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Impact of specific volatile thiols on varietal aroma of wines produced from Greek and some international grape varieties

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Submitted by

Andreas Kapaklis, M.Sc.

Born in Kalamata/Greece

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1st Supervisor:	**Prof. Dr. Karl-Heinz Kogel, Justus Liebig University Giessen**
2nd Supervisor:	**Prof. Dr. Doris Rauhut, Hochschule Geisenheim University**
Examiner:	**Prof. Dr. Sylvia Schnell, Justus Liebig University Giessen**
Examiner:	**Prof. Dr. Bernd Honermeier, Justus Liebig University Giessen**
Chairwoman:	**Prof. Dr. Gertrud Morlock, Justus Liebig University Giessen**

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Abstract

The volatile composition of young white wines from Greek, Swiss and German autochthonous varieties but also of the international variety Sauvignon blanc were studied using gas chromatography and other analytical techniques. The composition of the volatile constituents of the Greek varieties Malagousia, Asyrtiko and Roditis with special attention on varietal thiols was investigated. An analytical method was adapted and validated for their quantification. Wines of the Greek grape varieties, especially Malagousia, were proved to be rich in varietal thiols for the first time. Concentrations of up to 44.7 ng/L 4MSP (4-methyl-4-sulfanylpentan-2-one) and 1942 ng/L for 3SH (3-sulfanylhexan-1-ol) were measured, which are far above their odour threshold. Furthermore, the wines obtained from Malagousia showed high concentrations of monoterpenes (linalool, α-terpineol, hotrienol and geraniol) and C_{13} norisoprenoids (β-damascenone). In addition a scheme is proposed for the characterisation of wines from unknown grape varieties with focus on varietal thiols and other aromatic compounds. The characterisation scheme was developed using international and autochthonous varieties.

Zusammenfassung

Die Zusammensetzung von Aromakomponenten in Jungweinen von griechischen, Schweizer und deutschen autochthonen Rebsorten, sowie aus der internationalen Rebsorte Sauvignon blanc, wurden untersucht. Hierzu wurden gaschromatographische und andere analytische Verfahren eingesetzt. Für diese Studie wurden flüchtige Bestandteile der griechischen Rebsorten Malagousia, Asyrtiko und Roditis unter besonderer Berücksichtigung der rebsortentypischen Thiole gemessen. Für deren Quantifizierung wurde eine analytische Methode modifiziert und validiert. In den Weinen der griechischen Rebsorten, insbesondere Malagousia, wurden hohe Konzentrationen der rebsortentypischen Thiole zum ersten Mal festgestellt. Die gemessen Konzentrationen bis 44.7 ng/L 4MSP (4-Methyl-4-sulfanylpentan-2-on) und 1942 ng/L 3SH (3-Sulfanylhexan-1-ol) liegen weit über ihrem Geruchsschwellenwert. In den Proben von Malagousia wurden hohe Konzentrationen an Monoterpenen (Linalool, α-Terpineol, Hotrienol und Geraniol) und C_{13} Norisoprenoiden (β-Damascenone) nachgewiesen. Für die Charakterisierung von unbekannten Rebsorten wurde ein Schema mit Fokus auf die rebsortentypischen Thiole und andere Aromastoffe entwickelt. Zur Entwicklung dieses Schemas wurden internationale und autochthone Rebsorten verwendet.

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Abbreviations

% Vol.	Alcohol content in percent (v/v)
TRIS	2-Amino-2-hydroxymethyl-propane-1,3-diol
AOC	Appellation d'origine contrôlée
OPAP	Appellation of origin of superior quality (Greek wine legislation)
amu	Atomic mass unit
BHA	Butylated hydroxyanisole
cm	Centimetre
CI	Chemical ionisation
CIS	Cold injection system
Cys-4MSP	Cysteinylated precursor of 4MSP
Cys-3SH	Cysteinylated precursor of 3SH
°C	Degrees celcius
DCM	Dichloromethane
DNTB	5,5 – Dithio-bis(2-nitrobenzoic acid)
EI	Electron impact
eV	Electron volts
FTIR	Fourier transfer infrared spectroscopy
GC-AED	Gas chromatography atomic emission detection
GC-MS	Gas chromatography mass spectrometry
GC-ITMS-MS	Gas chromatography tandem ion trap mass spectrometry
g	g force
G-4MSP	Glutathionylated precursor of 4MSP
G-3SH	Glutathionylated precursor of 3SH
HS-GC-PFPD	Headspace-gas chromatography-pulsed flame photometric detection
ha	Hectare
hL	Hectolitre
HPLC	High performance/pressure liquid chromatography
h	Hours
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
IS	Internal standard
LAB	Lactic acid bacteria
LVI	Large volume injection
LOD	Level of detection
LOQ	Level of quantification
L	Litre
MLF	Malolactic fermentation
MSD	Mass selective detector
MeOH	Methanol
4MSB	4-Methoxy-2-methyl-2-sulfanylbutane
4MSP	4-Methyl-4-sulfanylpentan-2-one
m	Metre
µg/L	Microgramme per litre
µL	Microlitre
µm	Micrometre
mg/L	Milligramme per litre
mL	Millilitre
mm	Millimetre
mM	Millimolar
min	Minute
M	Molarity
MPS	Multi-purpose sampler
MWD	Multi wavelength detector

n.d.	Not detectable
ng/L	Nanogramme per litre
nm	Nanometre
nmol	Nanomole
NCI-MS	Negative chemical ionisation mass spectrometry
NOPA	Nitrogen by OPA (o-phthaldialdehyde)
n.q.	Not quantifiable/trace
TNB	2-nitro-5-thiobenzoic acid
OAV	Odour Activity Value
p-HMB	para-Hydroxy mercury benzoate
PLS	Partial least square
PFFBr	2,3,4,5,6-Pentafluorobenzyl bromide
PCA	Principal component analysis
PTV	Programmed temperature vaporising
RI	Refractive index detector
%RSD	Relative standard deviation (in percent)
RT	Retention time
rpm	Revelations per minute
s	Second
SIM	Selected ion monitoring
SPE	Solid phase extraction
SPME	Solid phase micro extraction
SIDA	Stable isotope dilution assay
SBSE	Stir bar sorptive extraction
3SH	3-Sulfanylhexan-1-ol
3SHA	3-Sulfanylhexyl acetate
TDN	1,1,6-Trimethyl-1,2-dihydronaphtalene
TDU	Twister desortion unit
VA	Volatile acidity in g/L acetic acid
YAN	Yeast assimilable nitrogen

General introduction

Varietal thiols are extremely odoriferous compounds that are contributing to the aroma of certain fruits like blackcurrant (Rigaud *et al.*, 1986), passion fruit (Engel & Tressl, 1991), grapefruit (Demole *et al.*, 1982) and guava (Idstein & Schreier, 1985) but have also been found to play a key role in the aroma of certain grape varieties (Tominaga *et al.*, 1996; 1998a; 2000; 2003). These varietal thiols give to the wines an exotic and fruity note of 'passionfruit', 'grapefruit' and 'cassis' but can also be unpleasant at high concentrations with descriptors like 'catpiss' (Darriet *et al.,* 1995; Dubourdieu & Tominaga, 2009).

These varietal thiols exist in the grapes as odourless precursors and are only released during alcoholic fermentation by the yeast (Tominaga *et al.*, 1998c; Peyrot des Gachons *et al.*, 2000; 2002a; 2002b) although some small amounts seem to exist already in a free state in unfermented musts (Capone *et al.*, 2011).

For the past twenty years since the detection of varietal thiols in wines (Darriet *et al.*, 1995) the interest of both research and the industry has grown significantly (Roland *et al.*, 2011a) as by the number of publications dealing with their viticultural, oenological and wine ageing aspects and has been demonstrated by the extensive and meticulous search for a powerful and practical approach for quantifying them (please see an extensive reference list in **Chapter 5**).

Despite these twenty years of development and research, there is still great potential for new discoveries in the field of varietal thiols as still many aspects on how they are influenced during the winemaking process are not clear (Roland *et al.*, 2012) as well as the interactions of these compounds in the wines, alternating some sensorial properties are not yet completely investigated (Lund *et al.*, 2009; Nikolantonaki *et al.*, 2010; Roland *et al.*, 2011b).

1. Literature review

1.1 Introduction

The flavour of a wine has been shown to play a decisive role in the buying decision of the consumer (Yegge & Noble, 2001). The meaning of flavour is the experience of the consumer of smelling and tasting the wine and is attributed to a large number of compounds that are volatile and non-volatile (Clarke & Bakker, 2004). Flavour in a wine comes from many different aspects of the production starting from the raw material, the grapes, over to the treatments and the fermentation of the must, to the post-fermentative treatments and the ageing on the final product.

1.2 Wine aroma

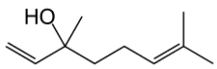
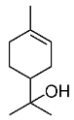
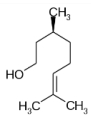
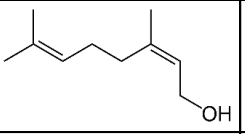
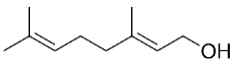
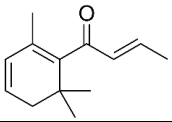
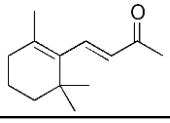
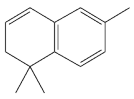
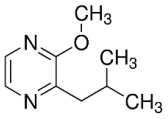
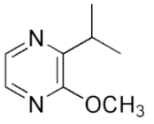
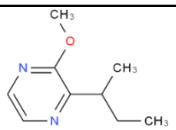
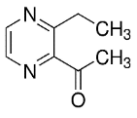
Wine is considered as one of the most complex aromatic products, with more than 1000 volatile compounds identified so far in various concentrations, from a few ng/L to hundreds of mg/L (Bertrand, 1983; Gómez-Míguez *et al.*, 2007; Roland *et al.,* 2012). These volatile compounds belong to many different chemical groups like alcohols, esters, acids, thiols, terpenes, aldehydes and have a major or minor contribution to the aroma. This contribution of each compound can be measured by the OAV (Odour Activity Value), which is the ratio of the concentration found in the analysed wine versus the odour threshold of the compound (Gómez-Míguez *et al.*, 2007).

According to Drawert (1974) there are four categories of wine aroma compounds:

1. Prefermentation aromas: These are compounds that are released when the berries are crushed during the pre-fermentative stage. These are found in free forms.
2. Fermentation aromas: These aromas are produced during the alcoholic and malolactic fermentation and are products of the metabolism of yeast and bacteria. The main members of this category are esters and higher alcohol and contribute to the fruitness and ‘wine character’ of the wine.
3. Postfermentation aromas: The aromas are developing during maturation and ageing of the wine, could involve microorganism activity and are forming the bouquet of old wines. One source of such aromas are the barriques and barrels used for maturation.
4. Varietal aromas: The category is more complex and includes: **(1)** compounds that are odoriferous and are present in the grapes in free form, like monoterpenes **(2)** compounds that are in odourless precursor form in the grapes and with a treatment or fermentation they can be set free. The difference between fermentation and varietal aromas is that the first ones are produced by the yeast with complex biochemical reactions and the original compounds are not identifiable anymore whereas in the later ones the original skeleton of the compound is preserved as it was synthesised by the plant and can be recognised (Cheynier *et al.*, 2010). Compounds that belong to this category are terpenes, varietal thiols and pyrazines.

1.3 Varietal aroma

Table 1-1: The main compounds –excluding thiols- involved in varietal aroma (adapted from Ribéreau-Gayon *et al.,* 1999; Ferreira *et al.*, 2000) * Odour threshold in water/alcohol solution ** Odour threshold in water *** Odour threshold in wine.

Group	Compound	Structural formula	Descriptor	Odour threshold
Monoterpenes	Linalool		Rose	25 µg/L***
	α-Terpineol		Lily of the Valley	250 µg/L***
	Citronellol		Citronella	18 µg/L***
	Nerol		Rose	400 µg/L***
	Geraniol		Rose	130 µg/L***
C_{13} norisoprenoid derivatives	β-Damascenone		Tropical fruit, stewed apple	140 ng/L***
	β-Ionone		violets	800 ng/L*
	1,1,6-Trimethyl-1,2-dihydronaphthalene (TDN)		"Petroleum" smell	20 µg/L***
Methoxypyrazines	2-Methoxy-3-isobutylpyrazine (IBMP)		Green pepper	2 ng/L *
	2-Methoxy-3-isopropylpyrazine (IPMP)		Green pepper, earthy	2 ng/L**
	2-Methoxy-3-*sec*-butylpyrazine		Green pepper	1 ng/L**
	2-Methoxy-3-ethylpyrazine		Green pepper, earthy	400 ng/L**

Varietal aroma originates from compounds that are present in the grapes and are reflecting the particular variety, climate and soil. It has been shown that varietal aroma is playing the most important role in the character of a wine than any other aroma compounds (Ribéreau-Gayon *et al.*, 1999). These aromas although originating from the grapes are many times found in an odourless precursor form and they are released with the processing after harvest. Such examples are the glucosidically bound terpenols, C_{13} norisoprenoids and varietal thiols (**Table 1-1**). There are some terpenes in free state in the grapes of some aromatic varieties such as muscats (Mateo & Jiménez, 2000).

It should be noted that the term "varietal aroma" does not follow a rule ‚specific compounds of specific varieties'. These compounds are quite ubiquitous among the various varieties but also in other fruits and plants. What makes the character of each wine is the combination and the concentration levels of each compound (Ribéreau-Gayon *et al.*, 1999).

1.3.1 Terpenes

Terpenes belong to a large family of organic compounds that are widespread within plants. From this large family the most odoriferous ones are the group of monoterpenes and sesquiterpenes. The first group has 10 carbon atoms and is built up by two isoprene units and the second has 15 carbon atoms and is built up by three isoprene units (Ribéreau-Gayon *et al.*, 1999). In wine the most important terpenes are monoterpenes (Mateo & Jiménez, 2000). On the other hand the role of the sesquiterpenes in wine has not been thoroughly investigated (Bayonove, 1993).

1.3.1.1 Monoterpenes

The first three monoterpenes were identified by Cordonnier (1956) in Muscat grapes and since then a lot of work has been done, which has been extensively reviewed (Rapp & Mandery, 1986; Mateo & Jiménez, 2000). The main monoterpes are shown in **Table 1.1** along with their aroma descriptors and odour thresholds. It can be seen from **Table 1.1** that the odour threshold for some of these monoterpenes is rather low, as little as a few µg/L. These compounds play a major role in Muscat wines where they were first identified but have been proved also to play a role in other varieties that exhibit 'Muscat' type aroma. The varieties are Gewürztraminer, Scheurebe, Riesling, Auxerrois, Müller-Thurgau, Pinot gris, Sylvaner and Kerner (Mateo & Jiménez, 2000).

Up to now about 50 monoterpene compounds have been identified with monoterpene alcohols being the most odoriferous (linalool, geraniol, nerol, citronellol and α-terpineol). Monoterpenes are classified into three categories according to Mateo & Jiménez (2000): **(1)** Free monoterpenes, which have the main involvement in the aroma, **(2)** polyhydroxylated forms that do not readily influence the aroma but are reactive and produce oxides that can contribute to the aroma, like nerol oxide (Williams *et al*., 1980) and **(3)** non-volatile glycosidically conjugated forms that have no effect in the aroma of wines. The glycosidically conjugated forms are more abundant in grapes than the free forms (Mateo & Jiménez, 2000) and under normal winemaking practices they cannot be released with the activity of *β*-glycosidases (Ribéreau-Gayon *et al.*, 1999).

Grape varieties have been classified according to their total free monoterpene concentration into three different categories: **(1)** Intense flavoured muscat varieties with total free monoterpenes up to 6 mg/L

(2) non-muscat aromatic varieties with a concentration of free monoterpenes 1-4 mg/L and **(3)** non-muscat varieties in which their aroma is not attributed solely to free monoterpenes (Rapp, 1990; Mateo & Jiménez, 2000).

1.3.1.2 C_{13} Norisoprenoid derivatives

C_{13} norisoprenoid derivatives originate from the degradation of carotenoids. Carotenoids are terpenes with 40 carbon atoms and can produce with oxidative degradation derivatives with 9-13 carbon atoms (Enzell, 1985). The derivatives with 13 atoms (C_{13} Norisoprenoid) are odoriferous compounds that have been proved to contribute to the aroma of wine (Schreier *et al.,* 1976; Sefton *et al.*, 1989).

These C_{13} norisoprenoids can be divided chemically into two different categories: Megastigmane and non-megastigmane. Both categories include a large number of compounds. Megastigmane are oxygenated C_{13} norisoprenoids with a benzene cycle, which has an unsaturated chain with four carbon atoms attached to it. The two most important megastigmane C_{13} norisoprenoids identified are β-damascenone and β-ionone. β-Damascenone was first identified in Scheurebe (Schreier *et al.*, 1976) but it seems to be ubiquitous in many varieties (Baumes *et al.*, 1986; Sefton *et al.*, 1993). Due to its low odour threshold it can play a major role in the aroma of certain wines especially in sweet fortified wines made from Muscat varieties (Ribéreau-Gayon *et al.*, 1999; Pineau *et al.*, 2007). β-Ionone has also been found in various grape varieties (Schreier *et al.*, 1976). While the impact of β-ionone is not so important in the aroma of white wines it seems to play an important role in the aroma of red wines (Pineau *et al.,* 2007). The most important non-megastigmane C_{13} norisoprenoid indentified is TDN (1,1,6-trimethyl-1,2-dihydronaphtalene), which gives the characteristic ‘petroleum’ nose in aged Riesling wines (Sacks *et al.*, 2012). TDN is not present in young wines but develops after bottling and can reach concentrations of more than 200 µg/L, which is many times above the odour threshold of 20 µg/L (Winterhalter *et al*., 1990).

1.3.2 Methoxypyrazines

Methoxypyrazines are nitrogenated heterocycles originating from the metabolism of amino acids (Ribéreau-Gayon *et al.*, 1999). Four methoxypyrazines have been identified in wines and are presented in **Table 1-1**. The main aroma descriptor of methoxypyrazines is green pepper and they have a very low odour threshold as low as 1 ng/L (Ribéreau-Gayon *et al.*, 1999). It has been shown that methoxypyrazines are ubiquitous in nature and have been identified in peas (Murray *et al.*, 1970; 1975) and green peppers (Buttery *et al.*, 1969). Methoxypyrazines were also identified in grapes from different varieties: Cabernet Sauvignon (Bayonove *et al.*, 1975; Allen *et al.*, 1994), Sauvignon blanc (Harris *et al*., 1987), Cabernet Franc and Merlot (Allen *et al.*, 1994) and some others. The highest concentrations occur in the first three and sometimes in Merlot (Riberau-Gayon *et al.*, 1999). Underripe grapes are richer in methoxypyrazines and higher concentrations are found in press wines (de Boubée, 1996). Concentrations between 0.5 and 50 ng/L 2-methoxy-3-isobutylpyrazine have been found in wines from Sauvignon blanc and Cabernet Sauvignon and in juices from Sauvignon blanc up to 78.5 ng/L (Lacey *et al.*, 1991; Allen *et al.*, 1994). In the study by Lacey *et al.* (1991) it was also shown that wines that originated from cooler regions had higher contents. Concentrations of the other methoxypyrazines,

2-methoxy-3-isopropylpyrazine and 2-methoxy-3-*sec*-butylpyrazine are found in much lower concentrations and do not play a significant role in the aroma of wines (Lavin & Acree, 1992).

1.3.3 Varietal thiols

Thiols and general sulphur compounds in wines have been mostly associated with off-flavours. Such off-flavours are the following: **(1)** hydrogen sulphide (rotten egg); **(2)** methanethiol (rotten cabbage); **(3)** ethanethiol (smoky) and **(4)** methionol (potato) (Dubourdieu & Tominaga, 2009). However there are also thiols that have a positive contribution in the aroma of certain fruits, plants, food and in wines from several grape varieties (**Table 1-2**). The odour impression is also very much related to the concentration of the specific sulphur compound.

Table 1-2: Occurrence of thiols in different fruits, food and plants

Product	Reference
Blackcurrant	Rigaud *et al.* (1986)
Passionfruit	Engel & Tressl (1991)
Grapefruit	Demole *et al.* (1982)
Guava	Idstein & Schreier (1985)
Box Tree	Tominaga & Dubourdieu (1997)
Roasted coffee	Tressl & Silwar (1981)
Beer	Vermuelen *et al.* (2006)
Wine	Darriet *et al.* (1995); Tominaga *et al*. (1996; 1998b)

Several of these highly odoriferous volatile thiols were identified in wines from the grape variety Sauvignon blanc (Tominaga *et al.*, 1998b) and have been found to be in correlation with the aroma descriptors ‘boxwood’, ‘cassis’, ‘passionfruit’, ‘grapefruit’, ‘gooseberry’ (Dubourdieu & Tominaga, 2009). Before the identification of varietal thiols the only compounds that had been identified to contribute in the varietal aroma of this variety were methoxypyrazines and are responsible for the green pepper aroma as mentioned in **Chapter 1.3.2** (Augustyn *et al.*, 1982; Allen *et al.,* 1991).

The first odoriferous varietal thiol that was identified was 4-methyl-4-sulfanylpentan-2-one (4MSP) (Darriet *et al.*, 1995) and has been measured in Sauvignon blanc wines from the Loire valley up to 200 ng/L (Dagan *et al.* 2014), which is more than 200 times of its perception threshold (0.8 ng/L in water, Tominaga *et al.*, 1998b). More varietal thiols have been identified in Sauvignon blanc wines: **(1)** 3-sulfanylhexyl acetate (3SHA) (Tominaga *et al.*, 1996), **(2)** 4-methyl-4-sulfanylpentan-2-ol (Tominaga *et al.*, 1998b), **(3)** 3-sulfanylhexan-1-ol (3SH) (Tominaga *et al.*, 1998b) and **(4)** 3-methyl-3-sulfanylbutan-1-ol (Tominaga *et al.*, 1998b).

It was shown by Tominaga *et al.* (1998b) that the compounds that are contributing more to the varietal aroma of Sauvignon blanc are 4-methyl-4-sulfanylpentan-2-one, 3-sulfanylhexyl acetate and 3-sulfanylhexan-1-ol. The other two identified varietal thiols contribute less or nothing due to their odour threshold and concentrations found in wines. 3-Methyl-3-sulfanylbutan-1-ol was measured at a maximum of 128 ng/L and has an odour threshold of 1500 ng/L in wine so it does it does not play a role in the aroma. 4-Methyl-4-sulfanylpentan-2-ol was rarely measured above the odour threshold of 55 ng/L in wine and it rarely plays a role in the varietal aroma (Tominaga *et al.*, 1998a; 1998b).

1.3.3.1 Biogenesis of varietal thiols

During the winemaking process four classes of aromas have been described by Drawert (1974) as mentioned in **Chapter 1.2**. Pre-fermentation (also known as primary aromas), fermentation (secondary aromas), post-fermentation (tertiary aromas) and varietal aromas. Varietal aromas are pre-existing in the form of odourless precursors in the grapes and are released during fermentation by enzymatic activity of the yeast (Roland *et al.*, 2012). Glucosidically bound precursors are the most common form in nature (Hösel, 1981) but more recently cysteine and glutathione precursors have been discovered (Tominaga *et al.*,1995 & 1998c; Peyrot des Gachons *et al.*, 2000; 2002a; 2002b; Thibon *et al.,* 2008b; Subileau *et al.,* 2008a; Fedrizzi *et al.,* 2009). These precursors are the bound form of 4MSP and 3SH. The biogenesis of 3SHA is different as it is being formed by esterification of 3SH during fermentation (Swiegers & Pretorius, 2007a). It has to be noted that in nature these volatile thiols are in free form but in grapes they are found in cysteinylated and glutathionylated forms (Peña-Gallego *et al.*, 2012). These precursors are in the grapes mostly in low concentration but higher concentrations, above 100 µg/L have been found for the cysteine conjugate of 3SH by Thibon *et al.* (2008b) and for the glutathione conjugate of 3SH by Capone *et al.* (2010). Although such concentrations of the precursors have been reported in the grapes, an experiment by Peyrot des Gachons *et al.* (2000) showed that only a small percentage of the conjugates are cleaved for the release of free thiols. The average release measured was 1.4 % for the Cys-4MSP and 4.2 % for the Cys-3SH precursors.

Three biogenesis pathways have been proposed for the release of varietal thiols in wine. The first involves the cysteinylated precursors of 3SH and 4MSP. These are cleaved by the activity of *β*-lyase produced by yeasts (Tominaga *et al.*, 1998c). The second pathway involves the glutathionylated precursors. The mechanism of release has been investigated only for 3SH (G-3SH) by Peyrot des Gachons *et al.* (2002b) although the glutathionylated precursor of 4MSP (G-4MSP) has also been identified by Fedrizzi *et al.* (2009). It was shown that when the musts from Sauvignon blanc and Gros Manseng were percolated through a column with immobilized *γ*-glutamyltranspeptidase the levels of Cys-3SH increased significantly (49-71 % increase for Sauvignon blanc and 537 % for Gros Manseng) (Peyrot des Gachons *et al.*, 2002b). This suggests that the G-3SH is a pre-precursor. It is known that such precursors are abundant in nature and are involved in the detoxification of the systems of living organisms. This also suggests that these two precursors (Cys-3SH & G-3SH) are part of such a cell detoxification mechanism (Peyrot des Gachons *et al.*, 2002b). Recently it was shown that the cells of *Vitis vinifera* can produce Cys-3SH from G-3SH and that when there is an infection with *Botrytis cinerea* the production of Cys-3SH is stimulated up to a thousandfold (Thibon, *et al.* 2011). The last pathway was suggested by Schneider *et al.* (2006) and involved conjugated carbonyl compounds, (*E*)-2-hexanal and mesityl oxide, with the addition of sulphur and reduction by the yeast during fermentation for the production of 3SH and 4MSP respectively. It was suggested by the study that this could explain the ubiquity of 3SH in wine although under laboratory conditions only 10% of the total concentration of the compound was formed with this pathway (Schneider *et al.*, 2006; Roland *et al.*, 2010a).

1.3.3.2 Factors affecting the release of varietal thiols

The release of varietal thiols can be affected by a number of parameters during the winemaking process as it has been shown by a number of studies. One of the first factors that was proven to affect the release of thiols were the different yeast strains. Howell *et al.,* (2004); Dubourdieu *et al.*, (2006), Swiegers *et al.,* (2006 & 2009) tested a number of yeast strains. It was shown that the tested strains had different capacity of releasing the different varietal thiols. Furthermore, the esterification capacity of each yeast strain was different for the production of 3SHA. The yeast strain VIN7 released about the same amount 3SH like yeast strain VIN13 but the esterification capacity was more than double (Swiegers *et al.*, 2009). This could be attributed to the fact that probably the release mechanism involves multiple carbon-sulfur lyases enzymes (Howell *et al.*, 2005) and could be overexpressed or suppressed in each strain individually. Another aspect that was tested was the use of non-*Saccharomyces* yeast in co-fermentation with the commercial yeast VL3 in different ratios by Anfang *et al.* (2008). It was shown that the co-fermentation of *Pichia kluyveri* with VL3 at an initial ratio of 9:1 increased the concentration of 3SHA significantly although the interaction mechanism is not known. This co-fermentation synergies could be employed in the future to enhance the release of varietal thiols without the use of genetically modified yeast as shown by Swiegers *et al.* (2007b).

Fermentation temperature also seems to have an effect in the concentration of varietal thiols (Swiegers *et al.*, 2006). Experiments carried out in model medium and Sauvignon blanc must, showed that the concentration of all varietal thiols (4MSP, 3SHA, 3SH) increased when the fermentation was conducted at 20℃ instead of 13℃. Higher temperatures are increasing the concentrations but only for a short time because their concentration was shown to decline towards the end of the fermentation (Masneuf-Pomarède *et al*., 2006).

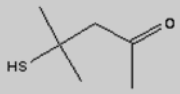
Other factors that affect the release and final concentration of varietal thiols is the addition of SO_2 in the must, prior to fermentation (Coetzee, 2011). Musts that were treated with SO_2 in moderate concentrations had higher concentrations of thiols than the untreated ones. In addition to that the combination of the addition of O_2 and SO_2 seems to increase the concentrations of the 3SH precursors leading to higher concentrations of 3SH and 3SHA (Roland *et al.*, 2010b). Maceration has also been shown to affect the concentration of the precursor Cys-3SH. It has been suggested that a long maceration period increases the concentration of the precursor and potentially the concentration of 3SH (Maggu *et al.*, 2007). Prefermentative cold soaking of Sauvignon blanc grapes also showed a significant increase in the concentrations of 3SH and 3SHA without increasing the concentrations of the precursor, but a decrease in (E)-2-hexenal and (E)-2-hexenol was observed supporting the theory that these two compounds are also have a role as precursor for 3SH (Schneider *et al.*, 2006; Roland *et al.*, 2011b). Finally it was also observed that wines that are produced from juices pressed at higher pressures had higher concentrations of 3SH. This is simply to explain that higher pressures result to higher extraction of precursors (Roland *et al.*, 2011b). This oenological practice can have a significant drawback though due to the fact that it can also increase the extracted oxidizable polyphenolics that in turn are reacting with the thiols decreasing their concentrations (Roland *et al.*, 2011b; Nikolantonaki *et al.*, 2010). Furthermore, it was shown that phenolics can have an influence in the perception of

varietal thiols by increasing the odour threshold of 3SH and decreasing the odour threshold of 3SHA (Lund *et al.*, 2009).

1.3.3.3 4-Methyl-4-sulfanylpentan-2-one (4MSP)

4MSP is the most powerful varietal thiol identified, with an odour threshold of 0.8 ng/L in water (Tominaga *et al.*, 1998a) and 3 ng/L in wine (Darriet *et al.*, 1995). This mercaptoketone is responsible for the aroma descriptors 'box tree', 'broom', 'passionfruit' and 'blackcurrant' (Dubourdieu & Tominaga, 2009) but in high concetrations it can be described as 'catpiss', which is perceived aromatically unpleasant (Darriet *et al.*, 1995). Besides Sauvignon blanc it has also been identified in wines from the varieties Colombard (Du Plessis & Augustyn, 1981), Scheurebe (Guth, 1997b), Gewürztraminer, Riesling, Muscat, Petit Manseng, Semillon (Tominaga *et al.,* 2000; Dubourdieu & Tominaga, 2009); Maccabeo (Escudero *et al.*, 2004) and recently it was also shown that significant concentrations can be found in red wines blended from the varieties Grenache, Syrah, Mourvèdre, Cinsault and Carignan (Rigou *et al.,* 2014).

Table 1-3: Data for 4MSP.[a] NIST Chemistry Webbook; [b] Darriet *et al.*, (1995); [c] Tominaga *et al.*, (1998a). * in water, ** in wine.

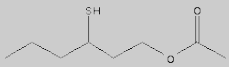
Compound	Abbreviation	Structure [a]	Descriptor [b]	Odour threshold (ng/L) [b,c]
4-Methyl-4-sulfanylpentan-2-one	4MSP		Blackcurrant, passion fruit, box tree, broom	0.8* (3.0**)

Normal concentrations of 4MSP can be as high as 40 ng/L (Tominaga *et al.,* 1998b). Concentrations of up to 400 µg/L in Scheuerebe wines were reported by Guth (1997b), which is extremely high and probably could be at around 400 ng/L as mentioned by Roland *et al.* (2011a). A recent study with 18 young Sauvignon blanc wines from the Loire Valley showed average concentrations for 4MSP of 80 ng/L with three of the wines showing a very high content of up to 200 ng/L (Dagan *et al.*, 2014).

1.3.3.4 3-Sulfanyl-hexyl acetate (3SHA)

The second most powerful varietal thiol was identified by Tominaga *et al.* (1996) but probably the first account of it was by Engel & Tressl (1991) were they described the compound in passionfruit. The compound has a low odour threshold of 4.2 ng/L and it has been found in Sauvignon blanc wines up to several hundred ng/L (Dubourdieu & Tominaga, 2009). Besides Sauvignon blanc it has been identified in several other varieties like Colombard, Riesling, Muscat, Petit Manseng, (Tominaga *et al.,* 2000; Dubourdieu & Tominaga, 2009) but also in rosé and some red wines from Merlot and Cabernet Sauvignon (Bouchilloux et al., 1998; Murat *et al.*, 2001a). 3SHA shows a complex aromatic description: 'box tree', 'grapefruit zest' and 'passionfruit' (Dubourdieu & Tominaga, 2009) (**Table 1-4**).

Table 1-4: Data for 3SHA. [a] NIST Chemistry Webbook; [b] Tominaga *et al.*, (1998a)

Compound	Abbreviation	Structure [a]	Descriptor [b]	Odour threshold (ng/L) [b]
3-Sulfanyl-hexyl acetate	3SHA		Passion fruit, grapefruit, box tree, gooseberry	4.2

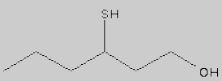
It was shown that its impact on varietal aroma of wines is highly variable. It has more importance in young wines because it has the tendency to hydrolyze during ageing to 3-sulfanylhexan-1-ol (3SH) (Dubourdieu & Tominaga, 2009). Another interesting point is that 3SHA is not present in botrytized sweet wines (Tominaga *et al.*, 2006b).

A study was coducted by Tominaga *et al.* (2006b) for the stereoisomeric distribution of 3SHA in dry and sweet wines. Two grape varieties were studied (Sauvignon blanc and Semillon) and the ratios of the *R* and *S* enantiomers were determined through chiral chromatography. It was shown that the distribution of the *R* and *S* form was approximately 30:70 respectively for all the wines studied. This ratio was also observed as being constant during the entire alcoholic fermentation. The last point studied was the odour thresholds of both *R* and *S* enantiomers. It was shown that not only the odour threshold for each was different (9 and 2.5 ng/L respectively) but also the olfactory description was different as the first one was described as 'passionfruit' and the later as 'box tree'.

1.3.3.5 3-Sulfanylhexan-1-ol (3SH)

The most abundant varietal thiol is 3-sulfanylhexan-1-ol, which has been found to be always present in Sauvignon blanc wines (Dubourdieu & Tominaga, 2009) but has been identified in many other varieties including the Swiss autochthonous variety of Petite Arvine, which was shown as an aroma impact compound (Fretz *et al.*, 2005b). Sweet wines also have high concentrations of 3SH well above the odour threshold, as shown by Tominaga *et al.* (2000) with Semillon botrytized wines. Furthermore, in a recent study red wines from AOC Languedoc were analysed and some of them showed high concetrantions of 3SH up to ~11,500 ng/L (perception threshold 60 ng/L) (Rigou *et al.*, 2014). However during barrel-ageing of the red wines the 3SH decreases considerably and after 12 months of ageing it will be reduced to about 300-600 ng/L as it oxidises easily and has high reactivity with quinones (Dubourdieu & Tominaga, 2009).

Table 1-5: Data for 3SH. [a] NIST Chemistry Webbook; [b] Tominaga *et al.* (1998a).

Compound	Abbreviation	Structure[a]	Descriptor[b]	Odour threshold (ng/L)[b]
3-Sulfanylhexan-1-ol	3SH		Grapefruit, passion fruit, gooseberry, guava	60

A recent study revealed that 3SH is also present in unfermented musts up to 100 ng/L, which is above the odour threshold (Capone *et al.*, 2011). It seems that 3SH is more widely distributed in wines from

many varieties than 3SHA, which tends to decrease rapidly after bottling and compared with 4MSP, which is more rare (Roland *et al.*, 2011a).

Unlike 3SHA the enantiomers of 3SH are evenly distributed in dry white wines from the varieties Sauvignon blanc and Semillon (50:50). However this was not observed during the alcoholic fermentation were the *S* form represented more than 60 % at the beginning and only when the fermentation proceeded the ratio approached 50:50. Furthermore, the ratio of *R* and *S* enantiomers of 3SH in sweet botrytized wines (Semillon grapes) was 30:70 and does not seem to be dependend on the vintage. The perception thresholds of the two enantiomers were 50 ng/L for the *R* form and 60 ng/L for the *S* form. The aroma descriptor for the *R* form is fruiter and has a strong aroma of 'grapefruit' whereas the *S* form has more 'passionfruit' character (Tominaga *et al.*, 2006b).

1.4 Quantification of varietal thiols

Quantification of varietal thiols even with today's sophisticated analytical instruments poses a major challenge. The challenges faced are their very low concentrations (4MSP is only present in a few ng/L) (Fedrizzi *et al.*, 2007), the compounds showed a poor "detectability" (Mateo-Vivaracho *et al.*, 2007), their mass spectra lacks characteristic ions with high m/z and they have a high reactivity with oxygen and other oxidants (Fedrizzi *et al.*, 2007; Mateo-Vivaracho *et al.*, 2007). Varietal thiols can also react with metals and form complexes (Mateo-Vivaracho *et al.*, 2007). Due to these facts it is not surprising that only a few quantification methods have been developed, most of them using the affinity of thiol groups forming complexes with organic mercury, based on the research published by Boyer (1954). This original research showed that the use of *p*-mercuribenzoate is adequate to analyse thiols. In the recent years the *p*-hydroxy mercuribenzoate (Tominaga *et al.*,1996; 1997; 2006a) and agarose gel containing phenylmercuric ions (Affi-Gel 501, Biorad, Munich, Germany) (Schneider *et al*., 2003) have been employed for forming these complexes. Other approaches use the derivatisation of thiols with pentafluorobenzyl bromide (Mateo-Vivaracho *et al.*, 2007; Rodriguez-Bencomo *et al.*, 2009).

1.4.1 Extraction of thiols with agarose gel containing phenylmercuric ions

The first application for extraction of thiols using agarose gel containing phenylmercuric ions was published by Full & Schreier (1994). The two researchers used commercial blackcurrant concentrate and with Affi-Gel 501 (Biorad, Munich, Germany) they succeeded to purify and enrich the thiols. Their basic idea was the reversible binding of the thiols with the phenylmercuric ions containing agarose gel, their separation/purification at this bounded state, and their release with the use of another thiol in excess (Full & Schreier, 1994).

In 2003 Schneider *et al.* proposed a method for quantifiying 4MSP, 3SHA and 3SH with the use of liquid-liquid extraction as a first step and then a purification step with covalent chromatography. Affi-Gel 501 was used for the purification, as in the method of Full & Schreier (1994). The quantitation of the compounds was done with Stable Isotope Dilution Assay (SIDA). For this purpose, isotopes of the three compounds were synthesized in the laboratory according to the procedure published by

Kotseridis *et al.* (2000). Detection was carried out with GC-AED (Gas Chromatography-Atomic Emission Detection) and GC-ITMS-MS (Gas Chromatography-Ion Trap Tandem Mass Spectrometry).

Proposed sample preparation: 500 mL of the wine to be analysed was put in a 1 L Erlenmeyer flask and cooled down to 1 ˚C with the use of an ice bath and nitrogen. 50 µL of each isotope (d_{10}-4MSP, d_5-3SHA and d_2-3SH diluted in pentane) were added as internal standards. 100 mL of dichloromethane were added and the mixture is stirred for 15 min at 700 rpm. 100 mL of dichloromethane were added again to the mixture and stirred for further 15 min at 700 rpm. The organic phase was separated in a separatory funnel and centrifuged for 5 min at 9000 g (4 ˚C) dried over sodium sulphate and concentrated to about 2 mL under vacuum at 25 ˚C. A cartridge was prepared by loading 500 µL of Affi-Gel 501 into a Pasteur pipet with glass wool at the bottom. The conditioning of the cartridge was carried out as follows: 5 mL isopropyl alcohol and then 5 mL of a pentane/dichloromethane mixture (2:1) were added. The wine extract is diluted with 2 mL of pentane and percolated through the cartridge, which was then washed with 25 mL of a pentane/dichloromethane mixture (2:1). The thiols were eluted from the agarose gel with 5 mL of a 1,4-dithio-DL-threitol solution (5 mM in a dichloromethane/pentane mixture 2:1) and washed with 1 mL of ultrapure water, dried over sodium sulphate and concentrated to about 500 µL using a Dufton column. The extract was concentrated again to about 100 µL under a nitrogen flow (Schneider *et al.,* 2003; 2006). The final concentration factor was 5,000.

Chromatographic analysis: During the initial work of Schneider *et al.* (2003) GC-AED was proposed for detection and compared with GC-ITMS-MS. In this work it was demonstrated that the first detection method showed some limitations in the quantification of 3SHA and 3SH and the lack of identity confirmation, which could lead to false results. Also a coelution of d_2-3SH and 3SH was observed (Schneider *et al.*, 2003). For this reason, the review of this method will only present the second detection method. The analysis was carried out with a Varian 3800 GC coupled with a Varian Saturn 2000 mass spectrometer (Varian, Steinheim, Germany). The column used was a DB-WAX 30 m x 0.25 mm, 0.5 µm film thickness (J&W Scientific, Steinheim, Germany) and the carrier gas was helium at a flow of 1 mL/min. 2 µL of the extract were injected at 20 ˚C and the injector heated up with 180 ˚C/min to 250 ˚C and was held throughout the run. The oven temperature started at 60 ˚C for 3 min and with a rate of 3 ˚C/min to 245 ˚C and hold for 20 min. The temperature of the trap and the transfer line were 150 and 170 ˚C respectively. 3SHA and 3SH were detected in CI mode using methane as a reagent gas (0.35 bar). For the detection of 4MSP also CI was used but the reagent gas was isobutane. The quantitation of all compounds was done using SIDA and the calibration data are shown in **Table 1-6** (Schneider *et al.*, 2003 & 2006).

Table 1-6: Calibration data of the SIDA method (adapted from Schneider *et al.*, 2003)

Compound	Concentration range (ng/mL)	R^2	Detection limit (ng/L)
4MSP	1.4-52.9	0.990	15
3SHA	1.5-61	0.999	0.7
3SH	7.8-1500	0.996	1.0

When this method was published in 2003, Affi-Gel 501 was no longer commercially available from any manufacturer. This meant that the reagent was synthesised using Affi-Gel 10 (Biorad, Munich, Germany) and *p*-aminophenylmercuric acetate according to a procedure obtained from Biorad (Schneider *et al.*, 2003). This step in the preparation of the method together with the synthesis of the isotopically labelled standards leads to an extensive preparatory work. It also needs to be noted that a large sample volume had to be processed (in this case 500 mL), which was time-consuming. Furthermore the procedure was expensive and complicated and therefore a part of the thiols was lost during extraction (Mateo-Vivaracho *et al.*, 2007). It also must be taken into consideration the extensive use of solvents (200 mL of dichloromethane per sample) adds to the costs and poses a problem for waste management. Mercuric derivatives are carcinogenic and could be toxic for the operators (Rodriguez-Bencomo *et al.*, 2009). In the extraction performance it was noted by Schneider *et al.* (2003) that the recovery when usingAffi-Gel 501 compared to *p*-hydroxymercuribenzoate was lower.

Despite these drawbacks this method had the advantage of the SIDA quantification because the physicochemical properties of the isotopically labelled standards are very close to the compounds to be analysed (Schneider *et al.*, 2003). It has also been widely accepted that this approach is the most accurate method for quantitation (Blank *et al.*, 1998). Furthermore, Schneider *et al.* (2003) reported that the extraction of the natural and labelled thiols was without any interference of a detectable contaminant.

1.4.2 The use of *p*-HMB in combination with DOWEX basic anion exchange columns

The first application using *p*-HMB for the extraction of 4MSP was proposed by Darriet *et al.* (1995). In this first application 750 mL of Sauvignon blanc were extracted using 100 mL organic solvents (diethyl ether/pentane 1:1). The thiols were then purified with 10/5/5 mL of a 2.5 mM *p*-HMB solution. The aqueous phase was then evaporated to about 10 mL and 350 mg of glutathione were added for releasing the bound thiols. A second liquid-liquid extraction step with three additions of 2/1/1 mL of diethyl ether-pentane (1:1) was performed and the combined aliquots were concentrated under a stream of nitrogen to 500 µL. The extract was then analysed using GC-MS. It has to be noted that this was the first report for the presence of 4MSP in wines from the variety Sauvignon blanc. It was published that 3SHA had been identified with the same method in Sauvignon blanc wines (Tominaga *et al.*, 1996). Tominaga *et al.* reported again in 1998 the identification of 3SH in Sauvignon wines and published a separate paper on the method development with some significant improvements (Tominaga *et al.*, 1998b). In the original method reported by Tominaga *et al.* (1996) it was shown that the quantification of some thiols is difficult due to coelution of further compounds other than thiols in the aqueous phase of *p*-HMB. To overcome this and to eliminate most of the impurities that were extracted the *p*-HMB fraction was percolated through a Dowex 1X2-100 (Sigma-Aldrich, Steinheim, Germany) strong basic anion exchange column.

Proposed sample preparation: In 500 mL of wine 2.5 nmol of internal standard (IS), 4-methoxy-2-methyl-2-sulfanylbutane, were added and the pH was set at 7. The sample was successively extracted 2 times with 100 mL of dichloromethane each time in a 1 L flask by stirring for 5

min. The two aliquots of the organic phase were combined and then centrifuged at 3800 g for 5 min. The thiols were selectively extracted with two successive additions of 20 mL of *p*-hydroxymercurybenzoate (1mM *p*-HMB in Tris buffer 0.2 M) for 5 min each time. The two aliquots of the aqueous phase were then combined and percolated through the strong basic anion-exchange column (1.5 x 3 cm) (Dowex 1, Sigma, 1X2-100). Subsequently the column was washed with 50 mL of sodium acetate buffer (0.05 M, pH 6). The varietal thiols were then released from the column with 60 mL of cysteine solution (640 mg/60 mL). The collected eluate from the column was collected in 100 mL flasks and extracted twice with 4 and 3 mL of dichloromethane respectively. The extraction was under constant stirring. The aliquots were collected, dried over sodium sulphate and under a nitrogen flow they were concentrated in a 10 mL tube to about 500 µL. The extract was transferred to a 1 mL vial and concentrated to 25 µL (Tominaga *et al*., 1998a & b; 2003).

Chromatographic analysis: 2 µL of the extract were injected in an HP 5890 II GC coupled with a HP 5972 MSD in splitless mode. The column used was a BP-20 (SGE) 50 m x 0.22 mm with a film thickness of 0.25 µm and helium as a carrier gas. The oven temperature programme started at 35 ˚C for 10 min and with a rate of 3 ˚C/min to 230 ˚C. The injector temperature was set at 250 ˚C and the source temperature at 250 ˚C. The detection mode of the MSD was SIM. (Tominaga & Dubourdieu 1997; Tominaga *et al.*, 1998b). The quantifier ions and the R^2 of the calibration curve for each compound are presented in **Table 1-7**.

Table 1-7: Quantitation and calibration data for the detected thiols (adapted from Tominaga *et al*., 1998b)

Compound	Quantifier ion	R^2
4MSP	75	1.000
3SHA	116	0.996
3SH	134	0.998

1.4.3 Alternative method with *p*-HMB and DOWEX

Tominaga & Dubourdieu published in 2006a an alternative method using *p*-HMB and a Dowex anion exchange column to the initially applied one (Tominaga *et al.*, 1998b). This method was initially published for the quantitation of 2-methyl-3-sulfanylfuran and 2-furanmethanethiol and it is probably used at present by the Faculte d`Oenologie at the Université de Bordeaux and at SARCO Laboratoires for the quantification of 4MSP, 3SHA and 3SH. This method had some significant improvement, such as using only 50 mL sample and eliminating the initial liquid-liquid extraction step with dichloromethane.

Proposed sample preparation: 1.2 nmol of 4-methoxy-2-methyl-2-sulfanylbutane were added as internal standard to 50 mL of wine and the pH value was set to 7. 5 mL of *p*-HMB (2 mM in 0.1 M Tris) were added to the sample and stirred for 10 min. The sample was then loaded to a DOWEX (Sigma, 1x2-100) anion exchange column (conditioning: flushing with 0.1 M HCl and rinsed with ultrapure water until pH 5-6 was reached). The resin was loaded to a glass column and the water was drained. Subsequently the resin was washed with 50 mL of ultrapure water and was ready for use and

percolated for 10 min. The column was rinsed with 50 mL of sodium acetate buffer (0.1 M, pH 6) containing 0.02 mM of *tert*-butyl-4-methoxyphenol as antioxidant. The thiols were released from the *p*-HMB complex fixed on the column by eluting them with 50 mL rinsing buffer containing 500 mg of cysteamine. 0.5 mL of ethyl acetate were added to the eluate to improve extraction and the solution was extracted twice with 4 and 2 mL of dichloromethane respectively. The aliquots were collected, dried over sodium sulphate and under a nitrogen flow they were concentrated in a 10 mL tube to about 500 µL. Finally the extract was transferred to a 1 mL vial and concentrated to 25 µL (Tominaga & Dubourdieu, 2006a).

Chromatographic analysis: An HP 5890 II GC coupled with an HP 5972 MSD and a DB-XLB (60 m x 0.25 mm, 0.25 µm film thickness) column were employed. 2 µL of the extract were injected at 250 ˚C. The oven temperature was set at 40 ˚C for 1 min and with a rate of 3 ˚C/min to 230 ˚C. The carrier gas was helium.

The two methods presented in **Chapter 1.4.2** and **1.4.3** are the most often published methods since the first application by Darriet *et al.* (1995). These methods have also been used by other workgroups and published by Swiegers & Pretorius (2007a) and King *et al.* (2008). Furthermore, it is the only commercially available quantification method for varietal thiols offered by Laboratoire SARCO (Floirac, France) (Swiegers *et al.*, 2009). According to the website of SARCO ("Thiols Volatils." *Laboratoire Sarco*. Web. 16 Nov. 2014.) 125 mL of wine are needed to analyse thiols. When compared with the publication by Tominaga *et al.* (1998b; 2003) the smaller of sample volume offers a significant improvement that probably is attributed to the improvements by Tominaga & Dubourdieu (2006a).

From the published chromatograms of the method (Tominaga *et al.,* 1998b) the sample extracts appear to be clean and contain almost exclusively the thiols of the original sample (Mateo-Vivaracho *et al.*, 2007), but do not solve the problems of 'detectability' and instability. The mass spectras of the compounds lack the characteristic ions in high *m/z* ratios and because of the long and complicated extraction procedure they are prone to oxidation and reactions with metal ions (Mateo-Vivaracho *et al.*, 2007). The ion exchange columns have to be packed with a properly conditioned DOWEX resin by the operator (Tominaga *et al.*, 1998b; 2003) increasing the probability for a mistake. Finally, the point to be noted is that there are only partial validation data for this method published (Ferreira *et al.*, 2007). Despite this it has to be mentioned that SARCO states that the method has been validated according to OIV Oeno 10/2005 resolution and NF V03-110 standard validation ("Thiols Volatils." *Laboratoire Sarco*. Web. 16 Nov. 2014.).

With this method it was also possible to identify four further thiols. These four compounds were 4-sulfanyl-4-methylpentan-2-ol, furfurylthiol, 2-methyl-3-sulfanylfuran and the less powerful 3-sulfanyl-3-methylbutan-1-ol (Tominaga *et al.*, 1998b; Tominaga & Dubourdieu, 2006a). Furthermore, with this method the stereoisomeric distribution of 3SH and 3SHA in wines could be studied (Tominaga *et al.*, 2006b). A further positive aspect is that this method is based on standard equipment such as a Mass Selective Detector (Skoog, 1998). The other methods published more meticulous equipment like PTV injectors and ion trap mass spectrometers are used (Ferreira *et al.* 2007). Some of

these mass spectrometers use CI (chemical ionisation), which is not a standard configuration (Schneider *et al*., 2003; 2006; Mateo-Vivaracho *et al.,* 2006; 2007).

1.4.4 The use of *p*-HMB in combination with polymeric Solid Phase Extraction (SPE)

Gómez-Míguez *et al.* (2007) first published the use of *p*-HMB in combination with polymeric solid phase extraction (SPE) to analyse thiols as part of a research on wines from the Spanish autochthonous grape variety Zalema. A method specific paper was then published by Ferreira *et al.* (2007).

Proposed sample preparation: 200 mL of wine (added with 5 mg/L of BHA and 250 ng/L of 1-hexanethiol and heptanethiol) are percolated through a LiChrolut EN 1 g bed of resins at a maximum flow of 5 mL/min. The bed of resins has been previously conditioned with 10 mL of dichloromethane, 10 mL of methanol and 10 mL of a 13% Vol. aqueous solution of ethanol. The cartridge is rinsed with an equal volume of TRIS (0.2 M pH 7.2 containing 40% methanol) and subsequently with 5 mL of water. The bed of resins is then dried under a stream of nitrogen. The odourants are eluted with 10 mL of 99% dichloromethane and 1% of methanol. The varietal thiols are selectively extracted with three times 1 mL of *p*-HMB solution (1 mM in HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) 0.2M, pH 10.7). The aliquots are combined and the pH is adjusted with the addition of 60 µL of HCl 4.6 M. The thiols are released with the addition of 450 µL of 10mM dithioerythritol in HEPES. The free thiols are purified with liquid-liquid extraction using 0.75 mL DCM (dichloromethane) twice. The IS is added (2-octanol, 100 µg/L) and the extract is concentrated in a water bath (47 ˚C) to about 200 µL.

Chromatographic analysis: A Varian CP-3800 GC equipped with a PTV injector (Programmed Temperature Vaporising), a DB-WAX 60 m x 0.25 mm, 0.25 µm film thickness column (J&W scientific) and a Saturn 2000 ion trap mass spectrometer was used. The carrier gas was helium at a flow of 1 mL/min. The oven temperature was 40 ˚C for 6 min and then with a rate of 2 ˚C/min to 200 ˚C and hold for 180 min. 20 µL of the extract were injected in the PTV at 40 ˚C for 0.6 min and then heated with a rate of 200 ˚C/min to 250 ˚C holding until the end of the programme. The quantifier ions and the R^2 of the calibration curve for each compound are presented in **Table 1-8**.

This method is also hampered by the same drawbacks of the *p*-HMB/DOWEX method by Tominaga *et al.* (2003) and Tominaga & Dubourdieu (2006a), like “detectability” and stability of the samples and the loss of thiols during the long extraction process (Mateo-Vivaracho *et al*., 2007). Furthermore, another problematic practice in such a complex sample preparation is the addition of one of the IS at the end of the procedure before the concentration of the sample (Skoog, 1998). 2-Octanol as an IS lacks the same properties like the thiols to be analysed (Dagan *et al.*, 2014)

Table 1-8: Quantitation and calibration data for the detected thiols (adapted from Ferreira *et al.,* 2007).

Compound	Quantifier	R^2
4MSP	75	0.9900
3SHA	88	0.9917
3SH	82	0.9994

1.4.5 Derivatisation of volatile thiols with the use of pentaflurobenzyl bromide

Mateo-Vivaracho *et al.* (2006) published a method with the derivation of thiols using pentaflurobenzyl bromide for improving the detectability and stability of the compounds. Such an approach has been proposed by Hofmann *et al.* (1996) with the use of 4-vinylpyridine as the derivatisation agent. However this method by Hofmann *et al.* (1996) was not developed any further as the gain in detectability wasn't significant and the chromatographic properties of the pyridine derivatives were complicated (Mateo-Vivaracho *et al.*, 2007). Instead PFBBr (2,3,4,5,6-pentafluorobenzyl bromide) was proposed as a derivatisation agent and the derivatives showed excellent electron-capturing properties (Mateo-Vivaracho *et al.*, 2006). Due to these properties two chromatographic detections were studied. Electron-Capture-Detection (ECD) and Negative Chemical Ionisation-Mass Spectrometry (NCI-MS), which both have very sensitive response in electrophilic atoms like halogens coupled on the derivatives (Mateo-Vivaracho *et al.*, 2006). For the initial method published two internal standards were used, 1-hexanethiol and benzylthiol (Mateo-Vivaracho *et al.*, 2006; 2007). In the improved method published again by Mateo-Vivaracho *et al.* (2008) the internal standard 1-hexanethiol was replaced in favour of SIDA according to Schneider *et al.* (2003). Furthermore the original on fibre SPME derivatisation and the derivatisation in a vial was replaced by direct derivatisation on an SPE cartridge.

Proposed sample preparation: 10 mL of wine with 0.05 g EDTA and 0.156 g L-cysteine hydrochloride is put in a 20 mL vial and shaken for 2 min. The internal standards are added (400 ng/L benzylthiol, 384 ng/L d_{10}-4MSP, 800 ng/L d_5-3SHA and 54 µg/L d_2-3SH) with a short stirring and additionally 0.1 g of methylhydroxylamine. The vial was gently purged with nitrogen, and incubated at 55 ˚C for 45 min. A 50 mg Bond Elut ENV SPE cartridge was conditioned with 1 mL of dichloromethane, 1 mL of methanol and 1 mL of water. 6 mL of the incubated wine sample were percolated through the cartridge. The non-attached major volatiles were washed out with 4 mL of a 40% methanol solution in 0.2 M phosphate buffer at pH 7.7 and finally with 1 mL of water. The derivatisation of the thiols took place on the cartridge by passing 1 mL of DBU solution (6.7 %, 1,8-diazabicyclo [5.4.0] undec-7-ene) and 50 µL of PFBBr in hexane (2000 mg/L). The mix in the cartridge was incubated for 20 min at room temperature (25˚C). The excess reagent was then washed out with 100 µL of a 2000 mg/L mercaptoglycerol solution in 6.7% DBU and letting the cartridge again for 20 min at room temperature. Afterwards, the cartridge was washed again with 4 mL of a 40% methanol solution in 0.2 M phosphate buffer at pH 7.7 and finally with 1 mL of water. The derivatisation products were subsequently eluted with 600 µL of a solvent mixture (hexane:diethylether 1:3) containing 375 ng/L of the chromatographic internal standard,

octafluoronaphtalene. The eluate was washed five times with 1 mL of NaCl (200 g/L), transferred to a 2 mL vial and spiked with a small amount of sodium sulphate (Mateo-Vivaracho *et al.,* 2008).

Chromatographic analysis: A Shimadzu QP-2010 GC coupled with a quadrupole mass spectrometer and equipped with a LVI injector (Large Volume Injection) and a VF-5MS column (20 m x 0.15 mm, 0.15 µm film thickness) was used. 20 µL of the extract were injected at 55 ˚C hold for 17 s and then the injector was heated with 5 ˚C/s to 250 ˚C holding the temperature until the end of the run. The temperature of the column was initially 45 ˚C for 2 min, at 25 ˚C/min to 140 ˚C, at 15 ˚C/min to 180 ºC, at 30 ˚C/min to 210 ˚C and finally with 250 ˚C/min to 300 ˚C and holding for 10 min. The carrier gas was helium with an initial flow of 0.6 mL/min, 17 s after the injection it was increased to 1.8 mL/min and after 3 min it was set to 1 mL/min. The injection was splitless from the 17 s until the 3rd minute of the run. The mass spectrometer was operated in NCI mode using methane as reagent gas at 2 bar pressure. The source operated at a temperature of 220 ˚C and the transfer line at 290 ˚C. SIM mode was used for detection (Mateo-Vivaracho *et al.*, 2008). The quantifier ions, the R^2 and the calibration range for the curve of each compound are presented in **Table 1-9**.

Table 1-9: Quantitation and calibration data for the detected thiols (adapted from Mateo-Vivaracho *et al.,* 2008)

Compound	Quantifier	R^2	Range (ng/L)
4MSP/*d*$_{10}$-4MSP	160/170	0.9986	0-100
3SHA/*d*$_5$-3SHA	175/180	0.9981	0-200
3SH/*d*$_2$-3SH	133/135	0.9719	0-2500

This method showed to be quite powerful by detecting more thiols than the varietal ones (2-methyl-3-furanthiol and 2-furfurylthiol). It presented good calibration data for the compounds 2-furfurylthiol, 4MSP, 3SHA and 3SH. The results were poor for the linearity and repeatability of 2-methyl-3-furanthiol and 3SH (Rodriguez-Bencomo *et al.*, 2009). The employment of SIDA for the quantification of the varietal thiols was in any case an important factor for the success of the method, except as mentioned for 3SH. Another point that needs to be mentioned is that the amount of chemicals used for one sample are less than in the previously above described methods, which is a matter to consider in terms of cost and disposal.

It seems that this derivatisation process has a good potential to be developed in a stable and powerful method as more research work has been published recently (Rodriguez-Bencomo *et al.*, 2009; Dagan *et al.*, 2014). Rodriguez-Bencomo *et al.* (2009) modified the method after the elution of derivates from the SPE cartridge. Instead of concentrating the extract and injecting it to the GC the extract was evaporated to dryness in a 10 mL SPME vial and sealed under nitrogen. Afterwards the sample was extracted for 30 min with a SPME fiber (DVB/CAR/PDMS) at 110 ˚C and then injected to the GC-MS. With this technique it was possible to detect 4MSP, 3SHA and 3SH close to their odour threshold. It was also possible to improve the sensitivity, linearity and repeatability of the 3SH determination, which was one of the major drawbacks of the method by Mateo-Vivaracho *et al.* (2008). Another improvement was the use of SPME to inject the sample avoiding the use of LVI which damages the column more quickly and contaminates the MS more rapidly (Rodriguez-Bencomo *et al.*, 2009).

