04	1.04	1.03	1.03	nd ^b	1.03	1.03	1.03
2-Phenylethyl alcohol	5.86	8.49	12.2	10.3	9.72	12.5	9.01	9.95	11.1	7.26	11.0	8.05	9.10	8.16	6.34	10.5	6.73
cis-oak-lactone	nd ^b	0.971	0.972	0.995	nd ^b	0.992	0.989	2.69	0.970	0.969	1.01	0.968	0.986	0.967	0.964	0.972	0.973
o-Cresol	0.743	0.802	0.845	0.887	0.755	0.858	0.829	0.117	0.808	0.783	0.908	0.779	0.789	0.785	0.798	0.787	0.791
Phenol	0.785	0.282	0.650	1.33	0.897	0.838	0.633	2.94	0.304	0.703	1.56	0.212	0.505	0.375	0.602	0.374	0.489
4-Ethylguaiacol	0.344	0.348	0.354	0.370	0.346	0.355	0.362	0.125	0.351	0.353	0.369	0.344	0.346	0.341	0.353	0.350	0.358
Octanoic acid	1.57	1.99	3.04	2.77	1.42	3.30	3.21	1.98	2.28	1.75	4.38	1.55	3.23	1.59	1.95	2.11	1.55
p-Cresol	0.219	0.264	0.265	0.293	0.222	0.288	0.255	0.305	0.258	0.258	0.358	0.221	0.227	0.234	0.249	0.270	0.274
Eugenol	0.508	0.593	0.695	0.729	0.574	0.623	0.674	0.865	0.588	0.651	0.756	0.555	0.603	0.563	0.602	0.606	0.690
Decanoic acid	0.658	0.691	0.733	0.787	0.611	0.808	0.875	0.122	0.732	0.685	1.02	0.666	0.908	0.706	0.754	0.735	0.639
2,6-Dimethoxy phenol	4.12	2.61	5.22	10.7	4.03	13.4	7.67	4.24	4.13	10.7	16.1	4.13	5.37	5.97	6.445	6.71	5.37
5-Hydroxymethylfurfural	2.46	4.10	6.15	9.00	2.45	9.00	4.50	2.70	3.37	3.21	13.5	2.25	2.14	1.50	1.93	1.99	2.70
Vanillin	2.62	8.46	9.74	18.9	7.07	15.7	9.43	7.07	9.43	7.64	28.3s	7.44	11.3	6.28	6.58	7.07	14.1

Table 7 Continue >>>

Selected tables

COMPOUND	CH35	CH36	CH37	CH38	CH39	CH40	CH41	CH42	CH43	CH44	CH45	CH46	CH47	CH48	CH49	CH50	CH51
Ethyl acetate	139	116	148	70.9	122	84.6	117	134	210	134	142	85.7	151	108	126	169	152
Ethyl butyrate	0.556	0.456	0.757	0.421	0.439	0.541	0.503	0.544	0.604	0.469	0.532	0.367	0.520	0.451	0.679	0.567	0.724
1-Propanol	69.5	39.5	21.0	14.9	18.1	13.3	29.9	32.0	6.90	51.9	54.2	21.1	33.0	19.2	42.7	15.1	46.8
Isobutanol	27.2	34.2	22.3	19.7	6.57	1.27	33.8	30.7	21.0	18.4	37.8	11.4	24.7	8.81	34.2	25.1	47.7
Isoamyl acetate	6.32	4.05	6.62	5.02	7.55	7.62	4.85	6.10	7.62	7.06	5.22	5.00	6.84	9.27	5.66	7.01	7.64
n-Butanol	9.19	7.49	8.08	11.3	3.51	5.61	10.6	2.10	1.03	7.71	7.11	11.4	0.684	6.28	7.63	7.27	0.430
Isoamyl alcohol	120	106	135	104	111	113	107	115	117	113	125	96.2	116	146	110	108	135
Ethyl hexanoate	0.597	0.517	0.624	0.706	0.549	0.998	0.605	0.470	0.750	0.450	0.722	0.575	0.650	0.564	0.510	0.686	0.662
Hexyl acetate	67.4 ^a	41.9 ^a	0.144	25.0 ^a	71.5 ^a	91.5 ^a	66.5 ^a	85.3 ^a	68.6 ^a	0.149	24.4 ^a	68.7 ^a	65.3 ^a	76.3 ^a	83.0 ^a	64.5 ^a	91.8 ^a
Acetoin	39.5	40.7	62.8	14.8	22.2	6.93	56.2	47.0	15.0	35.8	56.2	28.7	49.0	62.6	31.8	2.06	43.3
Ethyl-D-lactate	13.8	12.4	7.97	0.718	13.4	1.85	0.995	8.50	4.12	5.50	19.1	11.3	96.0	13.2	7.38	37.1	94.9
1-Hexanol	1.00	0.972	1.05	0.407	0.816	0.899	0.482	0.971	0.783	0.834	0.406	0.994	0.723	45.1 ^a	0.109	0.643	31.3 ^a
Ethyl octanoate	92.0 ^a	71.9	0.125	65.3 ^a	97.1 ^a	0.137	87.5 ^a	78.1 ^a	0.118	68.7 ^a	0.115	83.1 ^a	0.105	91.9 ^a	86.8 ^a	0.112	0.107
Acetic acid	489	465	489	2864	561	648	301	821	468	211	421	842	443	1462	340	769	585
Furfural	13.5	15.7	18.7	8.68	6.91	7.75	15.8	18.0	5.56	12.7	2.66	18.8	13.2	30.0	12.7	21.4	10.1
Propionic acid	4.00	4.78	4.90	9.89	16.1	11.2	13.4	12.6	14.4	5.32	10.1	20.1	10.4	34.3	7.98	15.6	7.15
Isobutyric acid	1.29	0.858	0.721	5.01	0.351	0.178	1.08	1.24	2.06	1.07	2.08	1.79	1.60	4.30	1.30	0.365	0.268
5-Methylfurfural	0.579	54.9 ^a	0.693	0.717	0.487	0.582	0.563	0.625	0.538	0.572	0.423	0.413	0.693	0.958	0.474	0.660	0.447
n-Butyric acid	4.24	0.235	4.92	5.14	2.08	1.92	4.43	3.73	2.26	4.02	4.01	2.66	3.77	4.52	3.78	4.10	3.75