1.4.6 Various other proposed methods

In 2007 Fedrizzi *et al.* proposed two methods, one using SPE cartridges (Isolute ENV+, Biotage) and the other using a CAR/PDMS/DVB (50/30 µm x 2 cm) SPME fiber. With both methods it was only possible to detect 3SHA and 3SH. This can be considered as a major drawback because 4MSP is the most powerful of the varietal thiols.

Table 1-10: Quantitation and calibration data of the method proposed by Fedrizzi *et al.* (2007), adapted data.

Compound	Quantifier/Qualifier ions	LOD (ng/L)	LOQ (ng/L)	R^2
3SHA	116/67,83,88	83 (SPE)/ 57 (SPME)	207 (SPE)/ 136 (SPME)	0.995/0.992
3SH	134/67,82,100	74 (SPE)/ 69 (SPME)	183 (SPE)/ 151 (SPME)	0.993/0.993

As it can be seen from **Table 1-10** another drawback of this method are the high LOQs for both 3SHA and 3SH, which are ~45 times and ~3 times above the odour threshold. On the other hand this method could offer a simplified procedure for the quantitation of 3SHA and 3SH in wines that contain higher concentrations as no derivatisation or selective extraction with mercury derivatives are needed. An improvement was the use of 6-sulfanylhexanol (6SH) as an internal standard that has more similar properties to 3SH.

Another method published by Dagan *et al.* (2014) concentrated its efforts in a quick derivatisation process coupled with HS-SPME-GC-MS/MS for the detection of 4MSP in concentrations below the odour threshold. The improvement in comparison to the other published methods is the use of a low amount of sample (3 mL), the fully automated reaction and desorption of the analytes and the low LOQ of 0.64 ng/L (Dagan *et al.,* 2014). The quantification of the 4MSP as previously reported by Schneider *et al.* (2003; 2006) was carried out with SIDA.

1.5 Grape varieties of interest

Five grape varieties were chosen for the purpose of this study. They are shortly presented in the following chapters.

1.5.1 Malagousia

Malagousia is a variety with a very interesting story as it was almost extinct until the mid-1970s. The variety originated from Etolia-Akarnania (western part of central Greece) and was discovered by Prof. Dr. Vassilis Logothetis of the Agricultural University of Thessaloniki. Until then the variety was used exclusively for blended wines and the aromatic capacity of it was lost (Lazarakis, 2006; Tourtoglou
et al., 2014). In 1976 the Oenologist of Domaine Carras in Greece, Evangelos Gerovassileiou, tested the variety and refined its vinification and by the beginning of the 1990s he presented his first varietal Malagousia wines with great success (Robinson *et al.*, 2012). Malagousia has been planted in many different regions of Greece, from the south Peloponnese and Attica to the north of Greece, which the later also has the most extensive vineyards (Robinson *et al.*, 2012).

The Malagousia vines are not very resistant to drought and the maturation phase is being supported by one or two short, well planned drip irrigation periods (Lazarakis, 2006) The bunches can be easily infected with *Botrytis cinerea* and downy mildew (Robinson *et al.*, 2012). Furthermore, the variety is prone to viral infections so care must be taken when selecting young vines (Lazarakis, 2006). According to the maturation of the grape bunches great variations have been shown in the aromatic expressions of the variety. When the grapes are being harvested with a potential alcohol below 11.5 % Vol. the wines produced show little aromatic complexity (Robinson *et al.*, 2012). When the potential alcohol is between 12.5-13.5 % Vol. the aromatic complexity is at its maximum. For overripe grapes the primary aroma components like terpenes are concentrated and the wines tend to have a 'Muscat' character (Lazarakis, 2006).

The wines produced with Malagousia are exclusively local or table wines as no OPAP (Onomasia Proelefseos Anoteras Piotitas-High Quality Wines with Origin) wines are allowed to be made with this variety.

1.5.2 Petite Arvine

Petire Arvine is being characterised as the finest grape variety of the oenological region of Valais in Switzerland (Robinson, 2006). According to the report of the Swiss Ministry of Agriculture the total area of Petite Arvine in Switzerland in 2013 was 166 ha (BLW, 2014). Almost all of this area (165.5 ha) is in the Canton of Valais, which represents one of the old autochthonous varieties (Robinson *et al*., 2012).

The vines are productive and are budding very early but are ripening very late and sometimes they are the latest in the whole vineyards of Valais (Robinson *et al.*, 2012). For its planting inclined vineyards are preferred that offer good water drainage, enough sunlight, but also are protected from wind. Irrigation is essential to avoid water stress (Hansueli Pfenninger, *Pers. Comm.,* 2013). The bunches, which are compact with small berries, are sensitive against Botrytis and the vines to downy mildew and mites (Robinson *et al.*, 2012).

Petite Arvine is mainly used to produce dry wines and a small number of sweet ones. The dry ones have a characteristic 'grapefruit' note and a saltiness at the palate. Furthermore they are characterised with a finesse that gives the variety the reputation of the finest in Valais (Robinson *et al.*, 2012).

1.5.3 Asyrtiko

Asyrtiko is considered as one of the best Greek autochthonous varieties. It has the ability to produce wines with total acidity above 6.5 g/L (in tartaric acid) and pH values below 3.0 in one of the most warm and dry vineyards, in Santorini Greece (Lazarakis, 2006). The variety is indigenous in Santorini (Robinson *et al.*, 2012) but because it has excellent adaption to other climates it is cultivated in many parts of Greece (Lazarakis, 2006). In 2008, 1,704 ha of Asyrtiko were in Greece making it the third most planted white variety after Roditis and Savatiano (Robinson *et al.,* 2012).

The vines are resistant to almost all diseases and show a medium to high vigorousness at its growth (Robinson *et al.*, 2012). As already mentioned it is very resistant against drought and the stock of the vine is very robust in order to cope with the strong winds blowing on the Aegean islands. This

characteristic and the easiness to adapt to new environments has made the variety one of the most widespread in Greece, cultivated in many Aegean islands and in the continental parts stretching from the Peloponnese to the south up to Macedonia in the north (Lazarakis, 2006).

The most important wine produced with Asyrtiko is the OPAP wine 'Santorini'. The whole island is declared as an OPAP zone. Furthermore, the OPAP wine is also produced from Asyrtiko in co-vinification with Athiri and Roditis. An interesting aspect of Asyrtiko is the different character of the varietal wines from Santorini and continental Greece. The Santorini ones are acidic and have a strong 'minerality' when compared with the continental ones, which are more fruity and aromatic (Lazarakis, 2006; Robinson *et al.*, 2012).

1.5.4 Roditis

Roditis is one of the oldest and most widespread autochthonous varieties of Greece (Lazarakis, 2006). It is cultivated in almost whole continental Greece (except Epirus) and the west part of central Greece with a total area of 9,743 ha in 2008 (Robinson *et al.*, 2012). The name Roditis is used for a family of clones that share similar characteristics (Lazarakis, 2006).

Roditis is a very vigorous vine, which is resistant to drought but quite sensitive to downy- and powdery- mildew (Robinson *et al.*, 2012). The bunches are of medium size with medium weight berries and have a dark rosé colour even if they are not exposed to sunlight (Lazarakis, 2006). At very fertile and productive soils the production is very high and can exceed 120 hL/ha (Lazarakis, 2006) and the wines tend to be light and with little aromatic intensity. At higher altitudes (above 300 m), poorer-, light- and chalky soils the vines lose their vigorousness and the mean weight of the bunches drops below 450 g. The wines produced from these soils show a strong aromatic fruity character that has a touch of Sauvignon blanc exotic notes, melon and dense structure in their palate (Lazarakis, 2006).

The variety is used in a number of OPAP wines either as 100% Roditis for OPAP 'Patras' or blended with Savatiano at OPAP 'Aghialos' and with Asyrtiko and Athiri at OPAP 'Plagies Melitona' (Lazarakis, 2006). The variety is also extensively used for producing table and local wines, but is also involved in the production of Retsina, the famous Greek wine that has pine tree resin added (Lazarakis, 2006).

1.5.5 Scheurebe

Scheurebe is a crossing of Riesling with an unknown variety and was bred by Georg Scheu in 1916 at the Alzey research centre (Robinson *et al.*, 2012). A common synonym for this variety is Sämling 88. The main two countries that have the variety planted are Germany and Austria. The planted areas of Scheurebe are declining in Germany over the years – currently 1,672 ha and in 2003 the area was 2,200 ha (Robinson *et al.*, 2012). Austria had 511 ha planted in 2007. Small areas of Scheurebe are also planted in Switzerland, Canada and New Zealand (Robinson *et al.*, 2012).

Scheurebe shows late ripening and is sensitive to powdery mildew. Furthermore, it is not as cold resistant as Riesling. The grapes can be used for both dry- and sweet-wines. The wines are very aromatic and show a 'blackcurrant' aroma -in extreme cases 'catpiss' (high concentrations of 4MSP)- and 'grapefruit' together with a refreshing acidity (Robinson, 2006; Robinson *et al.*, 2012). The sweet

wines can be produced either from fully ripe grapes or grapes affected by noble rot. These wines have an excellent ageing potential (Robinson *et al.*, 2012).

1.5.6 Sauvignon blanc

Sauvignon blanc has been characterised as an international variety, which is a “classic variety with a long established reputation for making premium quality wines in locations across the globe” (Mac Neil, 2001). In spite to the common belief, Sauvignon blanc probably does not seem to have its origins in Bordeaux but in the Val de Loire in France where it is mentioned as early as 1534 (Robinson *et al.*, 2012). In the 1780s it is mentioned in the region of Sancerre, which is also today one of the most known region for these varietal wines (Robinson *et al.*, 2012). Recent studies have shown that Sauvignon blanc is related to Chenin Blanc and it seems to be genetically close to Semillon (Jahnke *et al.*, 2009). Besides the white bunches normally produced there are also two known colour mutations, one being Sauvignon gris and the other Sauvignon rouge. They are cultivated in a limited extend in France (Robinson *et al.*, 2012).

Countries that have significant areas of the variety planted are France (26,839 ha in 2009), USA (6,235 ha in California in 2010 and Washington State with 475 ha, followed by smaller areas in Oregon, New York, Virginia and Texas), Chile (7,922 ha in 2008), Australia (6,405 ha in 2008), South Africa (9,155 ha in 2008) and New Zealand (18,000 ha in 2011) (Robinson *et al.*, 2012).

Wines made out of Sauvignon blanc can show various characters, from ‘green paprika’ to exotic notes of ‘passionfruit’, ‘cassis’ and ‘grapefruit’. Intense research for the past 20 years has been carried out to identify the compounds that give this distinctive aroma to the wines from this variety and how it is being influenced (Robinson *et al.*, 2012). Two significant group of compounds have been identified: Methoxypyrazines that are associated with the green character of the wines (‘grass’, ‘leaves’, ‘nettles’) and varietal thiols that are associated with the exotic character (Cotzee & du Toit, 2012). All these aromas can be influenced in the vineyard and in the winery with the oenological practices (Robinson *et al.*, 2012)

1.6 Aim of this study

The aim of this research study is to focus on the hypothesis that varietal thiols are more widespread than assumed, in many other varieties with special focus on Greek autochthonous. The aims of this study can be summoned in three work packages in which each is a prerequisite for the other:

- Developing and establishing a powerful method for measuring certain varietal thiols (4MSP, 3SHA and 3SH) in concentrations down to a few ng/L. The problematic of already existing methods using gaschromatography and mass spectrometry have been described in **Chapter 1.4**.

- Conducting fermentation experiments for two consecutive years with an international grape variety (Sauvignon blanc) and a local variety (Scheurebe), which are known for their varietal character. This aims to establish and to adapt an adequate methodology and to induce high

concentrations of volatile thiols helping to explore the limitations of the measuring methods. Furthermore, these fermentations were used to optimise the tasting scheme.

- Carrying out a more thorough study on Greek autochthonous grape varieties (Asyrtiko, Malagousia & Roditis) with special focus on varietal thiols, sensory analysis of the wines and the other aromatic compounds that might contribute to the character of the wines.

Due to the increasing popularity of these aromatic varieties, e.g. Sauvignon blanc internationally and e.g. Malagousia in the domestic Greek market but also slowly international, the importance of characterising them is vital. This study aims at the characterisation of the Greek varieties and in further studies the optimisation of their aroma and the enhancement of their 'typicity' can be sought.

2. Experimental part

2.1 Experiment setup

The experimental part of this thesis is divided into four different work packages:

1. Development and validation of the method for the quantitation of varietal thiols. This was an on-going process and the experiments 2 and 3 were measured with the originally developed method described in **Chapter 2.2.2** and experiment 4 was studied with the improved method (**Chapter 2.2.3**).
2. Fermentation experiment with Scheurebe and Sauvignon blanc musts during the vintage 2008 and 2009 with different commercial wine yeast for validating the method to analyse varietal thiols and developing the tasting/analysis scheme.
3. Greek varieties pre-selection. Three different autochthonous grape varieties of Greece were selected and tested for their varietal thiols and other components in order to select the most promising for continuative research.
4. Research on the selected variety from experiment 3 (Malagousia) and use of an autochthonous and partially characterised variety (Petite Arvine) for validating the results.

2.1.2 Chemicals

The chemicals and reference standards used in the analytical methods:

Major components: Acetic acid and ethanol HPLC gradient were supplied by Carl Roth (Karlsruhe, Germany). L-Malic acid, shikimic acid, D-glucose and D-fructose were supplied by Sigma-Aldrich (Seelze, Germany). L-Tartaric acid and citric acid were supplied by Fluka (Seelze, Germany). L-Lactic acid lithium salt was supplied by AppliChem (Darmstadt, Germany).

'Kaltron' method: Acetic acid ethylester, i-Butanol, propionic acid ethylester, 3-methyl-butanol, 2-methyl-butanol, i-butyric acid ethylester, butyric acid ethylester, lactic acid ethylester, hexanol, acetic acid 3-methylbutylester, acetic acid 2-methylbutylester, caproic acid, caproic acid ethylester, acetic acid hexylester, *trans*-linalool oxide, *cis*-linalool oxide, linalool, 2-phenylethanol, caprylic acid, succinic acid diethylester, caprylic acid ethylester, α-terpineol, benzeneacetic acid ethylester, acetic acid phenylethylester, capric acid, capric acid ethylester and isopropylbenzene (IS) were supplied from Sigma-Aldrich (Seelze, Germany). 2,6-dimethyl-5-hepten-2-ol was supplied by Carl Roth (Karlsruhe, Germany).

Low boiling point sulphur off-flavours: sodium sulphide, methanethiol and diethyl disulphide were supplied from Sigma-Aldrich (Seelze, Germany). Ethanethiol, dimethyl sulphide, thioaceticacid-S-ethylester and methyl-iso-propyl sulphide (IS) were supplied from Alfa Aesar (Karlsruhe, Germany). Carbon disulphide, dimethyl disulphide, dimethyl trisulphide were supplied by Acros Organics (Geel, Belgium). Butylmethyl sulphide (IS) was supplied by Lancaster (Ward Hill, MA, USA).

Free monoterpenes and C_{13} norisoprenoids: Geraniol, linalool, nerol, 1,8-cineole, *trans*/*cis*-linalool oxide, α-terpineol, β-ionone, hotrienol and octan-3-ol (IS) were supplied by Sigma-Aldrich (Seelze, Germany). Nerol oxide was supplied by Interchim (Montlucon, France). β-Damascenone was gifted by Firmenich (Geneva, Switzerland). Vitispirane was gifted by Dr. A. Rapp (Sieberdingen, Germany). TDN (1,1,6-trimethyl-1,2-dihydro-naphtalene) was supplied by Faculté d'Oenologie (Bordeaux, France). DMH (2,6-dimethylhept-5-en-2-ol) was supplied by Carl Roth (Karlsruhe, Germany). Strata S-DBL cartridges were supplied by Phenomenex (Darmstadt, Germany).

Varietal thiols method: 4-Methoxy-2-methyl-2-sulfanylbutane, 4-methyl-4-sulfanylpentan-2-one and 3-sulfanylhexyl acetate were supplied by Chemos (Regenstauf, Germany). 3-Sulfanylhexan-1-ol was supplied by Acros organics (Geel, Belgium). *p*-Hydroxy mercurybenzoate was supplied by Sigma-Aldrich (Seelze, Germany). HEPES and TRIS were supplied by Carl Roth (Karlsruhe, Germany). Lichrolut EN SPE cartridges were supplied by Merck (Darmstadt, Germany).

2.2 Analysis of varietal thiols

The analytical determination of varietal thiols in wine has been a complex matter because of several reasons. Wine is considered as one of the most complex aromatic products (Gómez-Miguez *et al.*, 2007; Roland *et al.,* 2012) so it is difficult to isolate specific odourants. The extremely low concentrations found in wines (as low as a few ng/L for 4MSP) and the high reactivity potential with oxygen, metals and other factors (Rigou *et al.*, 2014).

Almost all of the methods for measuring varietal thiols are based on the reaction of sulphhydryl groups with mercury salt, first time shown by Boyer (1954) (**Chapters 1.4.1, 1.4.2, 1.4.3** & **1.4.4**).

2.2.1 Preparation of standards

The chemical standards were 4MSB, 4MSP and 3SHA supplied by Chemos (Regenstauf, Germany) and 3SH was supplied by Acros Chemicals (Geel, Belgium).

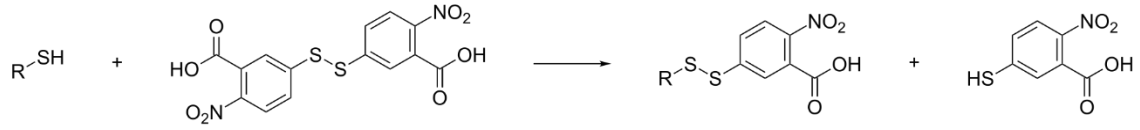
Table 2-1: Overview of the chemical standards used for the analysis of varietal thiols.

Compound Name	Abreviation	CAS	MW	Purity
4-Methoxy-2-methyl-2-sulfanylbutane	4MSB	94087-83-9	134	
4-Methyl-4-sulfanylpentan-2-one	4MSP	19872-52-7	132	99.02%
3-Sulfanylhexyl acetate	3SHA	136954-20-6	176	99.40%
3-Sulfanylhexan-1-ol	3SH	51755-83-0	134	98%

All the standard solutions were prepared in two sets. The first set of standards was prepared in dichloromethane and the second in ethanol (Both Carl Roth, Karlsruhe, Germany). Both solvents were of pure GC grade and were prior to use distilled for removing all the possible contaminant odourants. The different concentrations were prepared with serial dilutions. The standards in dichloromethane were used for direct injections in the GC-MS and the standards in ethanol were used for the additions for the calibration and for the analysis of the samples.

2.2.1.1 Determination of thiol concentration in the standards

Due to the fact that volatile thiols are prone to oxidation (Roland *et al.*, 2011a) the concentration of the standards was determined using the colorimetric Ellman's test (Ellman, 1959) prior to using them for analysis. The Ellman´s test is based on the reaction of the thiol group with the reagent DTNB (5,5´-dithio-bis(2-nitrobenzoic acid)) and the formation of a chromoform complex, TNB (2-nitro-5-thiobenzoic acid) that can be measured at 412 nm. The reaction is rapid and is stoichiometric.



Thiol + DTNB mixed disulphide + TNB

Figure 2-1: The reaction of a thiol with DTNB forming the chromophore TNB (adapted from Ellman, 1959)

Reagents: **1)** 3.24 g K_2HPO_4*$12H_2O$ and 0.19 g KH_2PO_4 are dissolved in 200 mL of distilled water and the pH adjusted to 8 **2)** 40 mg DTNB are dissolved in 10 mL of 0.1 M phosphate buffer (pH = 8)

Table 2-2: The volumes of each solution added to a 1 cm cuvette for the Ellman´s reaction

Blank	Sample
1000 µL H_2O	975 µL H_2O
500 µL DTNB solution	25 µL Thiols standard
500 µL Phosphate buffer	500 µL DTNB solution
	500 µL Phosphate buffer

The blank and samples were mixed and let for 15 min for the reaction to be completed. The absorbance is then measured at 412 nm with a spectrophotometer (Thermo Fischer, Schwerte, Germany).

The concentration of the thiol in the standard is being calculated with the following formula:

$$C = (Abs\ [\%]/\varepsilon)*M)*F$$

C = Concentration

ε = extinction factor (for nitrobenzene thiols the value is 13600)

M = Molecular mass of the measured thiol

F = Dilution factor (for this protocol 2000/25)

2.2.2 Initial method for the analysis of varietal thiols

The initial method used for the analysis of varietal thiols was adapted from Ferreira *et al.* (2007).

Purification of varietal thiols: 50 mL of wine containing 5mg/L of BHA and 200 ng/L of 4MSB as an internal standard were percolated through a bed of 500 mg LiChrolut EN resin (Merck, Darmstadt, Germany) at a maximum speed of 5 mL/min. The sorbent was conditioned prior to use with 10 mL of dichloromethane, 10 mL of methanol and 10 mL of a 13% (v/v) ethanol solution in ultrapure water. The

cartridge was rinsed with an equal volume of an aqueous TRIS buffer 0.2 M at pH 7.2 with 40% (v/v) methanol for washing out the non-retained compounds and after that with 5 mL of ultrapure water. The sorbent was dried under a gentle stream of nitrogen for 20 min. The retained odourants where then eluted with 10 mL of dichloromethane containing 1% of methanol (v/v). The organic phase was extracted with three successive additions of 1 mL of a 1 mM *p*-HMB solution in HEPES 0.2 M at pH 10.7 and stirred each time at 700 rpm for 5 min. The three aliquots where combined and the pH was adjusted to 7.5 with of 60 µL of HCl 4.6 M. The thiols-mercury-complexes where broken with the addition of 450 µL of a 10 mM dithioerythritol solution in HEPES at pH 10.2. The free thiols were extracted twice with 0.75 mL of dichloromethane. The two aliquots were combined and the extract was concentrated to about 50 µL in a water bath at 47 ˚C (Dünges, 1979). The samples were stored at -18 ˚C prior to chromatographic analysis.

Gas chromatography-mass spectrometry: The extracts were analysed by gas chromatography coupled with mass spectrometry. The unit consisted of an Agilent 6890N GC oven (Steinheim, Germany) fitted to an Agilent 5975C MSD (Mass Selective Detector). The GC oven was equipped with an MPS2 Autosampler and a CIS4 (Cooled Injection System) both from Gerstel (Mühlheim a. d. Ruhr, Germany). The column was a DB-WAX, 30 m x 0.32 mm x 0.25 µm (J&W scientific, Steinheim, Germany) with helium as a carrier gas. 3 µL of the extract were injected in spittless mode. The injector had the following injection programm: initial 40 ˚C for 0.2 min, raised to 250 ˚C at 12 ˚C/s and a hold time of 5 min. The oven program was set at initial 60 ˚C for 5 min and then the temperature was raised to 240 °C at a rate of 3 °C/min with a hold time of 40 min.

2.2.3 Improved method for the analysis of varietal thiols

The results of the initial method were not very satisfying with the 3SHA (R^2 = 0.9890) so the method was improved in some of the parameters.

Purification of varietal thiols: The method used was modified according to Ferreira *et al.* (2007). 50 mL of wine containing 200 ng/L of 4MSB as an internal standard were percolated through a bed of 500 mg LiChrolut EN resin (Merck, Darmstadt, Germany) at a maximum speed of 5 mL/min. The sorbent was conditioned prior to use with 10 mL of dichloromethane, 10 mL of methanol and 10 mL of a 13% (v/v) ethanol solution in ultrapure water. The cartridge was rinsed with an equal volume of an aqueous TRIS buffer 0.2 M at pH 7.2 with 40% (v/v) methanol for washing out the non-retained compounds and after that with 5 mL of ultrapure water. The sorbent was dried under a gentle stream of nitrogen for 20 min. The retained odorants where then eluted with 10 mL of dichloromethane containing 1% of methanol (v/v). The organic phase was extracted with three successive additions of 1 mL of a 1 mM *p*-HMB solution in HEPES 0.2 M at pH 10.7 and stirred each time at 700 rpm for 5 min. The three aliquots where combined and the pH was adjusted to 7.5 with of 60 µL of HCl 4.6 M. The thiols-mercury-complexes where broken with the addition of 450 µL of a 10 mM dithioerythritol solution in HEPES at pH 10.2. The free thiols were extracted twice with 0.75 mL of dichloromethane. The extract was concentrated to about 25 µL in a water bath at 47 ˚C (Dünges, 1979). The samples were stored at -18 ˚C prior to chromatographic analysis.

Gas chromatography-mass spectrometry: The extracts were analysed by a gas chromatography coupled with a mass spectrometry system. The unit consisted of an Agilent 6890N GC oven (Steinheim, Germany) fitted to an Agilent 5975C MSD (Mass Selective Detector). The GC oven was equipped with an MPS2 Autosampler and a CIS4 (Cooled Injection System) both from Gerstel (Mühlheim a. d. Ruhr, Germany). The column was a BP-20, 50 m x 0.22 mm x 0.25 µm (SGE, Victoria, Australia) and helium as a carrier gas. 7 µL of the extract where injected in spittless mode. The injector had the following injection programm: initial 30 ˚C, raised to 230 ˚C at 12 ˚C/s and a hold time of 30 min. The oven program was set at initial 40 ˚C for 4 min, raised to 200 ˚C at 3 ˚C/min and, finally, to 240 ˚C at 15 ˚C/min with a hold time of 60 min.

2.2.4 Data acquisition and calculation

Data acquisition of the MSD for both methods was in selected ion monitoring (SIM) mode. The peaks were identified according to their retention time (RT) and the ratios of the quantifier- and qualifier ions (**Table 2-3**). Peak area of the quantifier ions was used for calibration and quantification.

Table 2-3: The quantifier- and qualifier ions for each of the detected compounds

Compound	Quantifier	Qualifier 1	Qualifier 2
4MSB	134	100	-
4MSP	132	99	75
3SHA	116	101	-
3SH	134	100	-

All calculations for the calibration curves and for the quantitation of the compounds for the measured wines were done with Microsoft Excel 2013 (Microsoft, Redmont, Washington).

2.2.5 Method validation

Method validation was done according to DIN (32645 & 38402 A51) and ISO 5725 (Wellmitz & Gluschke, 2005; Kromidas, 2011) and the following six factors were taken into consideration:

1. Repeatability: By the injection of standards the method precision was measured by the total %RSD for three concentration levels. Both the instrument- and method repeatability were calculated.
2. Calibration & Linearity: The linearity of the method was tested in concentrations exceeding the normal concentrations found in wine. The %RSD for each level, the R^2 and the equation for the three compounds was calculated.
3. LOD/LOQ: The level of detection and level of quantitation were calculated according to DIN.
4. Recovery: The recovery rates for the extraction procedure were calculated for each odourant.
5. Correctness: By spiking an unknown wine with a known amount of the three thiols studied the the deviation from the theoretical expected value was calculated.
6. Robustness: The method was studied for its robustness with three factors that could affect the extraction of the varietal thiols: pH of the sample, elution solvent and speed of extraction.

2.3 Fermentation experiment 2008 & 2009

Fermentation experiments were conducted using two grape varieties, Scheurebe and Sauvignon blanc (**Table 2-4** & **2-5**), and six different commercial yeasts (**Table 2-6**) during harvest 2008 & 2009. 1.5 L green Schlegel bottles were used as fermentation vessels for the micro-vinifications. 1.3 L must were filled in each bottle. Each variant was carried out in triplicate.

Table 2-4: Properties of the Scheurebe musts for the vintages 2008 & 2009. Both Scheurebe vintages were sourced from Lergenmüller winery in Hainfeld (Germany).

Fresh Scheurebe must		
Parameter	**2008**	**2009**
Relative density	1.0739	1.0893
Reducing sugars (g/L)	181	221
pH	3.2	3.2
Total acidity (g/L tartaric acid)	8.4	8.4
Volatile acidity (g/L acetic acid)	n.d.	n.d.
Malic acid (g/L)	6	4.4
Tartaric acid (g/L)	4.5	3.3
Lactic acid (g/L)	n.d.	n.d.
Citric acid (g/L)	0.13	n.d.
Shikimic acid (mg/L)	45	39
NOPA (mg/L as isoleucine)	191	133

Table 2-5: Properties of the Sauvignon blanc musts for the vintages 2008 & 2009. The vintage 2008 was sourced from the DLR (Dienstleistungszetrum Ländlicher Raum) Oppenheim and the vintage 2009 of the Department of Grapevine Breeding of the Hochschule Geiseheim University.

Fresh Sauvignon blanc must		
Parameter	**2008**	**2009**
Relative Density	1.0729	1.0869
Reducing sugars (g/L)	215	230
pH	3.2	3.15
Total acidity (g/L tartaric acid)	5.7	6.9
Volatile acidity (g/L acetic acid)	0.1	n.d.
Malic acid (g/L)	4.5	4
Tartaric acid (g/L)	3.8	5.1
Lactic acid (g/L)	n.d.	n.d.
Citric acid (g/L)	0.31	0.25
Shikimic acid (mg/L)	27	22
NOPA (mg/L as Isoleucine)	181	207

Table 2-6: Specifications of the six selected commercial yeast strains.

Yeast strain	Manufacturer	Abbreviation	Fermetation Temperature (˚C)	YAN Requirement	Ethanol Tolerance (% Vol.)	Volatile acidity (in g/L acetic acid)	H_2S	References
X5	Laffort	X5	13 - 20	Medium	16	-	-	http://www.laffort.com/images/stories/telechargement/fiches%20commerciales/2%20-%20FC%20-%20ANGLAIS/FC_ANG_Zymaflore_X5.pdf
Cepage Sauvignon	DSM	SAU	15 - 25	-	15	< 0.2	-	http://www.vintessential.com.au/assets/datasheets/file/Data%20Sheets/Collection%20Cepage/cepage-sauvignon.pdf
VL3	Laffort	VL3	15 - 21	High	14.5	-	-	http://www.laffort.com/images/stories/telechargement/fiches%20commerciales/2%20-%20FC%20-%20ANGLAIS/FC_ANG_Zymaflore_VL3.pdf
Alchemy II	Anchor	ALII	13 - 20	Average	15.5	< 0.5	Very low	http://www.oenobrands.com/files/PDF/Anchor/Alchemy/Anchor-Alchemy-11-Product-Data-Sheet-EN.pdf
VIN13	Anchor	VIN13	15 - 20	Low	17	< 0.3	Very low	http://www.oenobrands.com/files/PDF/Anchor/Anchor-VIN-13-Product-Data-Sheet-EN.pdf
Oenoferm Bouquet	Erbsloeh	BOUQ	16 - 20	-	15	-	-	http://www.erbsloeh.com/product_datasheets/en/PMB_OenofermBouquetF3_GB_001.pdf

The dried yeasts were rehydrated according to the manufacturer's specifications and were added at a concentration of 20 g/hL. All the bottles were closed using air locks so that CO_2 from the fermentation could escape but no air could come into the bottle. The fermentations were carried out at 20 ˚C in a controlled environment.

2.3.1 Fermentation monitoring

The fermentations were monitored by loss of weight of the fermentation vessels from the production of CO_2. This loss of weight was measured during the beginning and the main phase of the fermentation every day and towards the end every other day. The scale (Kern, Balingen-Frommern, Germany) used was put into the fermentation room during the entire duration so that the environmental conditions are constant and the results consistent (acclimatisation of the scale).

2.3.2 Bottling of the wines

The finished fermentations were put into a temperature controlled room at 4 ˚C for 48h for clarification. Each replicate was portioned into one 0.75 L bottle, one 0.25 L bottle, one 0.18 L bottle and the rest in 50 mL bottles. The bottling took place under absence of air for preventing oxidation of the delicate aroma compounds. An apparatus was constructed and used N_2 overpressure (0.2 bar) on the surface of the liquid so that it can be pushed out of the fermentation bottle (**Figure 2-2**).

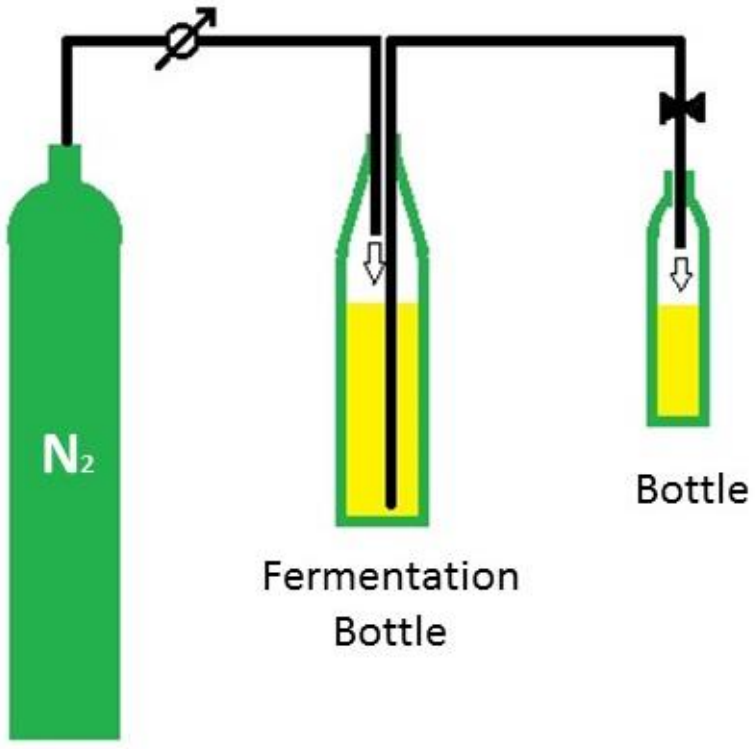


Figure 2-2: The configuration with N_2 overpressure used for bottling.

All clean bottles were flushed prior to bottling with N_2 and filled with minimum headspace. SO_2 (Kadifit, Erbsloeh, Geisenheim) was added at a concentration of 60 mg/L from a stock solution during bottling with a pipette. After bottling the wines were stored at 4 ˚C until analysis and tasting.

2.3.3 Sensory evaluation preparation

All wines and all replicates were tasted individually by trained testers at the Department of Microbiology and Biochemistry of the Hochschule Geisenheim University. Samples were discussed and a number of descriptors were noticed for each fermentation. The fermentations that were showing strong off-flavours and faulty character were not to be used in the tastings. From the pre-tastings, seven descriptors and 'typicity' were chosen to be used for sensory analysis.

The questionnaires for the tastings were prepared in the Department of Oenology of the Hochschule Geisenheim University with the programme FIZZ (Biosystemes, Couternon, France). The fermentation replicates were homogenised under nitrogen atmosphere so that oxidation of the delicate aroma compounds was minimised, and bottled in 0.75 L brown glass bottles with screw caps. One bottle was used for the sensory analysis.

2.4 Greek varieties pre-selection

Identification of varietal thiols in wines from Greek autochthonous varieties has not been previously reported by the literature. From discussion with Greek wine professionals and wine tastings during exhibitions (e.g. ProWein), a list of varieties could be eluted for further studies. The following criteria were used for this pre-selection:

- Description of wines for autochthonous Greek varieties were studied and comments like 'grapefruit', 'exotic notes', 'passionfruit' etc. were used as hints.
- Interviewing Greek wine professionals (e.g. Anestis Haitidis) with specific questions about aromatic profiles of Greek autochthonous grape varieties.
- These varieties should be readily available and if possible spread over Greece so that a number of wines could be collected and different areas could be studied.

After evaluating all the reports and the tasting notes a small list was created of autochthonous Greek varieties that would be used in this second test. These grape varieties were the following:

- Asyrtiko
- Malagousia
- Roditis

A total of 17 commercial wines were collected from the vintages 2008 & 2009 (5 Malagousia, 5 Asyrtiko, 4 Roditis, 1 blend Asyrtiko/Malagousia & 1 Sauvignon blanc from Greece as a benchmark wine) and 1 Malagousia from the vintage 2006 for evaluating the effect of ageing on the aroma of this variety (**Table 2-7**). The wines were tasted by a trained panel at the Department of Enology of the Hochschule Geisenheim University (**Chapter 2.6**) and analysed for their major components as well as for their aromatic compounds (varietal thiols, low boiling sulphur compounds and high boiling sulphur compounds).

Table 2-7: List of the wines used for the study.

Code	Producer	Variety	Vintage	Appelation
MA1	Lykos	Malagousia	2009	Ritsona
MA2	Claudia Papayanni	Malagousia	2009	Arnaia
MA3	Sokkos	Malagousia	2009	Attica
MA4	Gerovasiliou	Malagousia	2009	Epanomi
MA5	Porto Karras	Malagousia	2009	Sithonia
MA6	Gerovasiliou	Malagousia	2006	Epanomi
A1	Lazaridis	Asyrtiko	2009	Drama
A2	Tsantalis	Asyrtiko	2009	Santorini
A3	Sigalas	Asyrtiko	2009	Santorini
A4	Gaia	Asyrtiko	2009	Santorini
A5	Boutaris	Asyrtiko	2009	Santorini
R1	Palyvos	Roditis	2009	Nemea
R2	Monemvasia	Roditis	2009	Monemvasia
R3	Brintzikis	Roditis	2009	Pisatis
R4	Merkouri	Roditis	2008	Ilia
AM1	Gerovasiliou	Asyrtiko/Malagousia	2009	Epanomi
SB1	Evabelos Ghi	Sauvignon blanc	2008	Domokos

2.5 Malagousia & Petite Arvine analysis & sensorial experiment

After evaluation of the results of the Greek variety pre-selection process, it was decided to work only on the Greek variety Malagousia, which showed the highest content in varietal thiols (**Chapter 3.3.5**) and aromatic complexity. The Swiss grape variety Petite Arvine was also selected as its aromatics have been studied by Fretz *et al.* (2005a; 2005b). The wines from Petite Arvine were used mainly for validating the results for Malagousia but also for studying its aromatics.

The wines selected were all monovarietal and in both cases (Malagousia & Petite Arvine) all from the vintage 2013. All the wines were selected to cover the whole geographical area that they are planted For Malagousia this would be whole continental Greece (map in **Appendix 6-29**) and for Petite Arvine the whole Canton of Valais. A total of 18 Malagousia (**Table 2-8**) and 15 Petite Arvine wines (**Table 2-9**) were collected. Three bottles of each wine were purchased from specialised shops or were kindly donated by the producers.

2.6 Sensory evaluation

All sensory evaluation was conducted in the tasting room of Department of Oenology at the Hochschule Geisenheim Univesity.

2.6.1 Sensory panel

Sensory evaluation of all wines was carried out by a trained panel of wine professionals at the Hochschule Geisenheim University who participated in the study. All of them had an extensive experience in wine tastings and especially in descriptive wine tasting.

2.6.2 Tasting conditions

The tasting was conducted at the tasting room of the Department of Enology, Modeling & Systems Analysis of the Hochschule Geisenheim University. The temperature in the room (around 20 ˚C) and the lighting conditions were regulated. Each session lasted one hour with a maximum of 10 wines being tasted at each session.

2.6.3 Training of the panel

Prior to tasting a training for the key descriptors asked was carried out. An aromatilcally neutral wine was chosen from the Department of Grapevine Breeding and was spiked with aroma substances that corresponded to the aroma descriptors. For each of the substances one wine was spiked. The concentrations added in the training wines were adjusted to correspond with the concentrations found in normal wines. Varietal thiols were specially trained with various concentrations in 4MSP, 3SHA and 3SH that can be found in wines. During training the intensity of the descriptors was discussed with the tasters in order to have consistent results in the tastings. Furthermore, a commercial Sauvignon blanc wine was used a benchmark wine after the training (i.e., as a warm-up wine) as this variety is the reference when concerning varietal thiols since it was the first variety that these compounds were discovered (Tominaga *et al.*, 1996; 1997; 1998a).

Table 2-8: List of the 2013 Malagousia wines used in the study.

Code	Producer	Appellation	Remarks
M1	Alfa Estate	Florina	“Turtles” Vineyards
M2	Simeonidis	Pageo	-
M3	Fragou	Rafina	-
M4	Panagiotopoulos	Pyrgos Tryffilias	Organic
M5	Gerovassileiou	Epanomi	Single vineyard
M6	Celini	Pieria	-
M7	Lykos	Evia & Voitia	-
M8	Aslanis	Macedonia	-
M9	Antonopoulos	Patras	-
M10	Papagiannakou	Markopoulo	-
M11	Porto Carras	Sithonia	-
M12	Garypidis	Pydna, Pieria	Organic
M13	Papaioannou	Korinth	Organic
M14	Claudia Papayanni	Arnaia, Chalkidiki	-
M15	Ktima Roxani Matsa	Pallini	-
M16	Ampeloeis	Pageo	-
M17	Zafeirakis	Tyrnavos	-
M18	Texni Oinou	Drama	-

Table 2-9: List of the 2013 Petite Arvine wines used in the study.

Code	Producer	Appellation	Remarks
PA1	Hurlevent	AOC Valais	-
PA2	Bonvin	AOC Valais	-
PA3	Robert Gilliard	AOC Valais	-
PA4	Gerald Besse	AOC Martigny	-
PA5	Albert Mathier	AOC Salgesch	-
PA6	Caves Orsat	AOC Valais	-
PA7	Joseph Gattlen	AOC Valais	-
PA8	Nouveau Salquenen	AOC Molignon	-
PA9	Cave Fin Bec	AOC Valais	-
PA10	Provins	AOC Valais Fully	-
PA11	Provins	AOC Valais	-
PA12	Provins	AOC Valais	-
PA13	Robert Gilliard	AOC Valais	-
PA14	Robert Gilliard	AOC Valais	-
PA15	Provins	AOC Valais	-

2.7 Analytical methods

Apart from the development and validation of the method for measuring the varietal thiols a number of other analytical methods were employed for studying the wines.

2.7.1 Spectrophotometric analysis

Amino nitrogen: The primary amino nitrogen in the musts was determined with the NOPA ('Nitrogen by OPA') procedure (Dukes & Butzke, 1998). This assay is based on the derivatisation of the primary amino groups with an o-phthaldialdehyde/N-acetyl-L-cysteine reagent. The derivatives are produced quite rapidly and are stably absorbing at 335 nm. The results are expressed in mg/L of isoleucine equivalents. The analysis was performed at the Department of General and Organic Viticulture of the Hochschule Geisenheim University by staff members.

Total phenols: Total phenols for the commercial wines from the varieties Malagousia and Petite Arvine (all vintage 2013) were deterimined with the Folin method (Singleton *et al.*, 1999) at the Department of Wine Chemistry and Beverage Research at the Hochschule Geisenheim University by staff members.

2.7.2 HPLC

The analysis was performed at the Department of Microbiology and Biochemistry of the Hochschule Geisenheim University by staff members. For the analysis of the major organic acids, glucose, fructose and ethanol high performance liquid chromatography (HPLC) was employed, with a method modified from Schneider *et al.* (1987). An Agilent Series 1100 HLPC, equipped with a binary pump, autosampler, a Multi-Wavelength Detector (MWD) and a Refractive Index (RI) Detector (Agilent Technologies, Steinheim, Germany) was used. The MWD was set at a wavelength of 210 nm for the detection of organic acids and the RI was used for the detection of carbohydrates, organic acids and ethanol (**Table 2-10**).

Table 2-10: The list of detected compounds and the units.

Compound name	Unit
Tartaric acid	g/L
Malic acid	g/L
Shikimic acid	mg/L
Lactic acid	g/L
Acetic acid	g/L
Citric acid	g/L
Glucose	g/L
Fructose	g/L
Ethanol	g/L (Conversion to % Vol.)

As chromatographic separation column an Allure Organic Acids (300 mm x 4.6 mm, 5 µm particle size) was employed (Restek, Bad Homburg, Germany) with a C-18 4x3 mm as a guard column (Phenomenex, Aschaffenburg, Germany). The column temperature was set at 29 ℃ and the mobile phase was 0.0139% sulphuric acid and 0.5% ethanol diluted in ultrapure water at a flow rate of

0.6 mL/min. The samples were centrifuged for 10 min at 13000 rpm and diluted 1:1 with ultrapure water before injecting 5 µL in the HPLC.

2.7.3 FTIR

FTIR spectroscopy was employed parallel to the HPLC for the determination of the major organic acids, reducing sugars. Furthermore, the determination of glycerol, volatile acidity, pH and total acidity was also done with FTIR. The analysis was performed according to Baumgartner *et al.* (2001) and Patz *et al*. (1999) at the Department of Wine Chemistry and Beverage Research at the Hochschule Geisenheim University by staff members.

2.7.4 Analysis of esters, higher alcohols, fatty acids and monoterpenes

The analysis of the major volatile components was carried out with a modified method from Rapp *et al.* (1994) at the Department of Microbiology and Biochemistry of the Hochschule Geisenheim University by staff members.

Table 2-11: The list of compounds detected with the 'Kaltron' method.

Compound	Unit
Acetic acid ethylester	mg/L
i-Butanol	mg/L
Propionic acid ethylester	µg/L
3-Methyl-butanol	mg/L
2-Methyl-butanol	mg/L
i-Butyric acid ethylester	µg/L
Butyric acid ethylester	µg/L
Lactic acid ethylester	mg/L
Hexanol	µg/L
Acetic acid 3-methylbutylester	µg/L
Acetic acid 2-methylbutylester	µg/L
Caproic acid	mg/L
Caproic acid ethylester	µg/L
Acetic acid hexylester	µg/L
***trans*-Linalool oxide**	µg/L
***cis*-Linalool oxide**	µg/L
Linalool	µg/L
2-Phenyl-Ethanol	mg/L
Caprylic acid	mg/L
Succinic acid diethylester	µg/L
Caprylic acid ethylester	µg/L
α-Terpineol	µg/L
Benzeneacetic acid ethylester	µg/L
Acetic acid phenylethylester	µg/L
Capric acid	mg/L
Capric acid ethylester	µg/L

Sample preparation: 10 mL of the sample were transferred to a shaking tube. The two internal standards (5 µL 2,6-dimethyl-5-hepten-2-ol (1188 µg/L) and 5 µL of isopropylbenzene (112 µg/L)) and 2 g of NaCl were added. 100 µL of 1,1,2-trifluorotrichloroethane 'Kaltron' (Freon 113) were added as an extraction reagent and the tubes were shaken for 20 min with a mixer (Intelli Mixer, Neolab, Heidelberg, Germany). The content of the tube was centrifuged at 3000 rpm for 8 min. The supernatant was passed through a Na_2SO_4 cartridge for drying and the extract was then injected into the GC-MS system.

Chromatographic analysis: The extracts were analysed by gas chromatography coupled with mass spectrometry. The unit consisted of an HP 5890 Series II GC oven (Hewlett Packard, Steinheim, Germany) fitted to an HP 5972 MSD (working on EI 70eV and scan mode (range 35-250 amu). The GC oven was equipped with an MPS2 Autosampler and a CIS3 (Cooled Injection System) both from Gerstel (Mühlheim a. d. Ruhr, Germany). The column was a VF-5MS, 60 m x 0.32 mm x 1 µm (Varian, Steinheim, Germany) and helium as a carrier gas at a flow of 1 mL/min. 2 µL of the extract were injected in splitless mode (splitless time 1 min). The injector had the following injection programm: initial 30 ˚C, raised to 230˚C at 12 ˚C/s and hold time of 4min. The oven programm was set at initial temperature 40 ˚C for 5 min, raised to 125 ˚C at 3 ˚C/min and, finally, to 200 ˚C at 6˚C/m with a hold time of 14.2 min.

2.7.5 Analysis of low boiling point volatile sulphur off-flavors

The analysis was performed at the Department of Microbiology and Biochemistry of the Hochschule Geisenheim University by staff members. Low boiling point volatile sulphur off-flavours (**Table 2-12**) were analysed using HS-GC-PFPD (Head Space – Gas Chromatography – Pulsed Flame Photometric Detection) with a method developed by Rauhut *et al.* (2005).

Table 2-12: The list of compounds detected with the low boiling point sulphur compounds method.

Compound	Abbreviation	Unit
Hydrogen sulphide	H_2S	µg/L
Methanethiol	MeSH	µg/L
Ethanethiol	EtSH	µg/L
Carbon disulphide	CS_2	µg/L
Dimethyl sulphide	DMS	µg/L
Thioaceticacid-S-methyl ester	MeSAc	µg/L
Thioaceticacid-S-ethyl ester	EtSAc	µg/L
Dimethyl disulphide	DMDS	µg/L
Diethyl disulphide	DEDS	µg/L
Dimethyl trisulphide	DMTS	µg/L

Sample preparation: The wine samples were stored at 4 ˚C prior to analysis. The headspace sample vials were filled with 1.7 g NaCl, flushed with a stream of argon gas and sealed with parafilm (Bemis NA, Neenah, USA). 5 mL of the wine to be analysed were transferred to the vial and 5 µL 2,6-di-tert-butyl-4-methyl-phenol (4 mg/L) as an antioxidant, 20 µL ethylenediamine tetraacetic acid (0.2 g/L), 10 µL propanal (500 mg/L) as SO_2-binding compounds and finally 10 µL of internal standard

solution containing 2 standards (6 µg/L methyl iso-propyl sulfide and 6 µg/L butylmethyl suphide) were added. Care was taken at all time to minimise oxygen ingress into the vial. The samples were directly analysed with gas chromatography coupled with PFPD detection.

Chromatographic analysis: The unit consisted of an Agilent 6890N GC oven (Agilent, Steinheim, Germany) and fitted with an an OI5380 PFPD (Pulsed Flame Photometric Detector) working on sulphur mode (OI Analytical, Birmingham, Alabama, USA) at 250 ˚C. The GC oven was equipped with an MPS2 Autosampler and a CIS4 (Cooled Injection System) both from Gerstel (Mühlheim a. d. Ruhr, Germany). The column was an SPB-1 Sulphur, 30m x 0.32m x 1µm (Supelco, Munich, Germany) with helium as a carrier gas at a constant flow of 1.1 mL/min. The samples were kept in the MPS-2 at 60 ºC for 45 min under constant agitation and were injected in the GC with a 1000 µL headspace syringe at a split ratio of 10:1. The injector had the following injector program: initial -100 ˚C, raised to 180 ˚C at 12 ˚C/s and hold time of 8 min. The oven program was set at initial temperature of 29 ˚C for 7 min, raised to 180 ˚C at 10 ˚C/min with a hold time of 10.5 min.

2.7.6 Analysis of terpenic compounds

The terpenes were analysed with the use of SBSE (Stir Bar Sorptive Extraction) and GC-MS based on the method published by Günata *et al*. (1985), Kotseridis *et al.* (1999), modified by Schüttler (2012) and Fritsch *et al.* (2014). The analysis was performed at the Department of Microbiology and Biochemistry of the Hochschule Geisenheim University by staff members.

Table 2-13: The list of compounds detected with the terpenic compounds method.

Compound	Unit
1,8-Cineole	µg/L
***trans*-Linalool oxide**	µg/L
Nerol oxide	µg/L
***cis*-Linalool oxide**	µg/L
Vitispirane	µg/L
Hortrienol	µg/L
α-Terpineol	µg/L
TDN	µg/L
Myrtenol	µg/L
Nerol	µg/L
β-Damascenone	µg/L
Geraniol	µg/L
β-Ionone	µg/L

2.8 Statistical analysis

For all graphics and statistical analysis Excel 2013 (Microsoft, Redmont, Washington) and the statistical add-on package XLSTAT (Addinsoft, Witzenhausen, Germany) were used. Partial Least Square (PLS) regression, Principal Component Analysis (PCA) and Tukey Comparison test were chosen for the the statistical tests. The Tukey Comparison test was used with a $p = 0.05$. All PCA data were prior to analysis mean centered and autoscaled. For the aroma compounds the Odour Activity

Values (OAV) were used that allow to estimate the contributions to the final aroma of a wine (Gómez-Míguez *et al.*, 2007). The OAVs were calculated with the odour thresholds in **Appendix 6-28**.

3. Results and discussion

The following chapters give an overview on the conducted experiments and the obtained results. The experimental outline for all work packages can be found in **Chapter 2.1**. Firstly the method validation for the analysis of varietal thiols is presented in **Chapter 3.1**. Secondly the fermentation experiment with musts from the varieties Sauvignon blanc and Scheurebe from the vintages 2008 & 2009 are presented in **Chapter 3.2**. The pre-selection of wines from Greek varieties is reported in **Chapter 3.3**. Finally, the results from the analysed wines from the Greek variety Malagousia and the Swiss variety Petite Arvine are presented in **Chapter 3.4**. Because of the different nature of each package (method development, fermentation experiments, selection of varieties and sensory experiment with the Greek and Swiss grape variety) it was decided to present the discussion after each package and not at the end of all the results. This was also done for accessing and reviewing the results more easily. A summary of the results for all experiments is given in **Chapter 6 Appendix**.

3.1 Validation of varietal thiols method

The improved method for measuring varietal thiols (**Chapter 2.2.3**) was validated according to DIN (32645 & 38402 A51) and ISO 5725 (Wellmitz & Gluschke, 2005; Kromidas, 2011). The different aspects of the method that were validated are shown with their results in the following chapters.

3.1.1 Repeatability/Reproducibility

Method- and instrument repeatability were measured with the following procedure:

Three concentrations of standard solutions diluted in dichloromethane were directly injected into the GC-MS six times and the relative standard deviation was calculated. The concentrations chosen were within the concentrations that would be found in wine extracts. The results of instrument repeatability are shown in **Table 3-1**.

Table 3-1: Concentrations and relative standard deviations of instrument repeatability. Concentrations of the measured thiol standards are in ng/µL.

Level	4MSP		3SHA		3SH	
	Conc.	%RSD	Conc.	%RSD	Conc.	%RSD
1	0.773	6.83	0.867	3.61	0.771	6.71
2	0.387	3.62	0.433	1.34	0.386	3.38
3	0.193	7.26	0.217	4.69	0.193	9.52

For the method reproducibility an aromatically neutral wine was spiked with three concentration levels, extracted and injected into the GC-MS. Each level was prepared six times. The results of method reproducibility are shown in **Table 3-2**.

Table 3-2: %RSD for three concentration levels and average method reproducibility. Concentrations of the measured thiol standards in ng/µL.

Level	4MSP		3SHA		3SH	
	Conc.	%RSD	Conc.	%RSD	Conc.	%RSD
1	0.773	2.54	0.867	4.78	0.771	3.21
2	0.387	2.78	0.433	3.98	0.386	7.13
3	0.193	5.94	0.217	6.53	0.193	4.29
Total	-	3.53	-	6.53	-	3.89

When comparing the %RSD of the instrument repeatability and the method reproducibility (**Table 3-1** & **3-2**) it can be seen that all were below the accepted value of maximal 10 % RSD (Kromidas, 2011). For the instrument repeatability, it should be noted that because the analysis time was 120 min for each run and the solvent used was dichloromethane, which is highly volatile, it is possible to have deviation between the injections because of evaporation. Furthermore, the standards were not protected from oxidation so there may be a higher %RSD when compared with the method reproducibility.

3.1.2 Calibration & linearity

The calibration range (**Table 3-3**) was chosen according to the concentrations already cited in the literature for different varieties (Tominaga *et al.*, 1996; 1998a; Schneider *et al.*, 2003; Gómez-Míguez *et al.*, 2007; Mateo-Vivaracho *et al.*, 2006; 2007; King *et al.,* 2008; Rodríguez-Bencomo *et al.*, 2009; Dagan *et al.*, 2014; Rigou *et al*, 2014). The standard calibration curve had at least 5 points per curve and 5 replicates per point. For the calibration curves the standard addition method and linear regression were used for the calculations. Standard addition method was used minimize the matrix effect of the different varieties and because for the calibration a standard 'thiol poor' wine (selected by tasting) was used. It should be noted here that it was not possible to extract any thiols using a model wine (water, 10% ethanol, 6 g/L tartaric acid and pH set to 3).

Table 3-3 Linearity, calibration range and the average %RSD of the calibration. The values for the limited extended calibration of 3SH are in parentheses.

	4MSP	3SHA	3SH
Range	0-407 ng/L	21-419 ng/L	0-2210 ng/L (up to 14234 ng/L)
Equation	y=0.0198x-8^-15	y=0,0056x-2^-15	y=0,0031x
R^2	0.9985	0.9913	0.9857 (0.9987)
%RSD	3.53%	6.53%	3.89%

In the whole concentration range studied, the curve of each compound was linear with an average R^2 of at least 0.9857. The method showed a good linearity for all the compounds and was enough to measure the thiol content in most of the wines as already published by the literature without dilution steps (Tominaga *et al.*, 1996; 1998a; Schneider *et al.*, 2003; Gómez-Míguez *et al.*, 2007; Mateo-Vivaracho *et al.*, 2006; 2007; King *et al.,* 2008; Rodríguez-Bencomo *et al.*, 2009; Dagan *et al.*, 2014;

Rigou *et al.*, 2014). The %RSD was 6.53%, which shows good repeatability among other methods that have been published (Mateo-Vivaracho *et al.*, 2007).

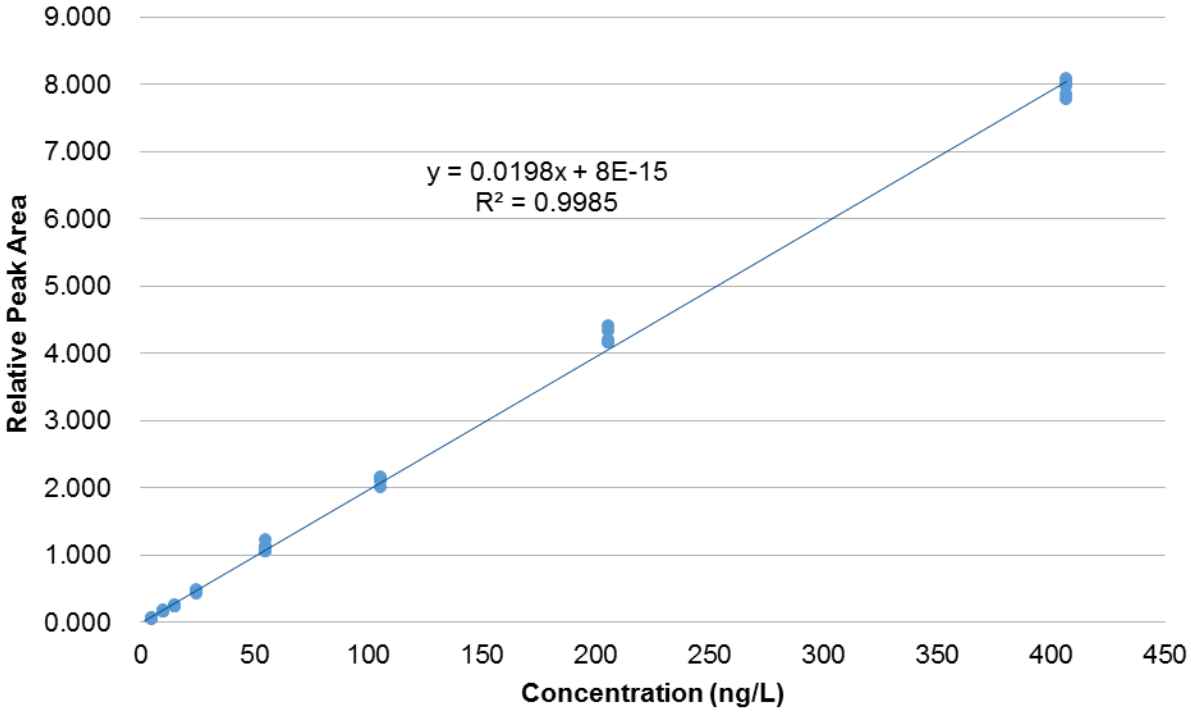


Figure 3-1 Calibration curve of 4-sulfanyl-4-sulfanylpentan-2-one (4MSP). The equation and R^2 of the curve are incorporated in the figure.

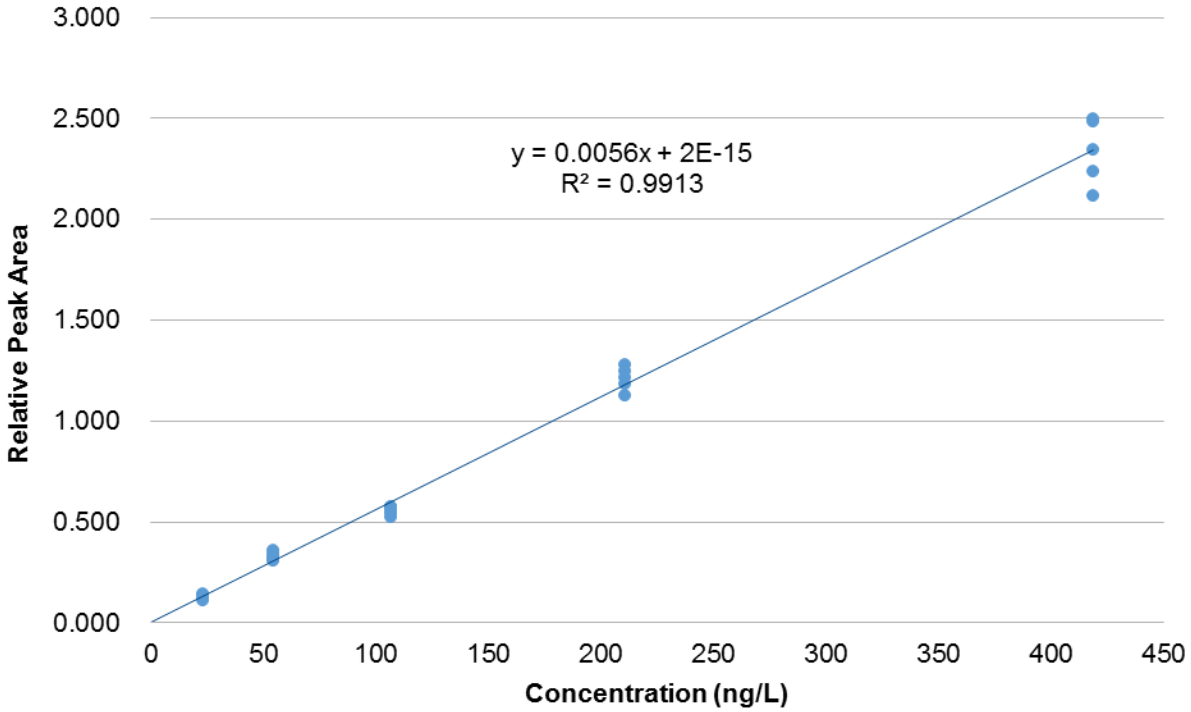


Figure 3-2: Calibration curve of 3-sulfanyl-hexyl-acetate (3SHA). The equation and R^2 of the curve are incorporated in the figure.

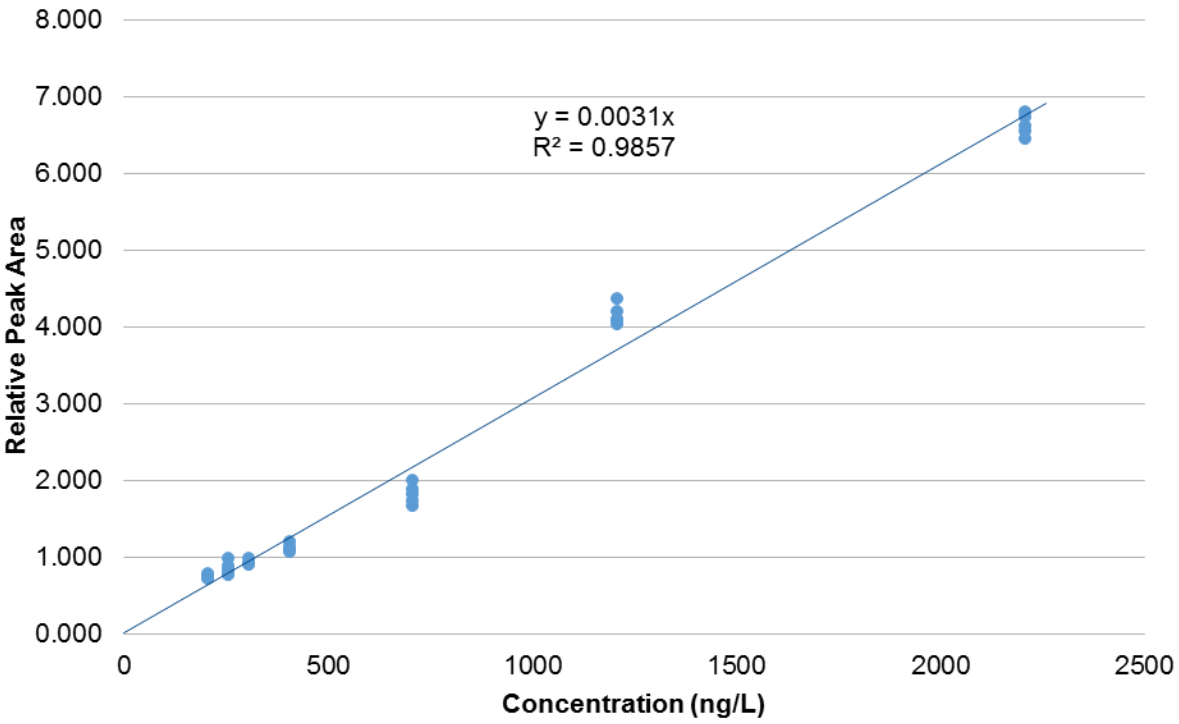


Figure 3-3 Calibration curve of 3-sulfanyl-hexan-1-ol (3SH). The equation and R^2 of the curve are incorporated in the figure.

Because of recent findings by Rigou *et al.* (2014) the calibration curve of 3SH was tested to a limited extend up to 14234 ng/L in order to determine that the linearity of the method was enough at these high concentrations. By plotting the two concentration ranges on the initial curve, it was shown that the linear regression model was plausible with an R^2 of 0.9987. Although this limited calibration showed good results it has not been integrated in the standard curve of 3SH because up to now a few publications have reported such high concentrations (Rigou *et al.*, 2014).

3.1.2.1 Problems during calibration

While extracting simultaneously high concentrations of 3SHA and 3SH, it was observed, that the calibration curve showed a worse R^2. It can be seen in **Figure 3-4** that the concentration level 208 ng/L is below the calibration curve when extracted together with 3SH. When extracted alone the calibrations points of that level were on the calibration curve (**Figure 3-5**). This meant that some of the 3SHA was 'disappearing'. By reviewing the extraction steps, it is probably attributed to the pH of the pHMB solution (pH 10.7) that hydrolyses partially the 3SHA to 3SH. The presence of 3SH in higher concentrations in the sample is probably propagating this hydrolysis. By plotting the results it was shown that the linearity was improved (from 0.9675 to 0.9913). This is the first time that this effect has been reported. 3SHA and 3SH were calibrated separately for improved calibration curves.

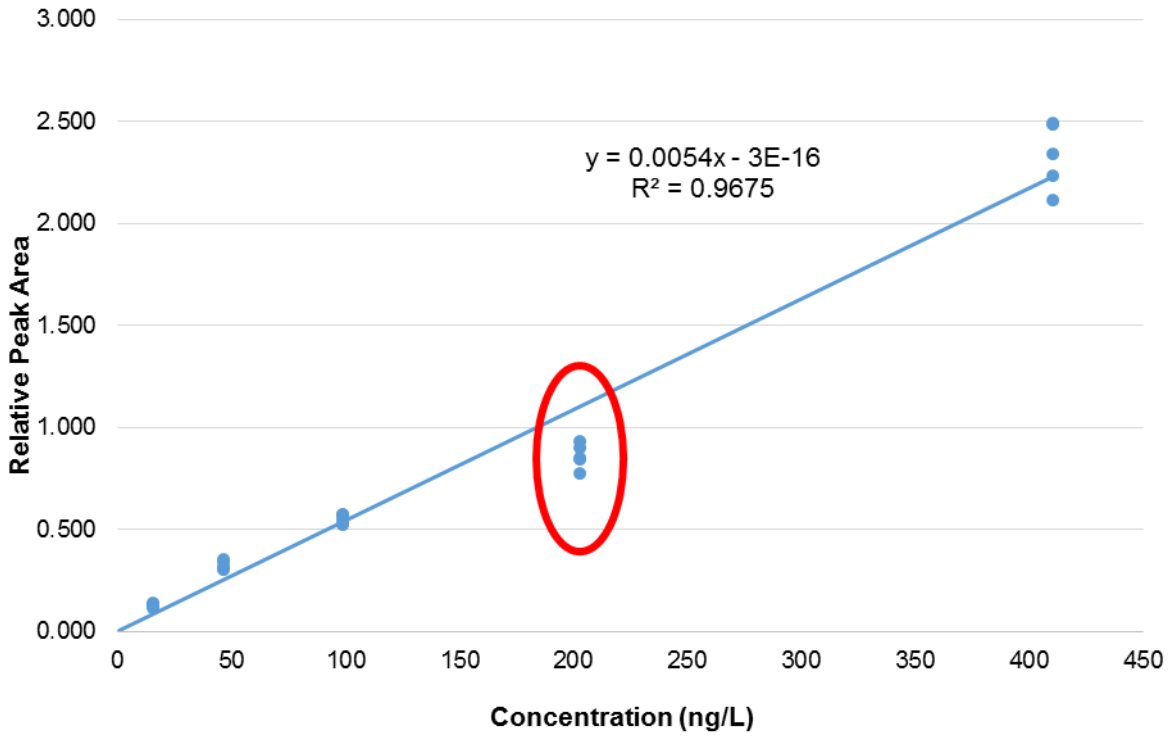


Figure 3-4: Calibration curve of 3SHA with simultaneous extraction of 3SH at the level 208 ng/L (red circle).

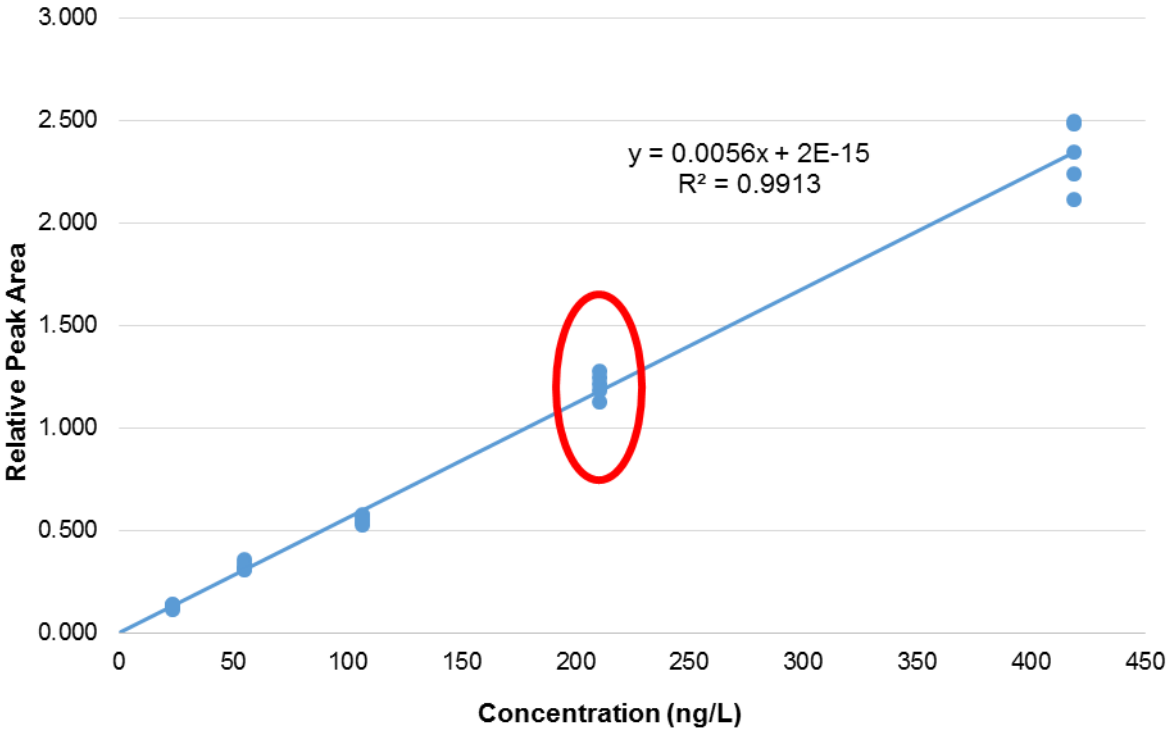


Figure 3-5: Calibration curve of 3SHA with the level 208 ng/L being calibrated separately (red circle).

3.1.3 Level of detection/Level of quantification

Both Level of Detection and Level of Quantification were determined according to DIN (Wellmitz & Gluschke, 2005):

$$LOD = Y_B + 3*S_B$$

$$LOQ = Y_B + 9*S_B$$

Y_B = Mean value of blind signal, S_B = Standard deviation of blind signal

Table 3-4 All LOD/LOQ limits are in ng/L

	4MSP	3SHA	3SH
LOD	1	27	54
LOQ	3	39	104

The method showed excellent LOD and LOQ for 4MSP (**Table 3-4**) with the later at around the odour threshold. The odour threshold of 4MSP was found to be 0.8 ng/L in model medium (Tominaga *et al.*, 2000) and 3 ng/L in wine (Darriet *et al.*, 1995).

In the case of 3SHA the LOD and LOQ are significantly above (**Table 3-4**) the odour threshold of 4.2 ng/L (Dubourdieu & Tominaga, 2009). This limitation of the method needs to be improved in the future in order to lower the values for both parameters. Nonetheless it has to be consider that 3SHA plays a role in the aroma of young wines and has the tendency to hydrolyse quickly during ageing (Dubourdieu & Tominaga, 2009). Furthermore, most of the published studies have shown that usually concentrations in wine are well above the calculated LOD_{3SHA} of 27 ng/L (Gómez-Míguez *et al.*, 2007; Mateo-Vivaracho *et al.*, 2006; 2007; King *et al.,* 2008; Rodríguez-Bencomo *et al.*, 2009). 3SHA showed an average %RSD of 6.53% for the standard calibration (**Table 3-3**), which is improved when compared with the published data (Mateo-Vivaracho *et al.*, 2007).

For 3SH the LOD is marginally below the odour threshold (**Table 3-4**) of 60 ng/L (Tominaga *et al.*, 1998). The LOQ is marginally above odour threshold but beacuse many varieties show concentrations of 3SH in amounts of a few hundred ng/L up to a few µg/L this should not be a great limitation.

3.1.4 Recovery

Recovery was measured by spiking a wine with three levels of thiol concentrations. The spiking concentrations for 4MSP were 101, 50 and 20 ng/L, for 3SHA were 208, 104 and 52 ng/L and for 3SH 1002, 501 and 200 ng/L. All the determinations were carried out in triplicate (**Table 3-5**).

Table 3-5 Summary of the recovery results for three calibration levels and average recovery.

Level	4MSP	3SHA	3SH
1	101.3%	102.6%	113.1%
2	102.5%	93.6%	83.3%
3	94.7%	106.9%	83.1%
Average	99.5%	101.1%	93.2%

The recovery for all the levels and the average mean recovery is comparable the published values (Mateo-Vivaracho *et al.,* 2006; Ferreira *et al.,* 2007). For 4MSP it is the highest reported recovery when compared with the only published findings from Ferreira *et al.* (2007).

3.1.5 Correctness

The correctness of the method was studied by spiking wines from two different varieties. The grape varieties used were Weißer Riesling (calibration wine) and Sauvignon gris. The first variety was perceived by tasting as 'thiol poor' and the second, being a Sauvignon clone (Robinson *et al.*, 2012), would be expected to have higher thiol content. Both wines were measured twice without the addition of thiols and then twice spiked with 20 ng/L 4MSP, 104 ng/L 3SHA and 500 ng/L 3SH.

Table 3-6: The deviation of the measured values (Measured value of the spiked wine versus the value of the non-spiked wine) in percent.

Compound	Calibration Wine	Sauvignon gris
4MSP	15.0%	20.0%
3SHA	-8.7%	-7.7%
3SH	10.4%	-13.3%

A significant deviation for 4MSP can be observed from **Table 3-6** though it would be difficult to achieve better values as the concentrations of 4MSP are very low. The values for the other compounds are between -13.3% and +10.4%, which given the complexity of the method and the difficulty for extracting and detecting the analytes at such low concentrations would be considered as normal. These values are comparable the published data by Ferreira *et al.* (2007) that reports a %RSD of 15%.

3.1.6 Robustness

Several factors affecting the robustness of the method were studied for evaluating their influence on the recovery of the varietal thiols during extraction.

As the pH value of wines range from 2.80 to 4.00 (Ribéreau-Gayon *et al.*, 1999), it was important to study how this affects the recovery of the varietal thiols. The initial pH of the calibration wine was 2.90. The pH of the calibration wine was adjusted to 3.10 and 3.30 as it should cover the pH range for most white wines. pH 7.00 was studied as an extreme case for other possible beverages of interest. All the deteriminations were carried out in triplicate (**Table 3-7**).

Table 3-7: Recovery for the variation in the pH of the sample. The mean recovery of the three triplicates is shown.

Variant	4MSP	3SHA	3SH
pH 3.10	97.2%	95.9%	96.2%
pH 3.30	102.5%	93.6%	83.3%
pH 7.00	101.3%	63.4%	75.5%

From **Table 3-7** it can be seen that the recovery of 4MSP is unaffected by a change in the pH. Between pH 2.90 and 3.10, the recovery of 3SHA is above 93.6 % but with an increase of the pH to 7.00 it decreases to 63.4 %. This could have an effect when extracting thiols from another matrix that

has a higher pH. 3SH seems to be more easily affected as the increase of pH to 3.30, decreases the recovery to 83.3%. At pH 7 the decrease is even more significant.

The composition of the solvent for eluting the varietal thiols from the SPE cartridges was tested. Instead of the 99% DCM and 1% MeOH mixture the elution was carried out with 100% DCM. Furthermore, the percolation speed was varied from the standard maximum 5 mL/min. Speeds of 2 mL/min and 8 mL/min were tested (**Table 3-8**). All the deteriminations were carried out in triplicate.

Table 3-8: Recovery for the variation of the extraction solvent and the speed of percolation. The average recovery of the three analyses is shown.

Variant	4MSP	3SHA	3SH
DCM only	92.4%	70.1%	91.2%
2 mL/min	95.9%	110.4%	97.0%
8 mL/min	103.0%	97.3%	75.9%

Similar to the pH variation (**Table 3-7**), 4MSP was almost unaffected by either the change of solvent nor by the percolation speed. 3SHA was affected only with the use of 100 % DCM but not from the speed of percolation. A similar decrease of the recovery was also reported by Ferreira *et al.* (2007). The speed of percolation seems to be critical for the extraction of 3SH as at 8 mL/min the recovery was as low as 75.9%.

Finally, wines from six different varieties from the Department of Grapevine Breeding of the Hochschule Geisenheim University were extracted and evaluated for matrix effects. In all the cases the chromatograms were clean, but no 3SHA was detected. This was probably attributed to the fact that the wines were from the vintage 2012 (two years old at the time of analysis) and it was shown that 3SHA is present only in young wines (Dubourdieu & Tominaga, 2009).

Figure 3-6: The concentrations of 4MSP measured in six different varieties.

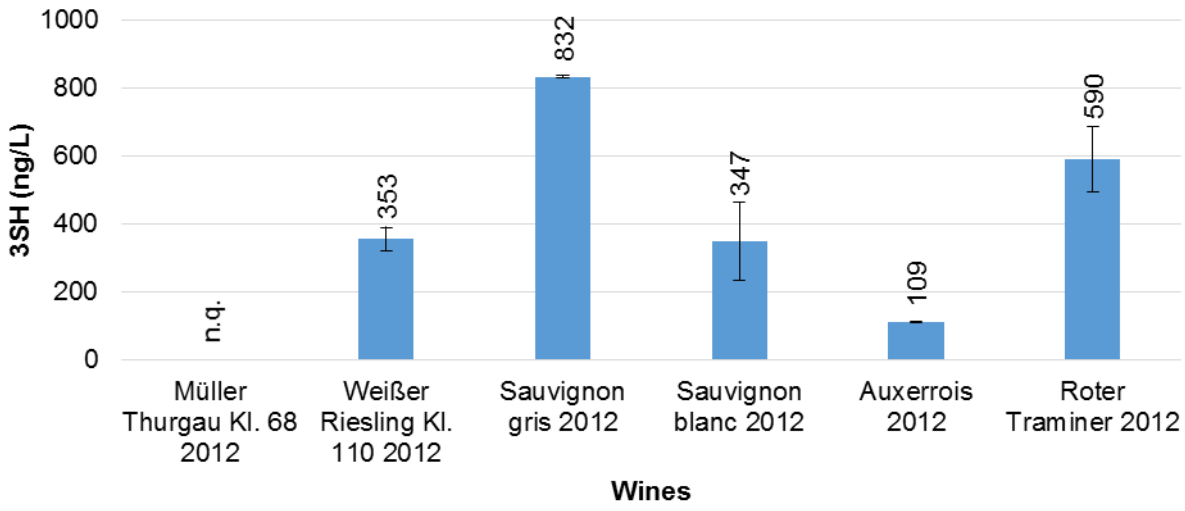


Figure 3-7: The concentrations of 3SH measured in six different varieties.

3.1.7 Discussion and conclusion

The goal of this first work package was to develop and implement a practical method for measuring the varietal thiols in wines, as well as to provide the accompanying validation data. The initial method described in **Chapter 2.2.2**, was developed further and its improved version (**Chapter 2.2.3**) was validated according to DIN (32645 & 38402 A51) and ISO 5725 (Wellmitz & Gluschke, 2005; Kromidas, 2011).

The GC-MS configuration was tested by calculating the instrument repeatability, which was measured to be below 9.52% (**Table 3-1**). The reproducibility of the method was within the accepted values of maximum 10% RSD (Kromidas, 2011); more specificically, it was calculated for 4MSP (3.53%), 3SHA (6.53%) and 3SH (3.89%) (**Table 3-2**).

The calibration range was chosen according to the concentrations published (**Table 3-3**). 3SH was calibrated up to 14234 ng/L, due to recent findings suggesting that such high concentrations are possible (Rigou *et al.*, 2014). Linearity was excellent in the whole concentration range and it was also shown that such high concentrations of 3SH are possible to quantify without the need of sample dilution. It was found that the simultaneous extraction of 3SHA and 3SH at high concentrations showed some irregularities due to interaction of these two compounds. Due to this effect, these compounds were calibrated separately in order to improve linearity (**Chapter 3.1.2.1**).

The method showed excellent LOD/LOQ for 4MSP (1/3 ng/L respectively) that were around the odour threshold of the compound in wine (Darriet *et al.*, 1995). The results for 3SH were also very satisfactory (54/104 ng/L), with the LOQ being marginally above the odour threshold. 3SHA showed high LOD/LOQ when compared with the odour threshold (27/39 ng/L), but the calibration curve above the LOD showed very satisfying results when compared to literature data (Mateo-Vivaracho *et al.*, 2007) (table 3-4).

Recoveries had average values for 4MSP 99.5 %, for 3SHA 101.1 % and for 3SH 93.2 % and were comparable with results published from other researchers (Mateo-Vivaracho *et al.*, 2006; Ferreira *et al.,*2007) (**Table 3-5**).

By spiking two different wines (Weißer Riesling & Sauvingnon gris) with known amounts of varietal thiols, the correctness for the method was evaluated (**Table 3-6**). A maximum deviation of 20 % was calculated for 4MSP in Sauvignon gris. This deviation seems to be high in percentage but in absolute values it would be 4 ng/L and at low concentrations it would be very difficult to obtain better results. For 3SHA and 3SH the values were better (**Table 3-6**) than the published 15 % deviation (Ferreira *et al.*, 2007).

Finally the method robustness was studied by altering various factors like pH of the sample, extraction solvent and speed of percolation (**Table 3-8**). At all the pH ranges which were chosen (3.10, 3.30 & 7.00) and other factors investigated (solvent and percolation speed), 4MSP showed to be almost unaffected, since the lowest recovery was 92.4 %. 3SHA was affected when having high pH values in the sample (63.4 % recovery at pH 7.00). This could be a limiting factor if the extraction method is applied in products that have higher pH values than wine. The extraction solvent was for 3SHA also detrimental as the recovery was as low as 70.1 %. On the other hand the speed of percolation did not affect its recovery as it was not below 97.3 %. 3SH showed to be more vulnerable to changes in the parameters of the extraction method requiring more care in its steps for minimizing mistakes. Both higher pH values and higher percolation speeds can decrease the recovery down to 75.5 %. Only the modification of the extraction solvent showed not to affect the recovery significantly, as this was still 91.2 %.

The aforementioned method was developed as a practical approach for measuring varietal thiols in wines. It offers some significant improvements in comparison to the initial method, which was modified and adapted accordingly (Ferreira *et al.,* 2007). The use of 4MSB as internal standard is more feasible as it has similar properties to the thiols to be analysed. This overcomes also the problem that some methods use SIDA (Stable Isotope Dilution Assay), which means that the standards need to be synthezised as they are not readily available. In this study, the internal standard is an off-the-shelf product supplied by Chemos (Regenstauf, Germany). The use of LVI also helped to cut down analysis time as it was not necessary to concentrate the sample significantly. The LOQ of 4MSP was shown to be excellent near its odour threshold in wine (Darriet *et al.*, 1995), the LOQ of 3SH was marginally higher; since 3SHA was high, more work needs to be done to overcome this difficulty.

Nevertheless there is still potential for method improvement, especially in the extraction of 3SHA as this is the most vulnerable compound (Dubourdieu & Tominaga, 2009), the goal being a lower LOQ. Furthermore, some automation in the part of extraction can be incorporated for decreasing labour and minimizing systematic mistakes. In combination with a decrease in the analysis time in the GC-MS by improving the oven programme or with the use of a shorter column, this could also increase the throughput of the method. One of such improvements would be the sourcing of an agitator that could mimic the hand shaking during the step of extracting the thiols from the aqueous *p*-HMB solution with DCM. This would give a homogenous shaking process and increase the capacity of this step.

Automation would also help during the percolation of the samples through the SPE cartridges. At this step of the process, their number is limited by the capacity of the operator performing the liquid-liquid extraction steps after the release of the thiols from the SPE cartridges. The advantage of such an automation would be the prevention of running the SPE cartridges dry.

3.2 Fermentation experiment 2008 & 2009

Musts from two grape varieties (Scheurebe & Sauvignon blanc) and two vintages were fermented (2008 & 2009) with six commercial yeast strains as described in **Chapter 2.3**.

The fermentations were carried out without the addition of extra nutrients and were only clarified by cooling down of the must after pressing and after the end of the fermentation. Also there was no addition of SO_2 before inoculation with the commercial yeast strains.

3.2.1 Scheurebe

The results from the fermentation experiment considering Scheurebe are presented and discussed in the following chapters.

3.2.1.1 Fermentation kinetics and major components

For both vintages, the fermentation took 13 days to complete (**Figure 3-8** & **3-9**). Fermentations were considered finished when there was no more significant weight loss (<0.1 g/day). After the fermentation the wines were transferred to a temperature controlled cellar at 4 ˚C for clarification.

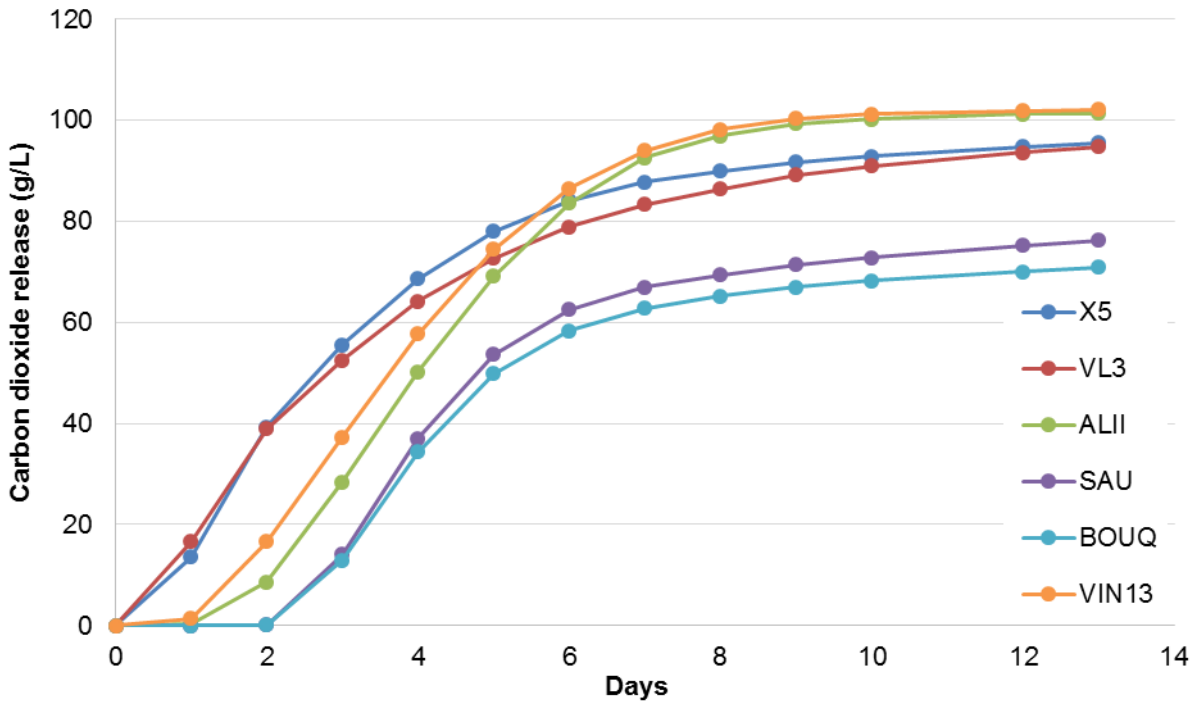


Figure 3-8: Fermentation kinetics of the six Scheurebe variants for the vintage 2008. Every curve represents the average value from the three fermentation replicates.

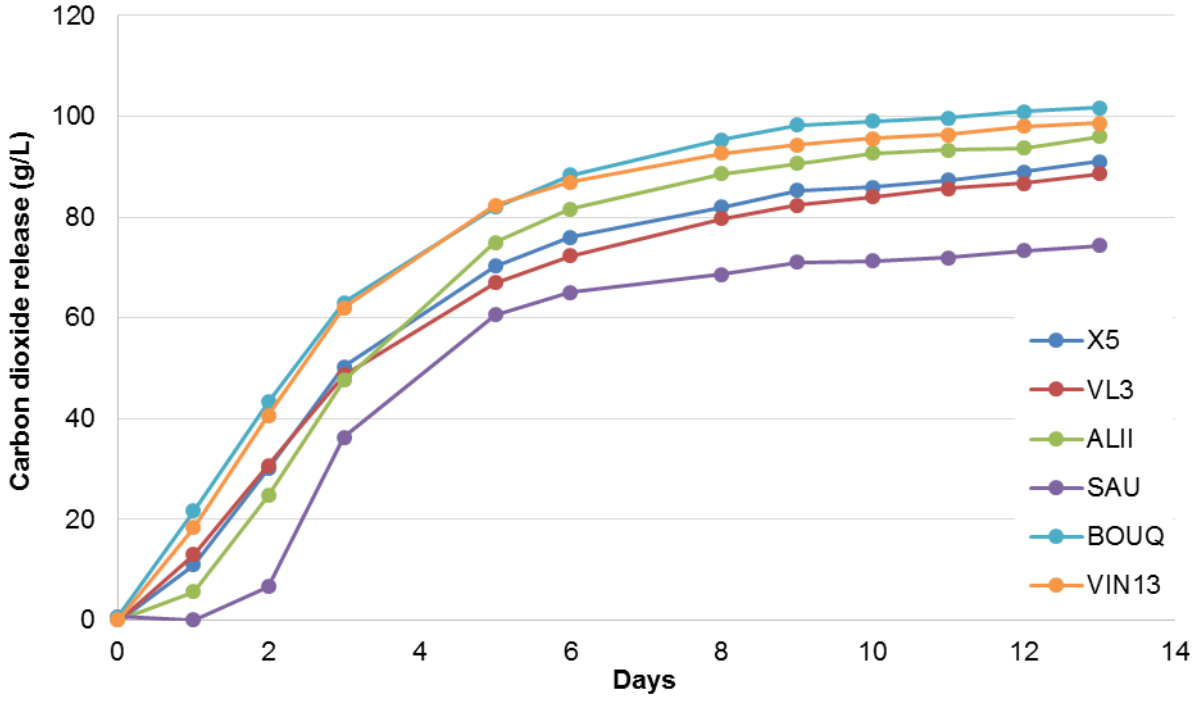


Figure 3-9: Fermentation kinetics of the six Scheurebe variants for the vintage 2009. Every curve represents the average value from the three fermentation replicates.

The fermentation kinetics of both Scheurebe musts indicate that the yeast SAU had a longer lag phase. For 2008, this was also observed for the BOUQ yeast, but the fermentation kinetics of the variant was normal with a very short lag phase for the 2009 Scheurebe must. The longer lag phases had also an effect in the wines as SAU (both for the 2008 and 2009 vintage) and BOUQ (only for the 2008 vintage), which did not complete the fermentations leaving residual sugars. These variants did not release as much total CO_2 as the other fermentations and they had residual sugar values ranging from 25.1 to 45.6 g/L (**Figure 3-11**). The tendency of not completed fermentations is also more evident for the vintage 2009 as all the wines had at least 7.5 g/L of residual sugars. This is probably attributed to the fact that the YAN for 2009 was 133 mg N/L with a sugar concentration of about 220 g/L. Dukes & Butzke (1998) suggested for avoiding a stuck fermentation for this sugar content a YAN of around 200 mg N/L, which was the case for 2009. For the aforementioned wines the alcohol content was also lower when compared with the other variants. Furthermore, a significant difference in the alcohol content of the wines for the vintage 2008 and 2009 could be observed (**Figure 3-10**). Volatile acidity (VA) values are the expected ones for young white wines (**Figure 3-12**) (Ribéreau-Gayon *et al.*, 1999; Dittrich & Grossmann, 2011). A summary of the results for the major components can be found in **Appendix 6-1**.

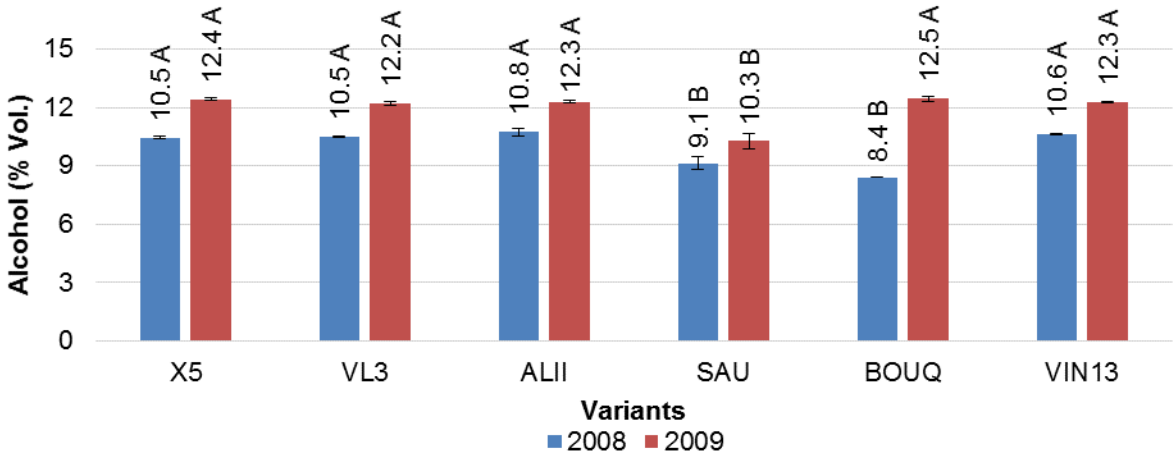


Figure 3-10: Alcohol content of the finished Scheurebe wines from six commercial yeast strains for the vintages 2008 and 2009. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

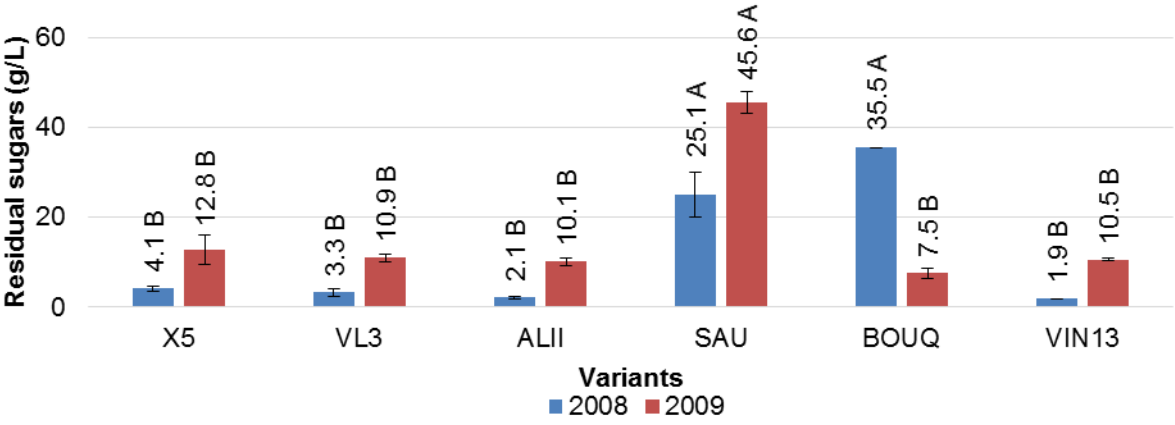


Figure 3-11: Residual sugars concentration of the finished Scheurebe wines from six commercial yeast strains for the vintages 2008 and 2009. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

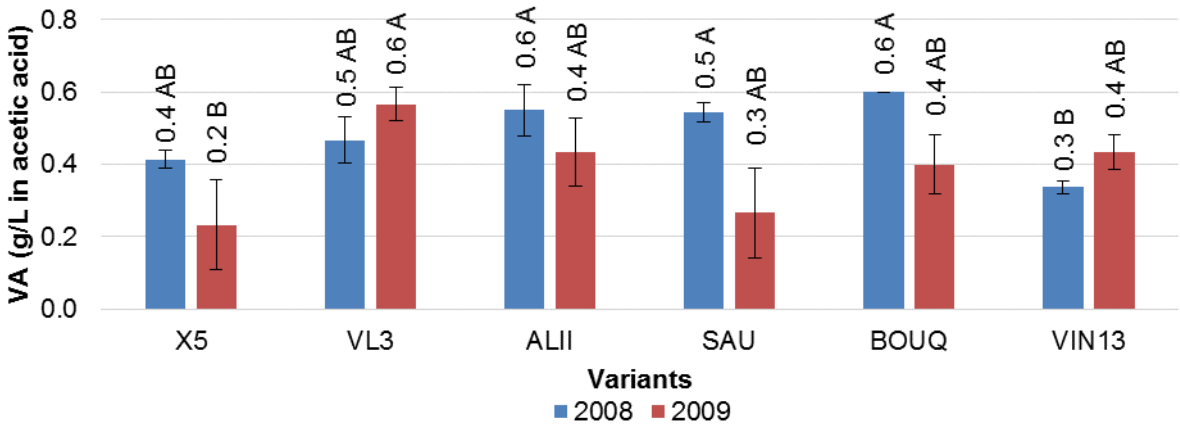


Figure 3-12: Volatile acidity (VA) of the finished Scheurebe wines from six commercial yeast strains for the vintages 2008 and 2009. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

Small amounts of malic acid found in the musts were utilised by the yeast and corresponds to the values published in the literature (**Figure 3-13**) (Ribéreau-Gayon *et al.*, 1999). Low concentrations of lactic acid were also produced (**Figure 3-14**). *Saccharomyces* yeasts are able to produce around 0.2 g/L of lactic acid during alcoholic fermentation (Dittrich & Grossmann, 2011).

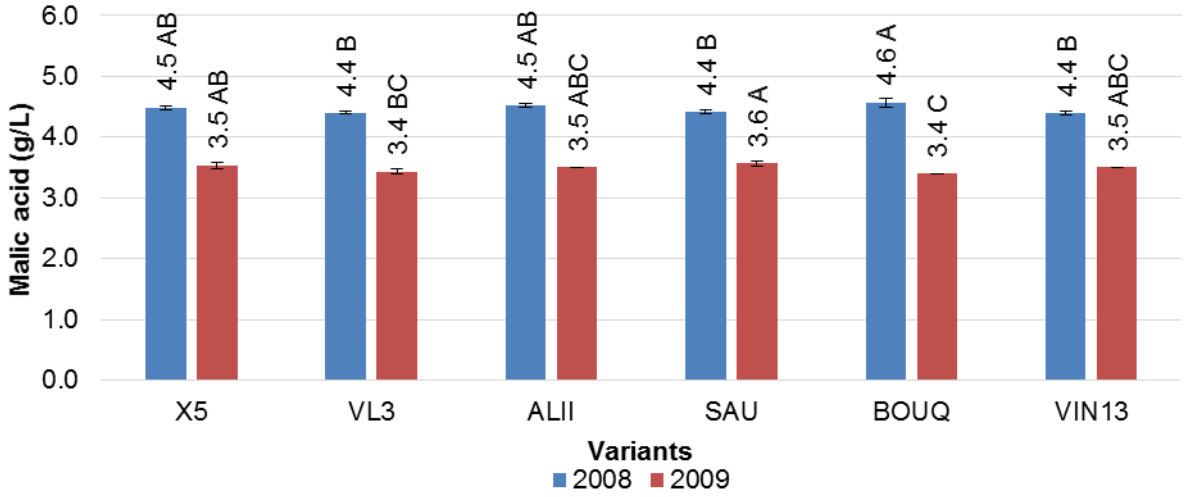


Figure 3-13: Malic acid concentration of the finished Scheurebe wines from six commercial yeast strains for the vintages 2008 and 2009. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

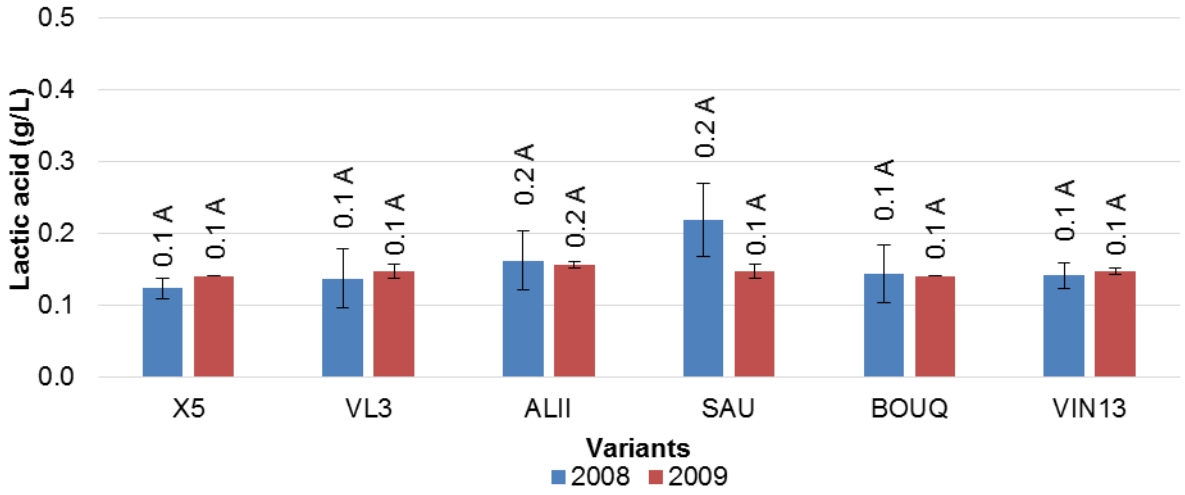


Figure 3-14: Lactic acid concentration of the finished Scheurebe wines from six commercial yeast strains for the vintages 2008 and 2009. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

3.2.1.2 Esters and higher alcohols

A summary of all the measured compounds with the 'Kaltron' method can be found in **Appendix 6-2**.

Esters: Acetic acid ethylester was produced by some yeast in concentrations high enough to play a role in the olfactory perception of the wines (Ribéreau-Gayon *et al.*, 1999). Wines fermented with ALII showed consistent concentrations of acetic acid ethylester for both vintages that were at the same time the highest of all the variants (**Figure 3-15**). The concentrations of acetic acid 3-methylbutylester

were well above the odour threshold value of 30 µg/L (Guth, 1997b) contributing to the fruity aromatics of the wines (**Figure 3-16**). Acetic acid 2-phenylethylester was found in all of the variants marginally below the odour threshold of 250 µg/L (Guth, 1997b) and probably it did not play a role in the aroma of the wines (**Figure 3-17**). Butyric acid ethylester showed variability in the detected amounts (**Figure 3-18**) for the different yeast strains. Yeast SAU produced the highest concentrations, which were about eleven times above the odour threshold (20 µg/L; Ferreira *et al.*, 2000; Fretz *et al.*, 2005a). Both lactic acid ethylester and succinic acid diethylester were found in concentrations significantly below their odour threshold (**Figure 3-19** & **3-20**).

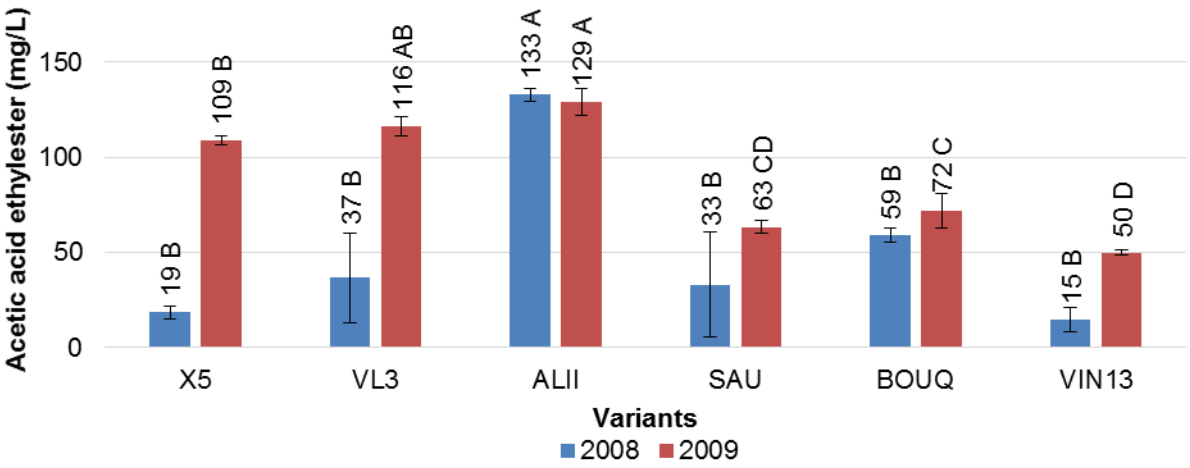


Figure 3-15: Acetic acid ethylester concentration of the finished Scheurebe wines from six commercial yeast strains for the vintages 2008 and 2009. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

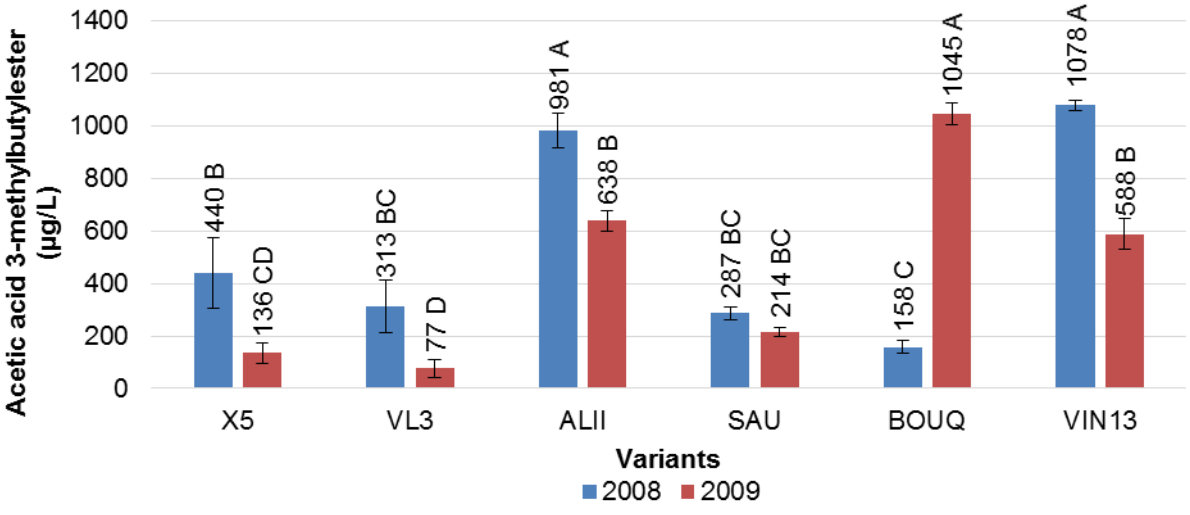


Figure 3-16: Acetic acid 3-methylbutylester concentration of the finished Scheurebe wines from six commercial yeast strains for the vintages 2008 and 2009. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

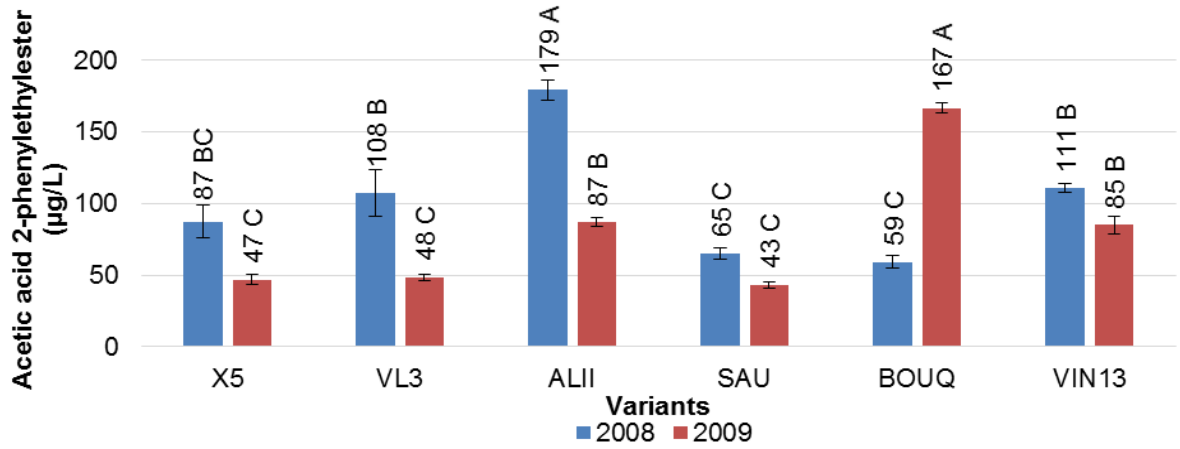


Figure 3-17: Acetic acid 2-phenylethylester concentration of the finished Scheurebe wines from six commercial yeast strains for the vintages 2008 and 2009. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

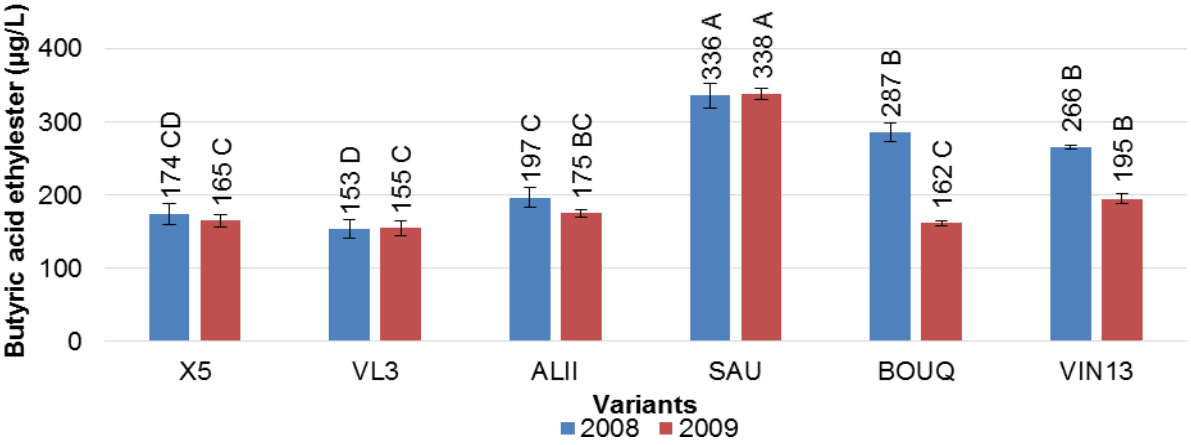


Figure 3-18: Butyric acid ethylester concentration of the finished Scheurebe wines from six commercial yeast strains for the vintages 2008 and 2009. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

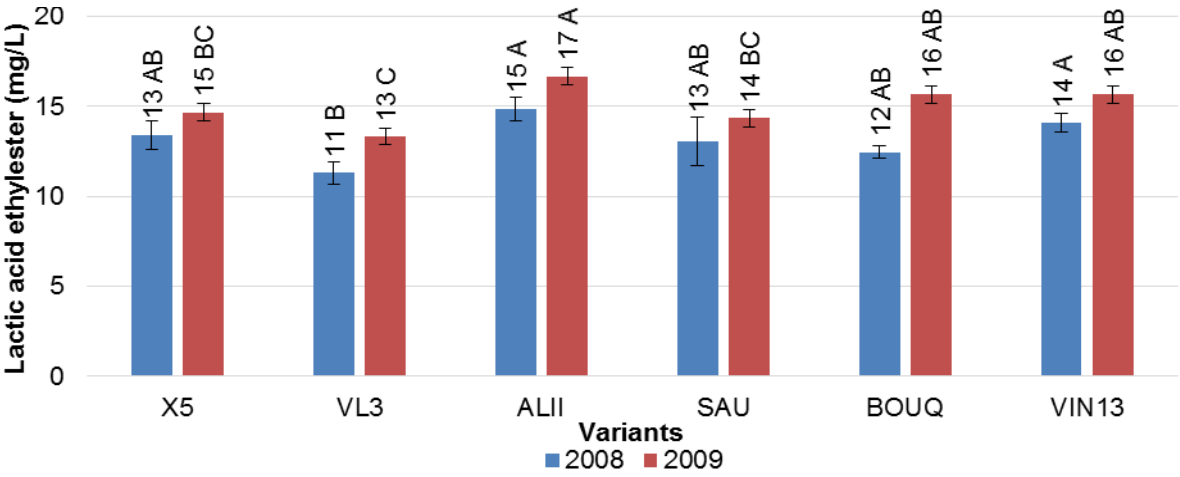


Figure 3-19: Lactic acid ethylester concentration of the finished Scheurebe wines from six commercial yeast strains for the vintages 2008 and 2009. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

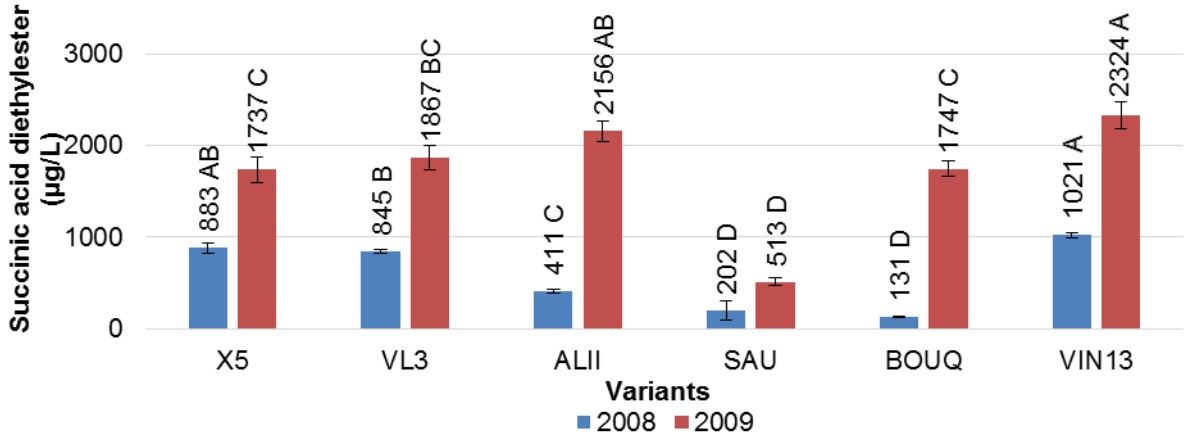


Figure 3-20: Succinic acid diethylester concentration of the finished Scheurebe wines from six commercial yeast strains for the vintages 2008 and 2009. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

Higher Alcohols: Significant concentrations of 3-methyl butanol were found, which were up to five times above the odour threshold (30 mg/L; Guth, 1997b) for variant BOUQ 2009 (**Figure 3-22**). This higher alcohol has a malty odour and contributes a vinous character to the wines (Guth, 1997b). 2-phenylethanol was measured in most of the wines around and marginally above the odour threshold of 14 mg/L (Ferreira *et al.*, 2000). BOUQ 2009 showed a much more significant concentration (**Figure 3-24**). 2-phenylethanol is adding to the 'floral' character of the wine as it has a characteristic rose smell (Ferreira *et al.*, 2000). i-Butanol and hexan-1-ol were found in low concentrations (**Figure 3-21** & **3-23**) and could not play a role in the aroma of the wines (Ferreira *et al.*, 2000).