Ethyl decanoate	8.21 ^a	1.11 ^a	34.8 ^a	1.82 ^a	3.81 ^a	5.41 ^a	7.00 ^a	9.18 ^a	18.4 ^a	2.64 ^a	12.6 ^a	1.51 ^a	14.2 ^a	1.58 ^a	6.06 ^a	13.8 ^a	10.3 ^a
Isovaleric acid	1.32	1.36	1.43	7.64	1.27	1.36	1.22	1.19	1.51	1.16	1.36	2.82	1.44	1.84	1.22	1.23	1.45
Diethyl succinate	4.48	5.96	4.32	5.15	4.66	4.50	5.07	4.68	4.48	4.55	5.00	4.48	5.10	4.87	4.27	4.50	5.20
n-Valeric acid	1.32	1.39	1.57	6.49	1.50	1.65	1.37	1.47	1.77	1.31	1.33	3.20	1.56	2.44	1.26	1.47	1.32
2-Phenethyl acetate	0.433	0.281	0.396	0.157	0.428	0.931	0.548	0.460	0.307	0.552	0.384	0.311	0.378	0.755	0.286	0.309	0.493
Hexanoic acid	4.58	3.94	8.24	9.93	5.25	5.63	3.98	3.66	7.45	3.77	5.01	5.71	4.64	7.72	3.90	5.84	5.24
Guaiacol	0.204	0.199	0.372	0.528	0.514	0.386	0.213	0.167	0.306	0.174	0.307	0.533	0.203	0.780	0.105	0.437	71.6 ^a
trans-oak-lactone	1.03	nd ^b	nd ^b	1.13	1.04	nd ^b	nd ^b	1.03	1.04	nd ^b	1.05	1.03	1.04	nd ^b	1.03	1.03	1.04
2-Phenylethyl alcohol	8.16	7.84	12.5	6.68	10.5	7.79	8.84	7.25	8.87	8.29	8.79	5.20	8.74	15.1	5.69	7.16	13.6
cis-oak-lactone	0.973	0.968	0.984	1.04	1.01	0.994	0.968	0.968	0.971	0.968	0.991	0.999	0.971	1.00	0.970	0.973	0.975
o-Cresol	0.773	0.790	0.824	0.891	0.854	0.845	0.792	0.788	0.876	0.763	0.798	0.936	0.761	0.961	0.762	0.834	0.737
Phenol	0.177	0.167	0.709	1.60	0.675	0.845	0.201	0.146	1.53	12.6 ^a	0.835	1.12	0.221	2.19	72.7 ^a	0.538	0.693
4-Ethylguaiacol	0.347	0.350	0.356	0.364	0.366	0.357	0.346	0.344	0.352	0.349	0.351	0.366	0.350	0.373	0.345	0.357	0.342
Octanoic acid	2.03	1.51	5.67	5.86	2.76	2.36	2.60	1.65	2.60	1.54	2.27	2.80	1.91	4.90	2.25	2.64	2.45
p-Cresol	0.238	0.266	0.261	0.455	0.283	0.295	0.222	0.228	0.326	0.218	0.249	0.352	0.246	0.388	0.230	0.256	0.195
Eugenol	0.598	0.575	0.634	0.734	0.670	0.654	0.678	0.574	0.612	0.593	0.665	0.702	0.556	0.771	0.537	0.711	0.501
Decanoic acid	0.674	0.668	0.889	4.03	0.750	0.810	0.734	0.652	0.796	0.628	0.687	1.09	0.686	1.84	0.709	0.696	0.698
2,6-Dimethoxy phenol	4.60	4.13	3.66	10.1	4.31	6.16	3.60	3.41	6.49	2.94	4.62	6.47	7.42	14.8	8.24	23.4	6.74
5-Hydroxymethylfurfural	2.65	3.00	3.07	4.50	10.9	8.27	2.71	4.34	4.57	2.17	2.12	8.68	3.75	15.0	7.89	1.75	3.00
Vanillin	9.43	10.1	27.9	58.7	33.2	33.2	20.3	26.1	19.2	12.6	19.2	36.5	11.7	23.4	10.6	45.5	6.32