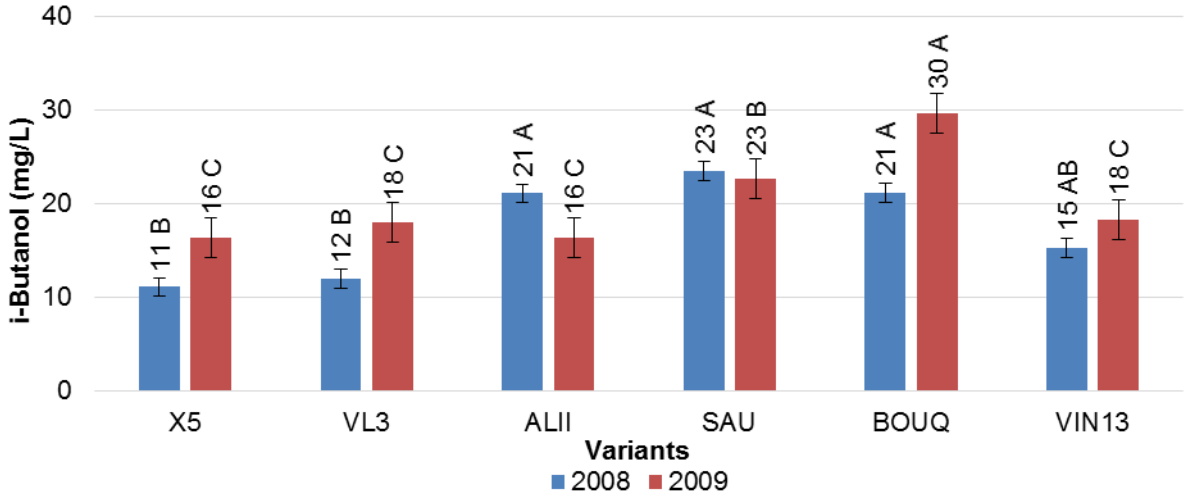


Figure 3-21: i-Butanol concentration of the finished Scheurebe wines from six commercial yeast strains for the vintages 2008 and 2009. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

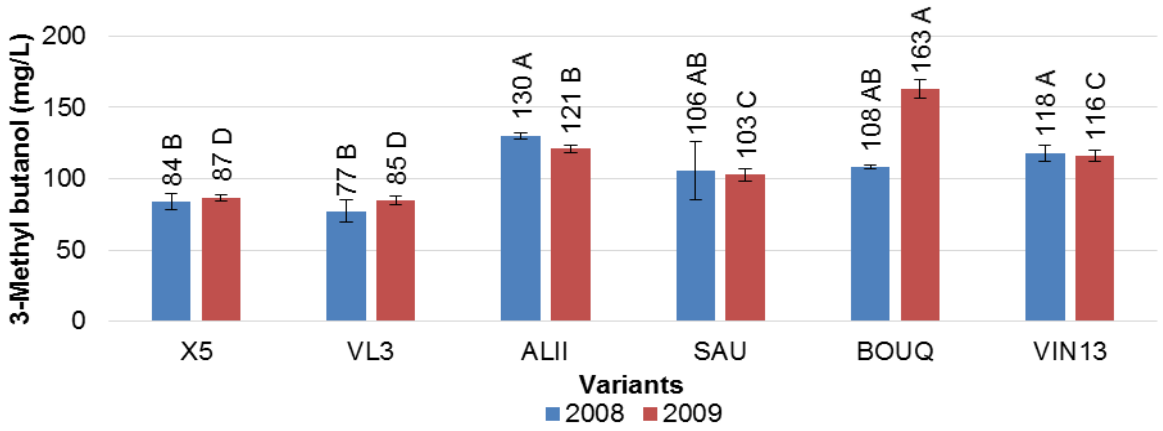


Figure 3-22: 3-methyl butanol concentration of the finished Scheurebe wines from six commercial yeast strains for the vintages 2008 and 2009. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

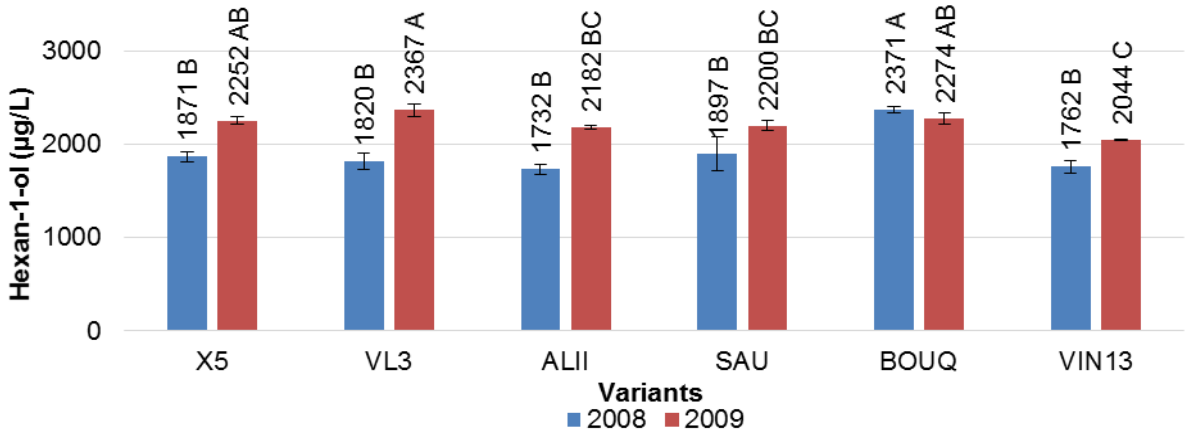


Figure 3-23: Hexan-1-ol concentration of the finished Scheurebe wines from six commercial yeast strains for the vintages 2008 and 2009. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

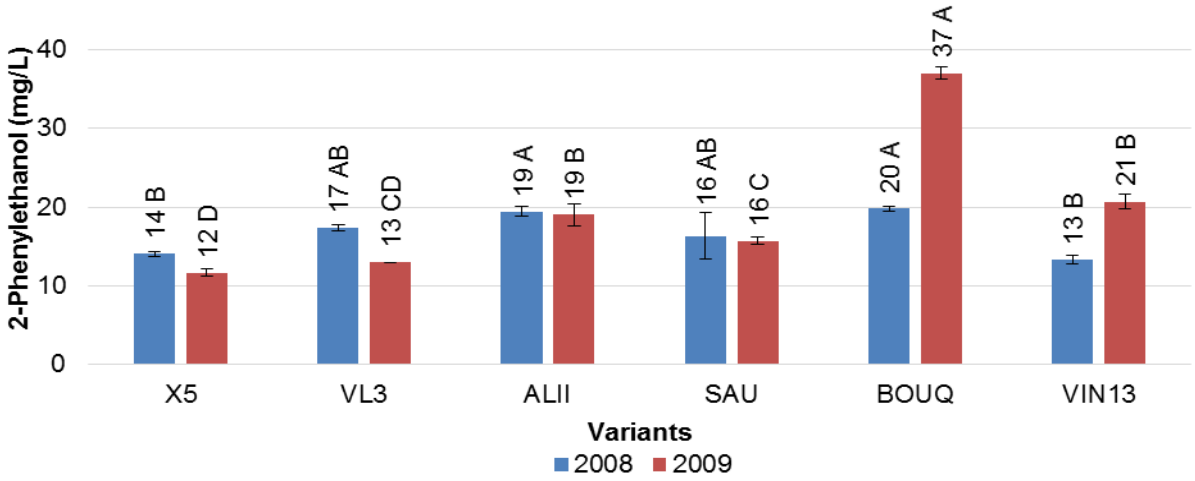


Figure 3-24: 2-phenylethanol concentration of the finished Scheurebe wines from six commercial yeast strains for the vintages 2008 and 2009. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

3.2.1.3 Monoterpenes

Among the studied terpenes the linalool oxides (*cis*- and *trans*-) are not being shown because they were quantified at a few µg/L, wheras their odour threshold is 3-5 mg/L (Mateo & Jiménez *et al.*, 2000). Linalool was found in the wines of both vintages in significant concentrations that contributed to their aroma (**Figure 3-25**). The compounds have been described to have a rose aroma and showed an odour threshold of 25 µg/L (Ferreira *et al.*, 2000). Guth (1997b) reported linalool contents up to 307 µg/L for Scheurebe wines. α-Terpineol was determined below its odour threshold of 250 µg/L (Ferreira *et al.*, 2000) for all of the variants (**Figure 3-26**). The wines from the vintage 2009 showed higher concentrations of monoterpenes. A summary of all the measured compounds with the 'Kaltron' method can be found in **Appendix 6-2.**

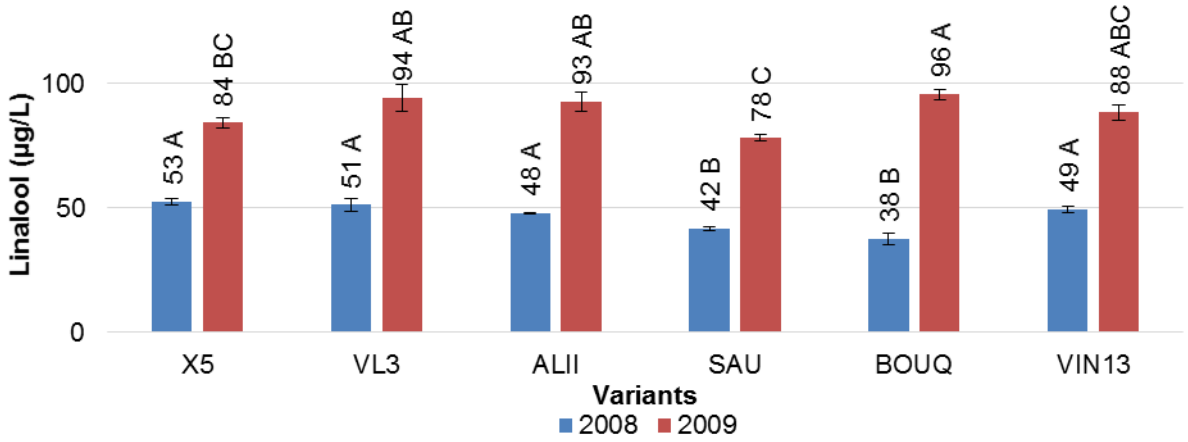


Figure 3-25: Linalool concentration of the finished Scheurebe wines from six commercial yeast strains for the vintages 2008 and 2009. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

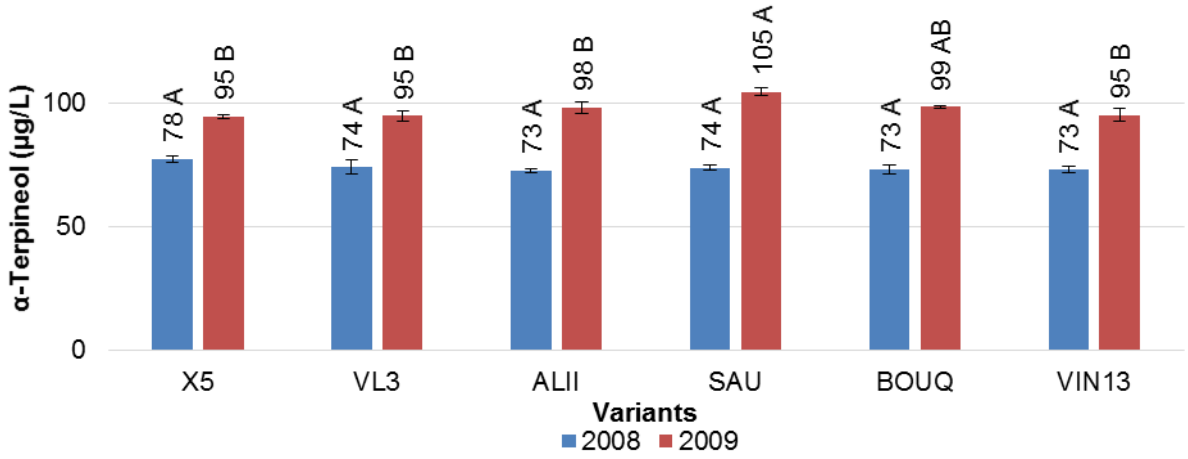


Figure 3-26: α-Terpineol concentration of the finished Scheurebe wines from six commercial yeast strains for the vintages 2008 and 2009. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

3.2.1.4 Low boiling point sulphur compounds

Low boiling point sulphur compounds comprise of off-flavour compounds that have a major impact on the aroma and are mostly correlated with a so-called 'reduced character' of a wine (Ribéreau-Gayon *et al.*, 1999). **Figure 3-27** showed that all wines from the vintage 2009 had H_2S detected but only the ALII variant was at concentrations that could have an impact on the aroma (Dittrich & Grossmann, 2011) and it was noted during the pre-tasting (**Chapter 2.3.3**). In two of the variants, ALII and BOUQ, MeSH was also measured at around the odour threshold (**Figure 3-28**) (Solomon *et al.*, 2010).

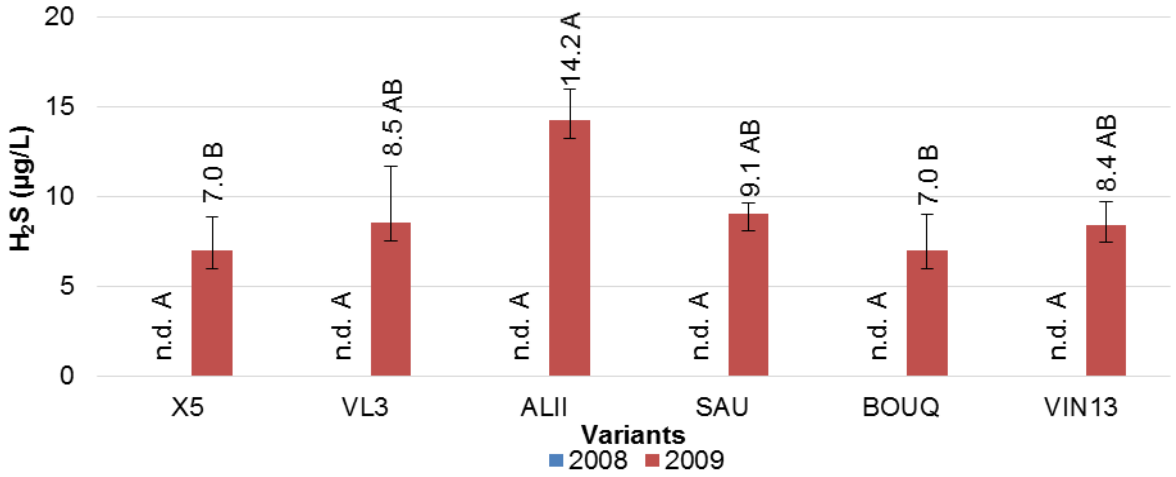


Figure 3-27: Hydrogen sulphide (H_2S) concentration of the finished Scheurebe wines from six commercial yeast strains for the vintages 2008 and 2009. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

This could be attributed to the fact that the must of the 2009 vintage showed a nitrogen deficiency with a YAN value of 133 mg N/L as opposed to the must of 2008, which contained 191 mg N/L. It has been suggested by Dukes & Butzke (1998) that for about 228 g/L reducing sugar in the must a concentration in YAN should be about 200 mg N/L (**Table 2-4**). These nitrogen dificiencies have been proved to propagate the higher production of sulphur off-flavours (Ribéreau-Gayon *et al.*, 1999).

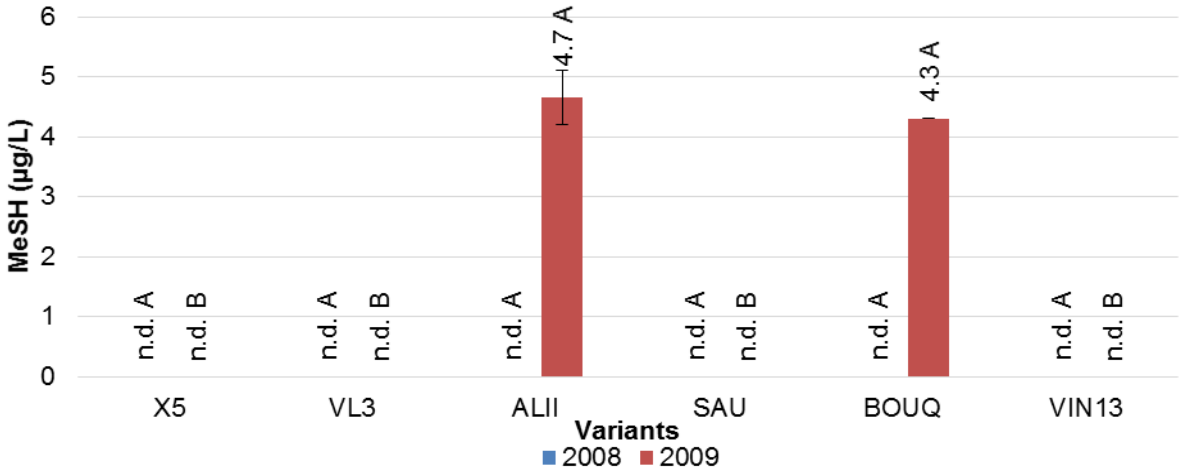


Figure 3-28: Methanethiol (MeSH) concentration of the finished Scheurebe wines from six commercial yeast strains for the vintages 2008 and 2009. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

DMS did not play a role as the wines were young and the concentrations below the odour threshold of 25 µg/L (**Figure 3-29**) (Goniak & Noble, 1987; Dittrich & Grossmann, 2011). A summary of the results for the low boiling point sulphur compounds can be found in **Appendix 6-3**.

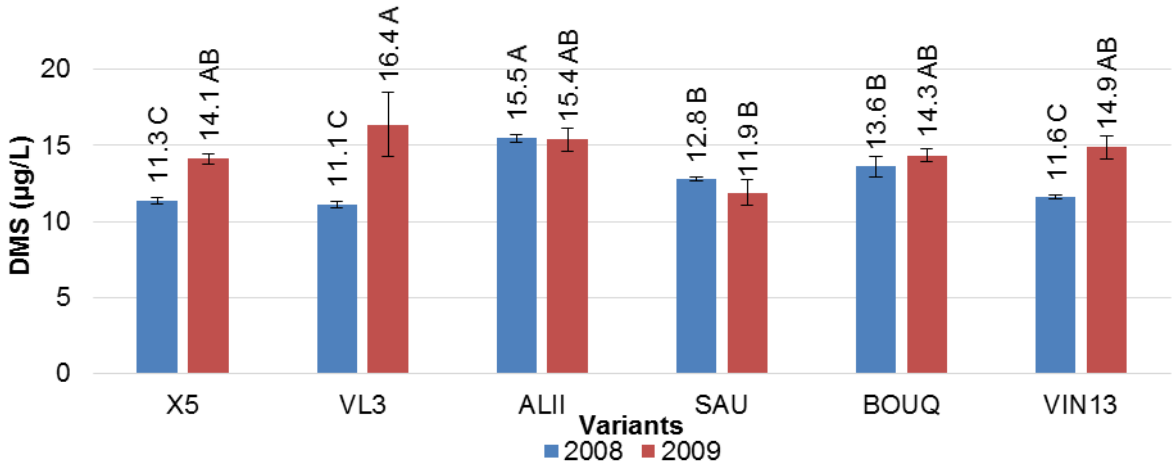


Figure 3-29: Dimethylsulphide (DMS) concentration of the finished Scheurebe wines from six commercial yeast strains for the vintages 2008 and 2009. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

3.2.1.5 Varietal thiols

Varietal thiols (4MSP, 3SHA, 3SH) for both vintages of Scheurebe were measured with the initial method as described in **Chapter 2.2.2**.

From **Figures 3-30, 3-31** and **3-32** some significant differences can be seen in the release of varietal thiols by the different yeast strains. These differences can also be observed for the two vintages. The wines produced with ALII for both vintages were amongst the highest measured. A concentration of 33.3 ng/L 4MSP for the ALII 2008 variant was quantified and was around eleven times above the odour threshold (Darriet *et al.*, 1995).

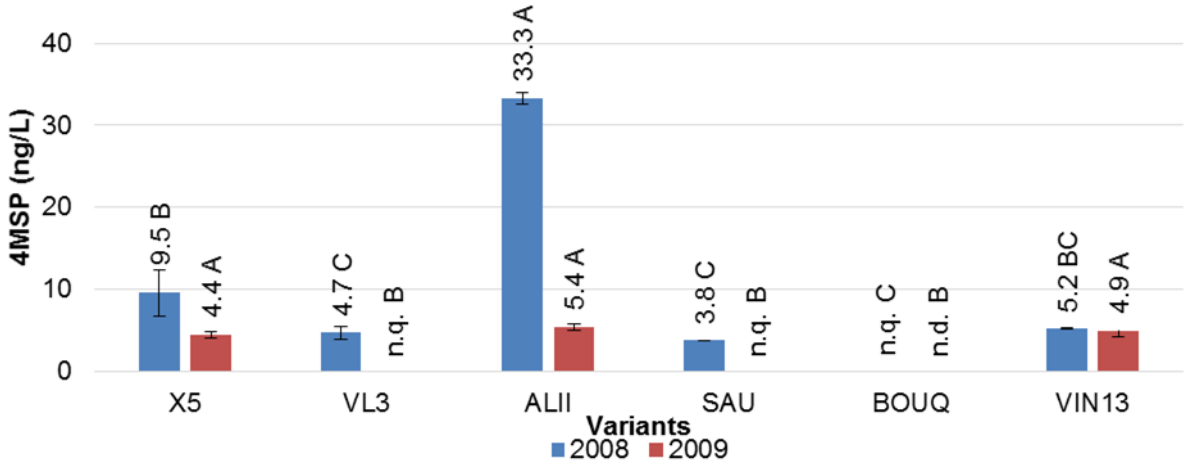


Figure 3-30: 4-Methyl-4-sulfanylpentan-2-one (4MSP) concentration of the finished Scheurebe wines from six commercial yeast strains for the vintages 2008 and 2009. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

The sensory analysis for the aforementioned wines showed a strong 'catpiss' character (**Figure 3-33**) that was also noted during the pre-tasting. This was in correlation with the publication by Darriet *et al.* (1995), in which was mentioned that high concentrations of 4MSP give to a wine an unpleasant odour.

For the wines from the vintage of 2009, 3SHA was quantified in concentrations about three times lower than the wines from the vintage 2008. 3SH with a concentration of 6372 ng/L for the variant ALII 2008 was 106 times above the odour threshold of the compound (Dubourdieu & Tominaga, 2009). A summary of the results for the varietal thiols can be found in **Appendix 6-3**.

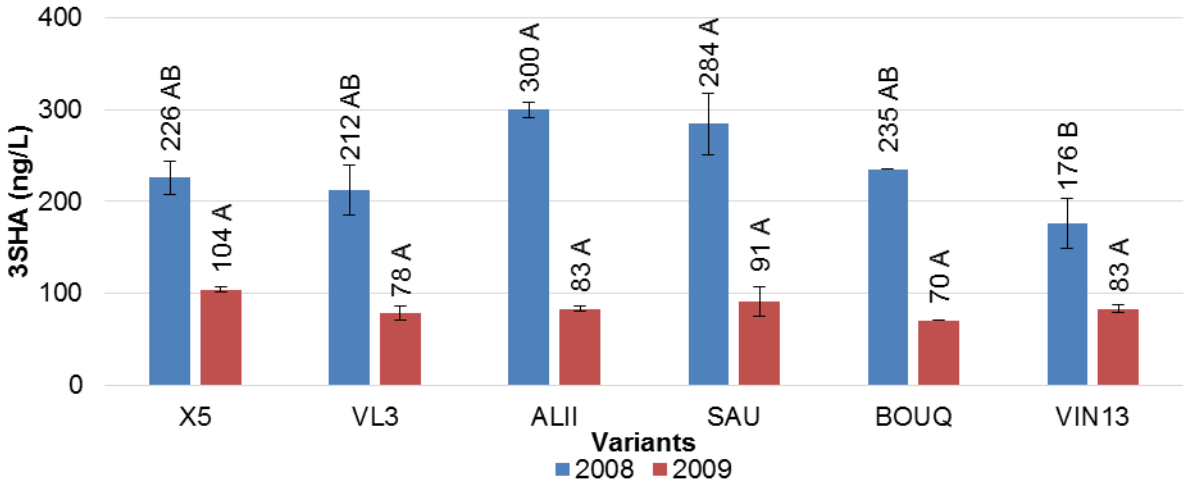


Figure 3-31: 3-Sulfanyl-hexyl acetate (3SHA) concentration of the finished Scheurebe wines from six commercial yeast strains for the vintages 2008 and 2009. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

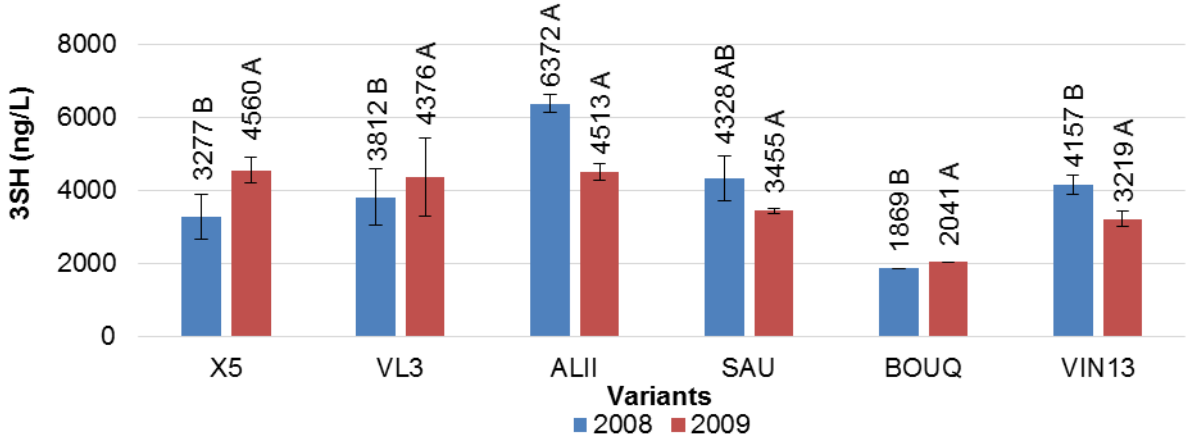


Figure 3-32: 3-Sulfanylhexan-1-ol (3SH) concentration of the finished Scheurebe wines from six commercial yeast strains for the vintages 2008 and 2009. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

3.2.1.6 Sensory analysis

Before the wines were prepared for the tasting by the expert panel, they were pre-tasted ruling out any of them that were faulty. Furthermore, the most common descriptors mentioned by the tasters were noted as a help for the development of a tasting scheme. This was done for the wines from the 2008

vintage. During the pre-tasting session two of the variants, SAU 2008 and BOUQ 2008 did not qualify for the tasting as off-flavours were determined. A ‚lactic', 'yogurt note', 'aldehydic-' and 'oxidative' characters were noted for the SAU 2008 variant. Similar characteristics were noted for the BOUQ 2008 variant. None of the wines from the vintage of 2009 showed off-flavours and all variants qualified for the tasting. This pre-selection was carried out in the Department of Microbiology and Biochemistry of the Hochschule Geisenheim University by a small panel of experts of the department.

It can be seen from **Figure 3-33** that the variant ALII 2008 was clearly described by the panel as 'catpiss' (also noted during the pre-selection), which was attributed to the high concentrations of 4MSP (Darriet *et al.*, 1995). It must be noted that for the vintage 2009 the variants X5, BOUQ and ALII were rated as more typical when compared to the other variants (**Figure 3-34**). The 'cassis' character that was attributed to the variant VL3 2009 was high although its concentration of 3SHA among the lowest measured and 4MSP could not be quantified. A summary of the tasting scores for all the wines can be found in **Appendix 6-4**.

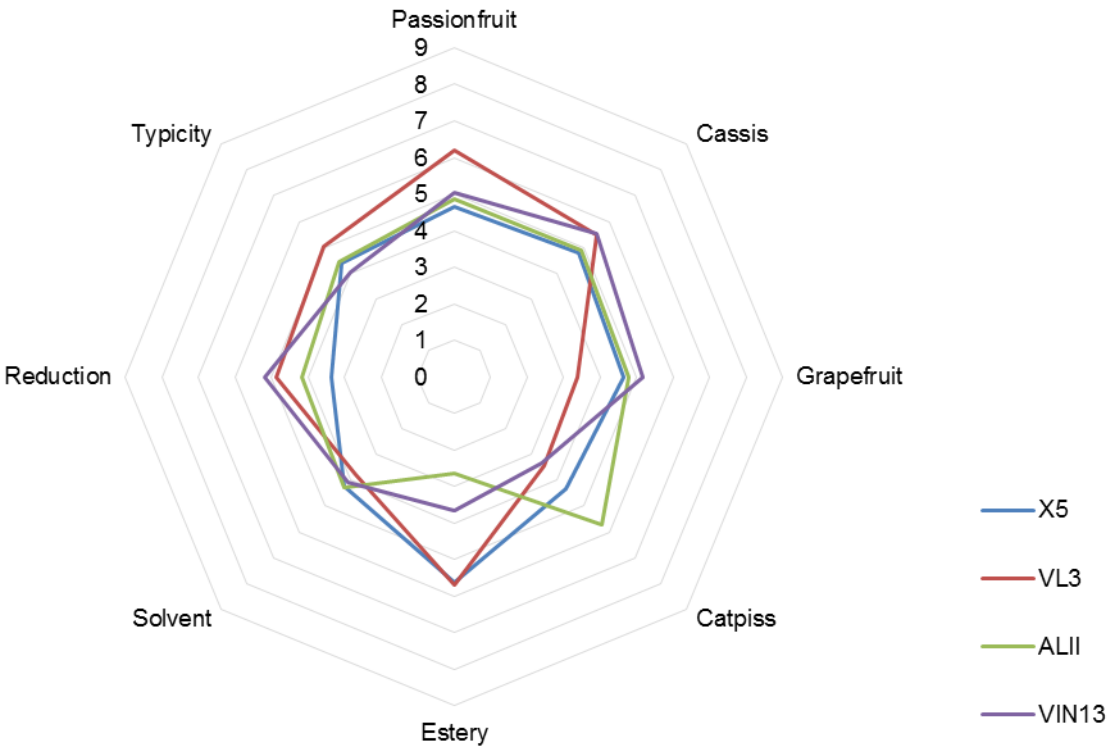


Figure 3-33: Spider plot for the four finished Scheurebe wines of the vintage 2008 that were tasted by the tasting panel.

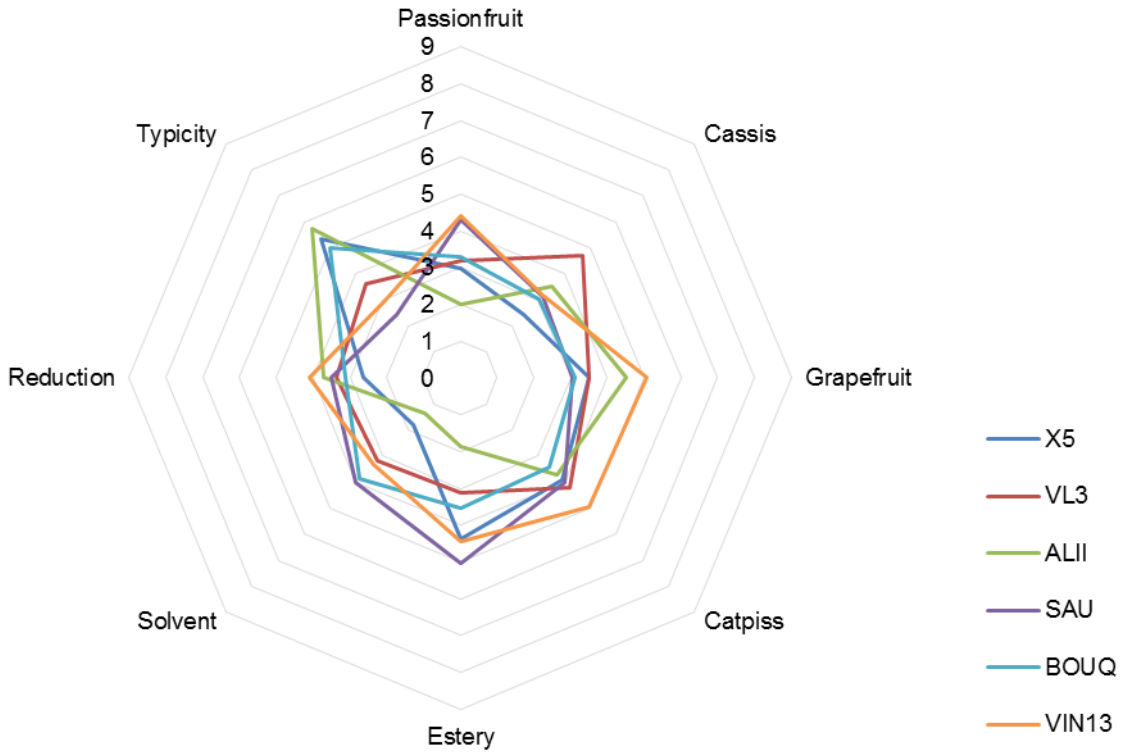


Figure 3-34: Spider plot for the six finished Scheurebe wines of the vintage 2009 that were tasted by the tasting panel.

3.2.1.7 Statistical analysis

PCA plotting was used for analysing the data statistically. The OAVs were used for estimating the contribution of the aroma compounds to the aroma of the wines (Guth, 1997b; Ferreira *et al.*, 2000; Gómez-Míguez *et al.*, 2007). The data were mean centered and autoscaled before analysis (van den Berg *et al.*, 2006). A summary of the OAVs can be found in **Appendix 6-5**.

The PCA for the sensory descriptors explained 62.92 % in the variability in the data (**Figure 3-35**). F1 accounted for 41.57 % of the variability and was heavily loaded with the descriptors ‘passionfruit’, ‘cassis’, ‘solvent’ and ‘reduction’ in the positive direction. F2 accounted for 21.35 % of the variability and was heavily loaded with the descriptor ‘grapefruit’ in the positive direction and ‘estery’ in the negative direction.

From the PCA plot it can be pointed out that most of the wines of the 2009 vintage were not correlating with the descriptors as well as the wines from the vintage 2008. The wines VIN13 2008 and X5 2008 were described by F1 and this could be attributed to the high concentrations of varietal thiols that were measured for the 2008 vintage. A point to mention was the correlation of the X5 2008 and VL3 2008 variants with the descriptor ‘solvent’ although the concentrations of acetic acid ethylester were very low. This could be attributed to a possible confusion of the tasters with the descriptors ‘estery’ and ‘solvent’. From **Figure 3-35** it could be seen that the descriptor ‘typicity’ does not correlate well with the descriptors of the varietal thiols (‘grapefruit’, ‘passionfruit’, ‘cassis’ and ‘catpiss’) so it was important to determine the relationship between ‘typicity’, the varietal thiols and their descriptors (**Figure 3-36**).

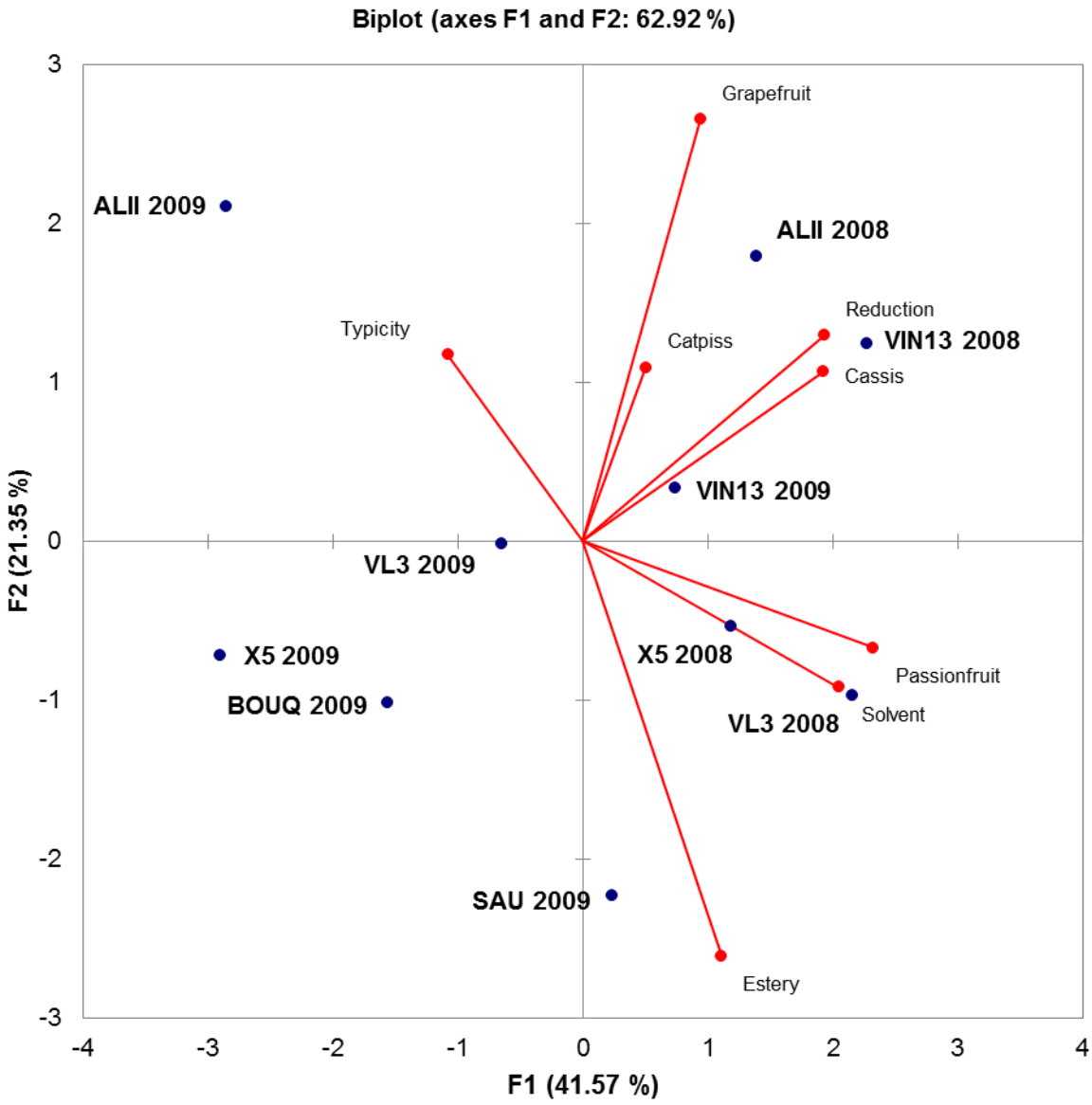


Figure 3-35: Principal Component Analysis (PCA) for the tasting results for the Scheurebe wines from the vintages 2008 & 2009. (The wines fermented with SAU and BOUQ from the vintage 2008 were not chosen to build the diagram because they were faulty and did not qualify to the tasting).

This PCA plotting explained 66.38 % of the variability of the samples (**Figure 3-36**). F1 accounted for 45.24 % of the variability and was heavily loaded with the varietal thiols (4MSP, 3SHA and 3SH) and the descriptor ‘cassis’ in the positive direction. F2 accounted for 21.13 % of the variability and was heavily loaded with the descriptor ‘typicity’ in the positive direction and ‘catpiss’ in the negative direction. None of the wines from 2008 were described by F2 and half of the wines from 2009 were described by the same factor (X5 2009, AL 2009, VIN13 2009) that made the wines perceived as more typical. Taking in account that the wines from 2009 had much lower concentrations of varietal thiols it could be probably hypothesised that high concentrations of varietal thiols do not enhance the typical character of wines. Probably their role would be complementary.

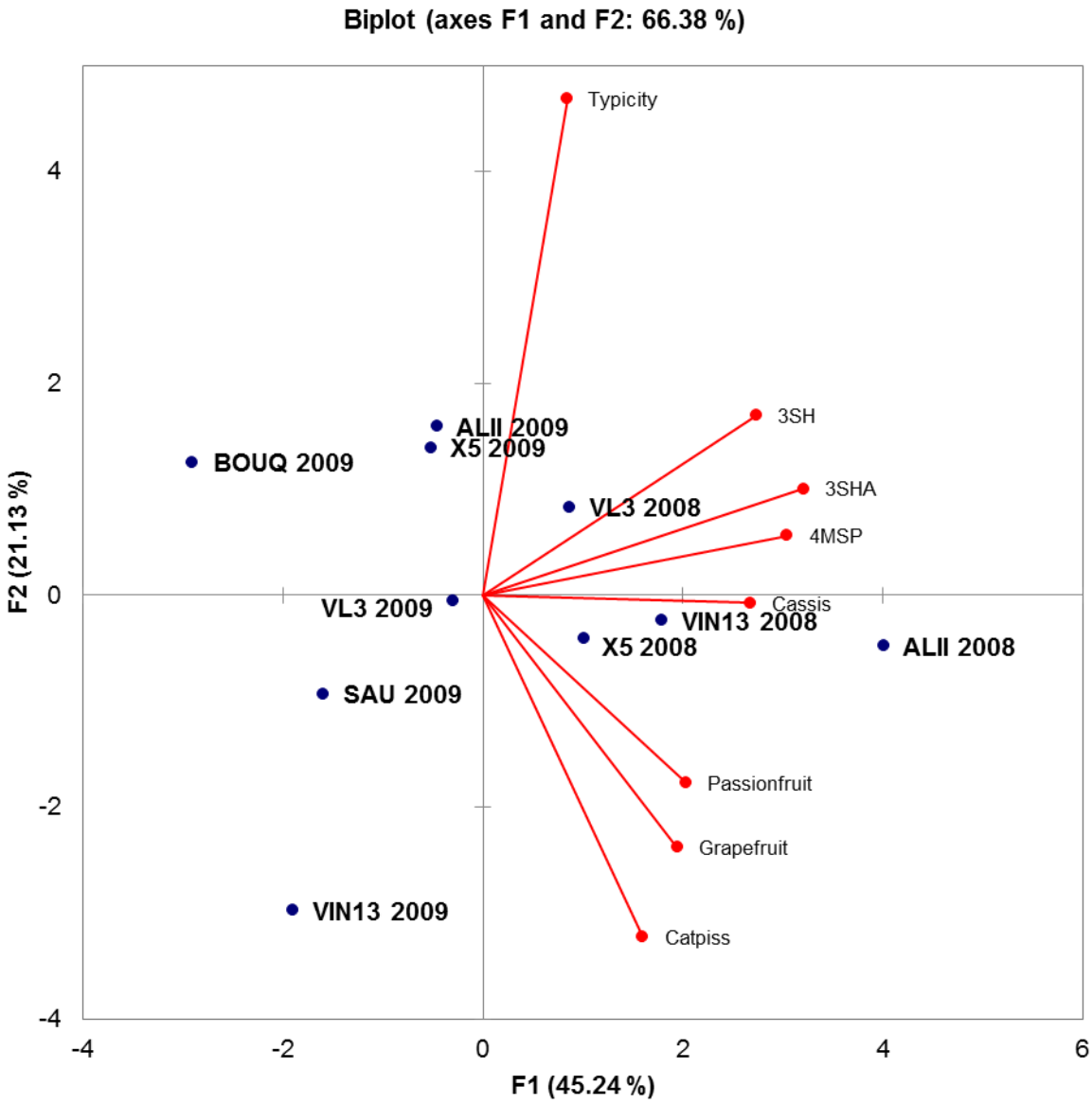


Figure 3-36: Principal Component Analysis (PCA) for the descriptor 'typicity', varietal thiols and their descriptors. The data were mean centered and autoscaled before analysis.]

For the PCA of the aromatic compounds and the tasting attributes, it was chosen to plot only the compounds that showed OAV values above 1 in at least one of the variants. The PCA on F1 and F2 explained 56.05% of the variability in the data. F1 accounted for 35.46% of the variability and was more heavily loaded with 'linalool', 'H_2S' in the positive direction and 'reduction', 'cassis' in the negative direction. F2 accounted for 20.58 % of the variability and was more heavily loaded with the attribute 'estery' in the positive direction and '4MSP', '3SH', 'acetic acid ethylester' in the negative direction.

From the plot (**Figure 3-37**) it can be seen that '4MSP' is closely related with 'catpiss' as described from the literature (Darriet *et al.*, 1995) and '3SH' with 'grapefruit'. 'Acetic acid 3-methylbutyl ester' although different from thiols contributes to the fruity character of a wine with an odour of banana

(Guth, 1997b). ‘Cassis’ and ‘3SHA’ also seem to have a relationship as deschribed by Dubourdieu & Tominaga (2009).

From **Figure 3-37** a clear separation of the 2008 and the 2009 wines can be seen. The wines from the vintage of 2008 are more close to the exotic descriptors like ‘passionfruit’ and ‘cassis’ whereas most of the wines from the vintage 2009 are correlating more with ‘floral’ (linalool) and ‘reductive’ (H_2S and other unpleasant sulphur compounds) notes.

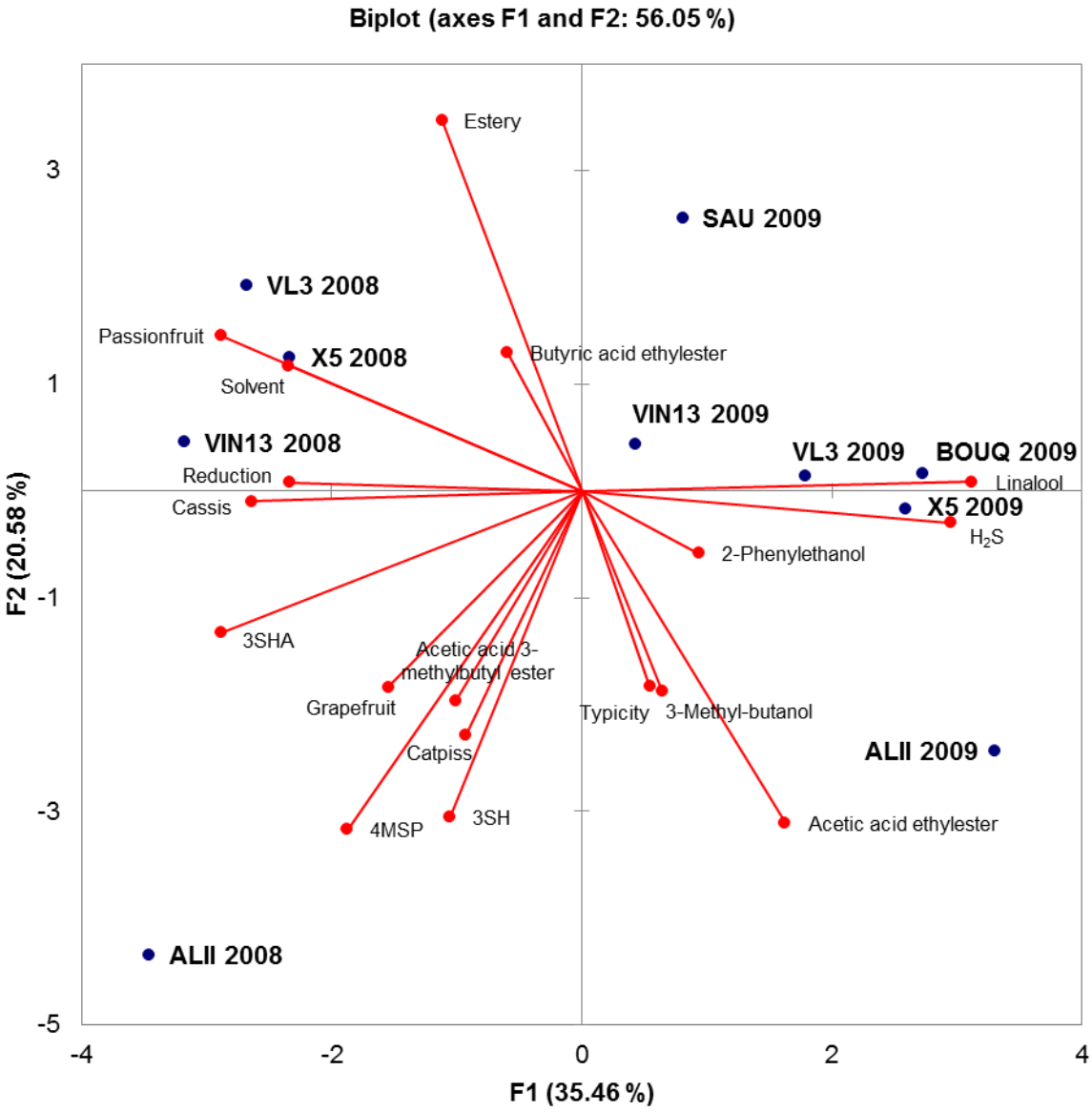


Figure 3-37: Principal Component Analysis (PCA) of the Scheurebe wines from the vintage 2008 & 2009 with the aromatic compounds that showed an OAV > 1 in at least one of the variants. The data were mean centered and autoscaled before analysis.

3.2.1.8 Discussion and conclusion

The goal of this work package was to ferment two Scheurebe musts of the vintages 2008 and 2009 with various commercial yeast strains, without the addition of nutrients, to induce high concentrations of varietal thiols and to develop a tasting scheme for further experiments.

During the vintage 2009 the Scheurebe must showed low values of YAN (133 mg N/L), which caused at least one yeast not completing the fermentation. Dukes & Butzke (1998) have suggested a YAN of about 200 mg/L for the sugar concentration of the vintage 2009 must. The strain SAU showed a stuck fermentation for the musts of both vintages and the strain BOUQ for the vintage of 2008. The fact that the strain SAU showed problems for the fermentations of both vintages probably suggests that is has a high requirement of YAN, although nothing could be found in the manufacturers specifications (**Table 2-7**). These stuck fermentations were evident in the results of both alcohol (**Figure 3-10**) and residual sugars (**Figure 3-11**).

Ester production was highly variable for the young wines of the two different vintages. ALII showed constant levels of acetic acid ethylester for both vintages, which were the highest amongst all the variants. Nevertheless its concentrations were marginally below the odour threshold, but were probably high enough to affect the perception of the wines positively (Ribéreau-Gayon *et al.*, 1999). Among the measured esters, acetic acid 3-methylbutylester and butyric acid ethylester seem to have played a role in the aroma of the wines. The first one has a characteristic odour of banana and contributes to the young fruity character of a wine with an odour threshold of 30 µg/L (Guth, 1997b). ALII and VIN13 produced constant high concentrations of acetic acid 3-methylbutylester in the wines of both vintages, between 21- and 36-fold above the odour threshold. Most of the other yeasts produced either highly variable concentrations for both vintages or significantly low concentrations when compared with ALII and VIN13. Butyric acid ethylester had more consistent results for all the variants for both vintages and significantly higher concentrations of 8-17 times above the odour threshold.

On the contrary to esters, higher alcohols were much more consistent in the young wines for both vintages. The variation of concentrations was not so substantial, neither between the strains nor between the young wines of both vintages. 3-Methyl butanol and 2-phenylethanol were in concentrations that contributed to the aroma of the wines. Especially 2-phenylethanol has a characteristic odour of roses and was found in all of the variants at least near the odour threshold of 14 mg/L (Ferreira *et al.*, 2000).

Linalool played a role in the aroma, with some significant concentrations almost four times above its odour threshold (25 µg/L; Ferreira *et al.*, 2000) being measured consistently for the young wines from the vintage 2009. These concentrations were below the reported values of about 307 µg/L reported by Guth (1997b). It was also noted that the wines from the vintage 2009 had significantly higher concentrations than the wines from the vintage 2008. This can be attributed to viticultural practice for linalool and to oenological practice for α-terpineol (Mateo & Jiménez, 2000). It has been suggested by Carrau *et al.* (2005) that higher concentrations of YAN are stimulating the formation of monoterpenes.

This is probably because a different pathway was suggested for their release (Novothy *et al.*, 1998; Vaudano *et al.*, 2004). This comes into contradiction with the observations in **Figures 3-25** and **3-26**.

Characteristic compounds that give reductive notes to the wines were only measured in wines from the vintage 2009. H_2S and MeSH were found in some of the variants above the odour threshold of the compounds (Solomon *et al.*, 2010; Dittrich & Grossmann, 2011). As mentioned also in **Chapter 3.2.1.4**, the most probable cause for the reductive notes of the wines from the vintage 2009 was the nitrogen deficiency of the musts.

The wines of vintage 2008 showed much higher concentrations of all the thiols when compared to the vintage 2009. It was found by Peyrot des Gachons *et al.* (2005) that higher nitrogen levels in the vines lead to higher concentrations of varietal thiol precursors. During the release of varietal thiols, ammonium is released and probably the nitrogen status of the musts plays a role (Bell & Henschke, 2005). Subileau *et al.* (2008b) published some insights on the release of varietal thiols during fermentation and showed that the nitrogen catabolic repression mechanism (NCR) modulated the production of varietal thiols. The addition of diammonium phosphate as a nutrient decreased the production of thiols. Furthermore, Thibon *et al.* (2008a) showed that the nitrogen catabolic repression mechanism mainly controlled the release of the thiols but not their uptake by the yeast cells. Probably the composition of the nitrogen source in musts plays a role and more studies will be necessary for understanding this complex mechanism. For 3SHA, a significant difference in the concentration between 2008 and 2009 was observed. The concentration in the finished wines was two to three times less and there is no consistency with the concentrations of 3SH. Probably the esterification of 3SH towards 3SHA is more complex and it is not only attributed to the capacity of each yeast (Howell *et al.*, 2005). Nevertheless the concentration of both 3SHA and 3SH are significantly above the odour threshold of the compounds. ALII 2008 showed concentrations of 3SH more than 105 times above the odour threshold giving a significant impact in the aromatics of the wines. Very significant was also 4MSP in the ALII 2008 variant with a concentration of 33.3 ng/L, which gave to the wine a strong ‘catpiss’ odour (Darriet *et al.*, 1995).

The sensory analysis of the wines revealed that the wines from 2009 were perceived as more typical by the tasters, although the concentration of varietal thiols for this vintage were lower. This observation, in combination with the statistical analysis, might lead to the result that ‘typicity’ of a wine is not correlated with high concentrations of thiols, with 4MSP especially playing a decisive role in that. From the Scheurebe experiment, ‘typicity’ appeared to be a balance between ‘floral’, ‘fruity’ and ‘exotic’ character.

Finally, by evaluating the training and tasting scheme for future tastings, the following conclusions could be drawn:

It could be seen from the PCA in **Figure 3-35** that the descriptors ‘estery’ and ‘solvent’ are close to each other. This could be attributed to the fact that many tasters confused these two descriptors, as sometimes the term ‘estery’ could be used for describing acetic acid ethylester. Due to this fact, for future tastings it was decided to use only the attribute ‘estery’ for describing the impression of acetic acid ethylester.

The descriptor ‘catpiss’ was only used for the description of ALII 2008. It seems that this attribute is possible but not very common so it was decided to remove it from the list of descriptors. Should a wine have such high concentrations of 4MSP, a field for comments was added to be filled out by the tasters.

The descriptor ‘typicity’ was confusing to the tasters and it was not clear which of the factors correlate with it. Finally, the descriptor ‘typicity’ for unknown varieties will not easy to use, as in many of the cases the tasters would be unfamiliar with the typical taste. Due to this fact, it was decided to remove it from further tasting sessions.

3.2.2 Sauvignon blanc

The results from the fermentation experiment considering Sauvignon blanc are presented and discussed in the following chapters.

3.2.2.1 Fermentation kinetics and major components

For both vintages the fermentation took around 19 days to complete (**Figures 3-38** & **3-39**). Fermentations were considered finished when there was no more significant weight loss (<0.1 g/day). After the fermentation the wines were transferred to a temperature controlled cellar at 4 ˚C for clarification.

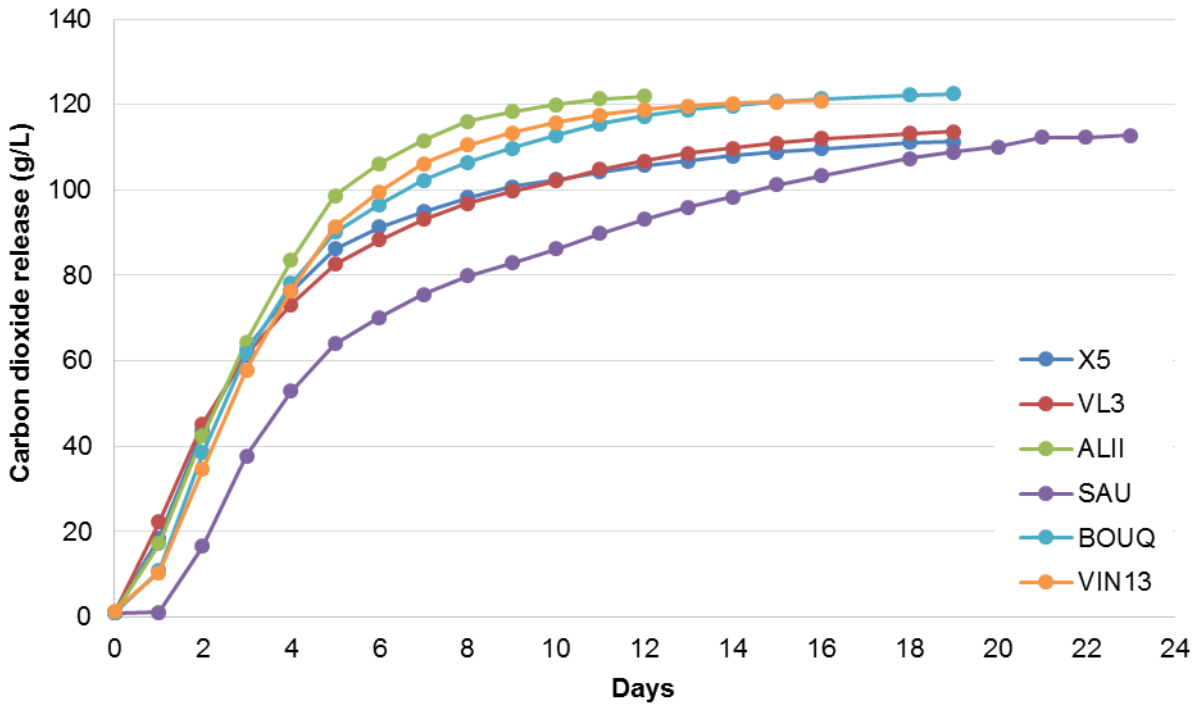


Figure 3-38: Fermentation kinetics of the six Sauvignon blanc variants for the vintage 2008. Every curve represents the average value from the three fermentation replicates.

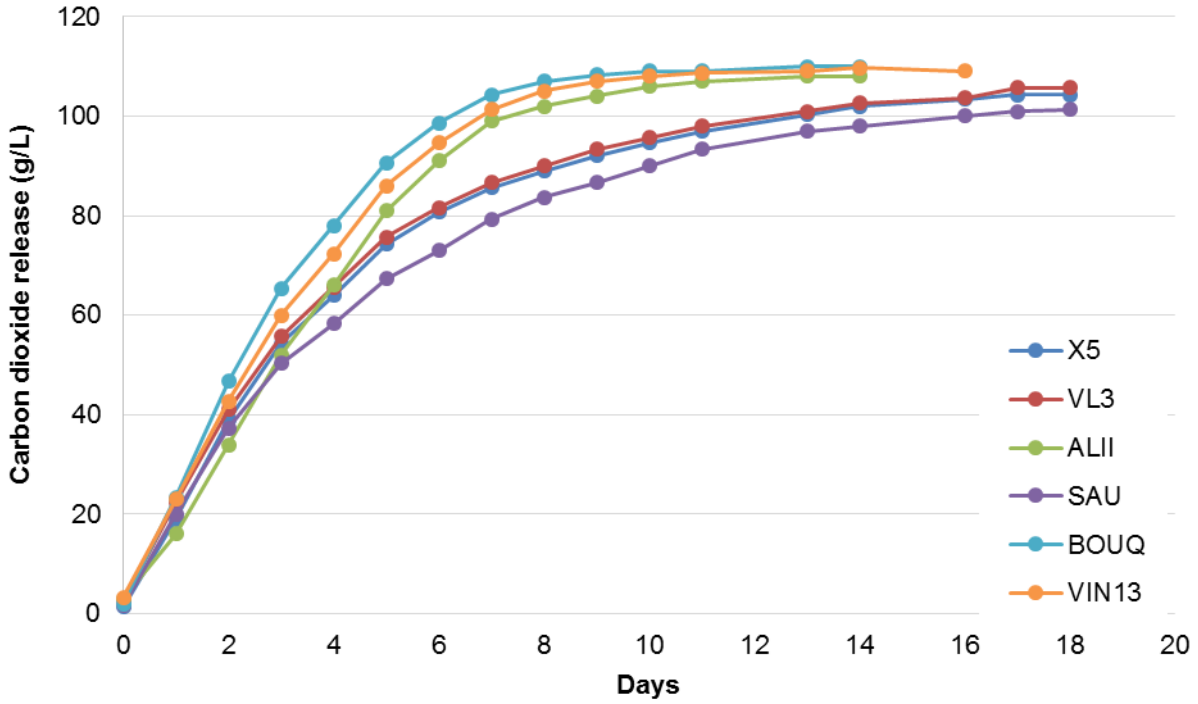


Figure 3-39: Fermentation kinetics of the six Sauvignon blanc variants for the vintage 2009. Every curve represents the average value from the three fermentation replicates.

Strain ALII had the shortest fermentation for both vintages when compared with the other yeast (**Figure 3-38** & **3-39**). From the fermerntation kinetics for both vintages it can be seen that yeast X5, VL3 and SAU were slower in completing the fermentations and the finished wines had residual sugars ranging from 8.4 g/L to 14.4 g/L. These values were significantly higher when compared with the other three strains (**Figure 3-41**). SAU showed for the vintage 2009 a partial MLF (malolactic fermentation) with an average lactic acid concentration of 0.9 g/L. The partial MLF had also a light impact on the VA as its concentration averaged 0.6 g/L and was marginally higher when compared to the other variants (**Figure 3-42**). This was probably attributed to a contamination during inoculation of the musts. SAU showed sluggish and uncompleted fermentations for both vintages like in the case of the Scheurebe variants (**Chapter 3.2.1.1**). This could be explained if the YAN requirements of this strain are high, though this could not be verified by the manufactures specifications (**Table 2-7**). The alcohol content is consistent for all of the variants of a vintage except SAU because of the fermentation being not completed (**Figure 3-40**). For the vintage 2008 the YAN of the must was 181 mg N/L and the sugar concentration at 215 g/L. The same parameters for the vintage 2009 must, were 207 mg N/L and 230 g/L respectively. Dukes & Butzke (1998) would suggest for the sugar concentrations for the vintages of 2008 & 2009 a YAN of 200 mg N/L and 275 mg N/L respectively. This was probably the cause for some of the stuck fermentations as not all the yeast strains have the same YAN requirements. A summary of the results for the major components can be found in **Appendix 6-6**.