Table 7 Continue >>>

Selected tables

COMPOUND	CH52	CH53	CH54	CH55	CH56
Ethyl acetate	108	133	141	144	142
Ethyl butyrate	0.739	0.685	0.397	0.618	0.380
1-Propanol	14.6	15.3	36.4	13.5	34.6
Isobutanol	47.8	31.7	25.5	36.5	50.5
Isoamyl acetate	5.13	4.99	4.38	8.86	3.83
n-Butanol	9.48	7.76	7.98	9.55	6.75
Isoamyl alcohol	152	119	96.9	137	112
Ethyl hexanoate	0.639	0.439	0.588	0.576	0.384
Hexyl acetate	38.2 ^a	46.1 ^a	38.7 ^a	0.105	62.9 ^a
Acetoin	8.89	32.5	49.2	66.6	43.1
Ethyl-D-lactate	66.9	8.45	1.76	9.03	33.1
1-Hexanol	0.351	0.201	0.659	27.4 ^a	0.278
Ethyl octanoate	84.1 ^a	81.9 ^a	82.4 ^a	0.101	66.4 ^a
Acetic acid	696	395	443	522	273
Furfural	1.16	13.3	14.8	0.733	10.5
Propionic acid	16.3	8.37	9.53	12.3	2.32
Isobutyric acid	1.08	0.519	0.796	1.13	0.717
5-Methylfurfural	20.9 ^a	0.655	0.574	63.8 ^a	0.361
n-Butyric acid	1.03	4.82	3.59	3.99	3.09
Ethyl decanoate	2.28 ^a	5.84 ^a	5.82 ^a	13.5 ^a	1.17 ^a
Isovaleric acid	1.65	1.37	1.46	1.38	3.79
Diethyl succinate	5.64	4.24	4.28	4.39	4.34
n-Valeric acid	1.52	1.33	1.34	1.57	1.22
2-Phenethyl acetate	0.232	0.172	0.174	0.953	0.250
Hexanoic acid	5.67	4.10	4.60	7.27	4.40
Guaiacol	0.353	0.156	0.186	0.302	0.120
trans-oak-lactone	1.03	nd ^b	1.03	nd ^b	nd ^b
2-Phenylethyl alcohol	16.9	6.50	6.03	16.2	7.08
cis-oak-lactone	0.964	nd ^b	0.968	0.971	nd ^b
o-Cresol	0.829	0.771	0.787	0.830	0.746
Phenol	1.00	0.234	0.216	0.636	0.263
4-Ethylguaiacol	0.354	0.346	0.347	0.351	0.341
Octanoic acid	2.11	2.04	1.94	3.46	2.53
p-Cresol	0.254	0.243	0.237	0.265	0.210
Eugenol	0.642	0.590	0.589	0.661	0.541
Decanoic acid	0.720	0.700	0.634	0.734	0.675
2,6-Dimethoxy phenol	12.4	5.11	7.81	11.4	3.97
5-Hydroxymethylfurfural	7.14	3.66	3.00	5.35	1.76
Vanillin	21.2	11.1	8.66s	19.5	2.45