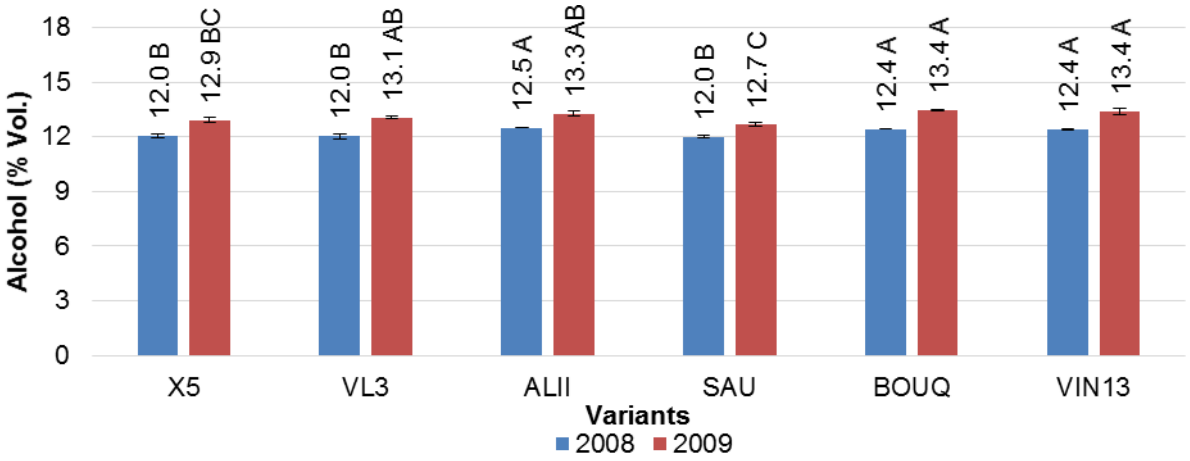


Figure 3-40: Alcohol content of the finished Sauvignon blanc wines from six commercial yeast strains for the vintages 2008 and 2009. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

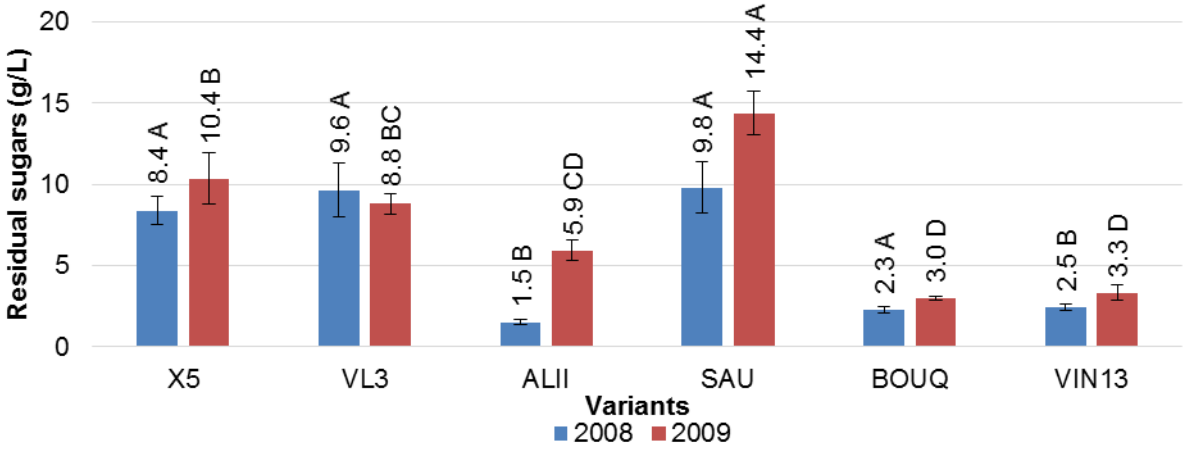


Figure 3-41: Residual sugars concentration of the finished Sauvignon blanc wines from six commercial yeast strains for the vintages 2008 and 2009. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

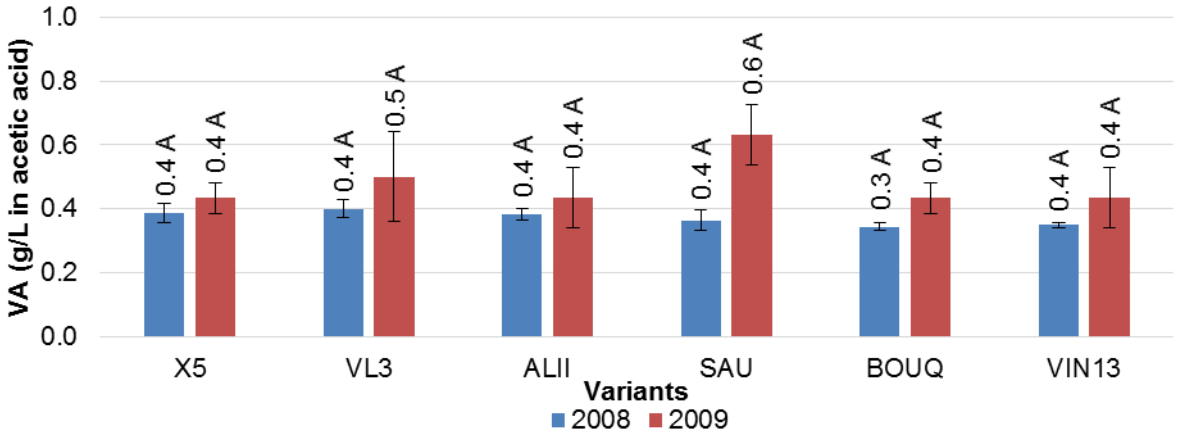


Figure 3-42: Volatile acidity (VA) of the finished Sauvignon blanc wines from six commercial yeast strains for the vintages 2008 and 2009. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

In **Figures 3-43** and **3-44** the effects of the spontaneous MLF for the variant SAU 2009 can be observed. There was a decrease in the concentration of malic acid and a significant concentration of lactic acid was produced. Probably the lactic acid bacteria utilised during this infection only malic acid because there were still redidual sugars left and the VA was not very high (homofermetative lactic acid bacteria).

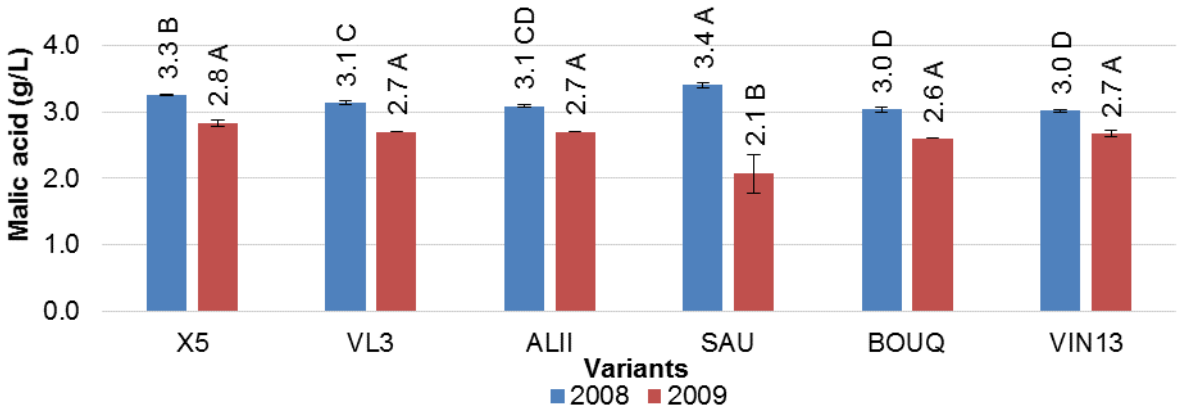


Figure 3-43: Malic acid concentration of the finished Sauvignon blanc wines from six commercial yeast strains for the vintages 2008 and 2009. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

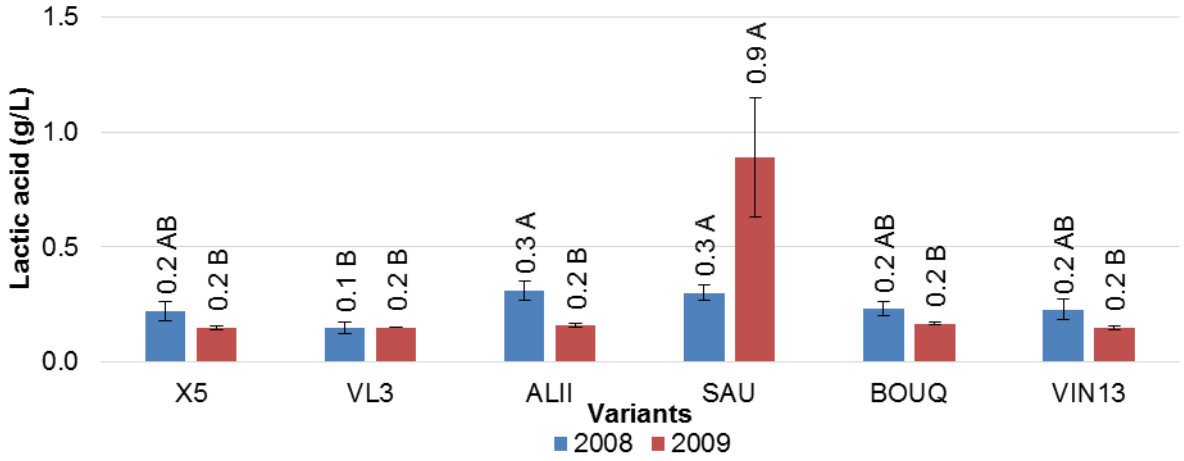


Figure 3-44: Lactic acid concentration of the finished Sauvignon blanc wines from six commercial yeast strains for the vintages 2008 and 2009. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

3.2.2.2 Esters and higher alcohols

A summary of all the measured compounds with the 'Kaltron' method can be found in **Appendix 6-7.**

Esters: Some of the esters were found in highly variable concentrations (**Figure 3-46** & **3-47**). Strain SAU seems to have produced the least amounts of these esters but this has to be treated with caution. As mentionend in **Chapter 3.2.2.1** the variant of the SAU 2009 was probably contaminated by bacteria during inoculation. This could also explain the high concentration of acetic acid ethylester for this variant. Furthermore, when comparing the wines from both vintages, the wines from 2009 showed higher content in acetic acid ethylester (**Figure 3-45**) with some of the variants exceeding the odour

threshold (160 mg/L; Ribéreau-Gayon *et al.*, 1999). For the fruity ester acetic acid 3-methylbutylester the fermentations with the yeast ALII, BOUQ and VIN13 showed very significant concentrations for the wines of the vintage 2008 but for 2009 the amounts measured were lower or none (**Figure 3-46**). Butyric acid ethylester contents were more consistent for the wines within the same vintage (**Figure 3-48**). Concentrations were high enough to contribute to the wines a sweet caramel scent (Ferreira *et al.*, 2000; Fretz *et al.*, 2005a). Similar concentrations in wines were also reported by Guth (1997b). The contamination of variant SAU 2009 was shown by the concentration of lactic acid ethylester (**Figure 3-49**) although this was below the odour threshold of 155 mg/L (Etiévant, 1991). Both acetic acid 2-phenylethylester and succinic acid diethylester were found in concentrations singnificantly below their odour threshold (**Figure 3-47** & **3-50**).

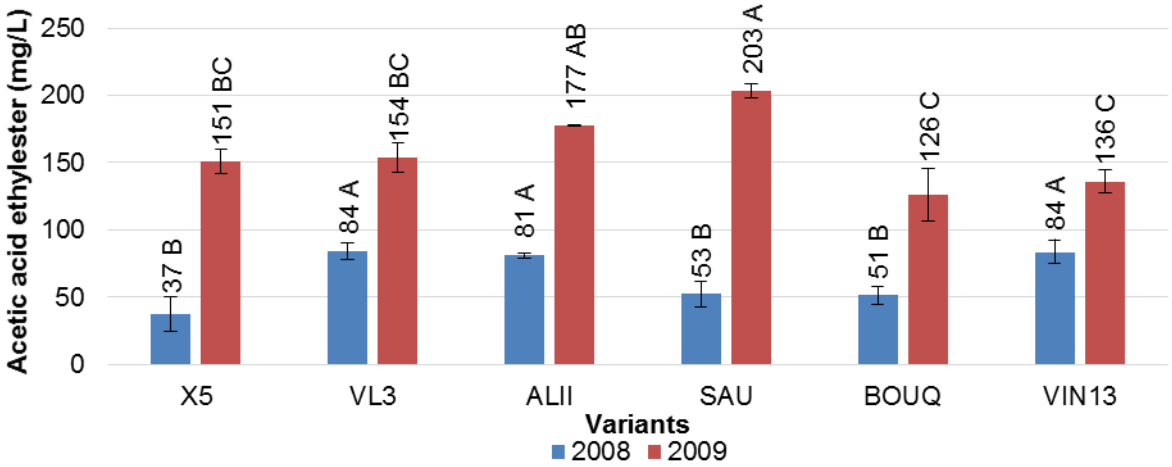


Figure 3-45: Acetic acid ethylester concentration of the finished Sauvignon blanc wines from six commercial yeast strains for the vintages 2008 and 2009. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

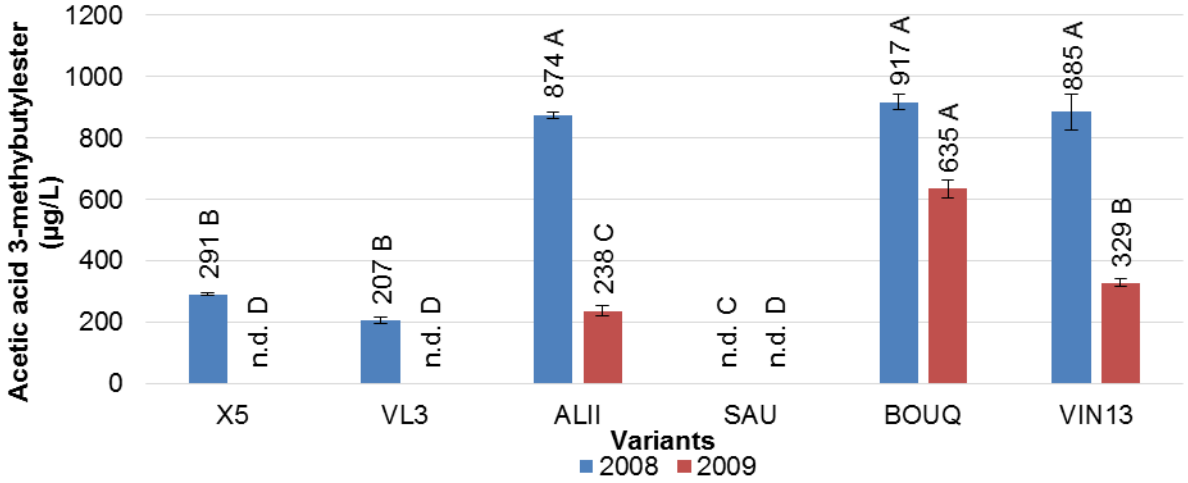


Figure 3-46: Acetic acid 3-methylbutylester concentration of the finished Sauvignon blanc wines from six commercial yeast strains for the vintages 2008 and 2009. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

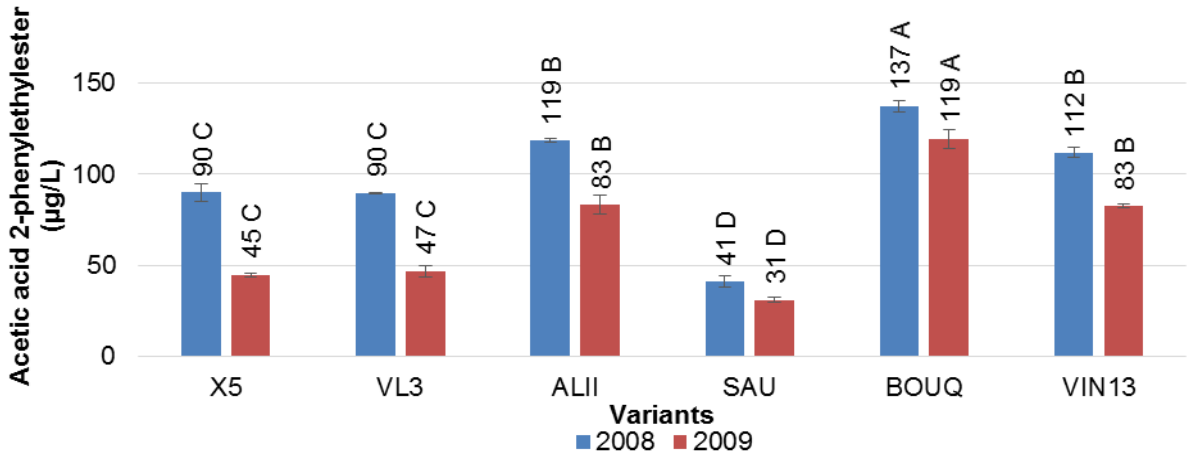


Figure 3-47: Acetic acid 2-phenylethylester concentration of the finished Sauvignon blanc wines from six commercial yeast strains for the vintages 2008 and 2009. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

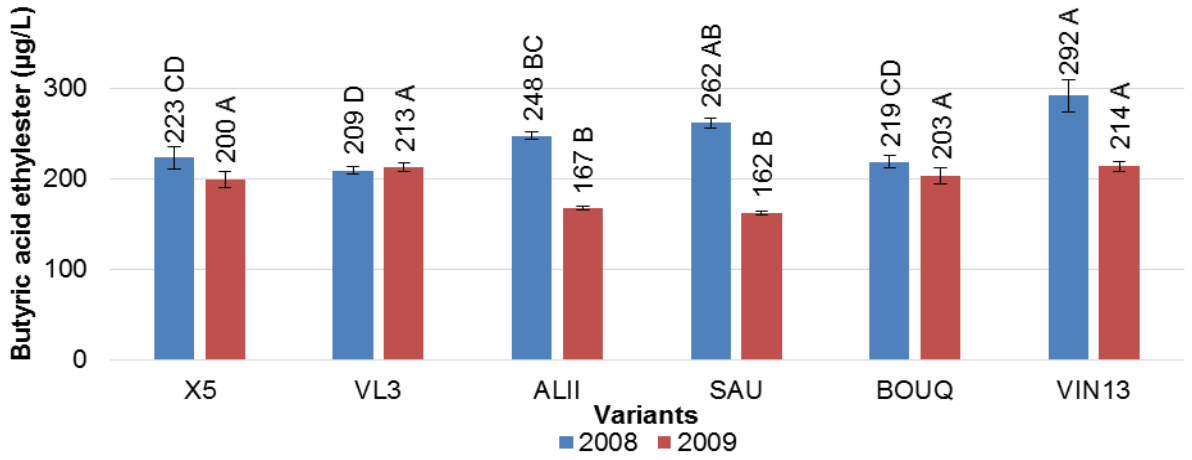


Figure 3-48: Butyric acid ethylester concentration of the finished Sauvignon blanc wines from six commercial yeast strains for the vintages 2008 and 2009. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

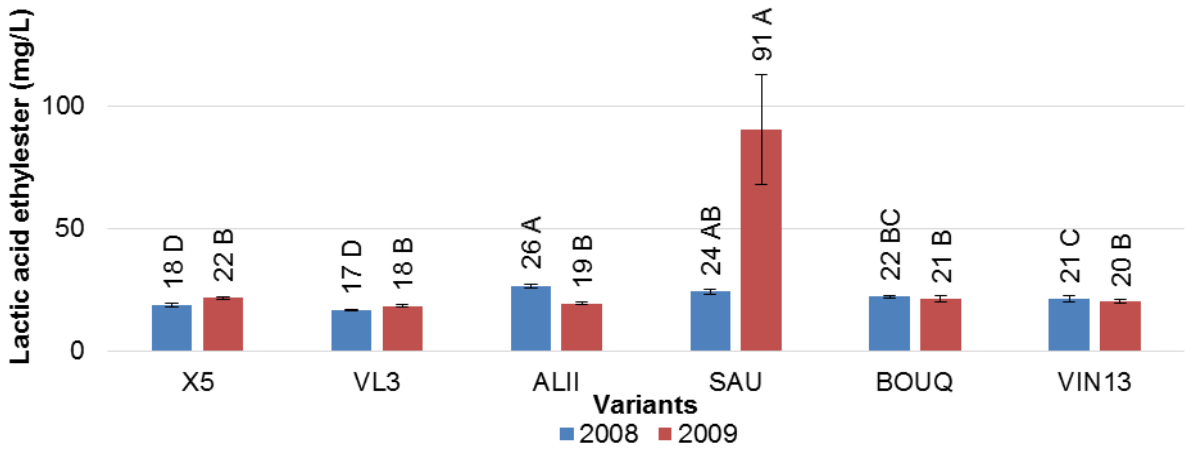


Figure 3-49: Lactic acid ethylester concentration of the finished Sauvignon blanc wines from six commercial yeast strains for the vintages 2008 and 2009. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

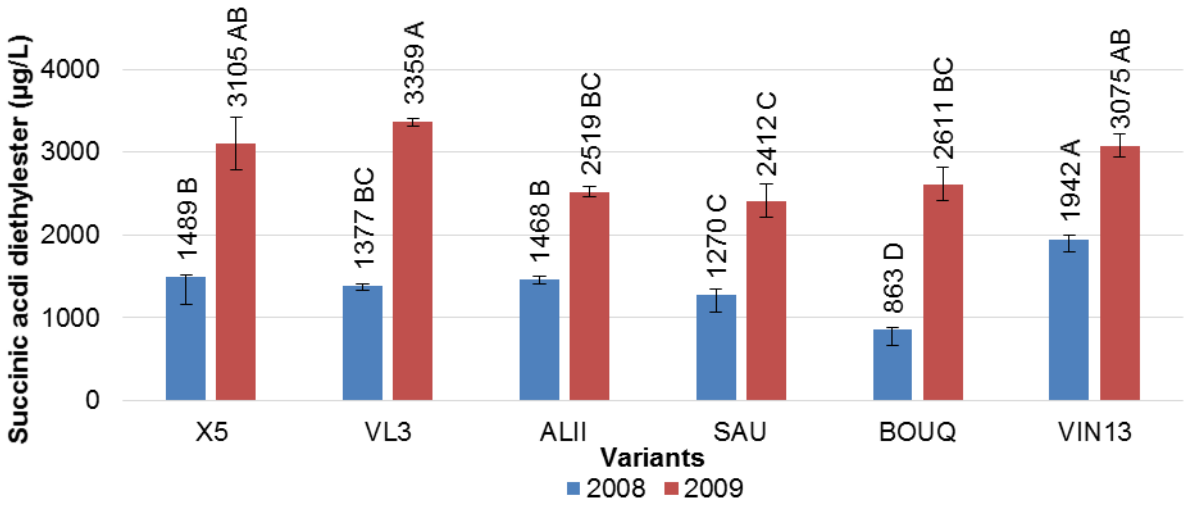


Figure 3-50: Succinic acid diethylester concentration of the finished Sauvignon blanc wines from six commercial yeast strains for the vintages 2008 and 2009. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

Higher alcohols: Higher alcohols showed to have similar concentrations to the Scheurebe wines (**Chapter 3.2.1.2**). 3-methyl butanol was measured in concentrations 2.5 to 5 times above the odour threshold (**Figure 3-52**) (30mg/L; Guth, 1997b). 2-phenylethanol was mostly found in the wines around its odour threshold (**Figure 3-54**) therefore it could be that it contributes in their bouquet a light scent of rose (14mg/L; Ferreira *et al.*, 2000). i-Butanol and hexan-1-ol were found in low concentrations (**Figure 3-51** & **3-53**) and could not play a role in the aroma of the wines (Ferreira *et al.*, 2000), although the amounts of the later were almost double for the vintage of 2009.

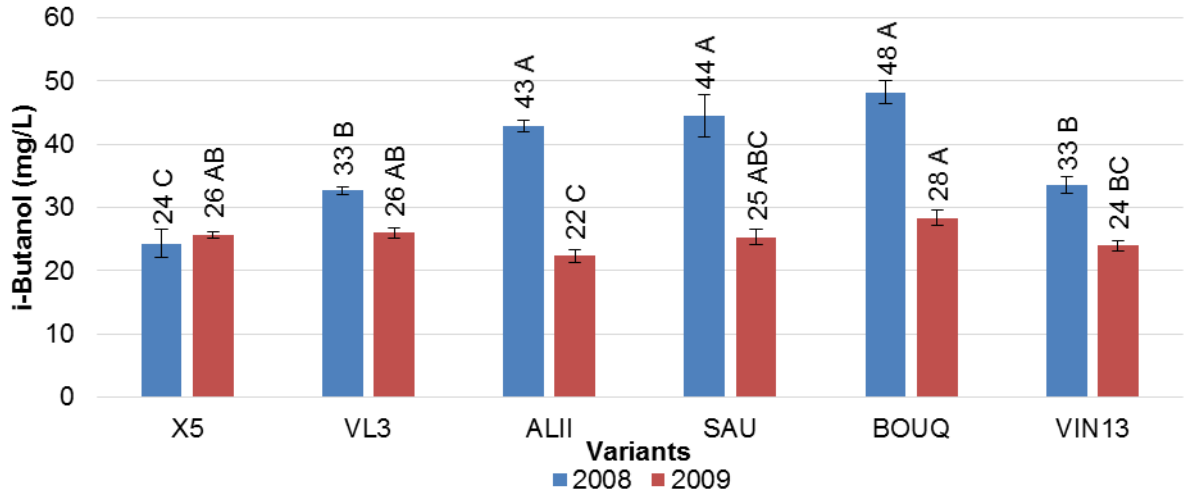


Figure 3-51: i-Butanol concentration of the finished Sauvignon blanc wines from six commercial yeast strains for the vintages 2008 and 2009. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

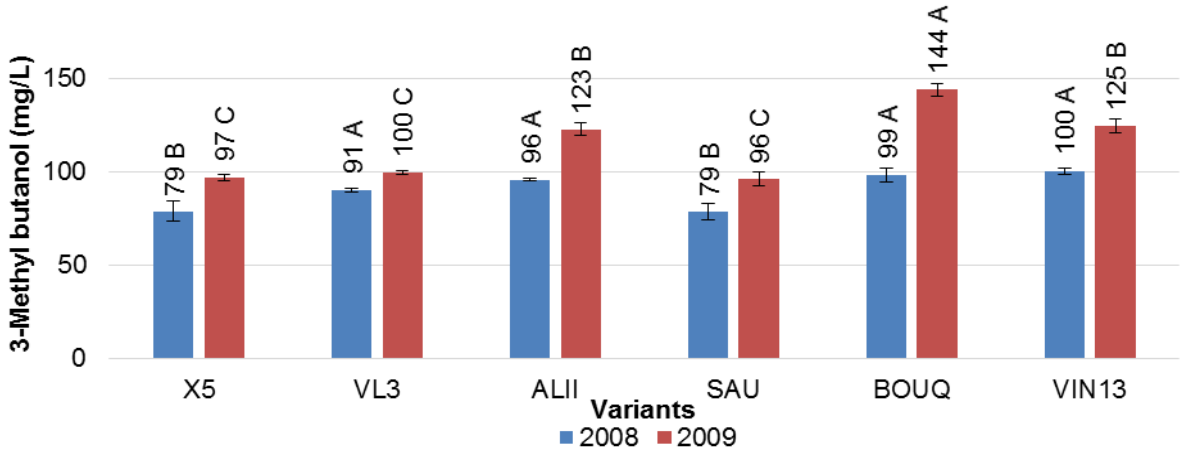


Figure 3-52: 3-methyl butanol concentration of the finished Sauvignon blanc wines from six commercial yeast strains for the vintages 2008 and 2009. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

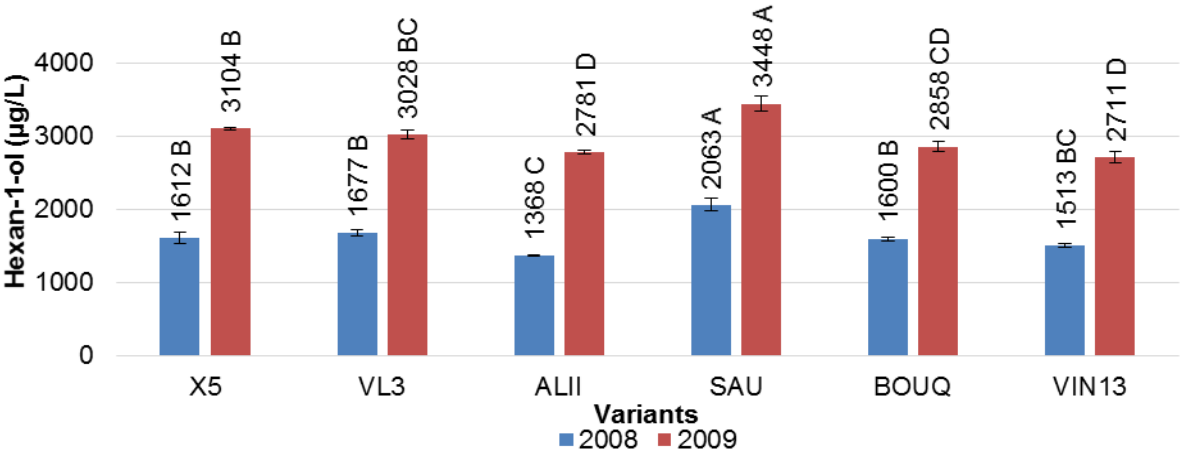


Figure 3-53: Hexan-1-ol concentration of the finished Sauvignon blanc wines from six commercial yeast strains for the vintages 2008 and 2009. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

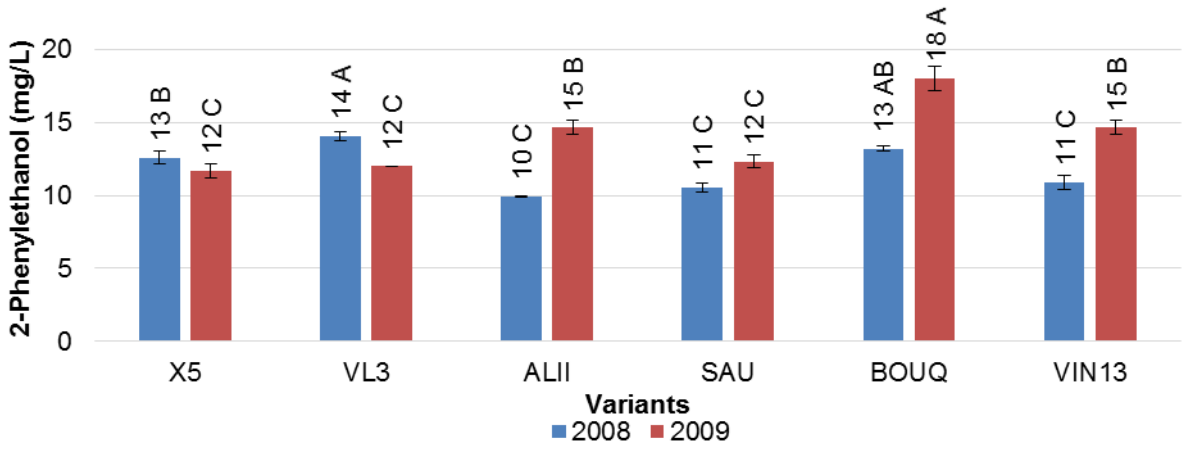


Figure 3-54: 2-phenylethanol concentration of the finished Sauvignon blanc wines from six commercial yeast strains for the vintages 2008 and 2009. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

3.2.2.3 Low boiling point sulphur compounds

Off-flavours showed a similar tendecy like in the wines from Scheurebe (**Chapter 3.2.1.4**). Therefore H_2S and MeSH were measured only in the wines from the 2009 vintage (**Figure 3-55** & **3-56**). Despite this fact the concentrations for both H_2S and MeSH were below their odour threshold. The only exception was X5 2009, which showed a MeSH content marginally above (Solomon *et al.*, 2010).

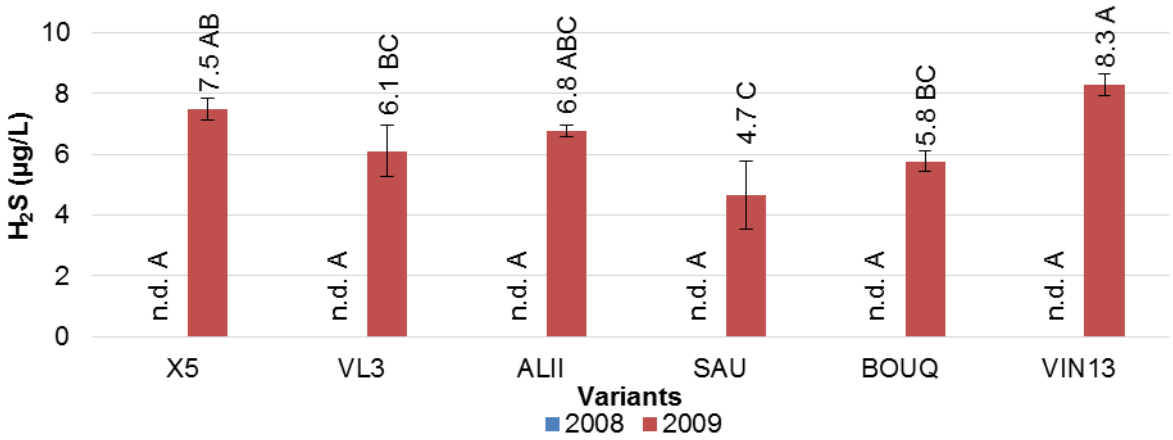


Figure 3-55: Hydrogen sulphide (H_2S) concentration of the finished Sauvignon blanc wines from six commercial yeast strains for the vintages 2008 and 2009. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

Taking into account the low YAN for 2009 (**Table 2-5**) this could offer an explanation why off-flavours were measured in the wines. The content of DMS found was below the odour threshold for all of the wines (**Figure 3-57**) (25 µg/L; Goniak & Noble, 1987). ALII 2008 and VIN13 2008 had significantly higher concentrations of DMS when compared but marginally below the threshold. The wines were not filtered prior to bottling and some variants could have had longer contact with that lees that could lead to higher concentrations of DMS as demonstrated by Niefind (1969). A summary of the results for the low boiling point sulphur compounds can be found in **Appendix 6-8**.

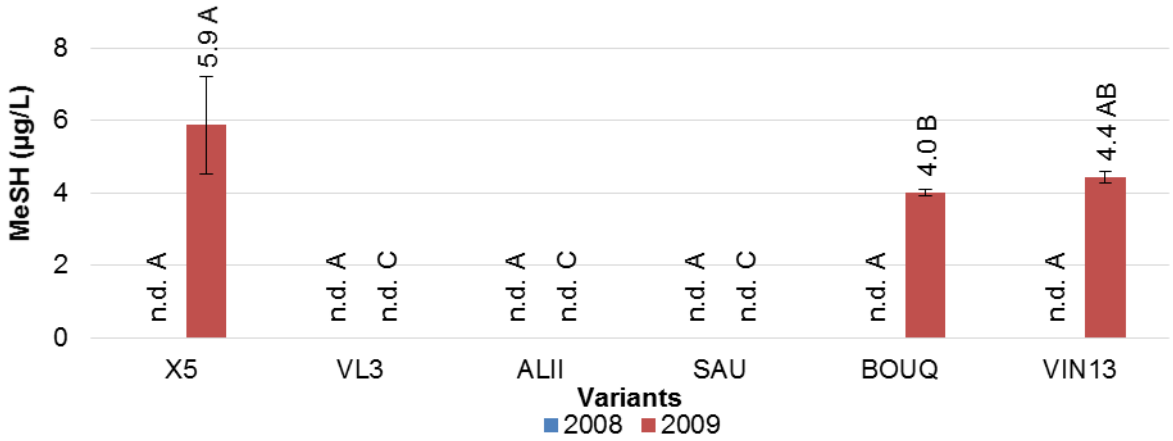


Figure 3-56: Methanethiol (MeSH) concentration of the finished Sauvignon blanc wines from six commercial yeast strains for the vintages 2008 and 2009. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

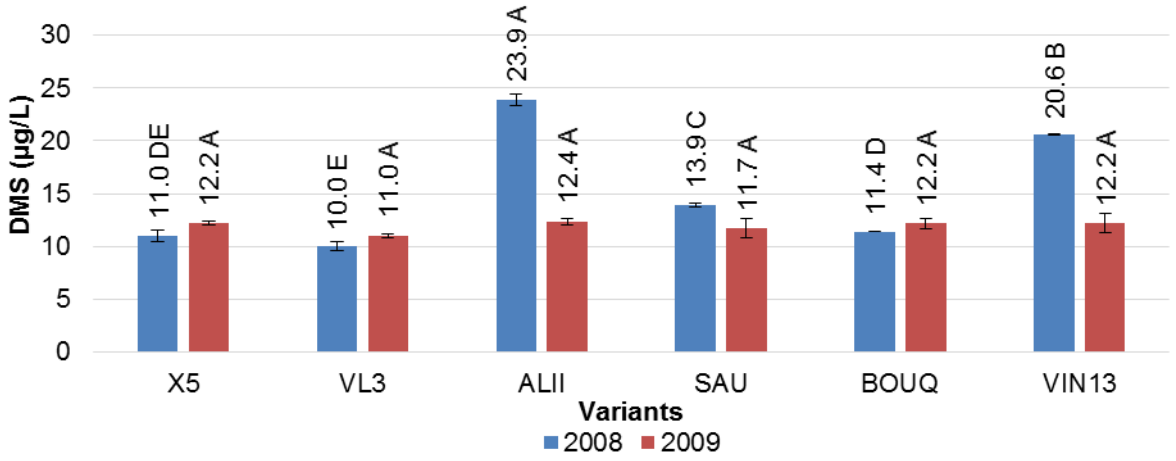


Figure 3-57: Dimethylsulphide (DMS) concentration of the finished Sauvignon blanc wines from six commercial yeast strains for the vintages 2008 and 2009. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

3.2.2.4 Varietal thiols

Varietal thiols (4MSP, 3SHA, 3SH) for both vintages of Sauvignon blanc were measured with the initial method as in described in **Chapter 2.2.2**.

The concentration of varietal thiols for the wines from Sauvignon blanc were much lower than those found in the wines from Scheurebe (**Chapter 3.2.1.5**). Similar to Scheurebe though, the wines from the vintage 2009 had much lower concentrations of 3SH and 3SHA when compared to the vintage 2008. Such high variability of varietal thiols has been reported by Dubourdieu & Tominaga (2009). 4MSP showed more consistent values over the two vintages (**Figure 3-58**). The amounts measured in the variants that 4MSP was detected were above the odour threshold of the compound 1 to 3 times (Darriet *et al.*, 2005). 3SH and 3SHA concentrations were significant for some of the variants reaching 76 and 67 times above their odour threshold respectively for the vintage 2008 (**Figure 3-59** & **3-60**). A summary of the results for the varietal thiols can be found in **Appendix 6-8**.

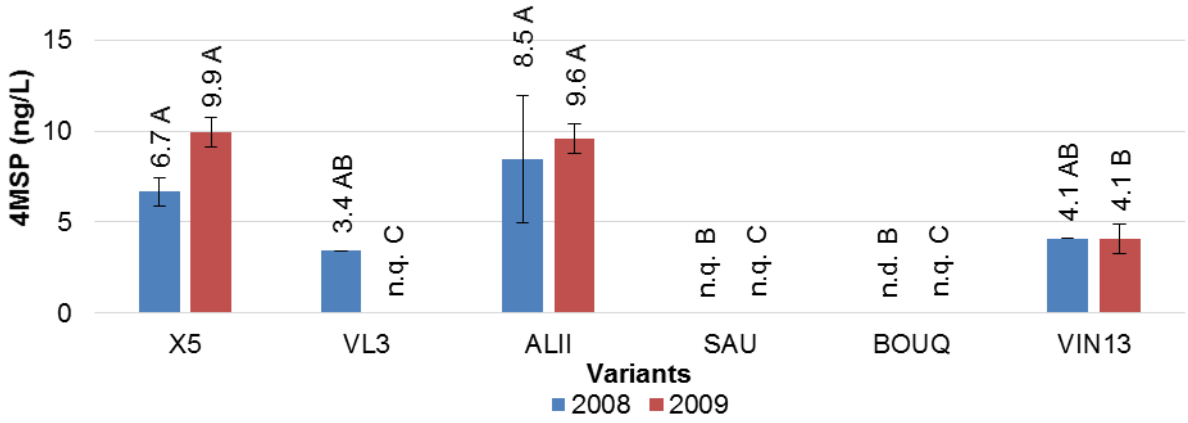


Figure 3-58: 4-methyl-4-sulfanylpentan-2-one (4MSP) concentration of the finished Sauvignon blanc wines from six commercial yeast strains for the vintages 2008 and 2009. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

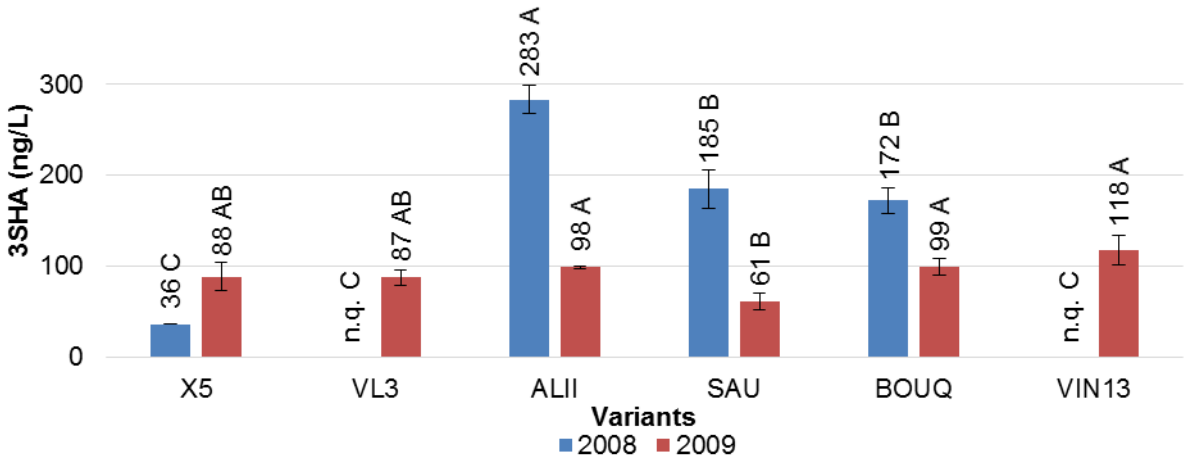


Figure 3-59: 3-sulfanyl-hexyl acetate (3SHA) concentration of the finished Sauvignon blanc wines from six commercial yeast strains for the vintages 2008 and 2009. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

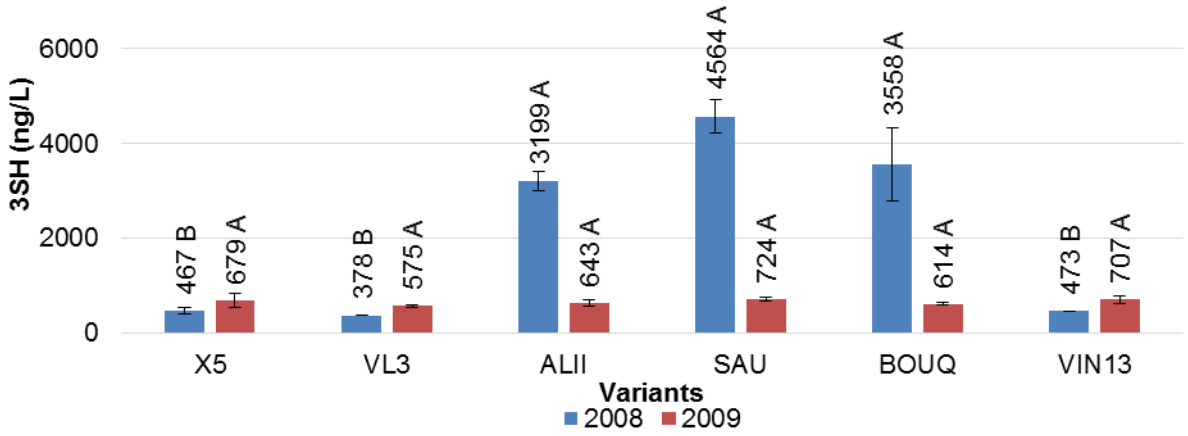


Figure 3-60: 3-sulfanylhexan-1ol (3SH) concentration of the finished Sauvignon blanc wines from six commercial yeast strains for the vintages 2008 and 2009. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

3.2.2.5 Sensory analysis

Before the wines were prepared for the tasting by the expert panel, they were pre-tasted ruling out any of them that were faulty. Furthermore, the most common descriptors mentioned by the tasters were noted as a help for the development of a tasting scheme. This was done for the wines from the 2008 vintage. All wines were found to be adequate for the tasting by the expert panel.

From **Figures 3-61** and **3-62** it can be seen the wines from the vintage 2008 and 2009 are very different in the intensity for some of the descriptors. There is variability for the descriptors ‘estery’ and ‘grapefruit’. As mentioned also for the tastings of the Scheurebe wines (**Chapter 3.2.1.7**) there was probably confusion among the tasters for the use of the descriptors ‘estery’ and ‘solvent’. It was shown from the analysis of the aroma compounds that the wines from 2009 had higher concentrations of acetic acid ethylester (**Figure 3-45**) and probably the tasters used this attribute to describe ‘estery’ instead of ‘solvent’. A further observation was that although the concentrations of varietal thiols was

lower for the wines from the vintage 2009, the perception of the tasters for these exotic notes were more intense for some variants when compared with the wines from 2008. Finally the descriptor ‘reduction’ may have not been used correctly because of the fact that all off-flavour compounds were below the odour threshould (**Chapter 3.2.2.3**). On the other hand a synergistic effect of these off-flavour compounds may intensify the reductive notes of a wine (Rauhut, 2009; Dittrich & Grossmann, 2011). A summary of the tasting scores for all the wines can be found in **Appendix 6-9**.

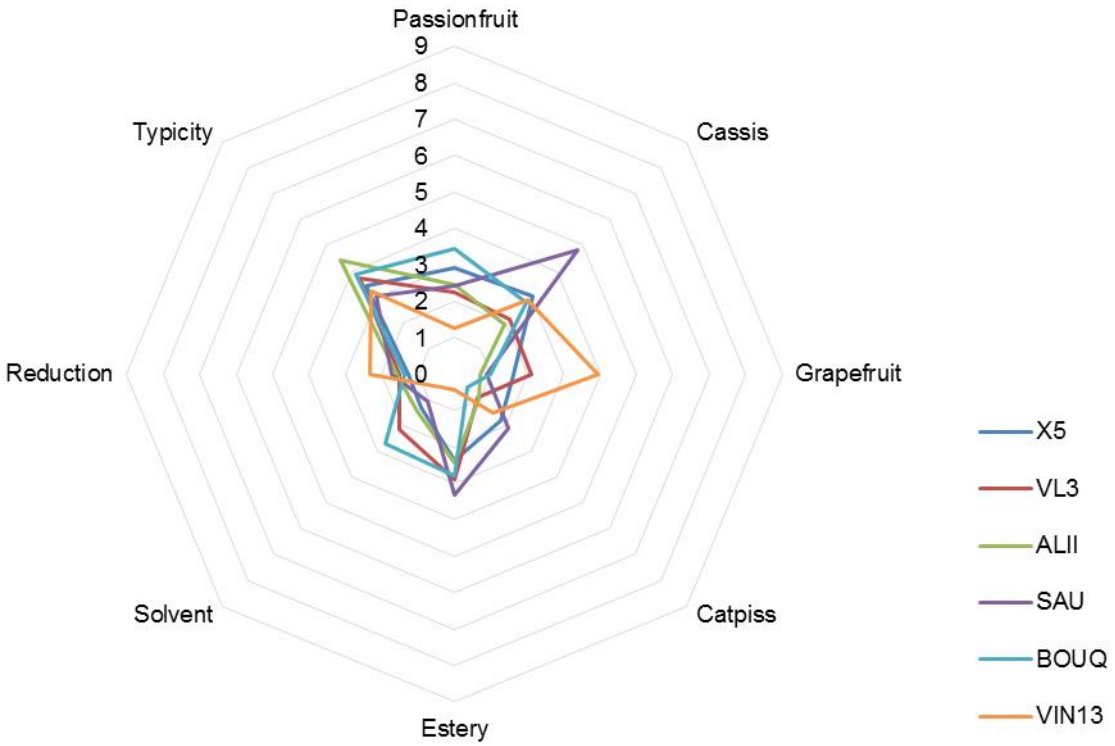


Figure 3-61: Spider plot for the six finished Sauvignon blanc wines of the vintage 2008 that were tasted by the tasting panel.

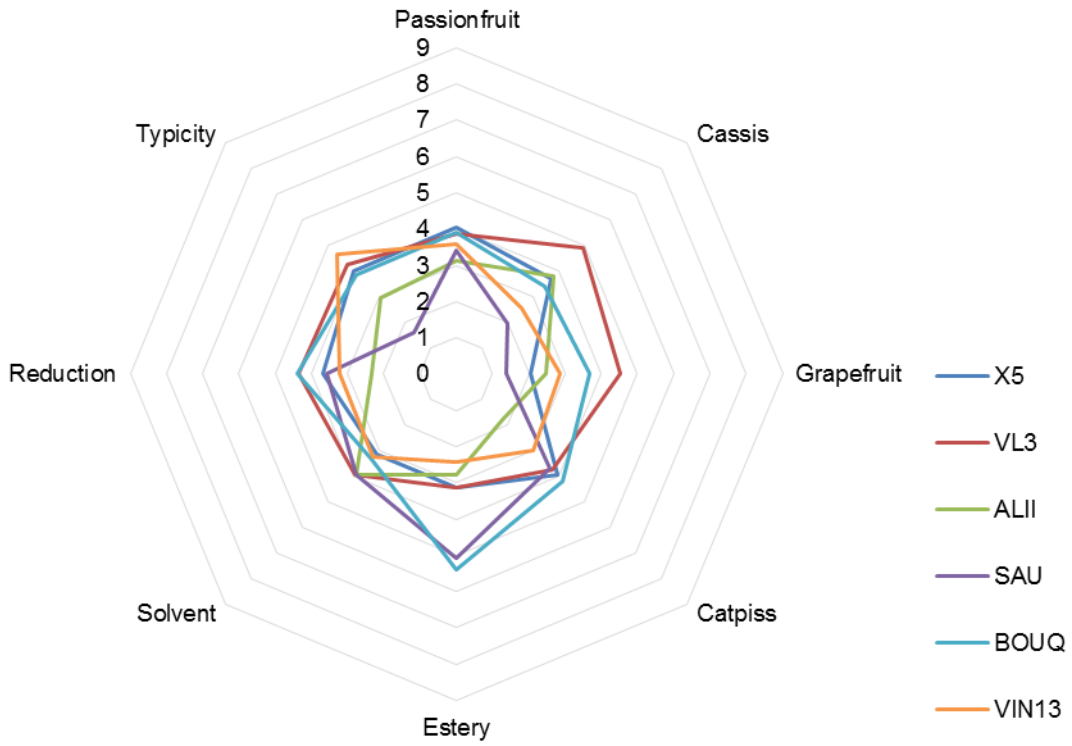


Figure 3-62: Spider plot for the six finished Sauvignon blanc wines of the vintage 2009 that were tasted by the tasting panel.

3.2.2.6 Statistical analysis

PCA plotting was used for analysing the data statistically. The OAVs were used for estimating the contribution of the aroma compounds to the aroma of the wines (Guth, 1997b; Ferreira *et al.*, 2000; Gómez-Míguez *et al.*, 2007). The data were mean centered and autoscaled before analysis (van den Berg *et al.*, 2006). A summary of the OAVs can be found in **Appendix 6-10.**

The PCA plot (**Figure 3-63**) for the sensory descriptors explained 69.55 % of the variability in the data. F1 accounted for 49.73 % of the variability and was heavily loaded with the descriptors ‘passionfruit’, ‘catpiss’, ‘estery’, ‘solvent’ and ‘reduction’ in the positive direction. F2 accounted for 19.82 % of the variability and was heavily loaded with the descriptor ‘grapefruit’ in the positive direction.

The wines from the vintage of 2008 built a cluster (except for VIN13 2008) and did not correlate with the descriptors. On the contrary the wines from the vintage 2009 have a better correlation with the descriptors (**Figure 3-63**). Variants X5 2009, VL3 2009 and BOUQ 2009 are well described by F1. This lack of correlation of the wines from the vintage of 2008 is probably similar to the hypothesis that was established for Scheurebe in **Chapter 3.2.1.7.** High concentrations of varietal thiols could lead to unpleasant aromatics for the wines (Darriet *et al.*, 1995). Finally the descriptor ‘typicity’ did not seem to be well understood by the tasters and it was decided to be abolished from further tastings.

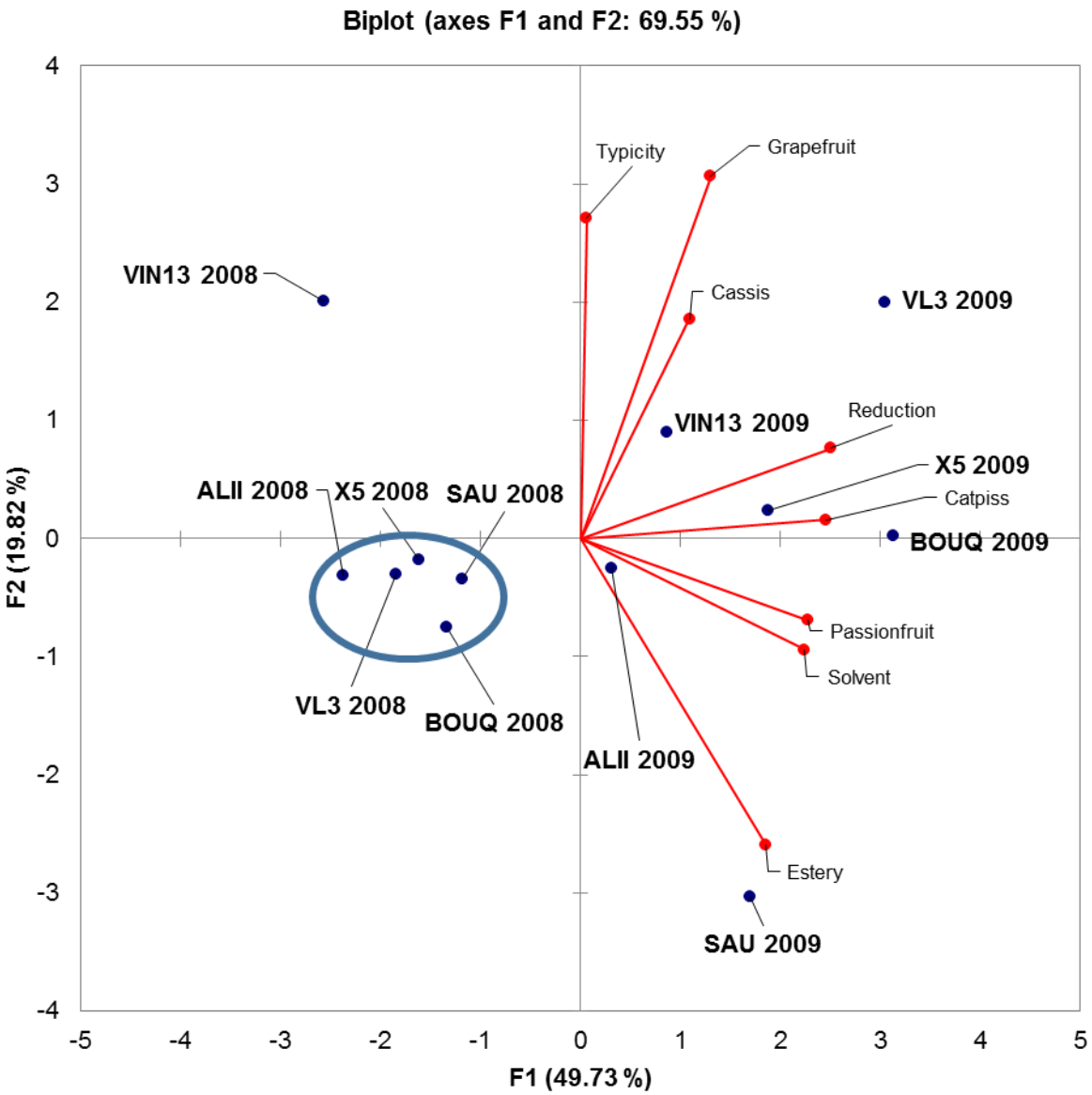


Figure 3-63: Principal Component Analysis (PCA) for the tasting results for the Sauvignon blanc wines from the vintages 2008 & 2009.

For the PCA for the aromatic compounds and the tasting attributes it was chosen to plot only the compounds that showed OAV values above 1 in at least one of the variants. The PCA on F1 and F2 explained 54.97 % of the variability. F1 accounted for 41.54 % of the variability and was more heavily loaded with the attributes 'reduction', 'catpiss' and 'H_2S' in the positive direction. F2 accounted for 13.43 % of the variability and was more heavily loaded with the aroma compound 'acetic acid 3-methylbutyl ester' and the attribute 'grapefruit' in the positive direction. From the plot (**Figure 3-64**) can be seen that the wines from the vintage 2008 and 2009 are clearly separated although no clusters were formed. Again 'typicity' posed a problem as none of the wines could be explained with that descriptor. F3 was heavily loaded with 'typicity' in the positive direction and VL3 2008 only was explained by F3 but in the negative direction (data not shown).

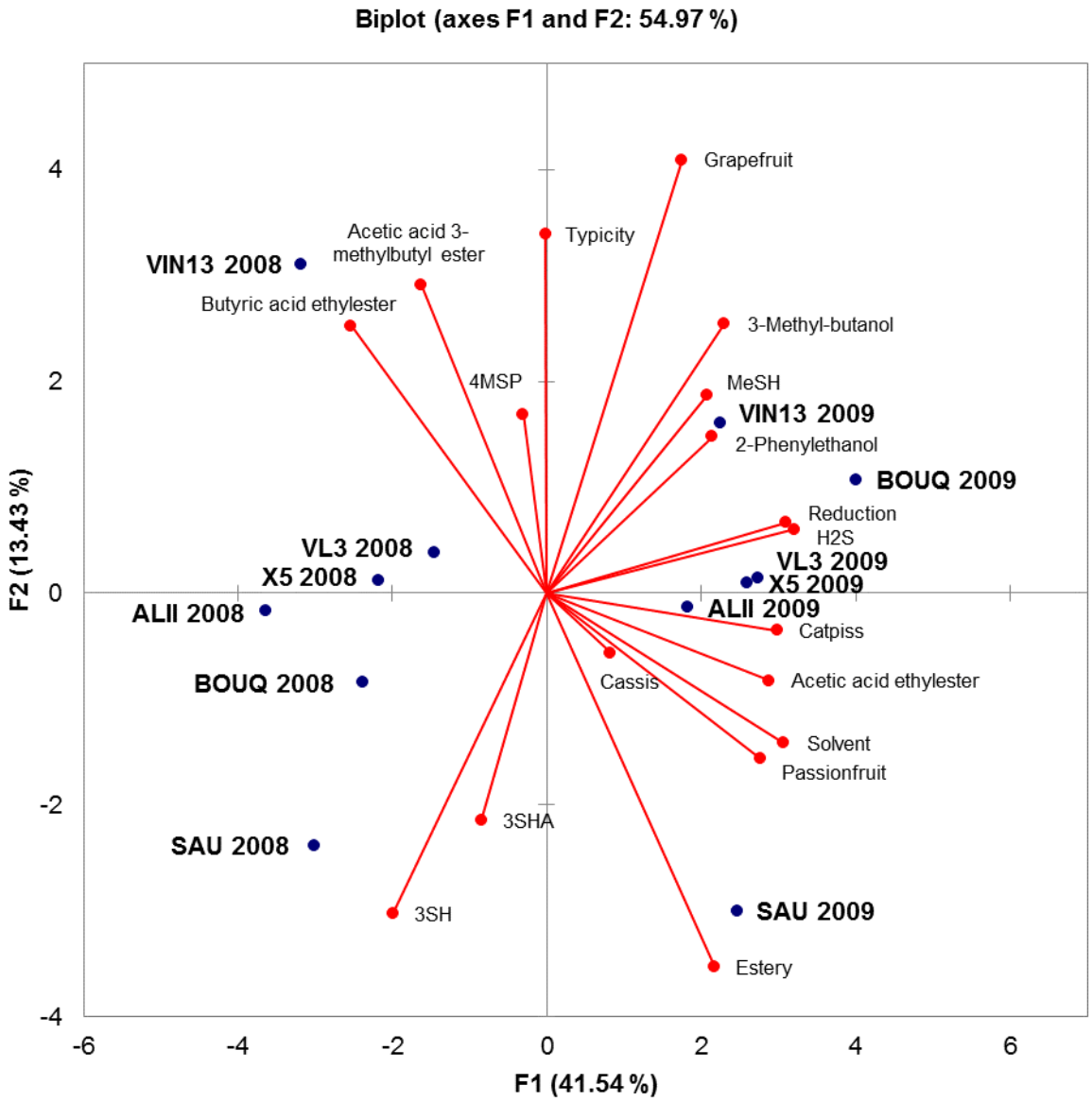


Figure 3-64: Principal Component Analysis (PCA) of the Scheurebe of the 2008 & 2009 vintage with the aromatic compounds that had an OAV > 1 in at least one of the variants. The data were mean centered and autoscaled before analysis.

3.2.2.7 Discussion and conclusion

The goal of this work package was to ferment two Sauvignon blanc musts of the vintages 2008 and 2009 with various commercial yeast strains, without the addition of nutrients, to induce high concentrations of varietal thiols and to develop a tasting scheme for further experiments.

The must from Sauvignon blanc showed for the 2009 vintage a YAN deficiency. The YAN was measured at 207 mg N/L, which was significantly less than the suggested 275 mg N/L by Dukes & Butzke (1998) for a sugar concentration of 230 g/L. This YAN deficiency was probably the reason for more than half of the fermentations not being completed (**Figure 3-41**), while also inducing the production of reductive sulphur compounds (**Figures 3-55** & **3-56**) (Ribéreau-Gayon *et al.*, 1999; Dittrich & Grossmann, 2011). The wines from the vintage 2008 also had incomplete fermentations, but

did not show residual sugar as the fermentations from 2009. Variant SAU 2009 was probably contaminated with bacteria during inoculation, and had elevated levels of lactic acid and significantly decreased levels of malic acid which suggest a partial MLF.