^a Measured in µg/L. ^b nd: not detected. For details of the wines refer to

Table 1. For analytical conditions refer to **chapter 6, section 6.2.**

Selected tables

Table 8 Quantitative (mg/l) data of 42 young Pinotage wines (PI50 – PI91) from vintage 2006.

COMPOUND	PI50	PI51	PI52	PI53	PI54	PI55	PI56	PI57	PI58	PI59	PI60	PI61	PI62	PI63	PI64	PI65	PI66
Ethyl acetate	164	146	184	192	153	218	181	216	206	214	211	152	177	183	200	236	240
Ethyl butyrate	0.308	0.443	0.257	0.357	0.502	0.466	0.283	0.412	0.432	0.367	0.407	0.306	0.620	0.363	0.286	0.376	0.475
1-Propanol	54.0	35.8	56.2	122	25.5	28.1	120	132	62.6	31.4	15.4	38.1	33.3	29.6	61.8	52.8	61.1
Isobutanol	54.5	39.2	65.6	52.3	37.0	34.3	35.8	38.1	63.1	38.8	42.6	52.7	30.9	53.1	52.5	60.4	35.6
Isoamyl acetate	6.01	4.89	2.60	5.65	6.88	9.05	6.05	6.22	6.56	7.02	10.9	6.83	6.30	3.18	3.96	6.30	9.38
n-Butanol	4.49	7.00	5.88	7.86	8.83	8.81	5.89	29.6	7.69	10.5	9.64	5.36	6.04	6.74	8.02	11.0 ^a	8.92
Isoamyl alcohol	147	140	147	151	161	161	127	132	157	153	157	142	154	139	151	155	144
Ethyl hexanoate	0.343	0.475	0.307	0.332	0.500	0.596	0.295	0.395	0.485	0.404	0.542	0.413	0.832	0.338	0.379	0.397	0.553
Hexyl acetate	41.5 ^a	18.9 ^a	23.9 ^a	32.1 ^a	23.1 ^a	83.8 ^a	52.3 ^a	27.6 ^a	37.0 ^a	60.5 ^a	81.2 ^a	80.2 ^a	16.4 ^a	15.9 ^a	21.5 ^a	24.1 ^a	46.3 ^a
Acetoin	69.0	91.8	8.57	43.1	29.3	37.7	82.9	53.1	46.7	36.7	73.8	20.7	52.2	28.1	26.2	37.8	219
Ethyl-D-lactate	346	275	275	351	208	152	169	384	284	352	375	298	242	249	416	486	227
1-Hexanol	0.325	0.171	1.62	8.69 ^a	1.03	1.08	0.117	1.03	0.131	1.11	0.235	0.610	0.928	0.208	0.101	1.04	0.687
Ethyl octanoate	90.9 ^a	0.127	67.3 ^a	91.5 ^a	0.120	0.166	89.2 ^a	0.115	0.134	0.107	0.148	0.120	0.220	89.8 ^a	0.100	0.125	0.153
Acetic acid	332	619	380	509	486	922	329	721	206	248	684	263	851	794	362	794	331
Furfural	18.0	10.4	17.8	1.26	20.4	1.22	3.74	4.71	9.64	13.7	19.1	5.22	20.9	8.75	0.252	9.09	16.5
Propionic acid	2.67	22.2	5.20	18.8	7.49	25.0	15.6	15.0	5.22	7.74	15.4	12.0	32.2	16.8	9.64	19.9	16.8
Isobutyric acid	1.63	2.25	1.62	1.72	2.16	1.38	2.06	2.22	2.32	1.78	1.38	1.40	2.79	6.10	3.19	2.02	2.81
5-Methylfurfural	31.0 ^a	0.416	0.722	0.141	70.0 ^a	93.0 ^a	0.366	0.424	0.550	0.632	0.662	0.260	0.824	0.505	0.225	0.399	0.181
n-Butyric acid	4.99	1.37	4.71	2.92	0.767	3.67	0.364	1.28	4.26	4.75	2.38	0.153	2.77	0.828	0.726	1.31	4.29