Ester production was variable in the young wines of the two different vintages. Acetic acid ethylester was in higher concentrations in the vintage 2009, with the variants ALII 2009 and SAU 2009 being above the odour threshold of 160 mg/L (Ribéreau-Gayon *et al.*, 1999). It has been shown that higher sugar concentrations in musts lead also to higher concentrations in acetic acid ethylester (Dittrich & Grossmann, 2011). SAU 2009 showed the highest concentration in acetic acid ethylester (203 mg/L; **Figure 3-46**) which is probably attributed to the contamination of the must during inoculation. It was suggested by Bandion & Valenta (1977) that wines containing more than 200 mg/L acetic acid ethylester should be considered as spoiled although their VA might not exceed 0.8 g/L. The fruity ester acetic acid 3-methylbutylester showed high variability, with some variants showing high concentrations and others none (**Figure 3-47**). Nevertheless, for the variants that this ester was detected it was above its odour threshold of 30 µg/L (Guth, 1997b) in concentrations up to 30-fold higher. Lactic acid ethylester was not found in relevant concentrations (odour threshold 155 mg/L; Etiévant, 1991) but the bacterial contamination for variant SAU 2009 was evident with a concentration about four times higher when compared with the other fermentations (**Figure 3-50**).

The measured higher alcohols were much more consistent than the esters for the young wines from both vintages. 3-Methyl butanol and 2-phenylethanol were in concentrations high enough to contribute to the aromatics of the wines. 3-Methyl butanol was measured between 2.6 and 4.8 times above its odour threshold, giving to the wine its vinous character (30 mg/L, Guth 1997b). 2-phenylethanol was found in all the variants around its odour threshold, so this compound probably contributes to the bouquet of the wines with a characteristic scent of roses (14 mg/L; Ferreira *et al.*, 2000).

Characteristic compounds that give reductive notes to the wines (especially H_2S and MeSH) were only measured in wines from the vintage 2009. As mentioned before, the most probable cause for that was the nitrogen deficiency of the must from the 2009 vintage. Nevertheless, all of the measured concentrations were below the odour threshold of the compounds and only MeSH in the variant X5 2009 was marginally above (5 µg/L; Solomon *et al.*, 2010). The concentrations of DMS were significantly below the odour threshold for all the wines (25 µg/L; Goniak & Noble, 1987) except for the variants ALII 2008 and VIN13 2008, which were measured in concentrations marginally below that. One possible explanation for this was the contact of the finished wines with the lees. The wines were not filtered prior to bottling, which means that some variants could have had longer contact with the lees leading to higher concentrations of DMS as demonstrated by Niefind (1969).

Varietal thiols were measured in significant concentrations in the young Sauvignon blanc wines from both vintages. Both vintages of X5 and ALII showed concentrations of 4MSP between two and three times above its odour threshold (3 ng/L; Darriet *et al.*, 1995) and were the higest among all the variants. Most of the wines from the vintage 2009 showed much lower concentrations of 3SH and 3SHA. For the yeasts ALII, SAU and BOUQ, the concentrations were two to six times lower for the vintage of 2009. Nevertheless, in the variants where 3SH and 3SHA was detected the concentrations

were well above their odour threshold. SAU 2008 showed concentrations 76 times above the odour threshold of 3SH and ALII 2008 67 times above the odour threshold of 3SHA (60 ng/L and 4.2 ng/L respectively; Dubourdieu & Tominaga, 2009).

As mentioned in **Chapter 3.2.1.8**, it can be noted that a significant nitrogen deficiency in the musts probably leads to lower concentrations of varietal thiols and higher concentrations of off-flavours. By adjusting the must to a certain but not significant deficiency, as was the case for the Sauvignon blanc must from 2008, the concentrations of varietal thiols seem to increase and off-flavours decrease or are not produced at all. Similar findings were published by Peyrot des Gachons *et al.* (2005), who stated that a higher nitrogen status in the vines leads to higher concentrations of varietal thiol precursors, although the role of nitrogen on thiol release is not known. During the release of varietal thiols, ammonium is released and probably the nitrogen levels of the musts plays a role (Bell & Henschke, 2005). The probable role of nitrogen in musts and its effects on the release of varietal thiols were addressed in Chapter **3.2.1.8**.

The sensory analysis showed that the wines from the vintage 2009 were aromatically more intense in many of the attributes asked to the tasters (**Figures 3-61** & **3-62**). Furthermore, the statistical analysis of the tasting scores (**Figure 3-63**) showed that the wines from the 2008 vintage did not correlate very well with the descriptors, whereas the wines of the 2009 vintage correlated better. For the wines from Sauvignon blanc the descriptor ‘typicity’ seems to have been confusing for the tasters or was not very well understood, proving to be almost not of use.

Finally, by evaluating the training and tasting scheme for future tastings the following conclusions could be drawn:

The descriptors ‘solvent’ and ‘estery’ were probably confusing for the tasters, as in the case of Scheurebe, although it was explained that ‘solvent’ is for acetic acid ethylester and ‘estery’ for the fruity and sweet esters (e.g. acetic acid 3-methylbutylester). Due to this fact, for future tastings it was decided to use only the attribute ‘estery’ for describing the impression of acetic acid ethylester. This was also decided for the descriptor ‘typicity’.

3.3 Greek varieties pre-selection

Seventeen Greek commercial wines were collected from specialized shops around Germany. Fifteen of the wines were from three autochthonous Greek grape varieties (6 Malagousia, 5 Asyrtiko, 4 Roditis), one was a blend of Asyrtiko/Malagousia and a monovarietal Sauvignon blanc also produced in Greece that was used as a control/benchmark wine (**Table 2-7**).

3.3.1 Major components

For Malagousia, Tourtoglou *et al.* (2014) reported alcohol contents ranging from 11.6 % Vol. to 13.9 % Vol. Therefore the values measured for the studied wines could be considered as normal (**Figure 3-65**). All of the wines have fermented to almost dry except for MA2 that had 10.9 g/L residual sugars (**Figure 3-66**). The residual sugars of MA2 was also reported during sensorial analysis by the tasters. Volatile acidity (VA) was measured at normal levels for white wines except for MA6 and R3 **(Figure 3-67)**. MA6 was from the vintage 2006 and the elevated value of VA of 0.8 g/L was probably

attributed to the age of the wine. Esters can hydrolyse during ageing and the respective acetates release small amounts of acetic acid increasing the VA (Ribéreau-Gayon *et al.*, 1999). R3 showed a VA value of 0.9 g/L, which is very high for a young white wine since at the time of tasting it was 12 months old. The probable cause for this increased value was a spontaneous MLF (**Figure 3-68** & **3-69**). A summary of the results for the major components can be found in **Appendix 6-11**.

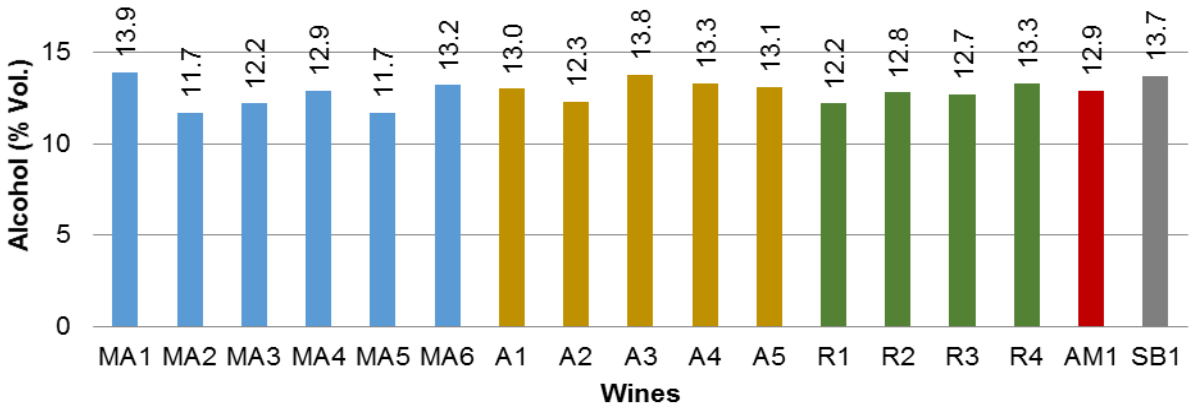


Figure 3-65: Alcohol content of the 17 Greek commercial wines. Each colour represents a different group of wines from different varieties.

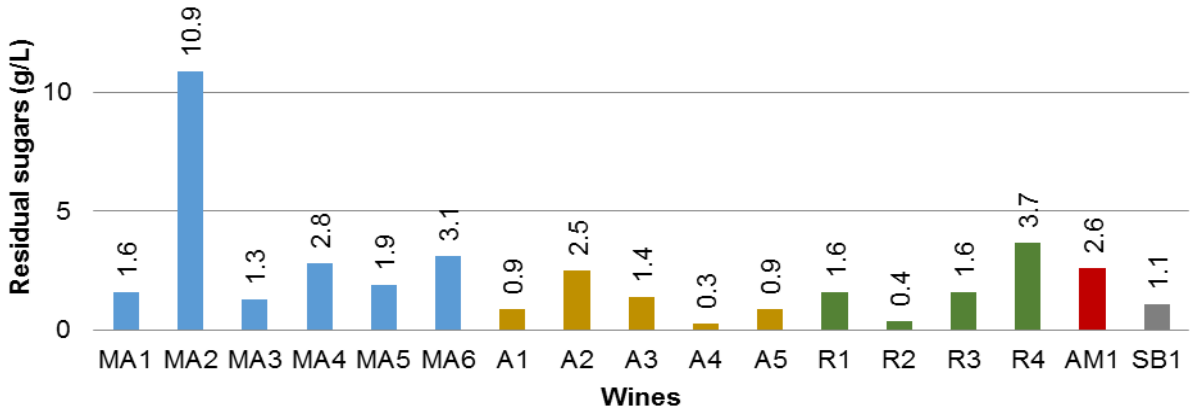


Figure 3-66: Residual sugar concentration of the 17 Greek commercial wines. Each colour represents a different group of wines from different varieties.

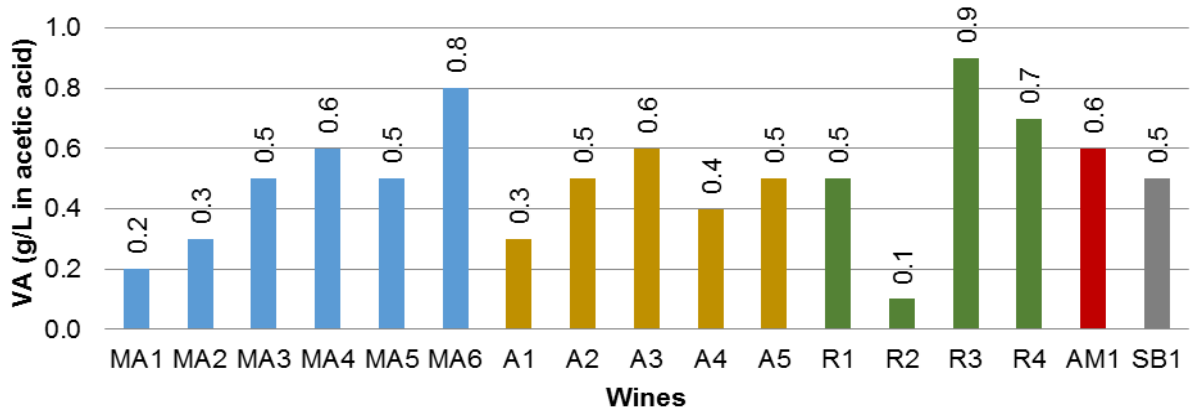


Figure 3-67: Volatile acidity (VA) of the 17 Greek commercial wines. Each colour represents a different group of wines from different varieties.

For wine R3 no malic acid was detected but a lactic acid concentration of 1.1 g/L was measured (**Figure 3-68** & **3-69**). These two facts and the elevated VA probably indicate that the MLF was carried out by heterofermentative lactic acid bacteria (Dittrich & Grossmann, 2011). For the rest of the wines no MLF could be determined as the values for lactic acid are very low.

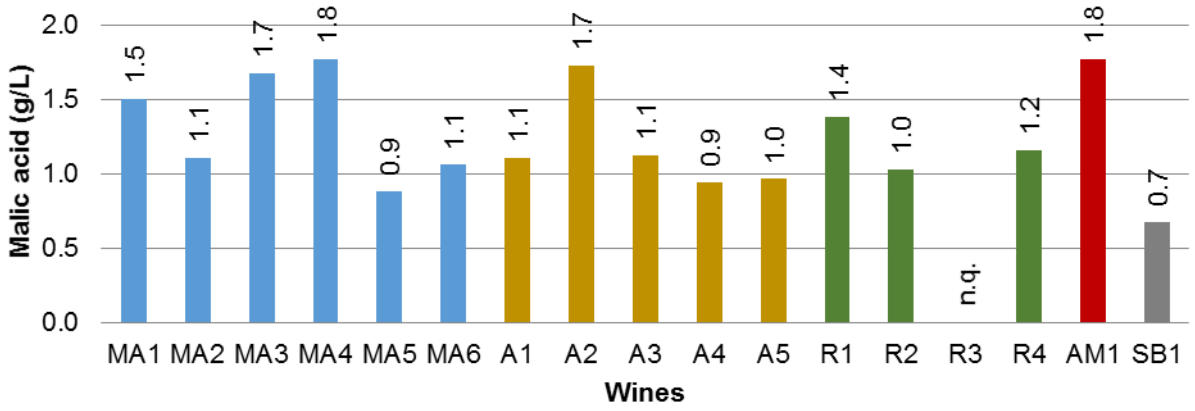


Figure 3-68: Malic acid concentration of the 17 Greek commercial wines. Each colour represents a different group of wines from different varieties.

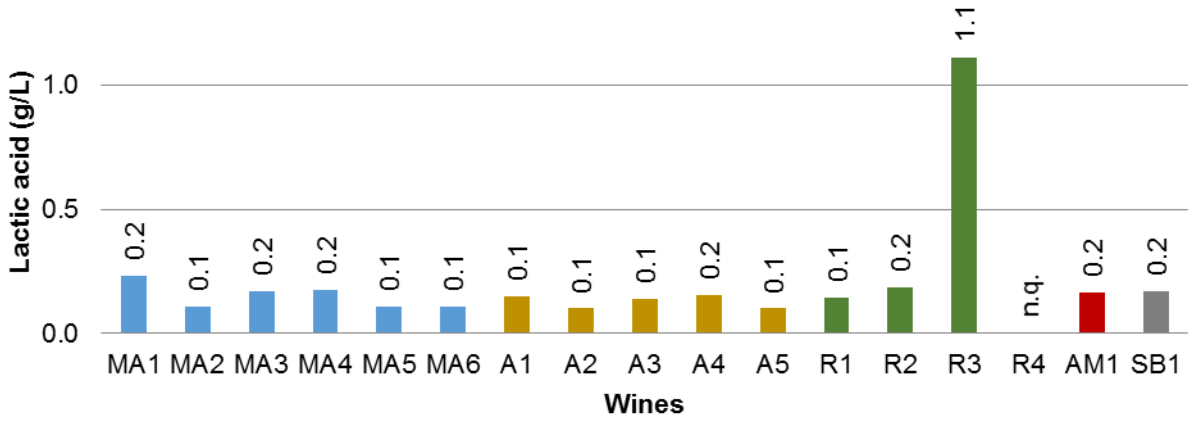


Figure 3-69: Lactic acid concentration of the 17 Greek commercial wines. Each colour represents a different group of wines from different varieties.

3.3.2 Esters and higher alcohols

A summary of all the measured compounds with the 'Kaltron' method can be found in **Appendix 6-12.**

Esters: Acetic acid ethylester concentrations were highly variable. All of the Malagousia wines showed concentrations constantly below the odour threshold (**Figure 3-70**). Despite this fact it has been mentioned that concentration of acetic acid ethylester below the odour threshold could contribute to the aromatic complexity of a wine (Ribéreau-Gayon *et al.*, 1999). Some of the wines though (A3, R3, R4 and AM1) had contents of acetic acid ethylester above the odour threshold (160 mg/L; Ribéreau-Gayon *et al.*, 1999). R3 showed the highest concentration with 203 mg/L and this was probably attributed to the spontaneous MLF. Acetic acid 3-methylbutylester concentrations also showed to be highly variable (**Figure 3-71**) and in most cases they were above the odour threshold (30 μg/L; Guth, 1997b). The probable spontaneous MLF of wine R3 was evident in the values of lactic

acid ethylester (**Figure 3-74**). R3 showed three times higher concentration of this particular ester, which is produced during MLF (Ribéreau-Gayon *et al.*, 1999; Dittrich & Grossmann, 2011), although its concentration was below the odour threshold (155 mg/L; Etiévant, 1991). Butyric acid ethylester showed some variability but all the wines showed contents above the odour threshold (**Figure 3-73**) (20 μg/L; Ferreira *et al.*, 2000; Fretz *et al.*, 2005a). Both acetic acid 2-phenylethylester and succinic acid diethylester were found in concentrations significantly below their odour threshold (**Figure 3-72** & **3-75**). What should be mentioned though was the significantly higher concentration of succinic acid diethylester in wine R3 when compared with the other wines.

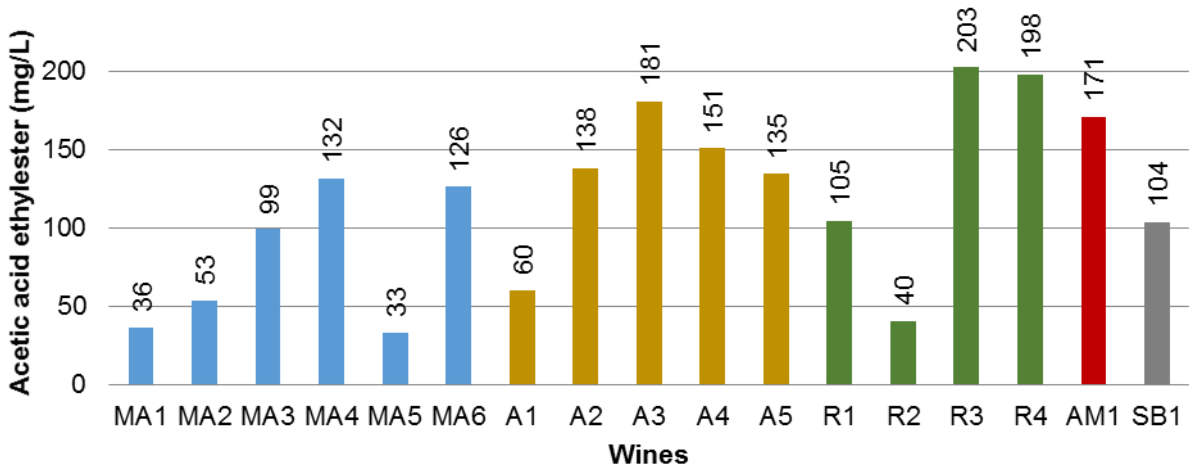


Figure 3-70: Acetic acid ethylester concentration of the 17 Greek commercial wines. Each colour represents a different group of wines from different varieties.

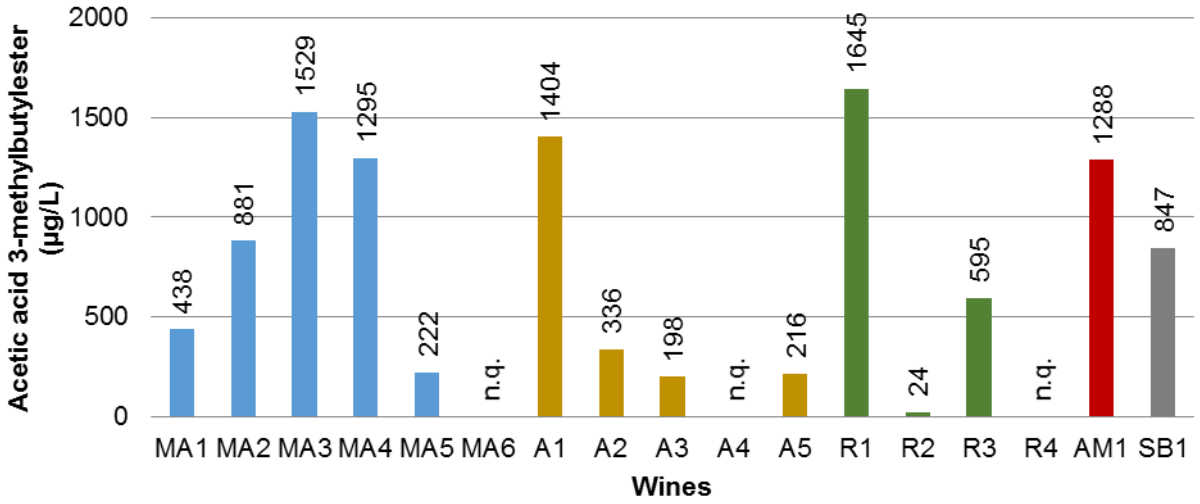


Figure 3-71: Acetic acid 3-methylbutylester concentration of the 17 Greek commercial wines. Each colour represents a different group of wines from different varieties.

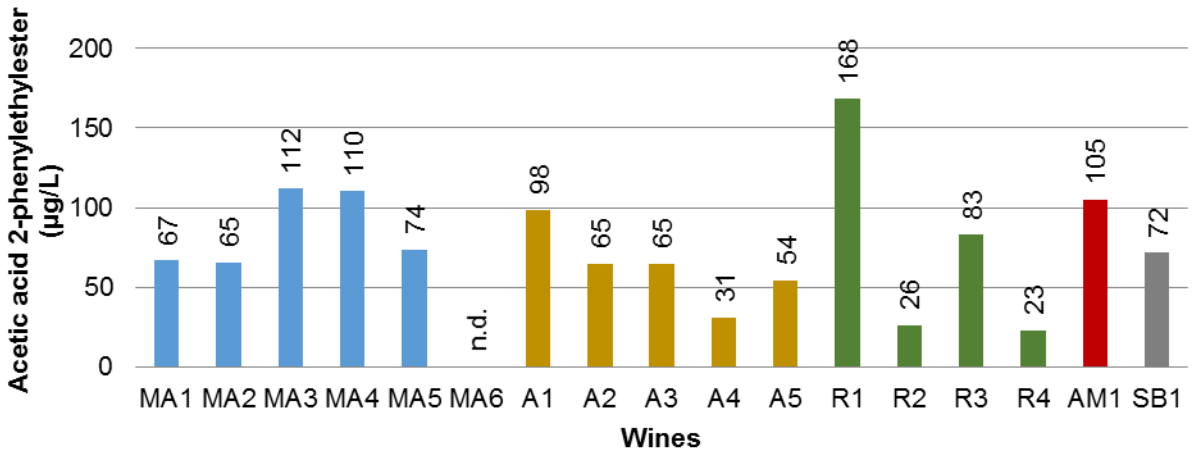


Figure 3-72: Acetic acid 2-phenylethylester concentration of the 17 Greek commercial wines. Each colour represents a different group of wines from different varieties.

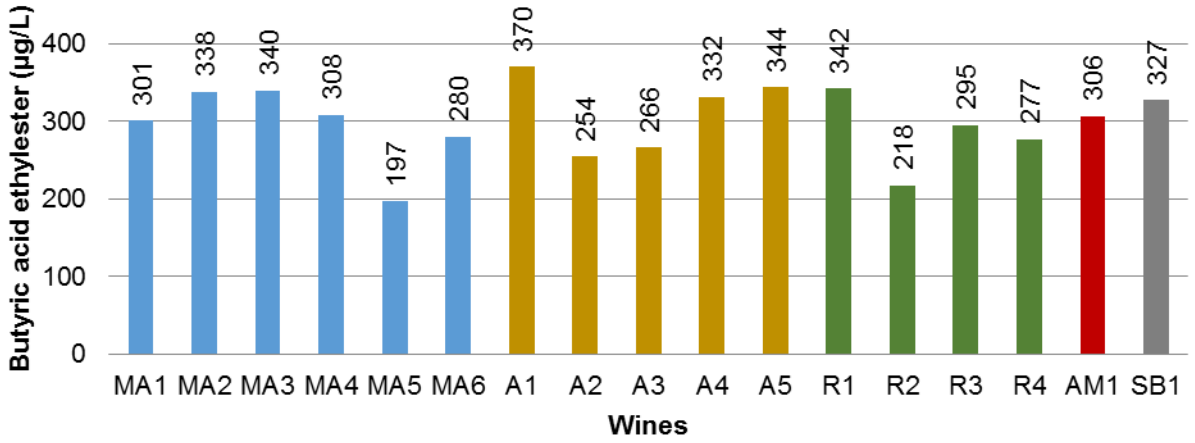


Figure 3-73: Butyric acid ethylester concentration of the 17 Greek commercial wines. Each colour represents a different group of wines from different varieties.

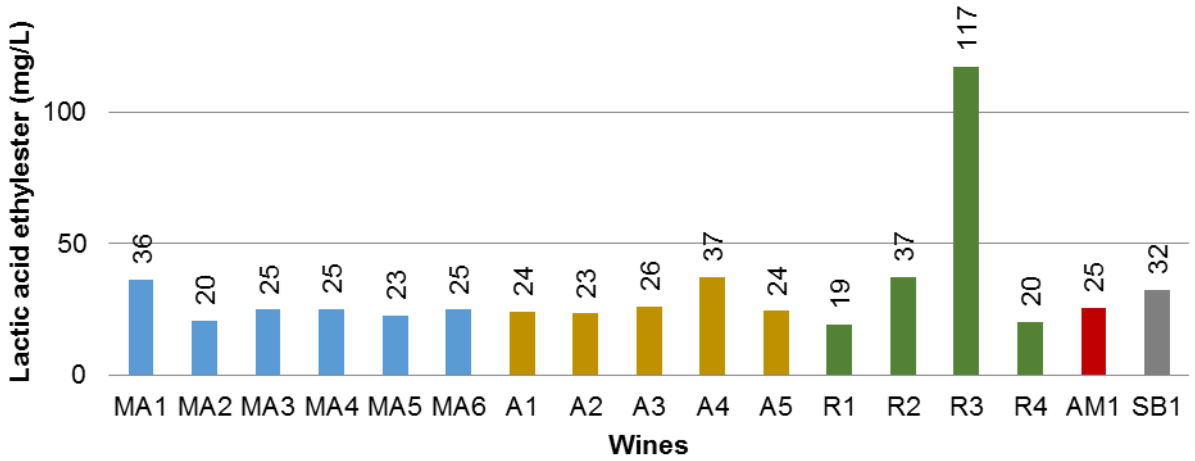


Figure 3-74: Lactic acid ethylester concentration of the 17 Greek commercial wines. Each colour represents a different group of wines from different varieties.

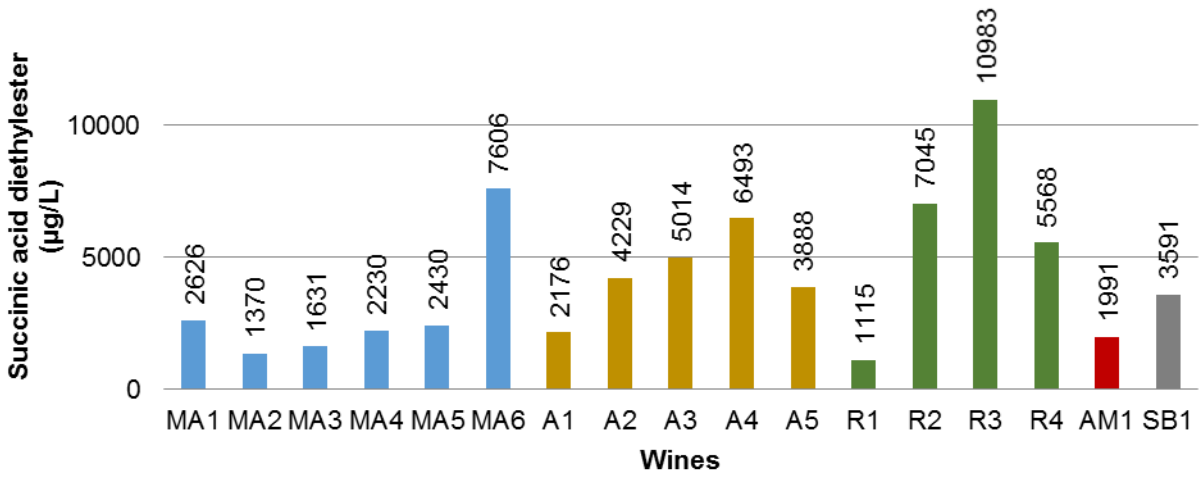


Figure 3-75: Succinic acid diethylester concentration of the 17 Greek commercial wines. Each colour represents a different group of wines from different varieties.

Higher alcohols: Two higher alcohols**,** 3-methyl butanol and 2-phenylethanol were measured in high enough concentrations to contribute to the aromatics of the wines (**Figure 3-77** & **3-79**). 3-Methyl butanol was measured in all of the wines at least 3.5 above the odour threshold of 30 mg/L and plays a role in the "vinous" character of a wine (Guth, 1997b). 2-phenylethanol was present in highly variable concentrations in all of the wines. Some wines had concentrations around- and others like MA5 had significant concentrations of about 3 times the odour threshold (14 mg/L; Ferreira *et al.*, 2000). i-Butanol and hexan-1-ol were found in concentrations singnificantly below their odour threshold (**Figure 3-76** & **3-78**).

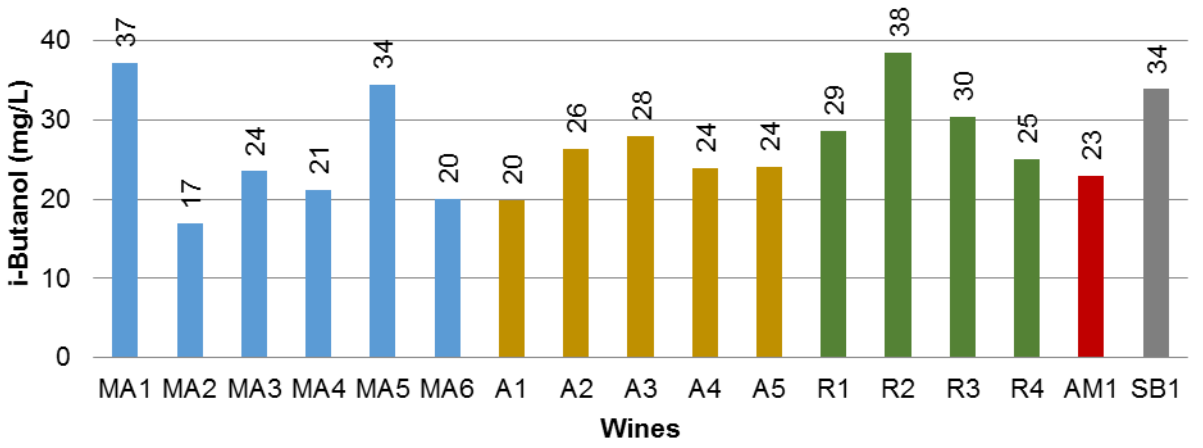


Figure 3-76: i-Butanol concentration of the 17 Greek commercial wines. Each colour represents a different group of wines from different varieties.

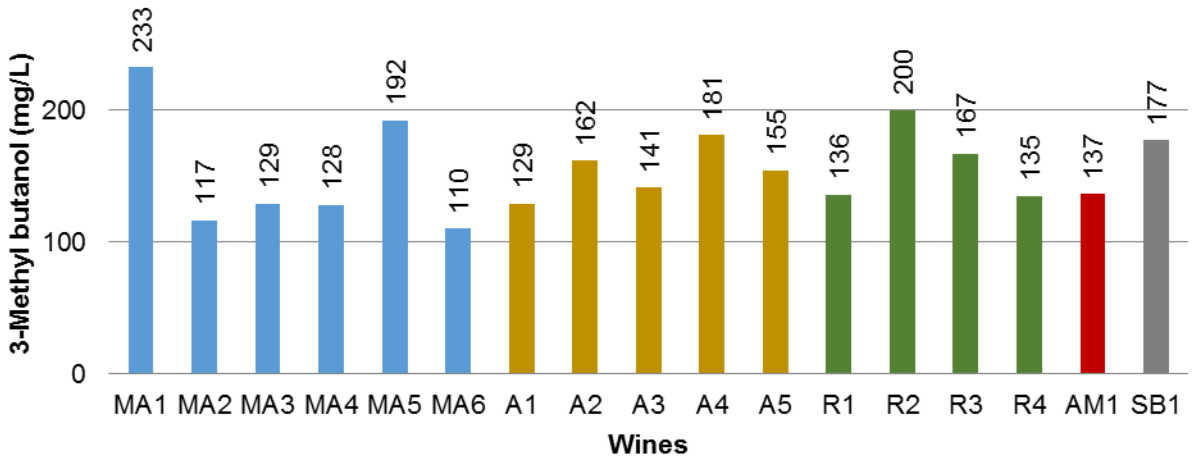


Figure 3-77: 3-methyl butanol concentration of the 17 Greek commercial wines. Each colour represents a different group of wines from different varieties.

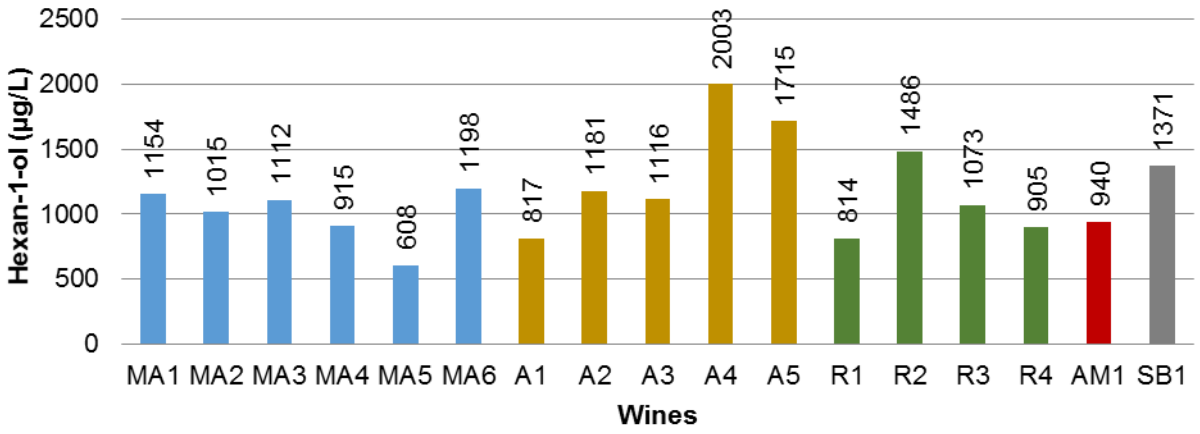


Figure 3-78: Hexano-1-ol concentration of the 17 Greek commercial wines. Each colour represents a different group of wines from different varieties.

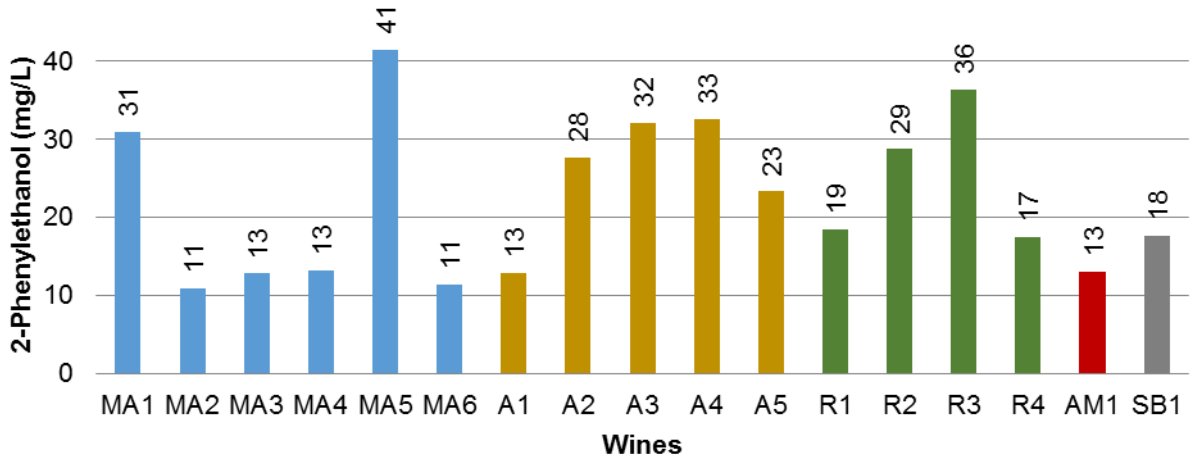


Figure 3-79: 2-phenylethanol concentration of the 17 Greek commercial wines. Each colour represents a different group of wines from different varieties.

3.3.3 Monoterpenes

Monoterpene concentrations in Malagousia wines have only been previously reported once, by a study from Metafa & Economou (2013). From the results below it can be seen that Malagousia wines showed high concentrations of monoterpenes except for wine MA6 (**Figure 3-80** & **3-81**). Wine MA2 showed not only significant amounts of linalool (8 times above odour threshold) but also α-terpineol (1.5 times above odour threshold) (25 µg/L and 250 µg/L respectively; Ferreira *et al.*, 2000). This was probably the reason for MA2 being characterized with a strong 'floral' character during sensory analysis (**Chapter 3.3.6**). Wines from the variety Asyrtiko and Roditis showed very low concentrations of monoterpenes that did not contribute to the aromatics of the wines. For wine AM1 the amounts of linalool measured probably are contributed from the Malagousia part of the wine. A summary of all the measured compounds with the 'Kaltron' method can be found in **Appendix 6-12.**

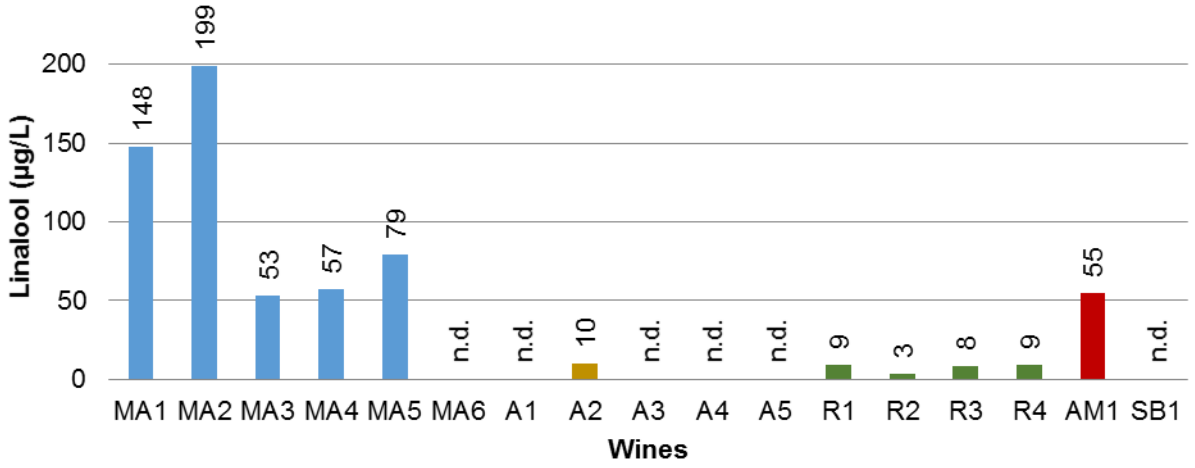


Figure 3-80: Linalool concentration of the 17 Greek commercial wines. Each colour represents a different group of wines from different varieties.

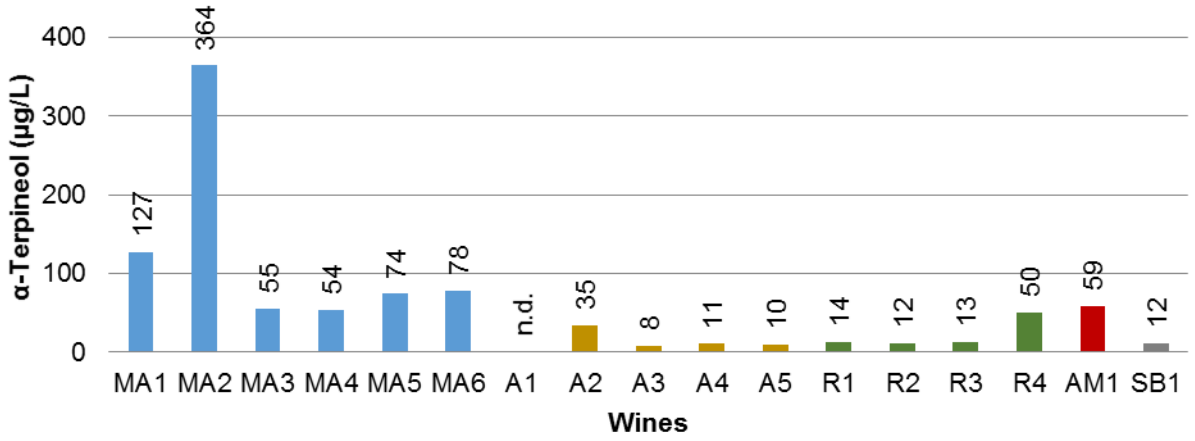


Figure 3-81: α-Terpineol concentration of the 17 Greek commercial wines. Each colour represents a different group of wines from different varieties.

3.3.4 Low boiling point sulphur compounds

The concentrations of low boiling point sulphur compounds were highly variable and some of the wines showed significant concentrations in both H_2S and MeSH. The highest concentrations for H_2S

were measured in wines MA4, R4 and AM1 (**Figure 3-82**), which were as high as 1.8 above the odour threshold (10 µg/L; Dittrich & Grossmann, 2011). MeSH was significant in wines MA4, R2, R3 and AM1 being around 2 times above the odour threshold (**Figure 3-83**) (Solomon *et al.*, 2010). Despite these concentrations in reductive notes none of the wines was characterised by a high score for the descriptor 'reduction' during sensory analysis (**Chapter 3.3.6**). DMS was found in significant amounts in wines MA6 and SB1 (**Figure 3-84**). Both of these wines were from older vintages (2006 and 2008 respectively) and the concentrations of DMS increase during ageing (Marais, 1979; Goto & Takamuto, 1987). In both wines DMS was measured above the odour threshold (25 µg/L; Goniak & Noble, 1987). A summary of the results for the low boiling point sulphur compounds can be found in **Appendix 6-13**.

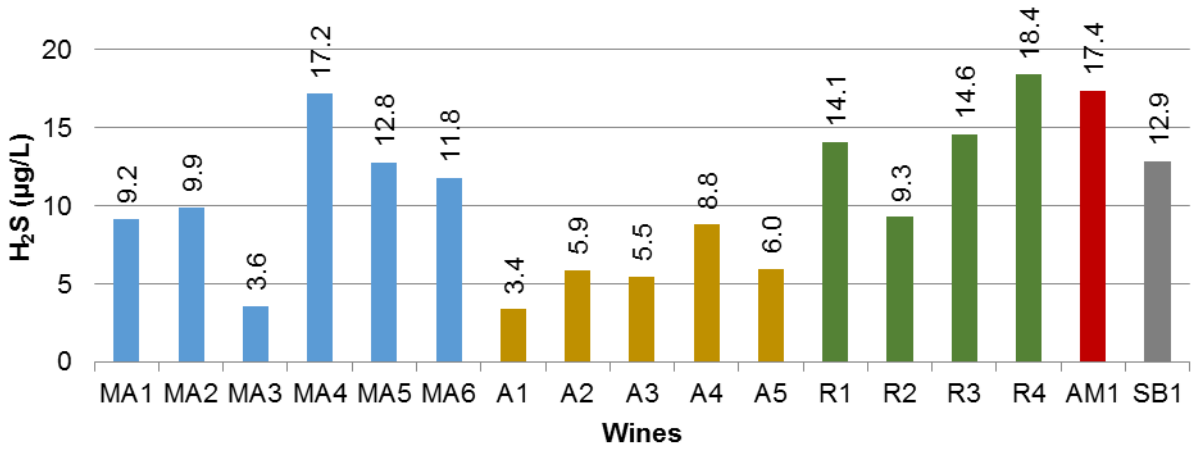


Figure 3-82: Hydrogen sulphide (H_2S) concentration of the 17 Greek commercial wines. Each colour represents a different group of wines from different varieties.

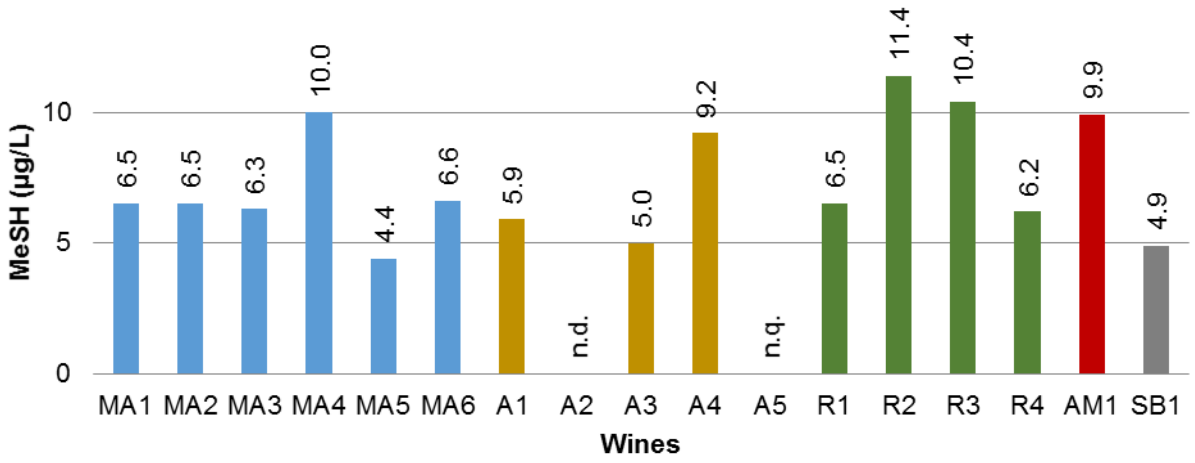


Figure 3-83: Methanethiol (MeSH) concentration of the 17 Greek commercial wines. Each colour represents a different group of wines from different varieties.

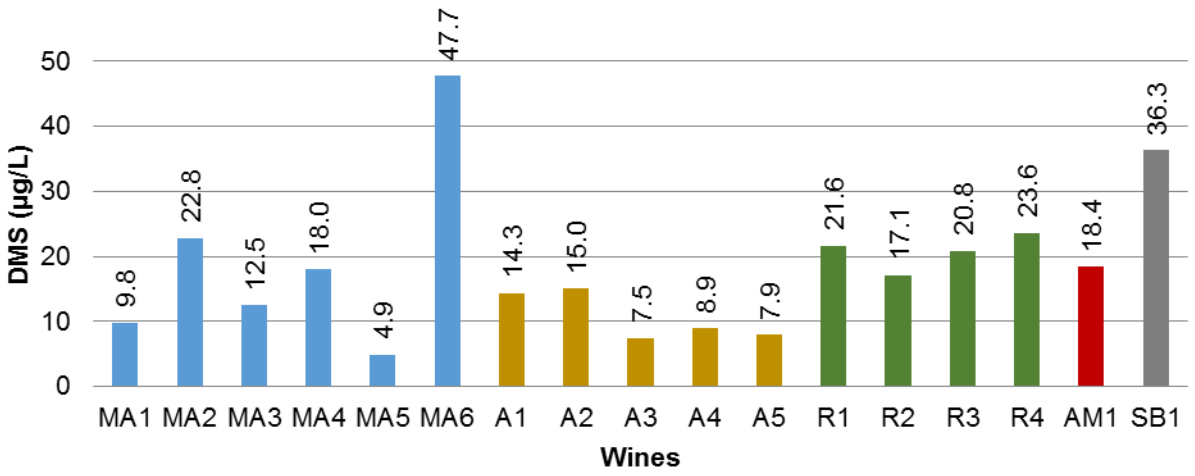


Figure 3-84: Dimethylsulphide (DMS) concentration of the 17 Greek commercial wines. Each colour represents a different group of wines from different varieties.

3.3.5 Varietal thiols

It is the first time results are presented considering varietal thiols in wines from Greek autochthonous grape varieties. Varietal thiols (4MSP, 3SHA, 3SH) for this work package were measured using the initial method described in **Chapter 2.2.2**. A summary of the results for the varietal thiols can be found in **Appendix 6-13**.

4MSP and 3SHA was only found in wines from the vintage 2009. Malagousia wines seem to be richer in 4MSP as in half of them the compound was identified in concentrations above the odour threshold (**Figure 3-85**) (3 ng/L; Darriet *et al.*, 1995). 3SHA was only found in few of the wines but in concentration that can play a role in the aromatics of the wines (**Figure 3-86**). 3SH is more ubiquitous and was identified in all of the wines (**Figure 3-87**). Half of the Malagousia wines showed significant concentrations above 1 µg/L and reaching almost 33 times the odour threshold (60 ng/L; Dubourdieu & Tominaga, 2009). An interesting observation from the results of 3SH was its presence in MA6, which at the time of analysis was four years old. These concentrations were comparable with MA4, which was from the same winery from the vintage 2009.

Asyrtiko wines showed the lowest concentration of 3SH (**Figure 3-87**) and none of the wines was above 700 ng/L, averaging 551 ng/L. On the other hand Malagousia wines had an average content of 1011 ng/L and Roditis 792 ng/L. Sauvignon blanc and the blend of Asyrtiko/Malagousia were also rich in 3SH.

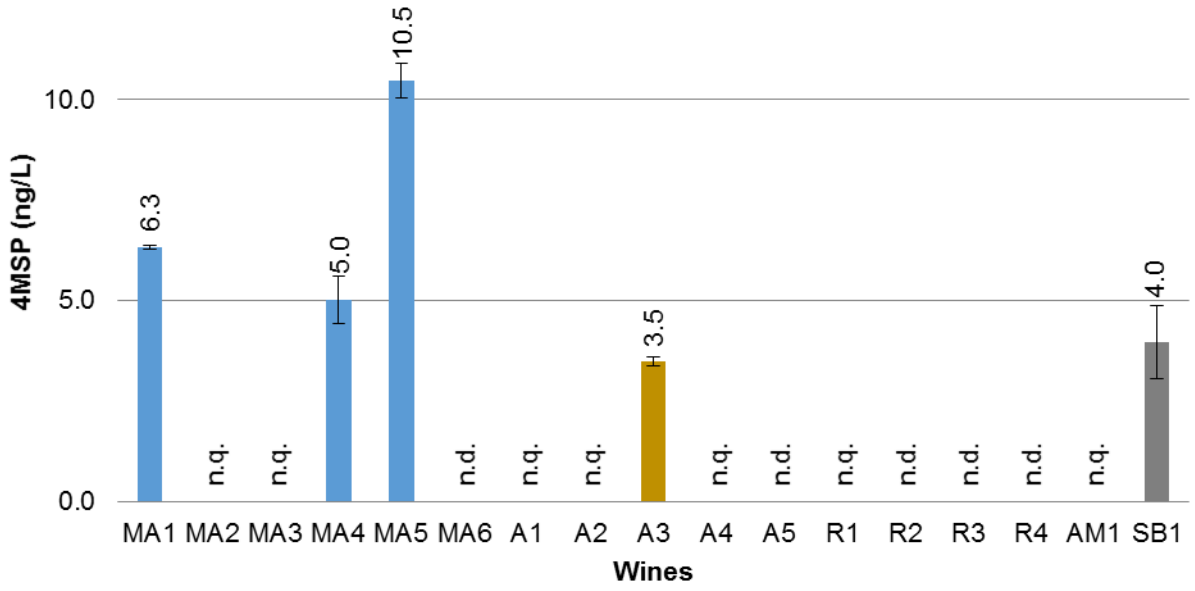


Figure 3-85: 4-methyl-4-sulfanylpentan-2-one (4MSP) concentration of the 17 Greek commercial wines. Each colour represents a different group of wines from different varieties.

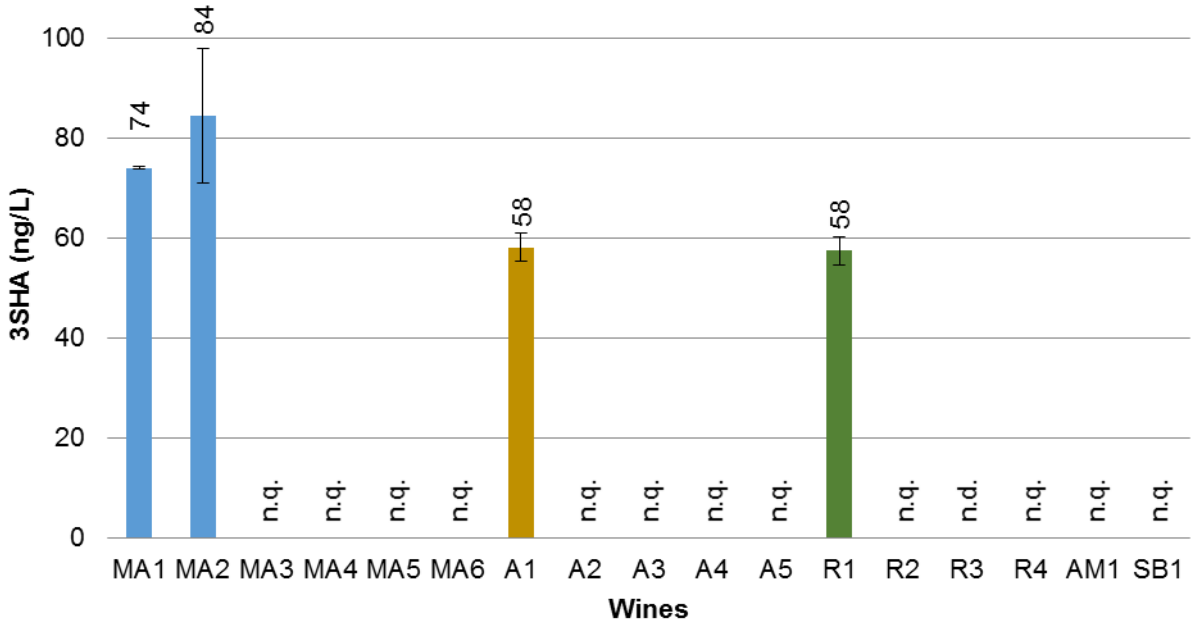


Figure 3-86: 3-sulfanylhexyl acetate (3SHA) concentration of the 17 Greek commercial wines. Each colour represents a different group of wines from different varieties.

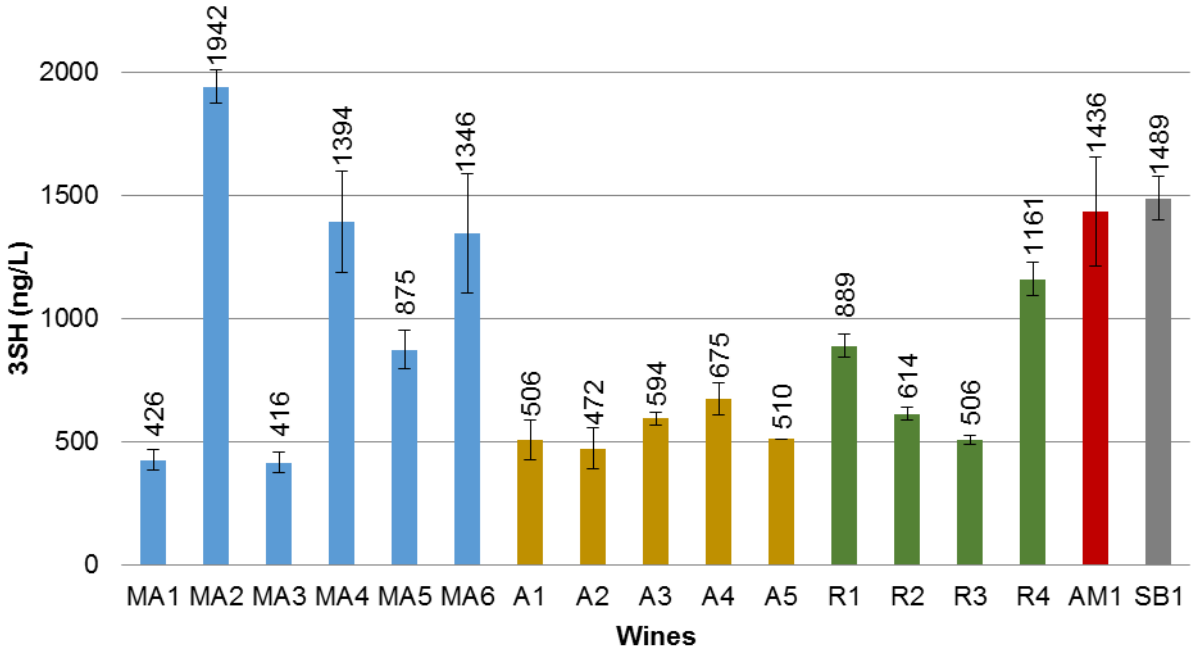


Figure 3-87: 3-sulfanylhexan-1-ol (3SH) concentration of the 17 Greek commercial wines. Each colour represents a different group of wines from different varieties.

3.3.6 Sensory analysis

For each group of wines (Malagousia, Asyrtiko, Roditis, Asyrtiko/Malagousia and Sauvignon blanc), the spider plots are presented individually for easiness of presentation. A summary of the tasting scores for all the wines can be found in **Appendix 6-14**.

Malagousia: Malagousia wines showed variability in the intensities for each descriptor. The wines from the vintage 2009 that had the higest concentrations of varietal thiols were characterised as intense in ‘passionfruit’ and ‘grapefruit’ (**Figure 3-88**). Wine MA2 was described by the tasters as intense ‘floral’ and probably correlates with the high concentrations of linalool and α-terpineol found (**Chapter 3.3.3**). MA5 was characterised with a strong ‘estery’ character although the concentration of acetic acid ethylester was the lowest amongst Malagousia wines (**Figure 3-70**). Probably the strong ‘floral’ and ‘passionfruit’ character of wine MA2 had a positive effect on the overall ‘impression’ giving the highest score for this attribute.

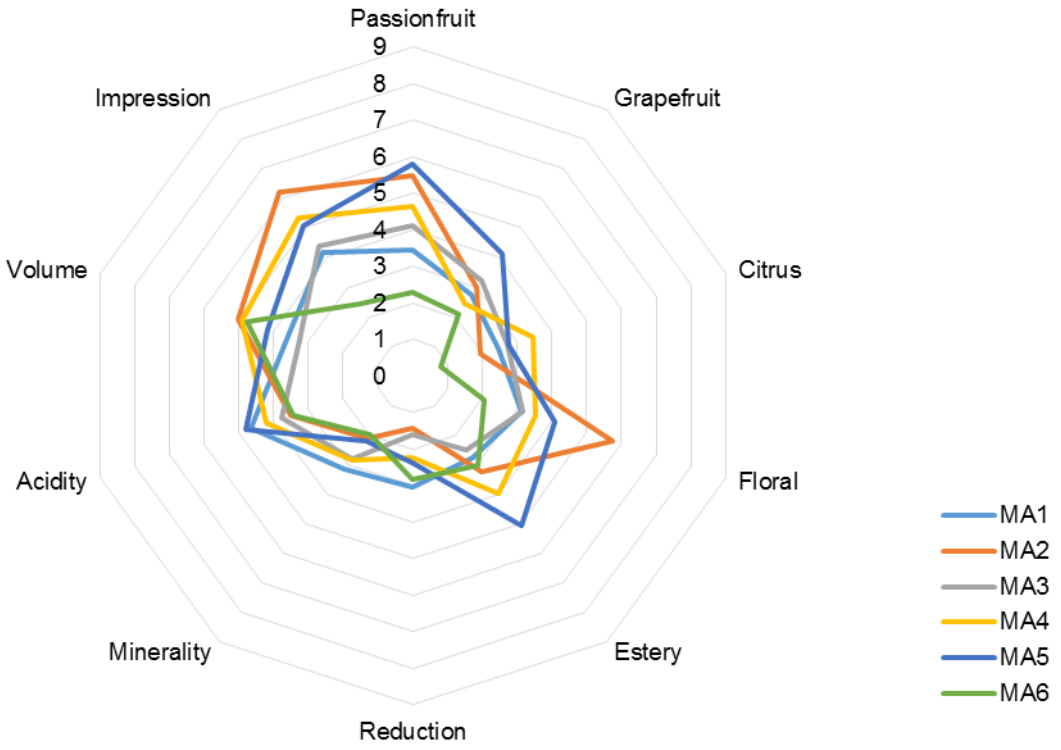


Figure 3-88: Spider plot for the six Malagousia wines tasted by the sensory panel.

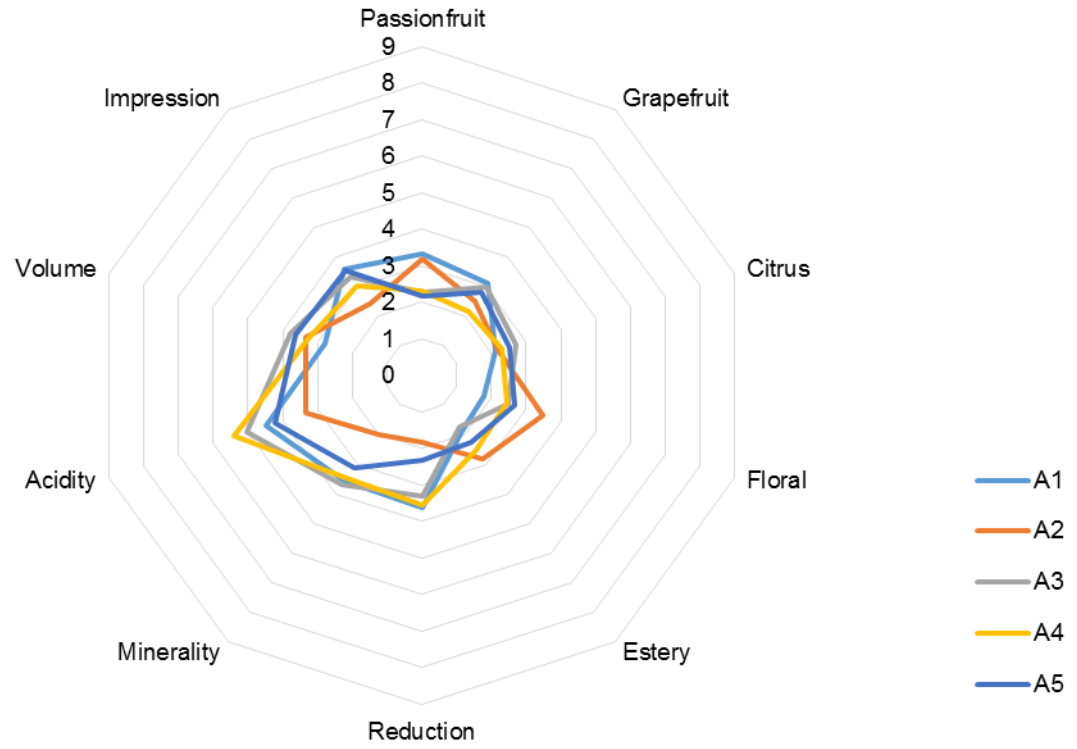


Figure 3-89: Spider plot for the five Asyrtiko wines tasted by the sensory panel.

Asyrtiko: Most of the wines from the variety Asyrtiko showed a strong acidic character, which is typical for wines from Santorini (**Figure 3-89**). It was mentioned that most of the times these wines show a pH below 3, which is very uncommon for such hot and dry region (Lazarakis, 2006). Wine A2 was an exception by being perceived more 'floral' and with a less 'acidic' character. The results from

the monoterpenes showed that A2 was the only wine from Asyrtiko that linalool was measured although under its odour threshold. It is known that this compound can contribute to the 'floral' charcter of a wine (Ferreira *et al.*, 2000).

Roditis: The variation in the intensities of the descriptors for the wines from the variety Roditis are much stronger when compared with Malagousia and Asyrtiko (**Figure 3-90**). R2 was marked with very low scores for all the aroma descriptors ('passionfruit', 'grapefruit', 'citrus' and 'floral') and showed stronger reductive notes. This can be explained by the fact that R2 showed H_2S value almost at the odour threshold and the highest MeSH concentration from all the wines tasted. It has been mentioned that these off-flavours act in a synergistic effect (Ribéreau-Gayon *et al.*, 1999; Rauhut, 2009). R1 and R4 had the highest 'impression' scores and were commented positively by the tasters. R1 was described by some of the tasters having a 'banana' nose. Acetic acid 3-methylbutylester, the aromatic compound, which is responsible for the banana aroma (Guth, 1997b), was measured in wine R1 in the highest concentration from all the wines studied (**Figure 3-71**).

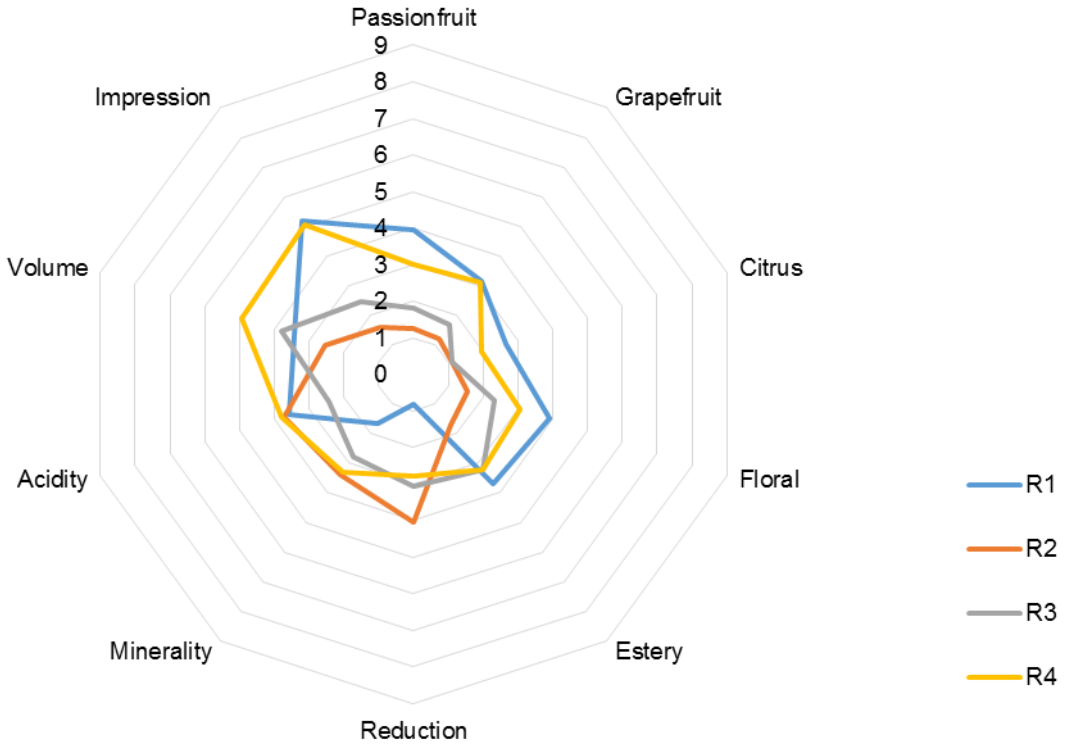


Figure 3-90: Spider plot for the four Roditis wines tasted by the sensory panel.

Asyrtiko/Malagousia: This blended wine showed a combination of the character described for the monovarietal wines from Asyrtiko and Malagousia (**Figure 3-91**). A light exotic nose ('passionfruit', 'grapefruit') and a 'floral' character probably originating from the part of Malagousia and a certain 'minerality' and 'acidity' probably contributed from Asyrtiko was described by the tasters.

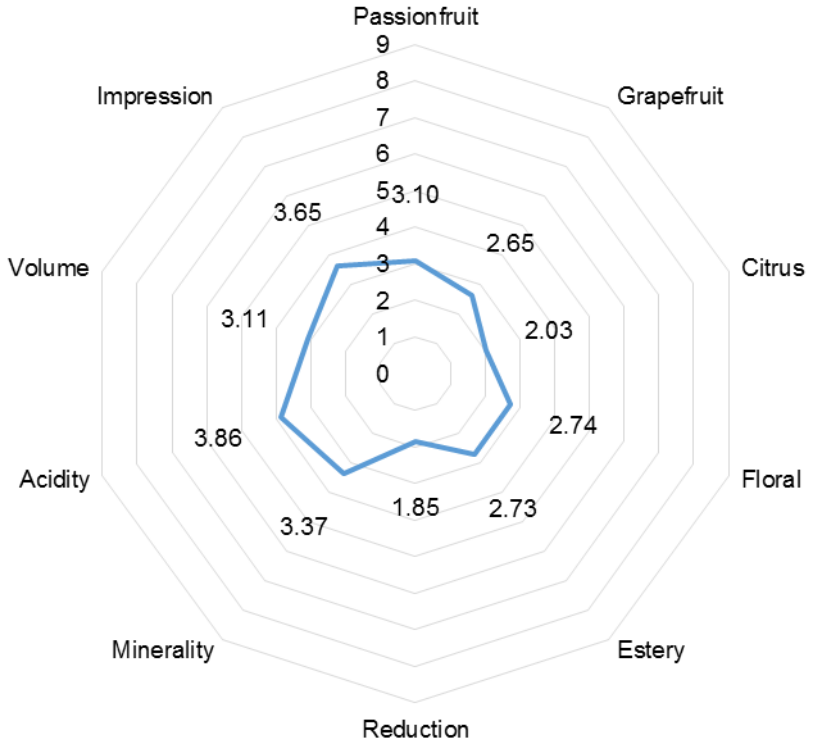


Figure 3-91: Spider plot of the Asyrtiko/Malagousia blended wine (AM1) tasted by the sensory panel.

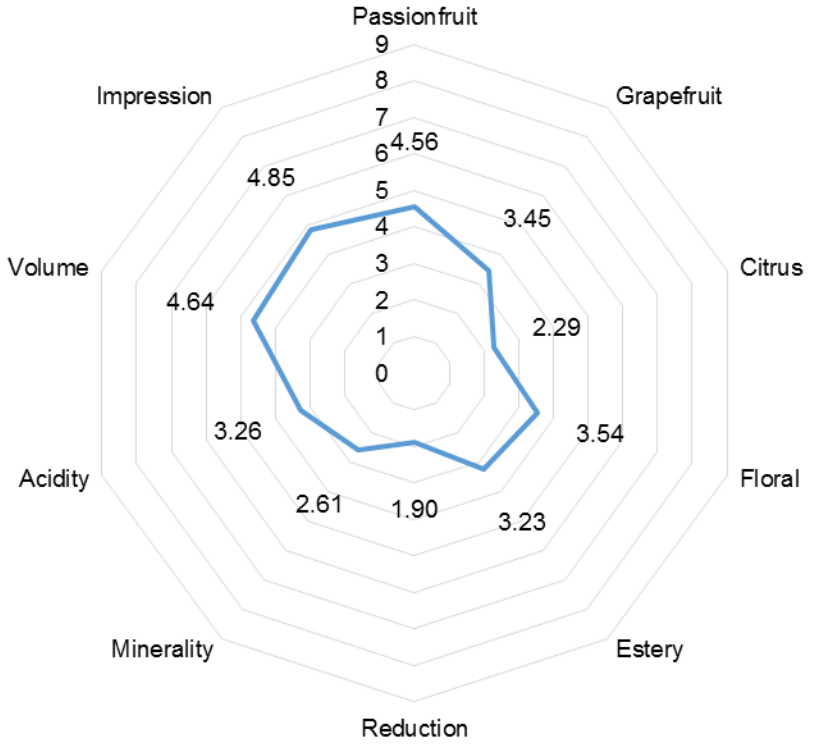


Figure 3-92: Spider plot of the Sauvignon blanc wine (SB1) tasted by the sensory panel.

Sauvignon blanc: Sauvignon blanc was used as a benchmark wine for the tasting as most of the professional attending the panel had experience with this international grape variety. The wine showed a strong 'passionfruit' and 'grapefruit' character comparable with the Malagousia wines (also with its 4MSP and 3SH concentrations). The reductive character of the wine was not as present to the tasters

probably because H_2S was around its odour threshold and MeSH had the lowest concentration from all the wines (**Figure 3-92**).

3.3.7 Statistical analysis

PCA plotting was used for analysing the data statistically. The OAVs were used for estimating the contribution of the aroma compounds to the aroma of the wines (Guth, 1997b; Ferreira *et al.*, 2000; Gómez-Míguez *et al.*, 2007). The data were mean centered and autoscaled before analysis (van den Berg *et al.*, 2006). A summary of the OAVs can be found in **Appendix 6-15.**

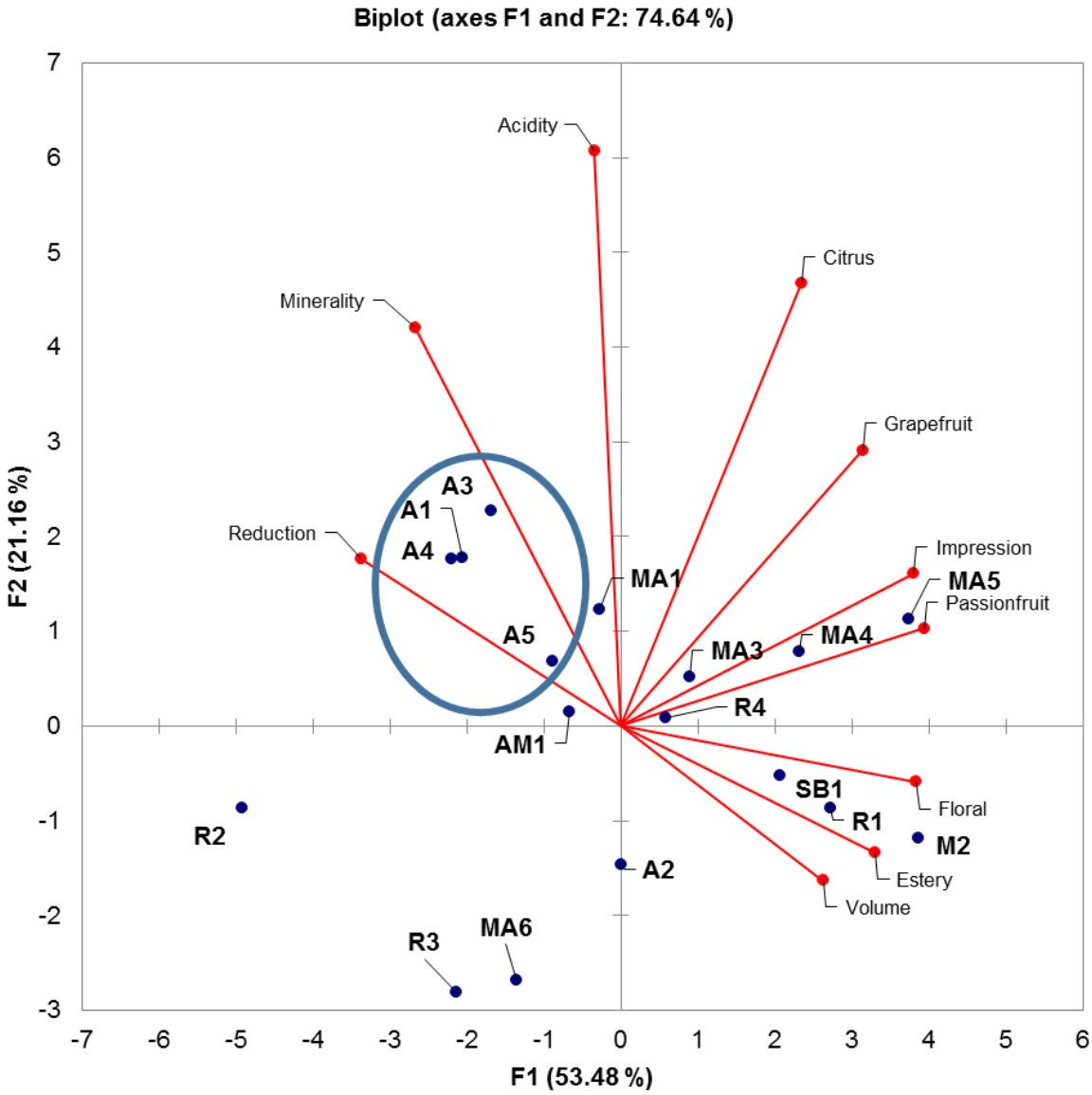


Figure 3-93: Principal Component Analysis (PCA) for the tasting results of the 17 Greek commercial wines.

The PCA for the sensory descriptors (**Figure 3-93**) explained 74.64 % of the variability in the data. F1 accounted for 53.48 % of the variability and was heavily loaded with the descriptors 'passionfruit', 'grapefruit', 'floral', 'impression', 'estery' in the positive direction and 'reduction', 'minerality' in the

negative direction. F2 accounted for 21.16 % of the variability and was heavily loaded with the descriptors ‘citrus’, ‘acidity’ in the positive direction.

Half of the Malagousia wines (MA2, MA4 & MA5) were explained by F1 and were also described as such by the tasters (**Figure 3-88**). Four out of five Asyrtiko wines (A1, A3, A4 & A5) formed a cluster around the descriptors ‘reduction’ and ‘minerality’, which correlates with the character described by Lazarakis (2006). The wines from the grape variety Roditis do not correlate well with the tasting scheme as only R1 is more close to the descriptors ‘floral’, ‘estery’ and ‘volume’. The other wines from this variety are either not well explained (R4) or have a negative correlation with factors F1 and F2.

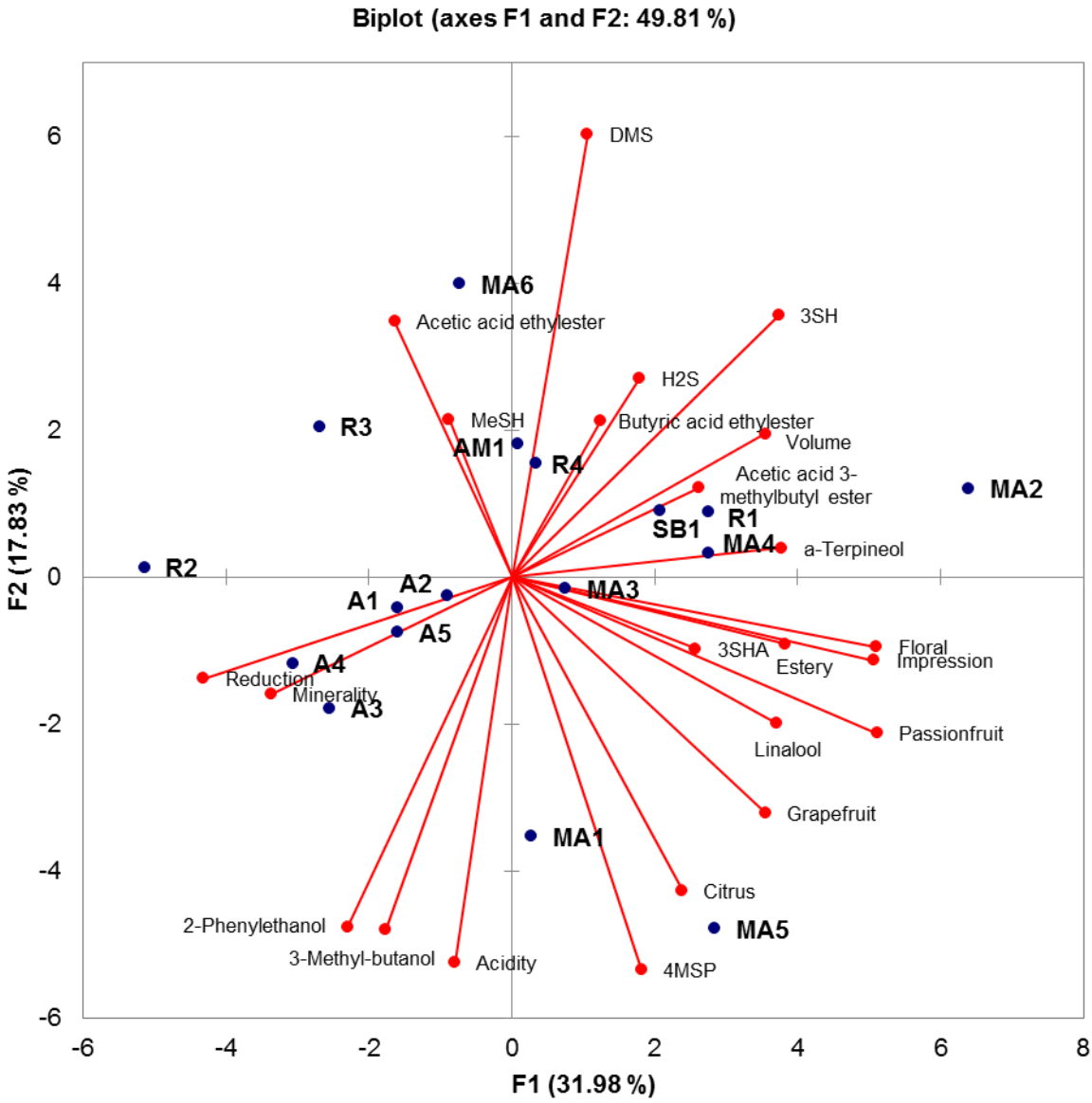


Figure 3-94: Principal Component Analysis (PCA) of the 17 Greek commercial wines with the aromatic compounds that had an OAV > 1 in at least one of the samples. The data were mean centered and autoscaled before analysis.

For the PCA of the aromatic compounds and the tasting attributes it was chosen to plot only the compounds that showed OAV values above 1 in at least one of the variants. The PCA on F1 and F2

explained 49.81 % of the variability in the data (**Figure 3-94**). F1 accounted for 31.98 % of the variability and was more heavily loaded with 'linalool', 'α-terpineol', '3SH', 'passionfruit', 'grapefruit', 'floral', 'impression' in the positive direction and 'reduction' in the negative direction. F2 accounted for 17.83 % of the variability and was more heavily loaded with 'DMS' in the positive direction and '4MSP', '3-methyl butanol', '2-phenylethanol', 'acidity', 'citrus' in the negative direction.

From the plot (**Figure 3-94**) it can be seen that it seems to be important for the 'impression' of the wines to have both 'floral' but also an exotic character. The fruity compounds ('3SH', 'acetic acid 3-methylbutyl ester' & '3SHA') and the 'floral' compounds (linalool, a-terpineol) closely relate with the descriptors 'floral' and 'passionfruit'. '4MSP' is less related with these descriptors and it is probably attibuted to the fact that higher concentrations can lead to a negative impression of the wines (Darriet *et al.*, 1995). Both MA1 and MA5 that had the highest content in 4MSP measured did not closely relate with the descriptor 'impression' as MA2 and MA4 that had lower concentration or none detected. As described for **Figure 3-93** the wines from Asyrtiko have formed a cluster around the descriptors 'reduction' and 'minerality'. In **Figure 3-94** A2 has also shifted into the cluster although it is more to the center of the plot correlating less with these descriptors. R1 correlated to a 'floral' and terpenic character, which cannot be explained by the measured aroma compounds (**Chapter 3.3.2 & 3.3.3**).

3.3.8 Discussion and conclusion

The goal of this work package was to study and select wines from Greek autochthonous varieties for further investigations. Wines from three autochthonous varieties were studied (Malagousia, Asyrtiko & Roditis). A blended wine from Asyrtiko/Malagousia and a monovarietal Sauvignon blanc produced in Greece were also used in the study. The later one was used for training and benchmarking purposes as it is considered as an international variety (Robinson *et al.*, 2012) and has been studied extensively for its aromatics (Allen *et al.*, 1991; Darriet *et al.*, 1995; Tominaga *et al.*, 1998a; 1998b; Dubourdieu & Tominaga, 2009).

From the three varieties used, Malagousia showed the most interesting characteristics and significant concentrations of both thiols and monoterpenes were found. Linalool concentrations in all the wines from Malagousia (except MA6) were between two to eight times above the odour threshold (25 µg/L, Ferreira *et al.*, 2000) and were comparable with the published data from Metafa & Economou (2013). Furthermore, MA2 showed a high concentration of α-Terpineol that contributes to the 'floral' character of the wine. The tastings showed that MA2 was characterised as significantly 'floral' by the tasters (**Figure 3-88**). Many of the wines showed off-flavours above the odour threshold for both H_2S and MeSH. However these were not perceived with reductive notes by the tasters, as it can be seen by the spider plots for the tastings (**Figure 3-88**). MA6 showed a significantly high concentration of DMS, which was almost two times above the odour threshold. These high concentrations are normal for aged wines, as DMS has the tendency to increase during storage (Marais, 1979; Goto & Takamuto, 1987; Dittrich & Grossmann, 2011). Varietal thiols were measured in concentrations of up to 10.5 ng/L for 4MSP, 84 ng/L for 3SHA and 1942 ng/L for 3SH. Furthermore, the 4 year old Malagousia MA6 (at the point of analysis) had significant concentrations of 3SH, which were similar to MA4. MA4 and MA6 was sourced from the same winery (same label) but from a different vintage, 2009 & 2006 respectively

(**Table 2-7**). Tominaga *et al.* (2006b) showed for the first time that varietal thiols could still be measured in three year old wines. It is the first time that significant concentrations of varietal thiols are found in a four year old wine and it could mean that these compounds, in this case 3SH, do not decrease so quickly but can be preserved for a number of years. This would not apply for 3SHA. Dubourdieu & Tominaga (2009) showed that it is present only in young wines and decreases rapidly with ageing probably by hydrolysis.

Wines from the variety Asyrtiko showed a less complex aromatic character than the wines from Malagousia. The concentrations of esters and higher alcohols were at the same levels as the wines from the other grape varieties (**Chapter 3.3.2**). Acetic acid ethyester was slightly increased, but only one wine (A3) exceeded the odour threshold of 160 mg/L (Ribéreau-Gayon *et al.*, 1999). Traces or very low concentrations of monoterpes were measured, components which are responsible for a 'floral' and 'fruity' character in the wines (Mateo & Jiménez, 2000). Low concentrations of monoterpenes in wines from Asyrtiko were also reported by Metafa & Economou (2013). These results were also reflected in the sensorial analysis, as the tasters noted the lack of 'floral' character in the wines (**Figure 3-89**). Only A2 was characterised lightly 'floral' although the concentrations of the monoterpenes found were very low. This leads to the hypothesis of a synergistic effect of the monoterpenes in increasing the perception of a 'floral' character of the wine. Asyrtiko showed the lowest concentrations of varietal thiols when compared with Malagousia and Roditis. Despite this fact 3SH was present between 7.8-11 times above the odour threshold (60 ng/L; Dubourdieu & Tominaga, 2009). 3SHA was found in wine A1 at almost 14 times above the odour threshold (4.2 ng/L; Dubourdieu & Tominaga, 2009). 4MSP was measured only in wine A3 around the odour threshold (3ng/L; Darriet *et al.*, 1995) and probably did not contribute to the exotic character of the wine. The wines were characterised as having notes reminiscent to unpleasant sulphur compounds by the tasting panel although they had low concentrations of off-flavours when compared with the other varieties. The island of Santorini is hot and lacks water (Lazarakis *et al.*, 2006) and in these climates YAN is much lower than in cooler and more moist climates, leading more often to reductive notes in the wines (Dittrich & Grossmann, 2011). It could probably be that the reductive character of the wines from Asyrtiko is influenced by other, not yet detected, compounds.

The wines from the variety Roditis showed similar results as the wines from Asyrtiko. Significant concentrations of varietal thiols were found but not as high as in the wines from Malagousia, alongside almost non-existend monoterpenes. It seems that the tasting scheme for wines from Roditis did not work as intented (**Figure 3-93**); moreover, at least one of the samples (R3) showed faults from a spontaneous MLF. Off-flavour wines had significant concentrations of H_2S and MeSH around or significantly above their odour threshold (**Figure 3-82 & 3-83**). Additionally, all of the wines from Roditis had elevated concentrations of DMS although most of them were young from the vintage 2009 (except R4). This could be attributed either to low YAN concentrations in the musts, to long contact times of the finished wines with the lees (Niefind, 1969; Dittrich & Grossmann, 2011) or to storage at higher temperatures in the shops prior to sale (Marais, 1979; Goto & Takamuto, 1987).

The blended wine from Asyrtiko/Malagousia showed a 'floral' and 'exotic' character, which probably originated from Malagousia, while the acid and mineral character probably originated from Asyrtiko

(**Figure 3-91**). Linalool concentration was two times above the odour threshold and since in the monovarietal Asyrtiko wines almost no linalool was found it can be concluded that Malagousia contributed more in the 'floral' character of the wine. The wine showed singnificant concentrations of off-flavours and had the second highest concentration in H_2S and MeSH from all the wines studied.

The wine from Sauvignon blanc that was used as reference, since it has been extensively studied (Allen *et al.*, 1991; Darriet *et al.*, 1995; Tominaga *et al.*, 1998a & b; Dubourdieu & Tominaga, 2009), proved to be helpful in validating the tasting results. The wine was described with a strong 'passionfruit' and 'grapefruit' character that was also reflected by the results for the varietal thiols (**Figure 3-85** & **3-87**). Reductive notes were low although it was shown to have significant concentrations of DMS (36.3 µg/L); this was probably because the wine was from the vintage 2008. Older wines tend to have increased concentrations of DMS (Marais, 1979; Goto & Takamuto, 1987).

From the tasting results, it could be concluded that the scheme and the training of the expert panel worked well for the wines from the varieties Malagousia and Asyrtiko. The wines from the variety Roditis showed almost no correlation with the tasting descriptors and cannot be considered further with this scheme. Asyrtiko wines (A1, A3, A4 and A5) showed clustering around the descriptors 'minerality' and 'reduction', as described by Lazarakis (2006). Wines from Malagousia tend to be more complex aromatically by having significant concentrations of monoterpenes and varietal thiols. Finally, the wines from Malagousia showed different characters from 'exotic' to 'floral' as seen in **Figure 3-94** (e.g. wines MA1 and MA2).

In conclusion wines from Asyrtiko showed significant concentration of varietal thiols but the reductive- and mineral character were not subject of this work. Furthermore, many Asyrtiko wines that are on the market are aged (Lazarakis, 2006) thus it would be difficult to collect many samples of young wines. It was decided not to study the variety further. The wines from Roditis showed some difficulties in quality (faulty sample R3). Furthermore, it was difficult to obtain monovarietal wines as this variety is mostly used for blending (Lazarakis, 2006). The tasting scheme proved not to be appropriate for these wines due to poor correlations and were also rejected from further investigations. The wines from Malagousia fitted well in the tasting scheme developed and the description of the variety with its aromatics was sufficient. Due to the complex character that the wines showed and the high concentrations of both monoterpenes and varietal thiols it was decided to study the variety further, as it was also easier to source young monovarietal wines from a lot of producers. Minor additions were made in the further studies, such as the extended spectrum of monoterpes and C_{13} norisoprenoids and the training of the expert panel for these compounds.

3.4 Malagousia & Petite Arvine analysis & sensorial experiment

Two grape varieties were studied: Malagousia from Greece and Petite Arvine from Switzerland. Malagousia was chosen from the previous study because of its richness in aroma compounds (**Chapter 3.3.3** & **3.3.5**) and complex sensory properties (**Figure 3-88**). Petite Arvine was chosen as it has been partially characterised in the literature (Fretz *et al.*, 2005a; 2005b; 2005c) and was used to validate the results for the wines from Malagousia.

3.4.1 Malagousia

Eighteen monovarietal Malagousia wines from the vintage 2013 were sourced from specialized shops in Germany and Greece. The wines were investigated for their constituents with special attention on their varietal aroma (terpenes and varietal thiols). Furthermore, a professional panel tasted the wines and evaluated them according to a scheme developed in the previous experiments (**Chapter 3.2.1, 3.2.2** & **3.3**). **Table 2-8** gives details about the origin of each wine and the producers. **Appendix 6-29** shows the origin of the wines on a map of Greece.

3.4.1.1 Major components

The alcohol content of the wines (**Figure 3-95**) was similar with the ones reported for Malagousia in the literature (Tourtoglou *et al.*, 2014). All the wines have been fermented to almost dry (**Figure 3-96**) and showed normal VA levels for young white wines (**Figure 3-98**) (Ribéreau-Gayon *et al.*, 1999; Dittrich & Grossmann, 2011). Total acidity showed comparable values (**Figure 3-97**) as published by Tourtoglou *et al.* (2014). A summary of the results for the major components can be found in **Appendix 6-16**.

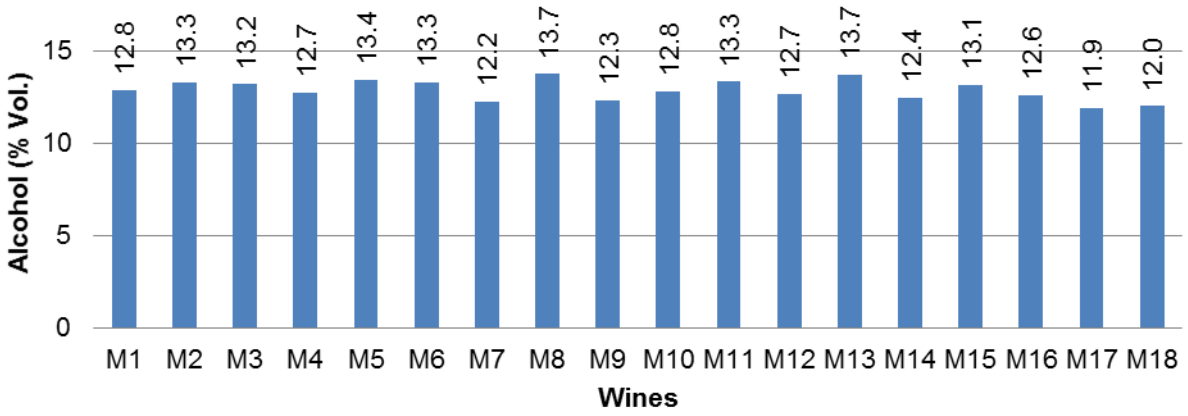


Figure 3-95: Alcohol content of the 18 commercial wines from the Greek variety Malagousia.

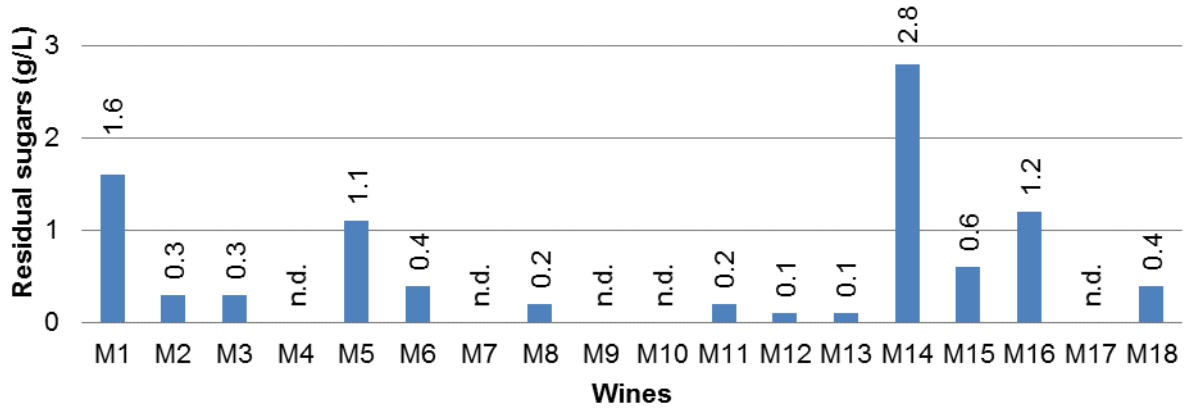


Figure 3-96: Residual sugars concentration of the 18 commercial wines from the Greek variety Malagousia.

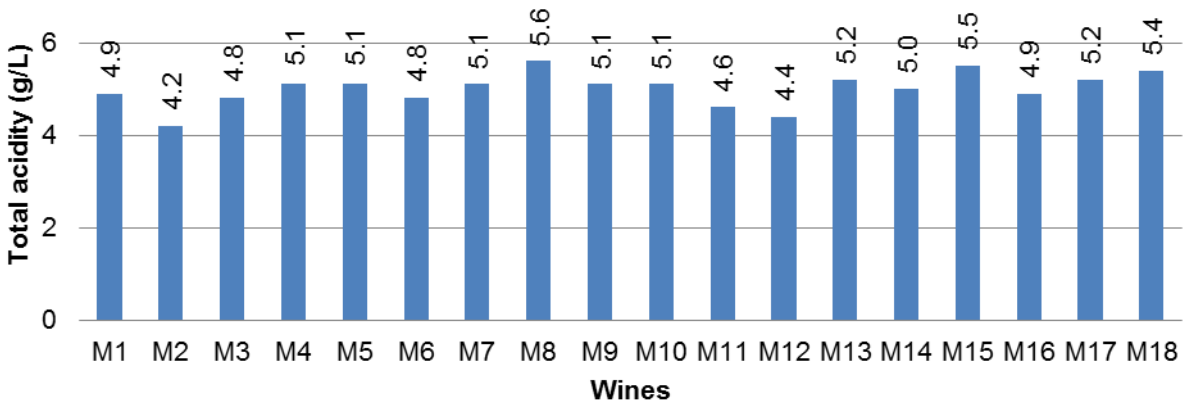


Figure 3-97: Total acidity of the 18 commercial wines from the Greek variety Malagousia.

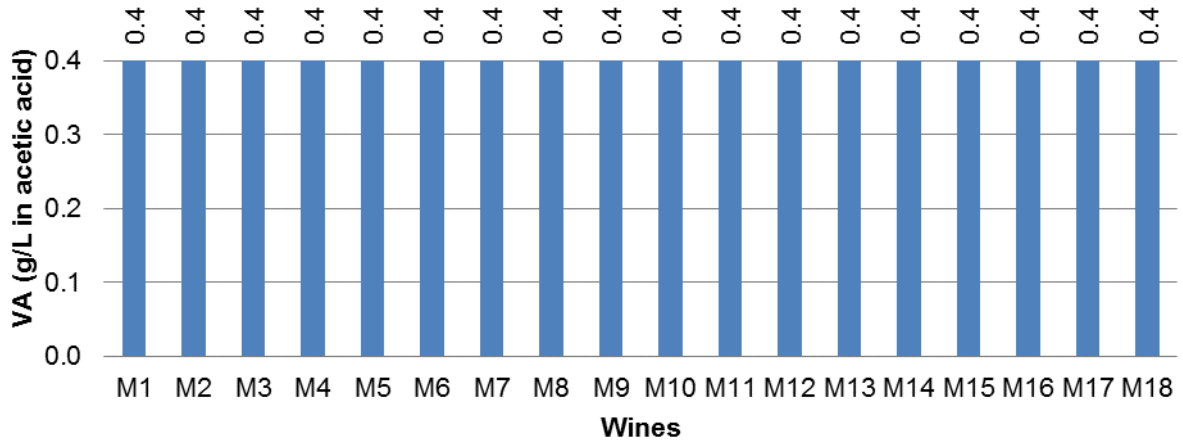


Figure 3-98: Volatile acidity (VA) of the 18 commercial wines from the Greek variety Malagousia.

Malic acid found in the wines (**Figure 3-99**) was also in accordance with the values published by Tourtoglou *et al.* (2014). For the case of M1, M4, M14 and M17 there could be a hint of contamination by lactic acid bacteria during fermentation as the concentrations of lactic acid (**Figure 3-100**) are marginally higher than the concentrations that yeast produce during alcoholic fermentation (Dittrich & Grossmann, 2011).

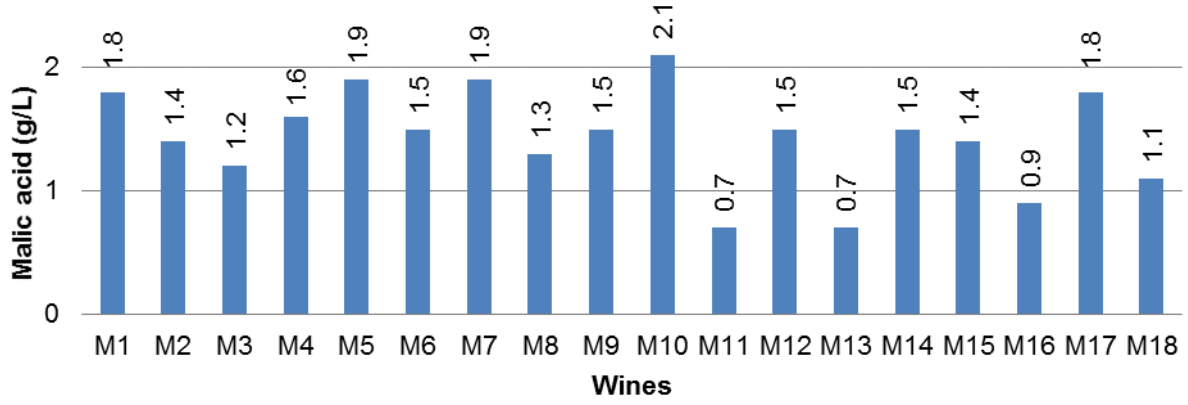


Figure 3-99: Malic acid concentration of the 18 commercial wines from the Greek variety Malagousia.

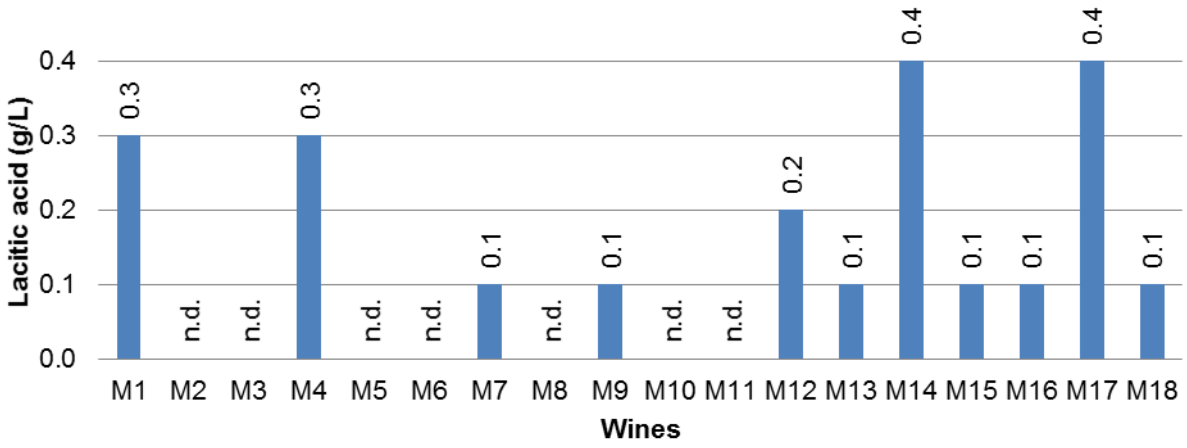


Figure 3-100: Lactic acid concentration of the 18 commercial wines from the Greek variety Malagousia.

Total phenols have been reported to affect the odour threshold of varietal thiols (Lund *et al.*, 2009) and their concentrations (Blanchard *et al.*, 2004; Nikolantonaki *et al.*, 2010). The total phenols measured in the wines (**Figure 3-101**) were similar to the concentrations reported for Malagousia by Tourtoglou *et al*. (2014). As Tourtoglou *et al.* (2014) mentioned the concentrations measured Malagousia can be compared with commercial wines from other varieties of the Greek vineyard. Additionally in this publication the spectrum of phenolics of Malagousia was also presented. It showed concentrations of epicatechin (average: 1.55 mg/L) and catechin (average: 3.75 mg/L), which are compounds that have been found to affect varietal thiols. Probably though the concentrations measured were very low to have an effect. Nikolantonaki *et al.* (2010) studied the influence of epicatechin and catechin on varietal thiols with a concentration of 500 mg/L each that is far too high.

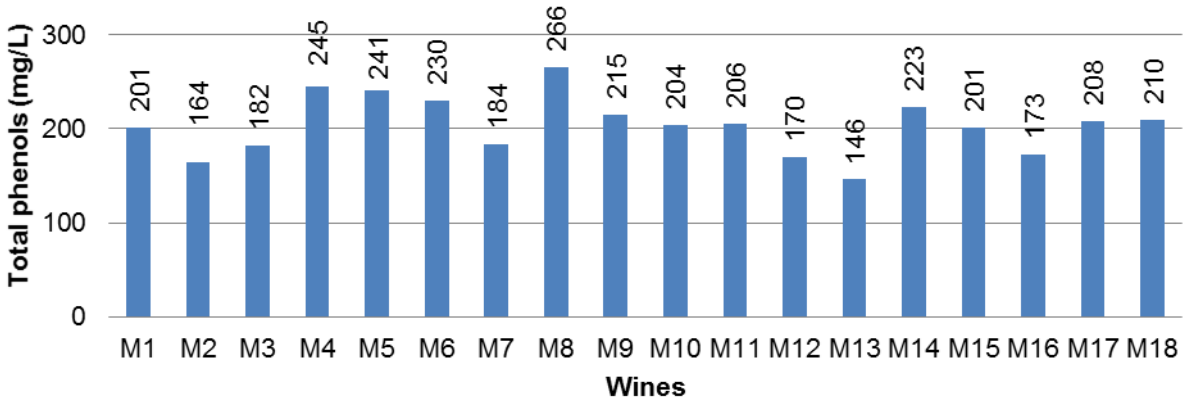


Figure 3-101: Total phenols concentration of the 18 commercial wines from the Greek variety Malagousia.

3.4.1.2 Esters and higher alcohols

A summary of all the measured compounds with the 'Kaltron' method can be found in **Appendix 6-17.**

Esters: Acetic acid ethylester concentrations were low, which suggest a clean fermentation of the wines (**Figure 3-102**). Very high concentrations of acetic acid 3-methylbutylester (aroma descriptor:

banana) were found in some of the wines reaching up to 100 times the odour threshold (**Figure 3-103**) (30 µg/L, Guth 1997b).

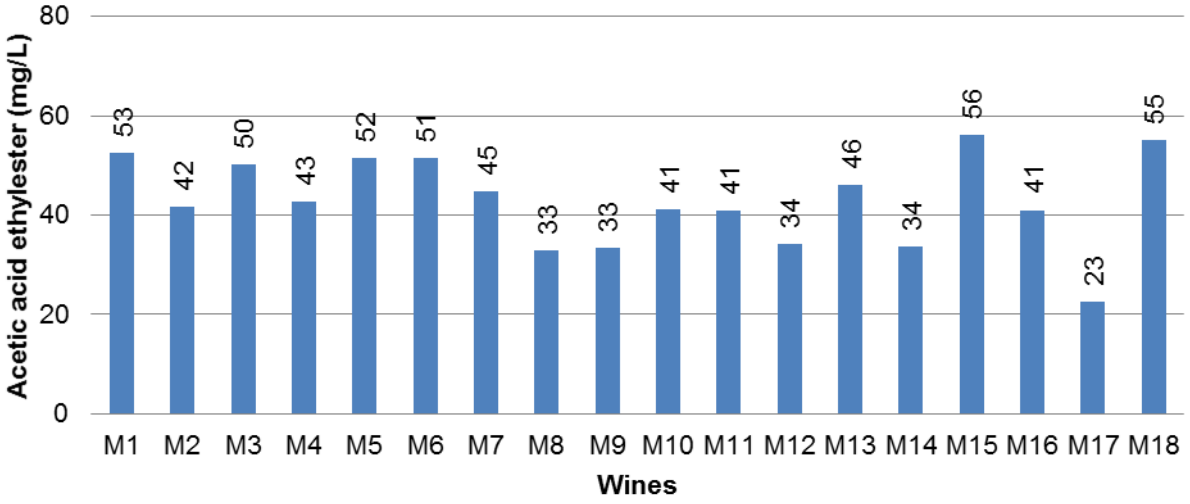


Figure 3-102: Acetic acid ethylester concentration of the 18 commercial wines from the Greek variety Malagousia.

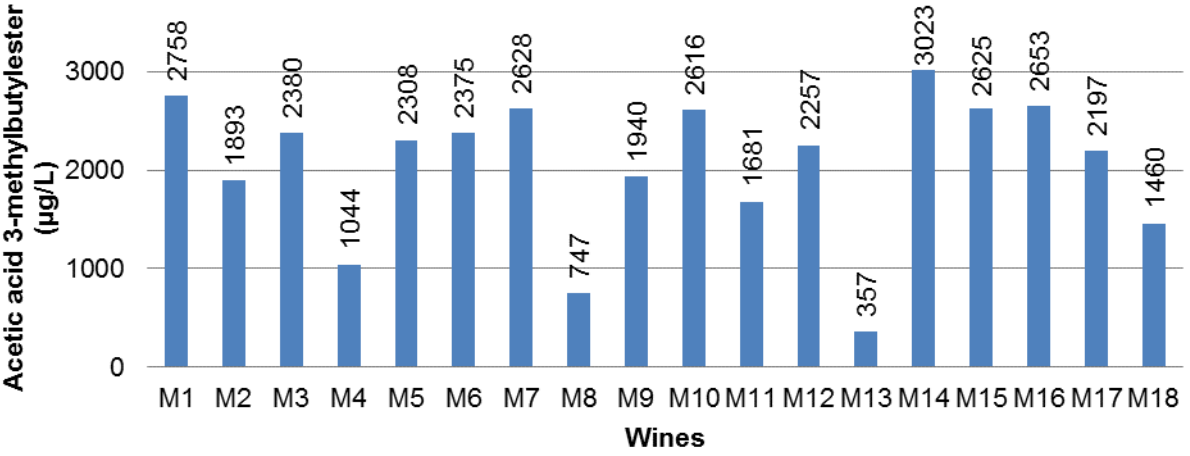


Figure 3-103: Acetic acid 3-methylbutylester concentration of the 18 commercial wines from the Greek variety Malagousia.

Acetic acid 2-phenylethylester was in most cases below the odour threshold of 250 µg/L (**Figure 3-104**) but some of the wines (M1, M11, M14 & M15) showed concentrations marginally above this (Guth, 1997b). Butyric acid ethylester was present in significant concentrations but did not show such a high variability as observed for other esters (**Figure 3-105**). Concentrations varied between 19- and 31 times above the odour threshold of 20 µg/L (Ferreira *et al.*, 2000; Fretz *et al.*, 2005a). Both lactic acid ethylester and Succinic acid diethyletster were below the odour threshold in all of the wines (**Figure 3-106** & **3-107**).

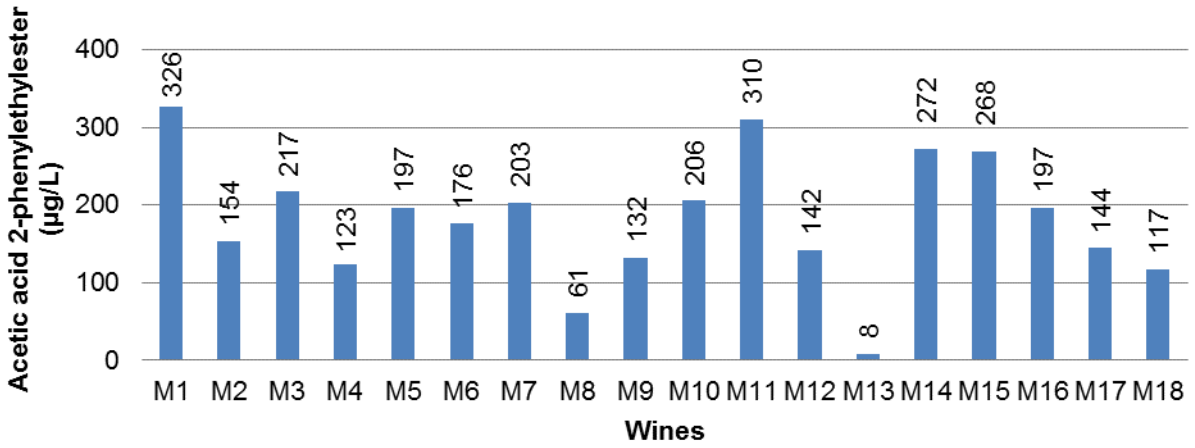


Figure 3-104: Acetic acid 2-phenylethylester concentration of the 18 commercial wines from the Greek variety Malagousia.

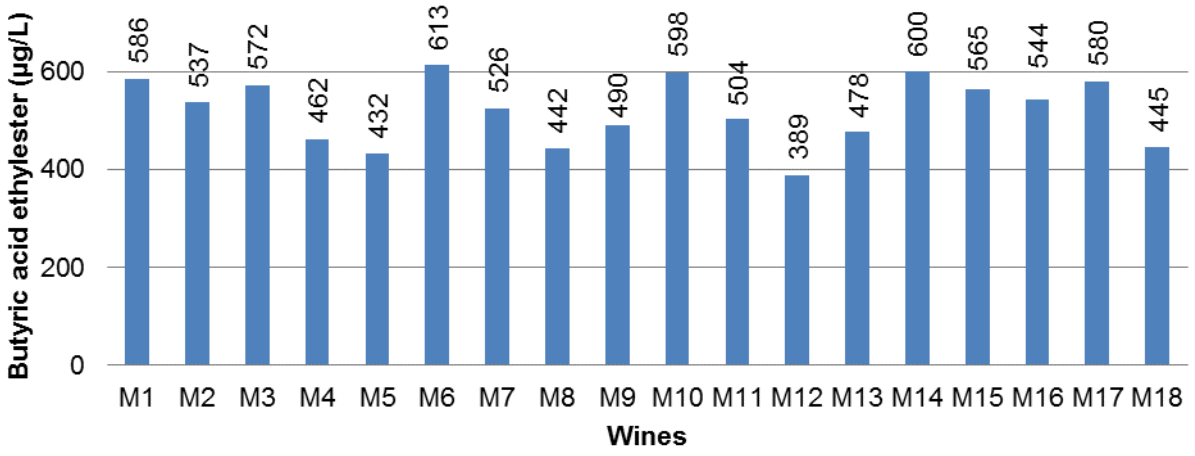


Figure 3-105: Butyric acid ethylester concentration of the 18 commercial wines from the Greek variety Malagousia.

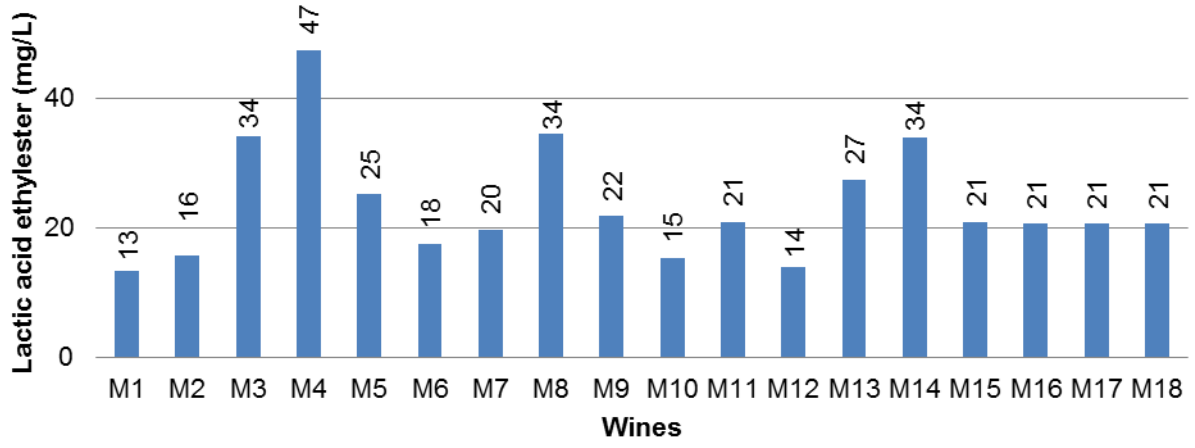


Figure 3-106: Lactic acid ethylester concentration of the 18 commercial wines from the Greek variety Malagousia.

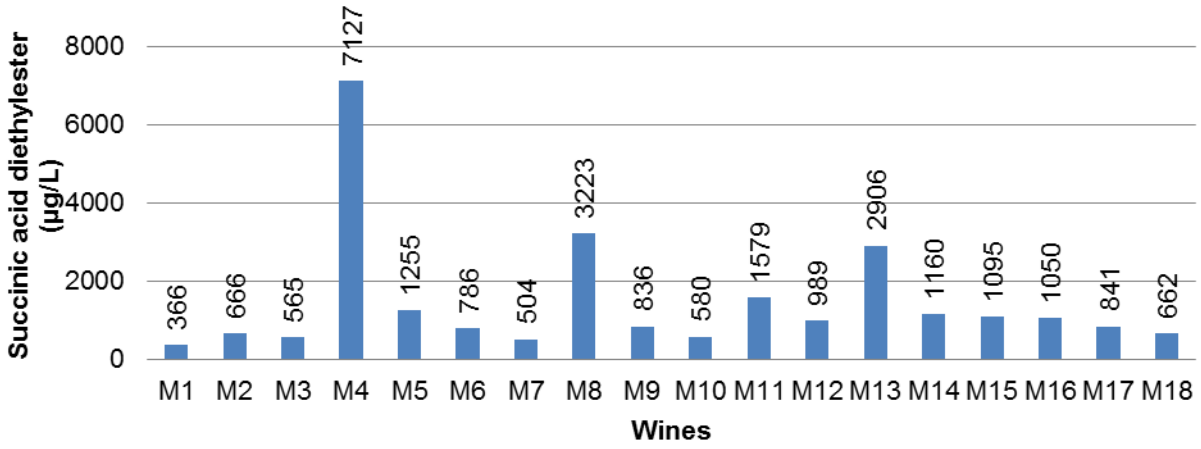


Figure 3-107: Succinic acid diethylester concentration of the 18 commercial wines from the Greek variety Malagousia.

Higher alcohols: From the higher alcohols quantified in the wines from Malagousia only 3-methyl butanol and 2-phenylethanol were in such concentrations to contribute to their aromatics (**Figure 3-109** & **3-111**). 3-methyl butanol was found in amounts three to six times above its odour threshold (30 mg/L; Guth, 1997b). 2-phenylethanol showed high variability and most of the wines showed concentrations above the odour threshold of 14 mg/L (Ferreira *et al.*, 2000). It should be mentioned that the wines M1, M2, M4 and M11 showed much higher amounts than the others. It could be possible that this compound could contribute to the 'floral' character of the wines, together with the terpenes, as it has a characteristic smell of roses (Ferreira *et al.*, 2000). i-Butanol and hexan-1-ol were found in such concentrations that could not contribute to the aroma of the wines (**Figure 3-108** & **3-110**).

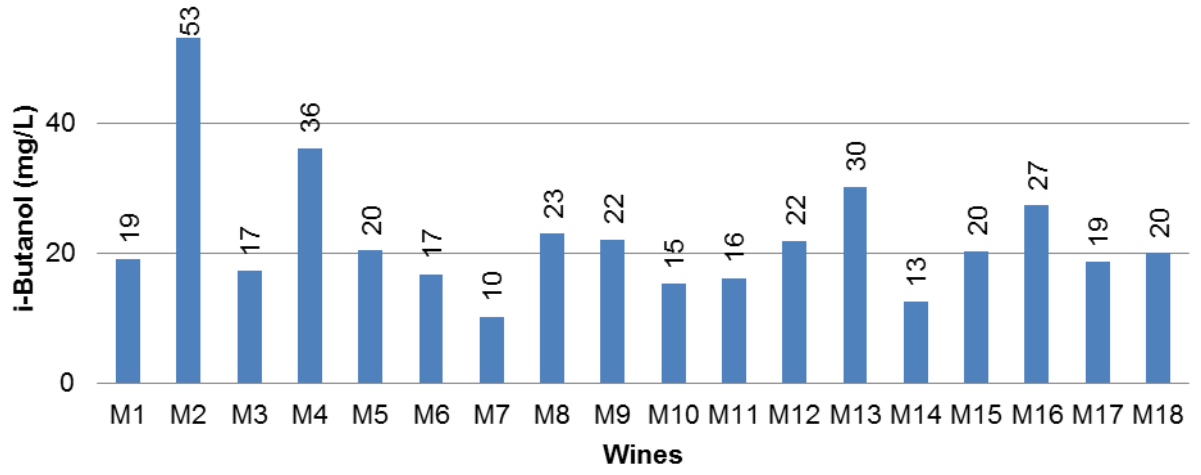


Figure 3-108: i-Butanol concentration of the 18 commercial wines from the Greek variety Malagousia.

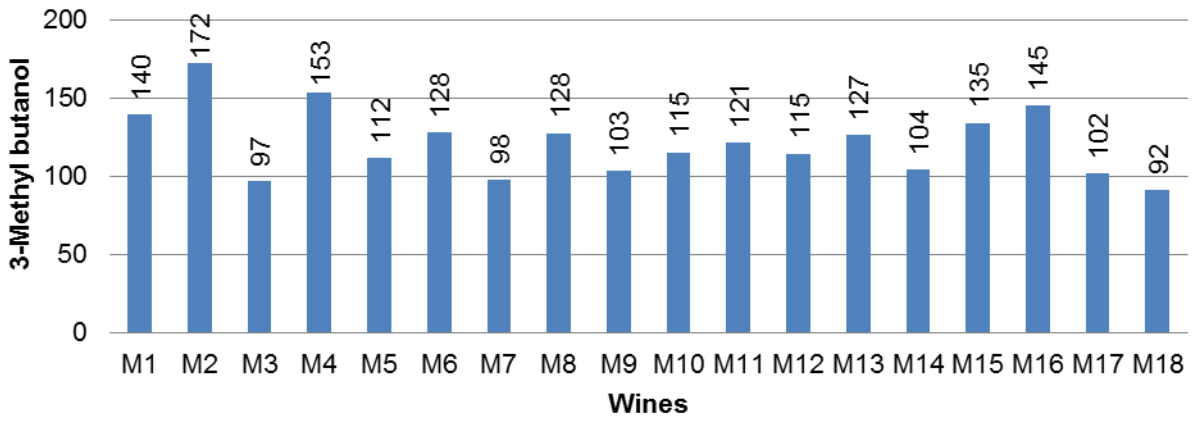


Figure 3-109: 3-methyl butanol concentration of the 18 commercial wines from the Greek variety Malagousia.