Ethyl decanoate	18.2 ^a	18.1 ^a	23.9 ^a	19.4 ^a	32.0 ^a	52.7 ^a	32.4 ^a	34.6 ^a	51.0 ^a	18.3 ^a	59.3 ^a	32.3 ^a	0.113	34.6 ^a	12.0 ^a	30.2 ^a	87.3 ^a
Isovaleric acid	1.75	1.62	1.51	1.35	1.72	1.64	1.44	1.47	1.54	1.55	1.52	1.58	1.54	1.39	1.69	1.81	1.52
Diethyl succinate	9.76	7.38	6.39	9.48	8.97	9.49	8.43	11.7	8.96	4.92	11.2	7.69	6.92	12.9	10.4	10.1	8.10
n-Valeric acid	1.58	1.68	1.38	1.61	1.55	1.86	1.62	1.70	1.56	1.55	1.54	1.41	1.72	1.49	1.70	1.80	1.82
2-Phenethyl acetate	0.273	0.230	0.162	0.262	0.177	0.309	0.338	0.210	0.192	0.204	0.460	0.422	0.136	0.217	0.284	0.230	0.432
Hexanoic acid	4.15	4.82	3.09	2.97	3.55	4.34	3.79	4.83	4.56	4.20	3.88	3.22	6.38	3.18	4.30	4.38	4.97
Guaiacol	0.371	0.539	0.259	0.446	0.403	0.53	0.440	0.388	87.2 ^a	0.185	0.279	0.563	0.858	0.541	0.369	0.983	0.528
trans-oak-lactone	1.04	1.04	nd ^b	nd ^b	nd ^b	nd ^b	1.04	nd ^b	1.03	nd ^b	1.03	1.04	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b
2-Phenylethyl alcohol	11.8	11.6	13.5	12.0	10.1	13.7	9.07	10.3	9.28	9.61	14.0	9.87	10.8	12.3	18.3	12.5	8.84
cis-oak-lactone	0.998	0.998	nd ^b	0.969	0.970	nd ^b	0.969	nd ^b	0.967	0.974	0.970	0.990	nd ^b	nd ^b	0.969	0.971	nd ^b
o-Cresol	0.785	0.853	0.787	0.849	0.812	0.852	0.837	0.841	0.741	0.767	0.810	0.838	0.905	0.834	0.819	0.854	0.821
Phenol	0.522	0.942	0.213	0.698	0.537	0.822	0.524	0.446	0.850	0.199	0.404	0.749	1.55	1.01	0.785	0.918	0.596
4-Ethylguaiacol	0.351	0.359	0.350	0.353	0.354	0.358	0.358	0.358	0.343	0.347	0.347	0.362	0.373	0.361	0.354	0.370	0.357
Octanoic acid	1.93	2.37	1.44	1.28	1.81	1.95	1.62	1.86	1.84	2.15	1.93	1.75	3.08	1.47	1.87	2.11	1.86
p-Cresol	0.258	0.301	0.246	0.272	0.268	0.314	0.291	0.296	0.249	0.250	0.254	0.281	0.310	0.278	0.295	0.315	0.302
Eugenol	0.616	0.682	0.567	0.610	0.620	0.641	0.631	0.605	0.496	0.546	0.550	0.623	0.782	0.704	0.620	0.836	0.605
Decanoic acid	0.818	0.749	0.729	0.689	0.737	0.812	0.741	0.738	0.751	0.774	0.855	0.741	1.02	0.721	0.748	0.779	0.778
2,6-Dimethoxy phenol	6.83	8.51	3.57	9.62	3.45	10.7	5.17	3.45	1.72	2.84	4.46	7.89	19.1	10.6	10.4	15.0	2.60
5-Hydroxymethylfurfural	0.331	0.663	0.672	0.438	0.877	0.909	1.32	1.64	0.188	0.429	0.891	2.05	12.9	1.06	1.89	1.64	0.546
Vanillin	69.0	83.3	8.99	18.0	20.6	31.7	27.0	10.56	6.75	10.9	13.7	91.8	175	61.0	14.3	91.8	13.3

Table 8 Continue >>>

Selected tables

COMPOUND	PI67	PI68	PI69	PI70	PI71	PI72	PI73	PI74	PI75	PI76	PI77	PI78	PI79	PI80	PI81	PI82	PI83
Ethyl acetate	277	261	209	154	140	154	167	188	242	170	199	167	141	213	186	171	172
Ethyl butyrate	0.354	0.536	0.468	0.373	0.208	0.279	0.315	0.380	0.277	0.245	0.336	0.308	0.237	0.429	0.316	0.334	0.406
1-Propanol	94.0	111	47.1	17.9	27.9	54.4	49.6	76.2	65.2	28.9	17.1	24.1	46.0	37.9	49.8	26.2	31.2
Isobutanol	7.63	36.7	69.4	47.0	43.3	86.3	70.3	53.2	23.3	79.6	41.7	53.9	49.2	64.0	66.6	44.0	43.0
Isoamyl acetate	0.681	9.54	8.33	5.96	1.42	2.86	4.65	9.93	9.35	3.00	2.43	6.64	2.58	8.83	0.399	2.89	8.18
n-Butanol	7.54	10.5	10.0	7.51	5.83	5.63	6.82	7.69	8.65	7.49	5.39	6.39	10.2	9.08	7.29	5.37	7.07
Isoamyl alcohol	117	156	185	192	141	175	167	184	141	182	128	122	152	174	147	127	156
Ethyl hexanoate	0.534	0.585	0.462	0.556	0.277	0.336	0.371	0.493	0.310	0.287	0.473	0.719	0.303	0.584	0.467	0.322	0.324
Hexyl acetate	4.58 ^a	25.9 ^a	23.3 ^a	23.5 ^a	5.39 ^a	18.3 ^a	20.4 ^a	75.9 ^a	40.9 ^a	14.8 ^a	17.1 ^a	42.7 ^a	21.8 ^a	88.8 ^a	3.78 ^a	4.50 ^a	32.6 ^a
Acetoin	138	96.8	79.1	26.9	44.7	68.5	97.3	150	28.9	121	53.7	70.6	56.0	67.6	118	16.1	23.1
Ethyl-D-lactate	384	328	388	352	1.04	269	281	383	344	296	335	214	331	366	235	32.6	225
1-Hexanol	0.512	0.882	0.800	0.742	1.01	0.823	0.669	0.609	0.612	0.970	0.187	0.833	0.668	0.582	0.498 ^a	1.01	0.780
Ethyl octanoate	0.183	0.154	0.142	0.136	67.1 ^a	70.4 ^a	86.3 ^a	0.134	0.110	76.3 ^a	0.123	88.5 ^a	68.4 ^a	0.174	0.119	91.6 ^a	68.3 ^a
Acetic acid	359	759	1082	1043	945	466	584	616	616	965	461	430	280	882	614	444	675
Furfural	20.3	1.22	4.89	10.8	8.70	15.3	13.6	1.76	3.54	3.40	2.20	5.05	20.9	4.65	5.65	15.0	6.99
Propionic acid	10.8	11.5	18.1	18.5	21.4	5.26	1.36	10.7	14.5	34.4	7.20	8.66	5.34	33.5	20.4	9.48	12.1
Isobutyric acid	1.25	1.73	2.16	1.96	7.14	3.34	2.87	1.81	1.46	4.36	3.12	4.16	1.90	2.77	2.25	2.30	2.24
5-Methylfurfural	0.183	0.237	0.165	0.513	0.511	45.3 ^a	0.135	0.178	0.105	0.166	0.346	0.351	0.166	0.375	0.314	48.4 ^a	0.242
n-Butyric acid	1.08	0.494	1.33	0.678	0.359	5.01	0.519	0.513	76.1 ^a	4.43	0.290	5.07	0.387	5.27	2.08	2.45	0.144
Ethyl decanoate	63.6 ^a	57.9 ^a	65.3 ^a	36.8 ^a	20.5 ^a	29.1 ^a	10.9 ^a	79.5 ^a	30.2 ^a	4.38 ^a	37.3 ^a	32.8 ^a	6.90 ^a	0.109	20.2 ^a	21.4 ^a	14.9 ^a
Isovaleric acid	1.51	1.47	1.68	1.67	1.60	1.77	1.67	1.74	1.53	2.00	1.47	1.40	1.60	1.54	1.68	1.56	1.46
Diethyl succinate	5.96	6.42	6.14	15.4	14.2	8.58	12.4	6.13	5.45	13.2	9.37	4.60	9.39	10.2	8.37	5.32	6.30
n-Valeric acid	1.60	1.57	1.53	1.55	1.41	1.56	1.69	1.74	1.55	1.72	1.62	1.38	1.64	1.90	1.71	1.75	1.41
2-Phenethyl acetate	28.5 ^a	0.288	0.275	0.326	0.114	0.214	0.194	1.04	0.725	0.242	82.9 ^a	0.397	0.188	0.462	30.9 ^a	0.124	0.561
Hexanoic acid	5.11	4.44	4.58	4.28	2.95	3.82	4.50	6.19	3.86	3.64	4.29	2.89	3.69	4.31	4.22	4.00	3.01
Guaiacol	0.298	0.473	0.459	0.745	0.604	0.267	0.281	0.255	0.402	0.458	0.298	0.371	0.376	0.647	0.440	0.344	0.552
trans-oak-lactone	1.03	nd ^b	1.03	1.04	nd ^b	nd ^b	nd ^b	1.04	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	1.04	nd ^b	nd ^b	nd ^b
2-Phenylethyl alcohol	7.66	9.00	14.9	22.7	13.3	16.0	20.1	21.9	12.7	24.3	11.0	6.76	19.4	14.9	14.1	11.3	9.91
cis-oak-lactone	0.977	nd ^b	0.968	0.992	nd ^b	nd ^b	nd ^b	0.973	nd ^b	nd ^b	0.999	0.986	0.970	0.970	0.972	nd ^b	0.992
o-Cresol	0.801	0.808	0.832	0.852	0.837	0.771	0.793	0.833	0.818	0.867	0.816	0.819	0.810	0.883	0.857	0.796	0.826
Phenol	0.437	0.459	0.926	1.02	1.09	0.250	0.514	0.574	0.412	0.990	0.412	0.879	0.494	0.973	0.683	0.572	0.855
4-Ethylguaiacol	0.352	0.357	0.355	0.367	0.363	0.353	0.353	0.353	0.355	0.355	0.348	0.354	0.355	0.697	0.360	0.353	0.358
Octanoic acid	2.70	2.01	2.15	1.75	1.40	1.49	1.67	2.76	2.21	1.99	2.14	1.57	1.65	2.13	2.00	1.56	1.58
p-Cresol	0.262	0.279	0.274	0.295	0.285	0.254	0.274	0.275	0.269	0.294	0.259	0.259	0.277	0.325	0.290	0.275	0.269
Eugenol	0.575	0.629	0.626	0.759	0.710	0.575	0.571	0.565	0.622	0.559	0.602	0.608	0.599	0.658	0.624	0.554	0.686
Decanoic acid	0.855	0.813	0.934	0.730	0.731	0.715	0.686	0.919	0.856	0.819	0.823	0.762	0.702	0.936	0.707	0.710	0.723
2,6-Dimethoxy phenol	8.32	10.9	6.72	15.3	9.65	5.00	5.17	7.32	10.9	9.63	2.70	18.6	6.08	4.82	4.71	8.00	11.6
5-Hydroxymethylfurfural	0.527	0.471	0.867	4.23	2.83	1.39	1.41	1.27	1.41	1.96	4.18	5.17	3.47	2.13	6.97	78.5 ^a	1.16
Vanillin	16.7	34.8	28.2	160	53.7 _s	28.5	26.1	24.7	32.8	26.9	46.4	5.10	14.4 _s	23.3	6.59	4.32	58.9

Table 8 Continue >>>

Selected tables

COMPOUND	PI84	PI85	PI86	PI87	PI88	PI89	PI90	PI91
Ethyl acetate	195	172	124	233	215	203	162	212
Ethyl butyrate	0.299	0.362	0.234	0.337	0.386	0.362	0.312	0.430
1-Propanol	40.4	41.9	23.5	152	45.3	26.6	36.1	41.1
Isobutanol	48.6	57.6	73.0	22.6	72.0	42.2	55.3	66.5
Isoamyl acetate	4.17	5.25	1.03	10.1	8.17	9.26	8.02	6.94
n-Butanol	8.84	7.84	6.59	8.25	9.30	10.8	6.34	7.23
Isoamyl alcohol	176	137	164	130	150	148	170	175
Ethyl hexanoate	0.442	0.328	0.316	0.420	0.409	0.369	0.510	0.533
Hexyl acetate	23.9 ^a	33.6 ^a	5.78 ^a	55.3 ^a	41.6	52.8 ^a	41.6 ^a	31.3 ^a
Acetoin	175	55.0	10.5	199	3.15	98.6	40.0	99.1
Ethyl-D-lactate	423	259	239	355	319	364	445	504
1-Hexanol	0.124	0.122	0.692	0.491	0.114	0.721	0.342	0.243
Ethyl octanoate	0.133	0.108	56.4 ^a	0.128	0.13	0.125	0.136	0.161
Acetic acid	499	188	718	2643	426	2132	319	816
Furfural	3.55	17.9	5.21	9.54	21.5	18.6	0.155	18.5
Propionic acid	15.2	13.3	7.88	34.4	7.57	39.3	8.03	18.3
Isobutyric acid	2.47	3.33	1.73	2.00	2.32	3.12	4.73	2.36
5-Methylfurfural	0.384	0.116	0.120	0.466	0.186	0.797	0.325	0.705
n-Butyric acid	2.02	0.595	4.63	4.98	0.862	3.24	1.15	3.26
Ethyl decanoate	14.4 ^a	37.1 ^a	11.3 ^a	59.6 ^a	45.6 ^a	39.1 ^a	29.3 ^a	37.9 ^a
Isovaleric acid	1.73	1.67	1.68	1.57	1.69	1.82	1.90	1.75
Diethyl succinate	11.7	10.8	12.9	17.1	11.3	9.11	6.58	11.4
n-Valeric acid	1.86	1.68	1.39	1.77	1.58	1.87	1.67	1.71
2-Phenethyl acetate	0.240	0.249	71.8 ^a	0.497	0.289	0.607	0.584	0.281
Hexanoic acid	4.14	4.68	3.15	4.48	4.01	4.29	5.15	4.31
Guaiacol	0.532	0.236	0.623	0.630	0.435	1.21	0.392	0.653
trans-oak-lactone	1.04	nd ^b	1.04	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b
2-Phenylethyl alcohol	15.9	11.2	21.0	14.4	10.8	14.1	21.9	20.0
cis-oak-lactone	0.976	nd ^b	1.00	0.971	0.971	nd ^b	nd ^b	nd ^b
o-Cresol	0.838	0.800	0.820	0.881	0.812	0.883	0.804	0.815
Phenol	0.745	0.399	1.43	1.44	0.909	1.55	0.813	0.888
4-Ethylguaiacol	0.365	0.352	0.361	0.360	0.358	0.388	0.353	0.361
Octanoic acid	1.85	1.91	1.49	1.79	1.81	2.12	2.45	2.28
p-Cresol	0.302	0.285	0.261	0.320	0.279	0.353	0.270	0.279
Eugenol	0.650	0.561	0.707	8.08	0.603	0.952	0.609	0.606
Decanoic acid	0.688	0.813	0.735	0.786	0.777	0.802	0.858	0.803
2,6-Dimethoxy phenol	20.0	5.67	18.8	21.8	13.1	37.0	11.8	4.90
5-Hydroxymethylfurfural	0.245	68.9 ^a	5.57	4.83	1.86	12.8	1.42	1.73
Vanillin	34.0	3.98	71.8	40.3	20.1	237	60.8	27.8

^a Measured in µg/L. ^b nd: not detected. For details of the wines refer to **Table 1**. For analytical conditions refer to **chapter 6, section 6.2**.