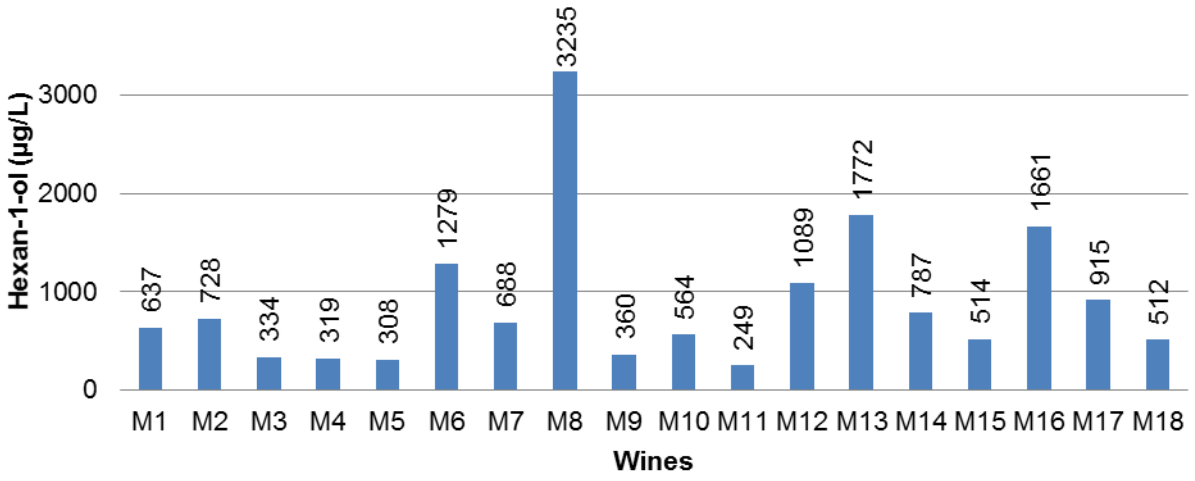


Figure 3-110: Hexan-1-ol concentration of the 18 commercial wines from the Greek variety Malagousia.

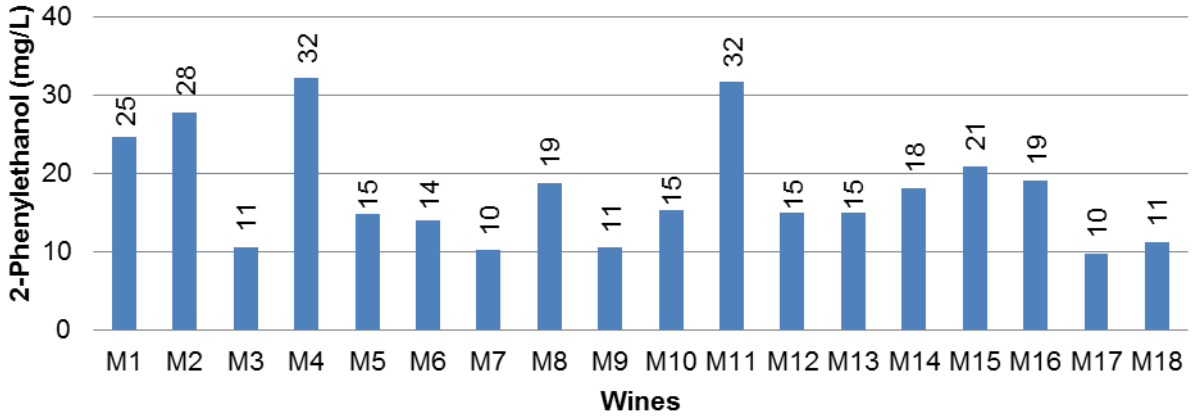


Figure 3-111: 2-phenylethanol concentration of the 18 commercial wines from the Greek variety Malagousia.

3.4.1.3 Terpenes

In **Chapter 3.3** it was shown that wines from the grape variety Malagousia had high concentrations of monoterpenes. Linalool was in almost all the samples above the odour threshold. α-Terpineol was in one of the samples significantly above the threshold (**Chapter 3.3.3**) This was in accordance with the published data by Metafa & Economou (2013). Because Metafa & Economou (2013) studied only experimental wines it was decided to quantify the free monoterpenes and the C_{13} norisoprenoid spectrum for commercial Malagousia wines (for method see **Chapter 2.7.5**). The results probably categorise Malagousia as a non-muscat but aromatic variety with total monoterpene concentration of 1-4 mg/L according to Mateo & Jiménez (2000). The most important terpenic compounds found in the wines from Malagousia were linalool and β-damascenone (**Figure 3-112** & **3-116**). Both were in all the wines far above the odour threshold of these compounds (linalool: 25 µg/L; Ferreira *et al.*, 2000; β-damascenone: 0.14 µg/L; Pineau *et al.*, 2007). The only wine that presented an exception was M1 that showed very low linalool concentrations. Hotrienol was measured in three of the wines (M4, M13 & M14) in high concentrations that should play a role in their 'floral' character (**Figure 3-113**). M13 showed amounts of more than four times the odour threshold (110 µg/L; Ribéreau-Gayon *et al.*, 1999). α-Terpineol was found in five of the wines in significant concentrations (M3, M4, M8, M13 & M14) and geraniol in M14 (**Figure 3-114** & **3-117**). Nerol was found not to contribute in any of the wines (**Figure 3-115**). A summary of all the measured terpenes can be found in **Appendix 6-18.**

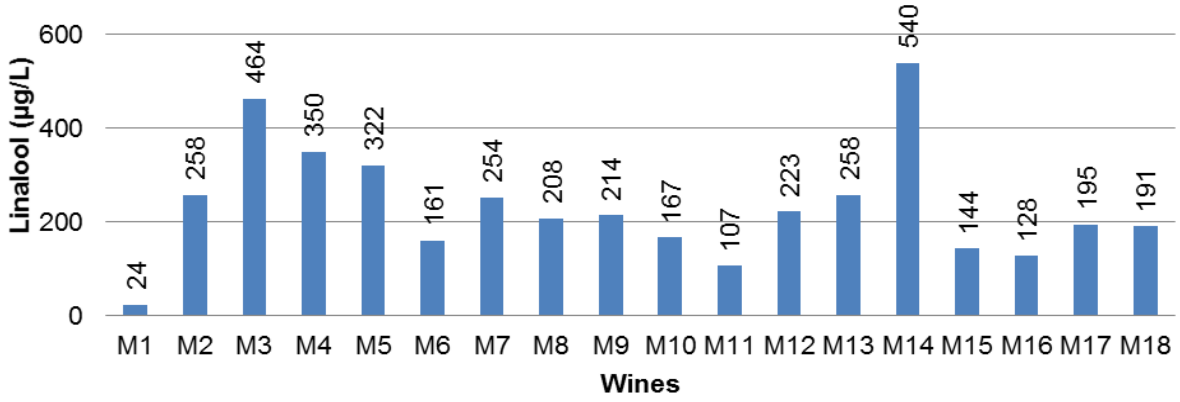


Figure 3-112: Linalool concentration of the 18 commercial wines from the Greek variety Malagousia.

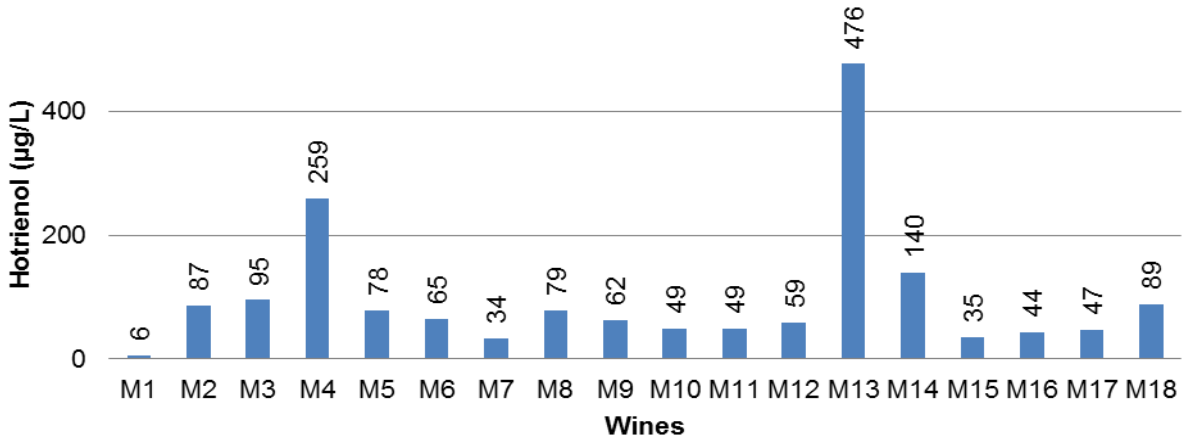


Figure 3-113: Hotrienol concentration of the 18 commercial wines from the Greek variety Malagousia.

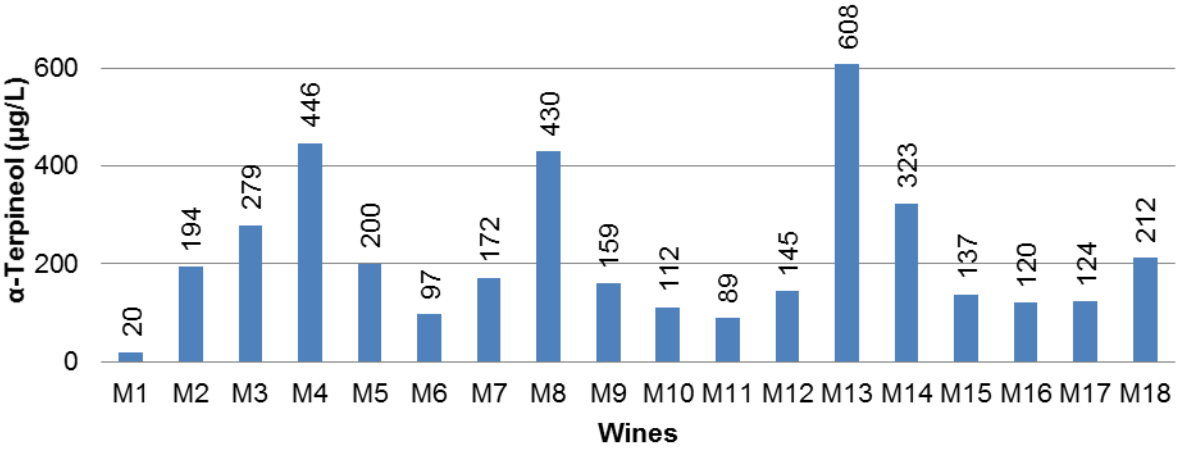


Figure 3-114: α-Terpineol concentration of the 18 commercial wines from the Greek variety Malagousia.

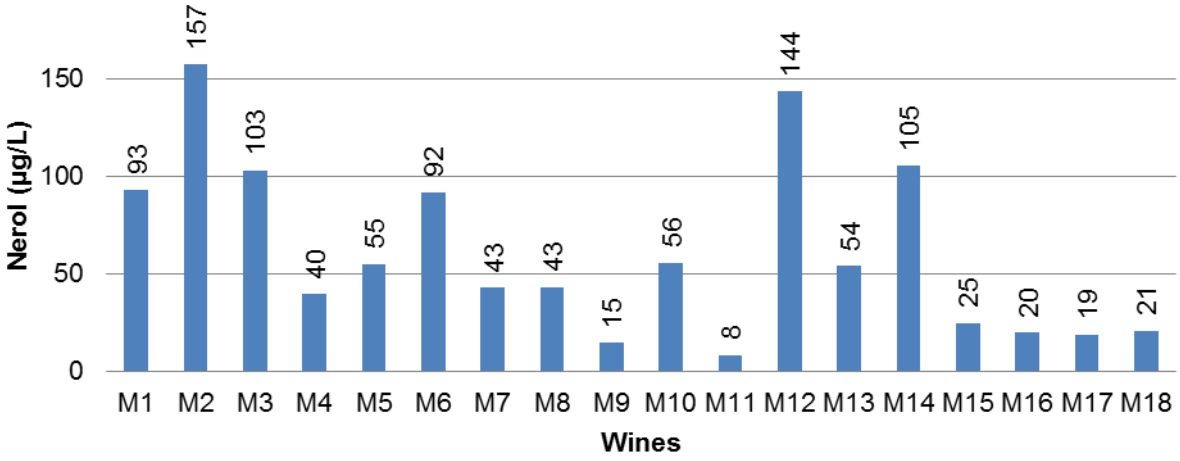


Figure 3-115: Nerol concentration of the 18 commercial wines from the Greek variety Malagousia.

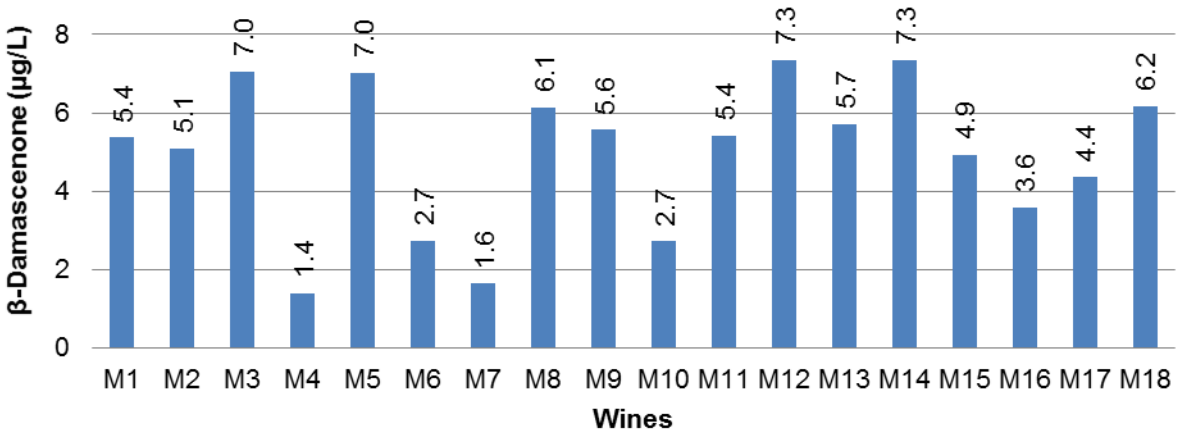


Figure 3-116: β-Damascenone concentration of the 18 commercial wines from the Greek variety Malagousia.

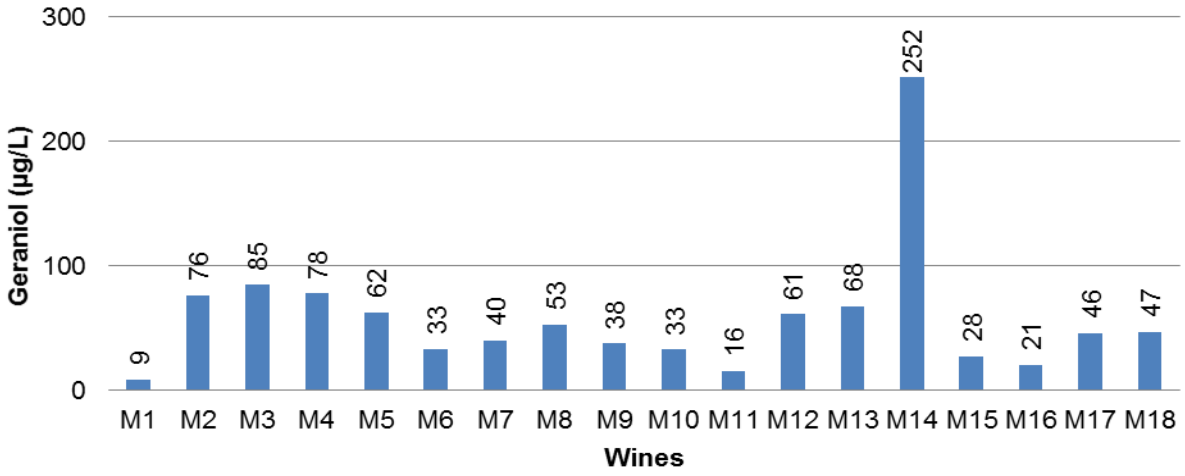


Figure 3-117: Geraniol concentration of the 18 commercial wines from the Greek variety Malagousia.

3.4.1.4 Low boiling point sulphur compounds

Low boiling point sulphur compounds do not seem to play a significant role in the aroma of Malagousia wines from the vintage 2013. Only wines M2, M7 and M17 had concentration of H_2S above the odour threshold (**Figure 3-118**) (10 µg/L; Dittrich & Grossmann, 2011). Despite this fact none of the wines was described to have reductive notes by the tasters. MeSH was marginally below (**Figure 3-119**) and DMS levels were significantly below the odour threshold (**Figure 3-120**), which means that the aromatics of the wines would not be affected (Segurel *et al.*, 2004; Solomon *et al.*, 2010; Dittrich & Grossmann, 2011). A summary of the results for the low boiling point sulphur compounds can be found in **Appendix 6-19**.

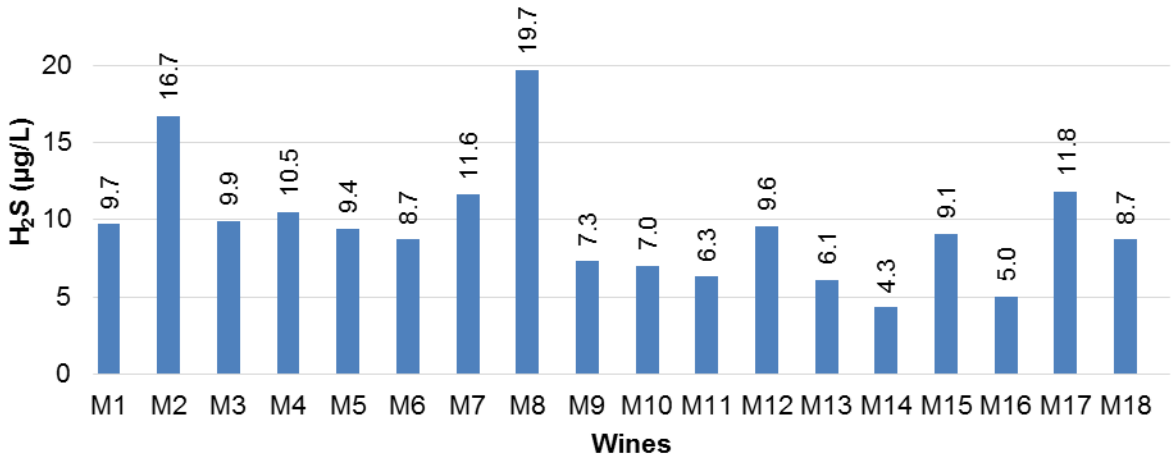


Figure 3-118: Hydrogen sulphide (H_2S) concentration of the 18 commercial wines from the Greek variety Malagousia.

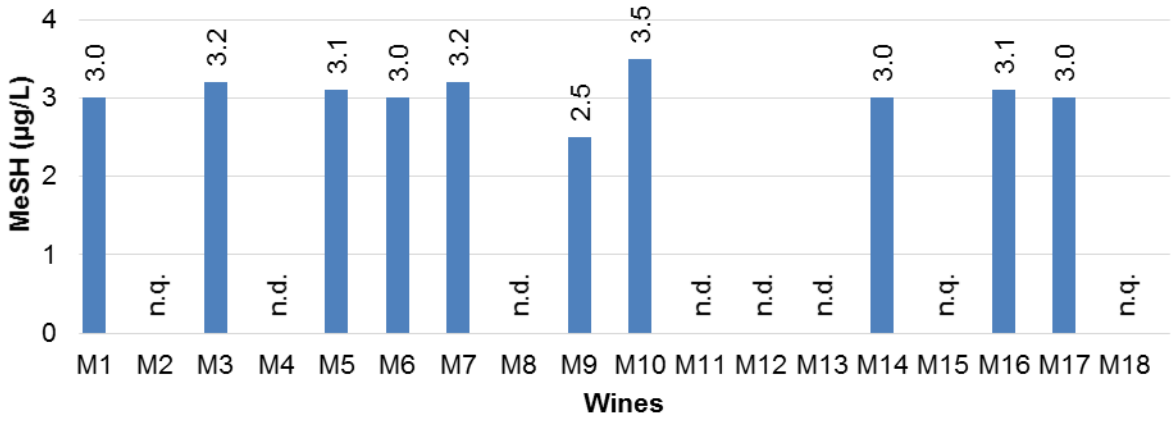


Figure 3-119: Methanethiol (MeSH) concentration of the 18 commercial wines from the Greek variety Malagousia.

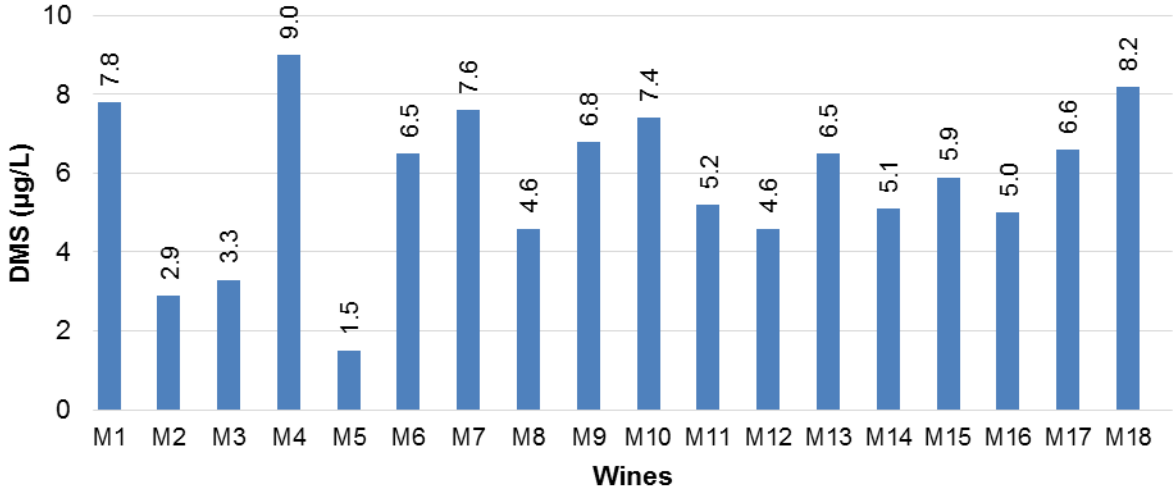


Figure 3-120: Dimethylsulphide (DMS) concentration of the 18 commercial wines from the Greek variety Malagousia.

3.4.1.5 Varietal thiols

The results presented in **Chapter 3.3.5** showed that wines from the variety Malagousia contained significant concentrations of varietal thiols. During sensory analysis of the Malagousia wines for the vintage 2013, some tasters reported for wine M4 attirbutes like ‘catpiss’ (x2), ‘cassis’ (x3) and ‘blackcurrant’ (x1). This was a strong indication singnificant concentrations of 4MSP should be found. Similar, wine M11 was reported by the tasters with descriptors ‘blackcurrant’ (x1), ‘cassis’ (x1) and ‘catpiss’ (x2). The results for the varietal thiols presented here confirmed the preliminary findings reported in **Chapter 3.3.5**. Malagousia wines were found to be very rich in varietal thiols with high concentrations of both 3SH and, the not so ubiquitous, 4MSP. 10 out of the 18 wines were found to have 4MSP above the odour threshold (3 ng/L; Darriet *et al.*, 1995). Wine M4 showed a 4MSP concentration of 44.7 ng/L and M11 15.9 ng/L (**Figure 3-121**). For 3SH only two wines showed concentrations below the LOQ of the method (M2 & M12) (**Figure 3-122**).

No 3SHA was found and could be attributed to the following two possibilities: **(1)** the concentrations of the wines in 3SHA were below the LOQ of 39 ng/L **(2)** the concentrations were below the LOD of the method. It was also shown in the previous study in **Chapter 3.3.5** that out of the six wines from Malagousia only two had 3SHA concentrations up to 84 ng/L (**Figure 3-86**).

The high concentrations of 4MSP for wines M4 and M11 corresponded to low 3SH concentrations although the opposite was to be expected. This tendency was observed also for wine M14 where 4MSP was marginally above the odour threshold and 3SH was found in the highest concentration of all the wines. Dubourdieu & Tominaga (2009) measured Sauvignon blanc wines from the vintage 1996 with concentrations of 4MSP 4 ng/L and 3SH 3736 ng/L whereas from the vintange 1995 the wines contained 4MSP 44 ng/L and 3SH 1686 ng/L. If there would be a correlation between the release of these two compounds it could possible to regulate the ratios of these varietal thiols during fermentation and regulate the impact on aroma. A summary of the results for the varietal thiols can be found in **Appendix 6-19**.

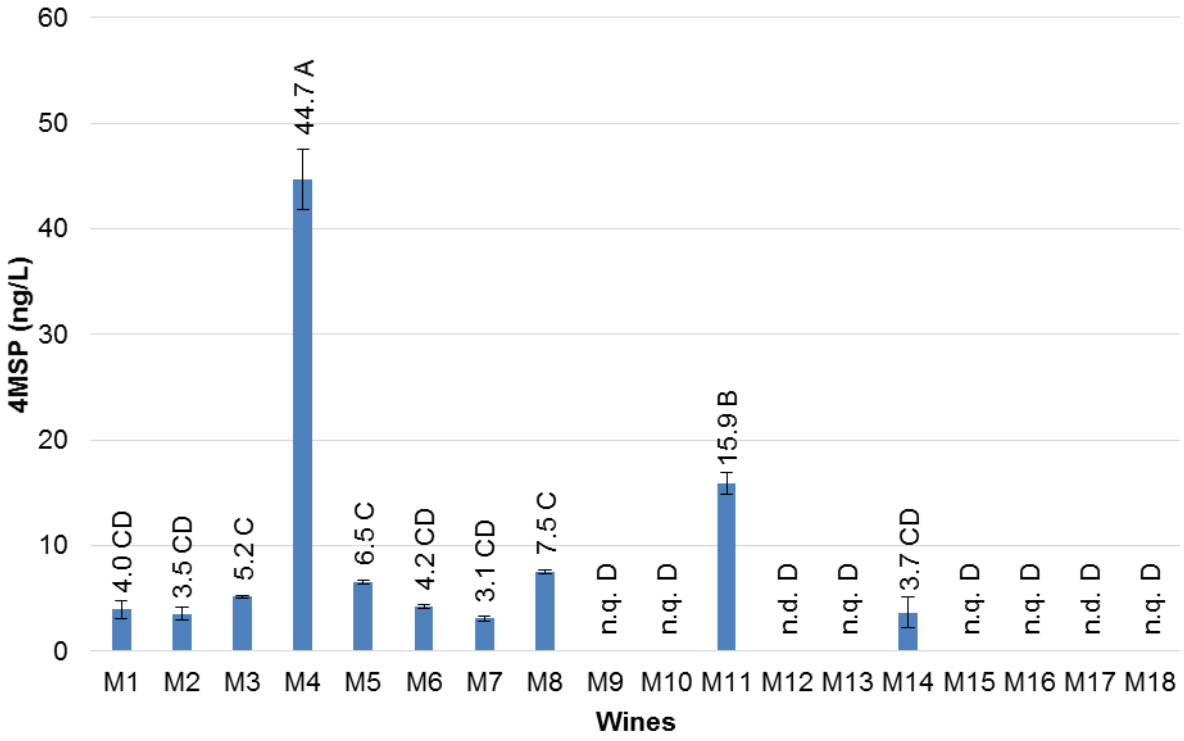


Figure 3-121: 4-methyl-4-sulfanylpentan-2-one (4MSP) concentration of the 18 commercial wines from the Greek variety Malagousia. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

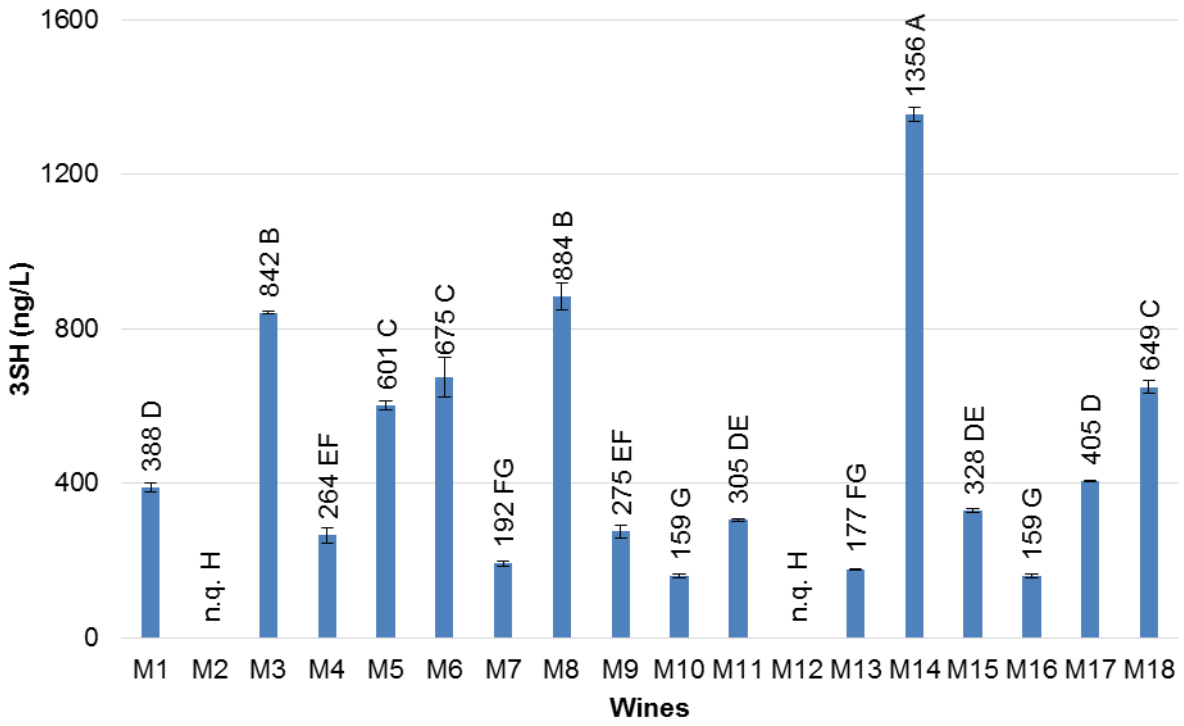


Figure 3-122: 3-sulfanylhexan-1-ol (3SH) concentration of the 18 commercial wines from the Greek variety Malagousia. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

3.4.1.6 Senosory analysis

Because of the large number of wines tasted it was decided to plot the samples with the highest and lowest 'impression' score. A summary of the tasting scores for all the wines can be found in **Appendix 6-20**.

Figure 3-123 showed a high variability in the perception of the descriptors for the wines from Malagousia. Substantial differences for the intensity scores were for the descriptors 'passionfruit', 'floral' and 'impression'. It could be that the overall 'impression' of the wines is associated with their 'floral' and exotic character. This is evident for wine M2 that showed high scores for the attributes 'passionfruit', 'grapefruit' and 'floral' but also showed the highest 'impression' score. This strong 'floral' character of wine M2 though cannot be explained by the quantified terpenes (**Chapter 3.4.1.3**) as these were not in highest concentration. On the other hand 2-Phenylethanol (descriptor: rose) was found in wine M2 at a concentration twice its odour threshold (Ferreira *et al.*, 2000) and could have an effect on the attribute 'floral'. Despite this fact there could still be further aromatic compounds in the wines from the variety Malagousia that have not been identified and contribute to the 'floral' character of the wines.

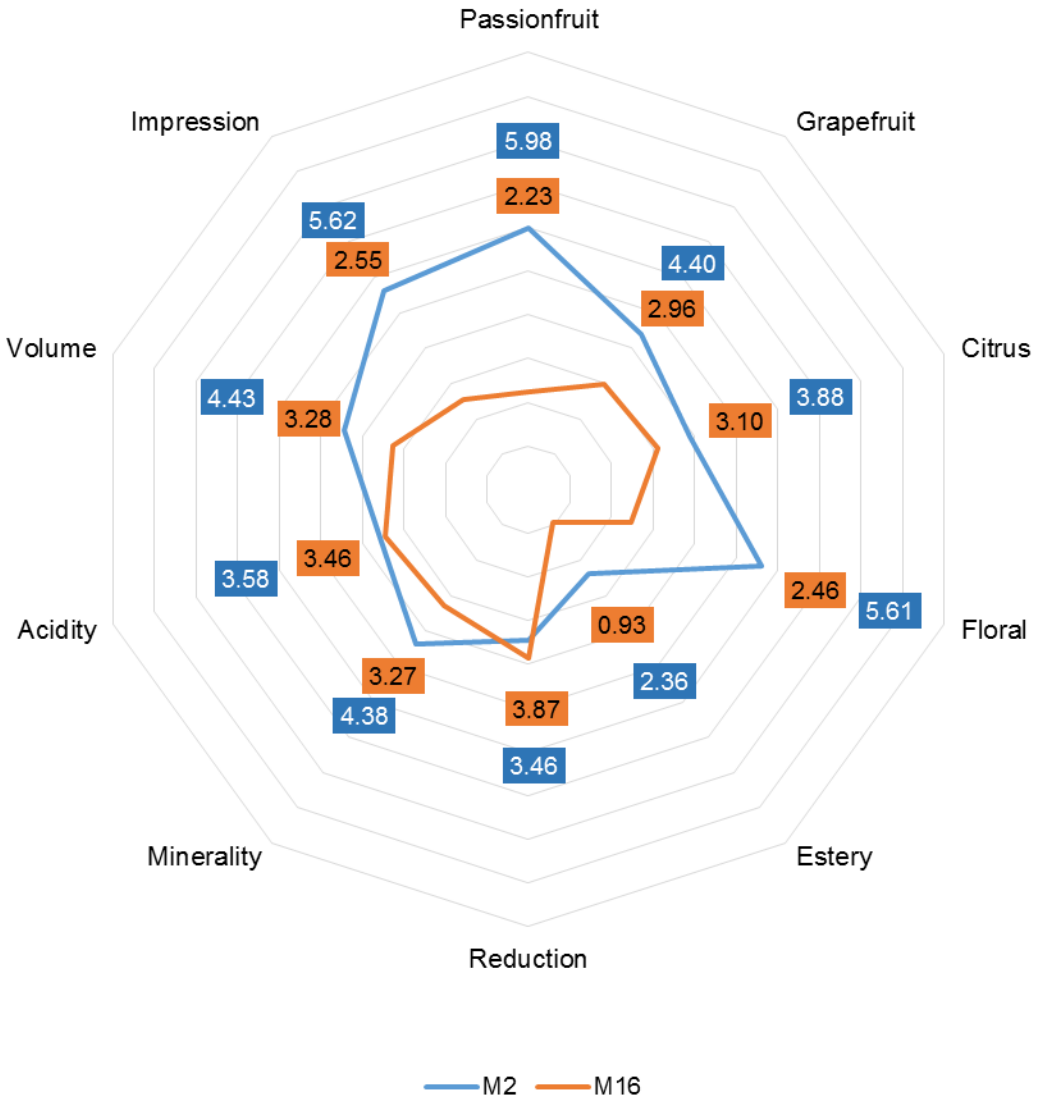


Figure 3-123: Spider plot for the sensory attributes of the 18 commercial wines from the Greek variety Malagousia. Only the wines with the highest and lowest 'impression' score were plotted. The blue line shows the wine with the highest 'impression' score and its values are in the blue boxes, whereas the wine with the lowest 'impression' score is represented with an orange line.

3.4.1.7 Statistical analysis

PCA plotting was used for analysing the data statistically. The OAVs were used for estimating the contribution of the aroma compounds to the aroma of the wines (Guth, 1997b; Ferreira *et al.*, 2000; Gómez-Míguez *et al.*, 2007). The data were mean centered and autoscaled before analysis (van den Berg *et al.*, 2006). A summary of the OAVs can be found in **Appendix 6-21.**

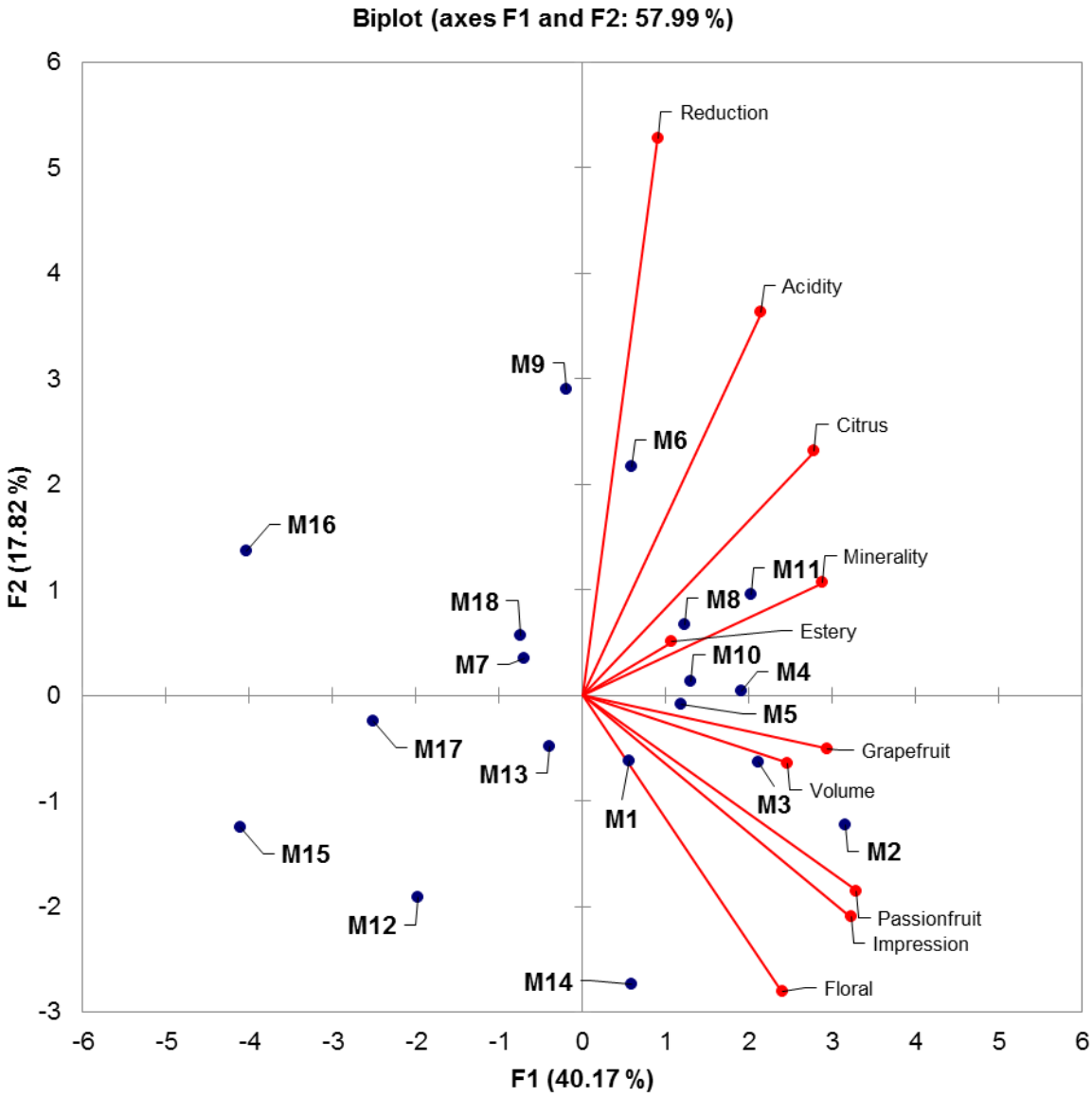


Figure 3-124: Principal Component Analysis (PCA) for the tasting results of the 18 commercial wines from the Greek variety Malagousia.

The PCA for the tasting results (**Figure 3-124**) accounted for 57.99 % of the variability in the data. F1 accounted for 40.17 % of the variability and was more heavily loaded with the descriptors 'passionfruit', 'grapefruit', 'citrus', 'floral', 'minerality' and 'impression' in the positive direction. F2 accounted for 17.82 % of the variability and was more heavily loaded with the descriptors 'acidity' and 'reduction' in the positive direction.

From **Figure 3-124** it can be seen that the wines from Malagousia can be perceived very differently. Nevertheless the 'impression' of the wines appears to have some correlation with the descriptors 'grapefruit', 'passionfruit' and 'floral'. Wine M4 does not seem to be well explained by the attributes although it was correlating with F1. Probably the high concentration of 4MSP and the description by the tasters as 'catpiss' (x2) could have dominated the aroma of the wine making its evaluation with the supplied questionnaire more difficult. This was though the first time that such high concentrations of

4MSP were found in Malagousia wines and it could be considered to add the descriptor ‘catpiss’ for future tastings. Wines M6 and M9 were well expaine by F2, which was correlating with ‘reduction’. The concentration of off-flavours in these two wines was found to be lower than most of the other wines measured (**Chapter 3.4.1.4**).

For determining which descriptors and compound that were in correlation with the ‘impression’ of the wines from Malagousia a Pearsons correlation test (p>0.05) was conducted in the total results.

Table 3-9: Correlation of the descriptor ‘impression’ with other descriptors/values. Only significant correlations are presented.

Descriptor	Correlation (p>0.05)
Passionfruit	0.687
Grapefruit	0.529
Floral	0.506
Minerality	0.569
Volume	0.643

Pearsons correlation showed that the descriptor ‘impression’ correlated only with descriptors (‘passionfruit’, ‘grapefruit’, ‘floral’, ‘minerality’ and ‘volume’). The aroma compounds that correlated with these descriptors were chosen also to be plotted (OAV>1) (**Appendix 6-28**). The PCA for the descriptor ‘impression’ (**Figure 3-125**) accounted for 50.33 % of the variability. F1 accounted for 34.39 % of the variability and was more heavily loaded with ‘linalool’, ‘α-terpineol’, ‘geraniol’, ‘3SH’, ‘passionfruit’, ‘grapefruit’, ‘floral’ and ‘impression’ in the positive direction. F2 accounted for 15.93 % of the variability and was more heavily loaded with the descriptor ‘minerality’.

From this PCA it could be said that the ‘impression’ of the wines from Malagousia probably correlates with the ‘floral’ and ‘exotic’ descriptors and not necessarily with specific compounds. It can be said that this attribute is more complex and further work will be needed to establish significant correlations.

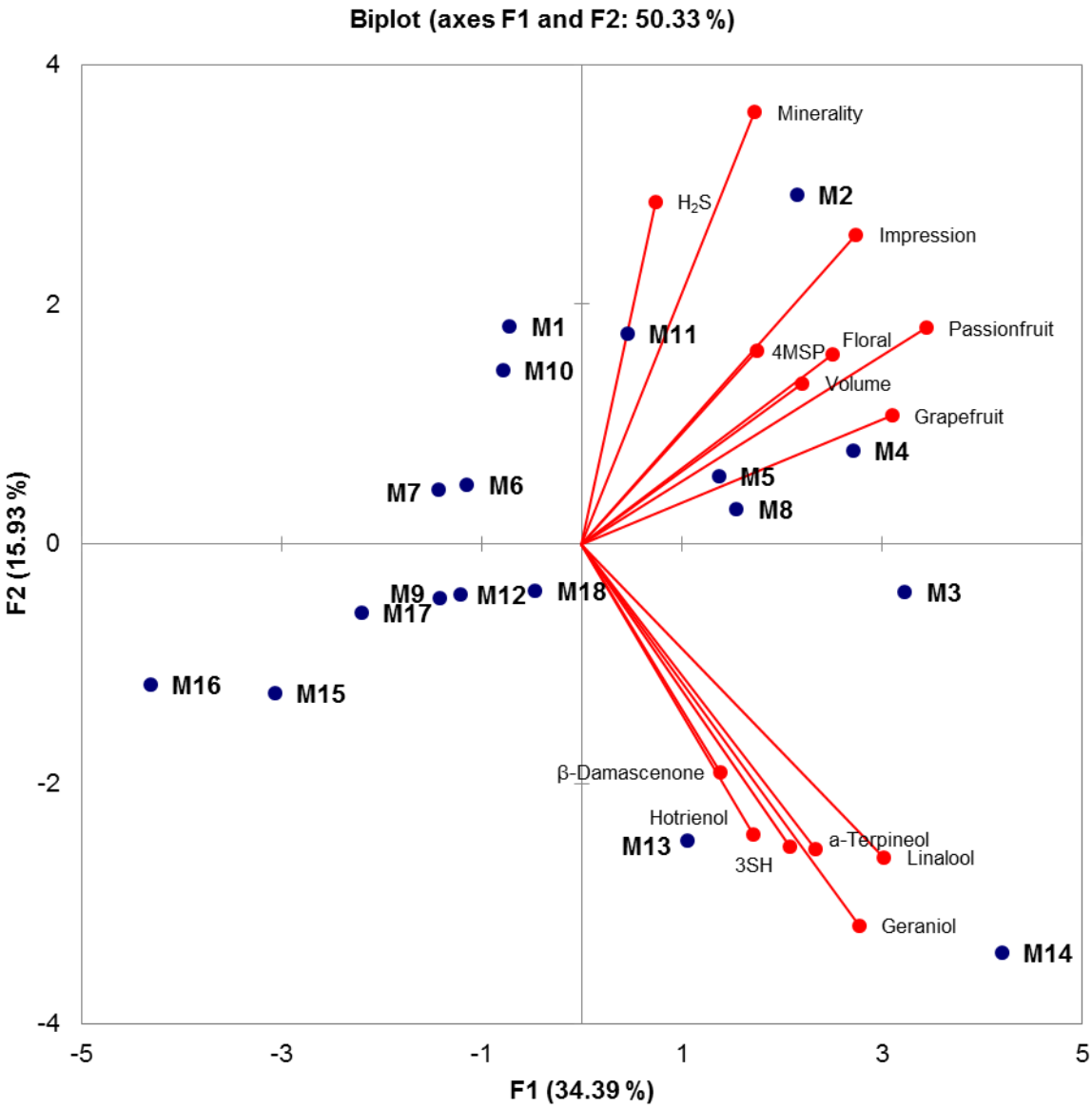


Figure 3-125: Principal Component Analysis (PCA) of the 18 commercial wines from the Greek variety Malagousia for the descriptor 'impression', the correlating descriptors and their compounds. The data were mean centered and autoscaled before analysis.

For the PCA of the aromatic compounds and the tasting attributes it was chosen to plot only the compounds that showed OAV values above 1 in at least one of the variants. The PCA on F1 and F2 accounted only for 38.28 % of the variability in the data, which is low (**Figure 3-126**). F1 accounted for 22.81 % of the variability and was more heavily loaded with '4MSP', 'passionfruit', 'grapefruit', 'minerality', 'volume' and 'impression' in the positive direction. F2 accounted with 15.47 % of the variability and was more heavily loaded with 'linalool', 'geraniol' and '3SH' in the positive direction. The rest of the attributes and compounds were scattered up to F7 (data not shown). As shown also in **Figure 3-125** the descriptors 'passionfruit' and 'floral' seem to be related with the descriptor 'impression'. 4MSP and 3SH are in equal distance from 'impression'. This could mean that both

compounds could play a role but none should be in very high concentrations or the ratio between them could be important.

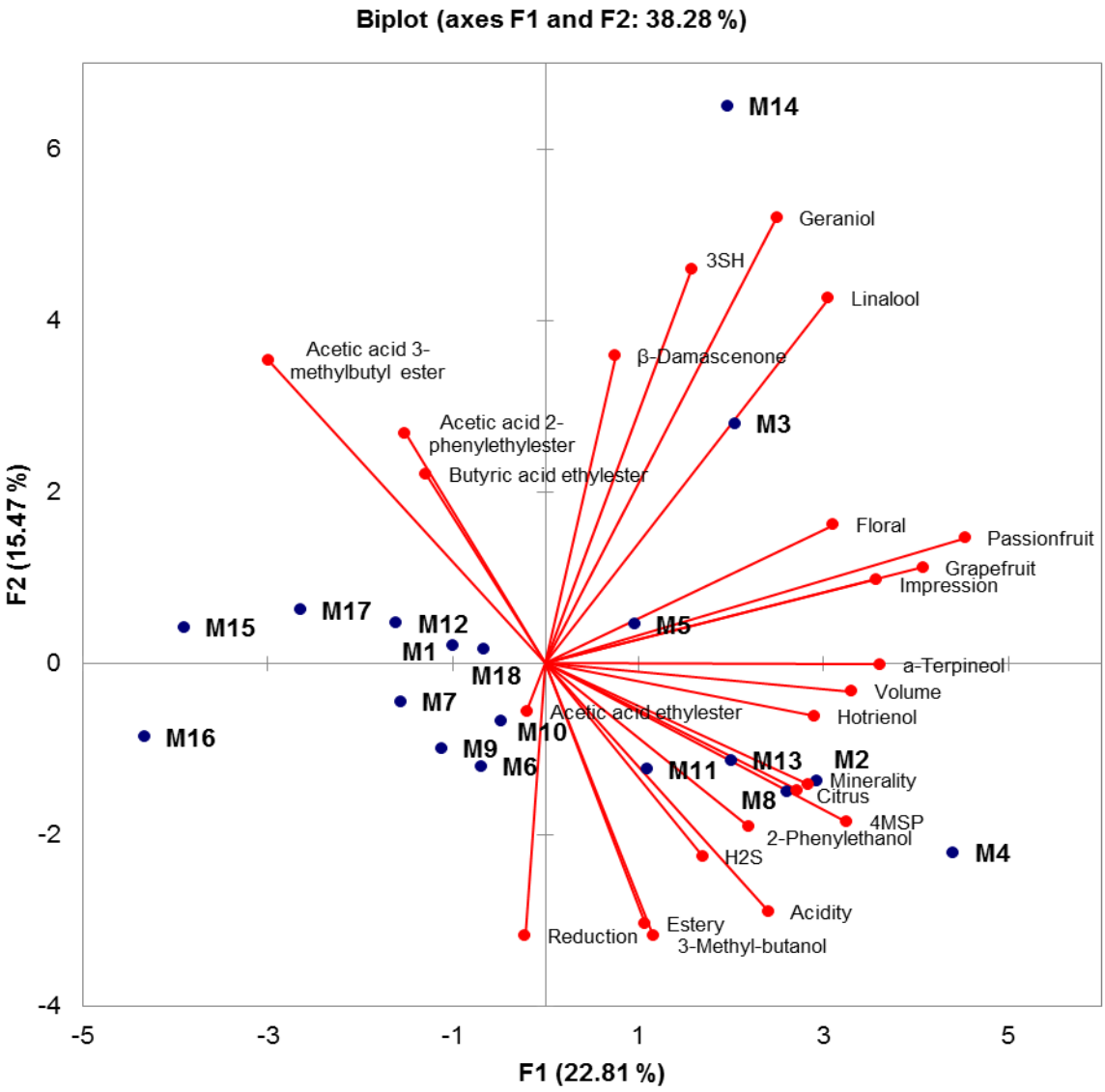


Figure 3-126: Principal Component Analysis (PCA) of the 18 commercial wines from the Greek variety Malagousia with the aromatic compounds that had an OAV > 1 in at least one of the samples. The data were mean centered and autoscaled before analysis.

3.4.1.8 Discussion and conclusion

The goal of this work package was to study the wines from the variety Malagousia in more detail, with special attention on the aromatic compounds (terpenes & varietal thiols). As it was mentioned in **Chapter 3.3**, the wines were found to be rich in terpenic compounds, so it was decided to study them more in depth with a specific method of analysis described in **Chapter 2.7.6**. Again the focus was also on varietal thiols. For this study, 18 commercial wines of the Greek autochthonous variety Malagousia from the vintage 2013 were collected from specialized shops in Greece and Germany.

The basic composition of the wines proved to be normal for young white wines from this variety (Tourtoglou *et al.*, 2014). All the wines had fermentation to almost dry with normal VA values. The levels of total phenols were similar to the values published for Malagousia and comparable to the other varieties of the Greek vineyards (Tourtoglou *et al.*, 2014). Furthermore, when comparing the values found in the specific phenolic compounds by Tourtoglou *et al.* (2014) with the concentrations needed to have an effect of the varietal thiols (Nikolantonaki *et al.*, 2010), the concentrations could be considered too low to have an effect.

The fruity ester acetic acid 3-methylbutylester was measured in concentrations almost 100 times above the odour threshold of the compound (30 μg/L; Guth, 1997b). Butyric acid ethylester was also measured in high concentrations, 19-30 times above the odour threshold (20 μg/L; Ferreira *et al.*, 2000; Fretz *et al*, 2005a). 3-methyl butanol and 2-phenylethanol were the two higher alcohols that were in concentrations high enough to have an effect in the aroma of the wines. The former was 3-5.7 above the odour threshold (30 mg/L; Guth, 1997b), while the latter was found in levels around the odour threshold and up to 2.3 times higher (14 mg/L; Ferreira *et al.*, 2000).

The results for terpenes showed significant concentration in almost all of the wines. Some empirical observations have been described by Lazarakis (2006) who stated that, if the grapes of the variety are harvested late the terpenic character of the wines becomes evident, giving to the wines almost a "muscat" character. Some data for experimental wines from Malagousia were published by Metafa & Economou (2013). This is the first time that data for the terpenic compounds in commercial wines from Malagousia are being published. As a prospect it could be interesting to link their release with the technological practice in the vineyard and the cellar, as some studies have shown for other varieties (Vaudano *et al.*, 2004; Carrau *et al.*, 2005). From the results it seems that the wineries are using a combination of: **(1)** viticultural practice enhancing the impact of terpenes like linalool, geraniol and nerol which could be in a free from in the grapes in combination with climate (Mateo & Jiménez, 2000; Carrau *et al.*, 2005) **(2)** winemaking practice for enhancing the flavour with additional terpenic compounds like α-terpineol and hotrienol that are affected by treatments (Mateo & Jiménez, 2000). The results would probably categorise Malagousia as a non-muscat but aromatic variety with total monoterpene concentration of 1-4 mg/L according to Mateo & Jiménez (2000).

The most abudand of the terpenic compounds found were linalool and β-damascenone. Both were present in all the wines in concentrations significantly above the odour threshold. Linalool was measured in wine M14 at 540 μg/L, which is almost 22 times above the odour threshold (25 μg/L, Ferreira *et al.*, 2000); the wine was characterized with the second highest score for the attribute 'floral' during the tasting by the expert panel (**Appendix 6-20**). Some of the wines also had significant concentrations of hotrienol (M4, M13 & M14), α-terpineol (M3, M4, M8, M13 & M14) and geraniol (M14) significantly above the odour threshold.

In the aspect of varietal thiols the preliminary results discussed in **Chapter 3.3** were confirmed. One of the wines (M4) showed a concentration of 4MSP of 44.7 ng/L and was commented by some tasters to have a 'catpiss' character. 10 out of the 18 wines studied had concentrations of 4MSP above the odour threshold (3 ng/L; Darriet *et al.*, 1995). Such high concentrations of 4MSP like in wine M4 have

not often been reported. Dubourdieu & Tominaga (2009) stated that 4MSP concentration may reach 40 ng/L in Sauvignon blanc. Two other reports of such concentrations found in dry white wines were published by Guth (1997b) for Scheurebe and by Dagan *et al.* (2014) for Sauvignon blanc from the Loire valley. High concentrations of 4MSP can give an upleasant odour of 'catpiss' (Darriet *et al.*, 1995) Furthermore, concentrations of 3SH up to 1356 ng/L were measured and were in similar levels as described in the preliminary test in **Chapter 3.3.5**. No 3SHA was found, which could be attributed to following two possibilities: **(1)** the concentrations of the wines in 3SHA were below the LOQ of 39 ng/L **(2)** the concentrations were below the LOD of the method. Due to these significant concentrations of varietal thiols it would be interesting to study the precursors in the musts from the variety Malagousia in the future and also determine their linking with the viticultural and enological practice, as it has been described by Peyrot des Gachons *et al.* (2005).

Exotic ('grapefruit', 'passionfruit') and 'floral' character seems to dominate the aroma of wines from Malagousia without significant reductive off-flavours. Furthermore, there was a significant correlation of the 'impression' of the wines with the descriptors 'passionfruit', 'grapefruit', 'floral', 'minreality' and 'volume'. These can be used as further hints for describing the variety more detailed in the future. Although removed from the tasting scheme in **Chapter 3.3** the descriptor 'catpiss' should be added in order to cover such extreme cases like the content of 4MSP in wine M4.

3.4.2 Petite Arvine

Fifteen monovarietal Petite Arvine wines from the vintage 2013 were either sourced from specialized shops in Switzerland or directly from the wineries. Three of the wines were a kind donation from Robert Gilliard SA in Sion, Valais. The pre-requisite for the wines was not be aged in barrels. **Table 2-9** gives details about the origin of each wine and the producer.

3.4.2.1 Major components

The Alcohol content was for young white wines elevated (**Figure 3-127**). Petite Arvine wines though are known for higher alcohol contents ranging from 12.5 to 14.5 % Vol. (Robinson *et al.*, 2012). Not all of the wines were fermented to dry and some had residual sugars left (**Figure 3-128**). PA1 showed 15.3 g/L of residual sugars, which was also commented by the tasters during sensory analysis. Other wines with notable residual sugar concentrations were PA4, PA6, PA7 and PA13 with 4.1, 6.8, 3.5 and 5.0 g/L respectively. PA2 appeared to have a lower total acidity when compared with the other wines and this could be explained by a partial MLF (**Figure 3-129**). VA of 0.4-0.5 g/L in acetic acid can be considered normal for young white wines (**Figure 3-130**) (Dittrich & Grossmann, 2011). A summary of the results for the major components can be found in **Appendix 6-22**.

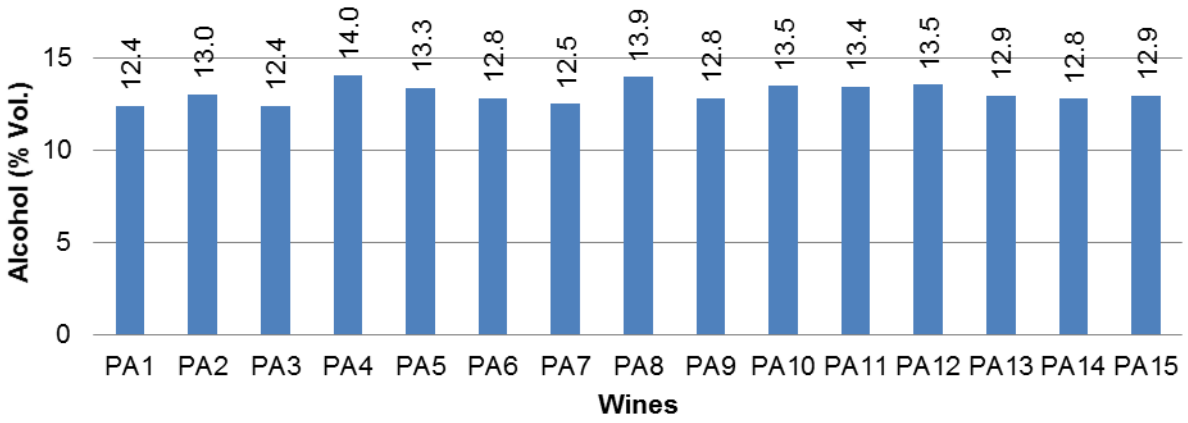


Figure 3-127: Alcohol content of the 15 commercial wines from the Swiss variety Petite Arvine.

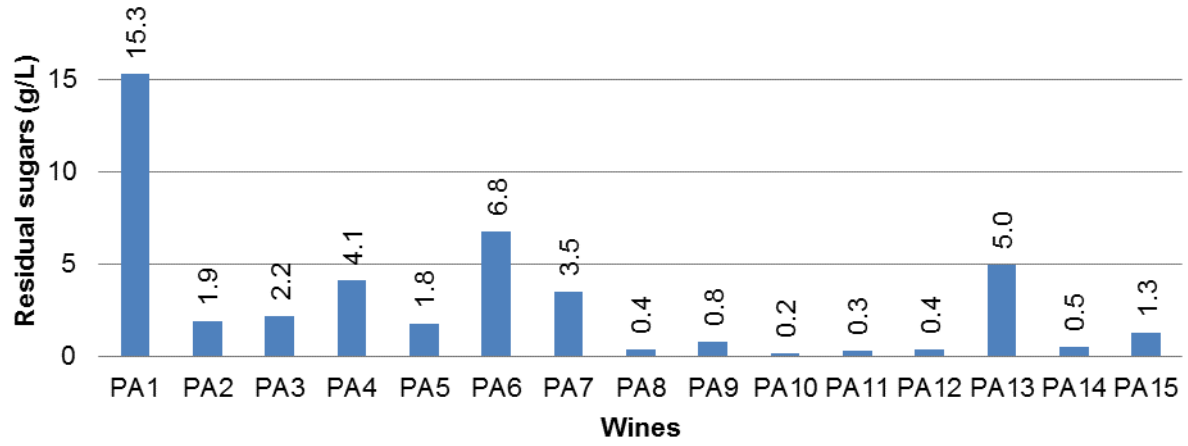


Figure 3-128: Residual sugars concentration of the 15 commercial wines from the Swiss variety Petite Arvine.

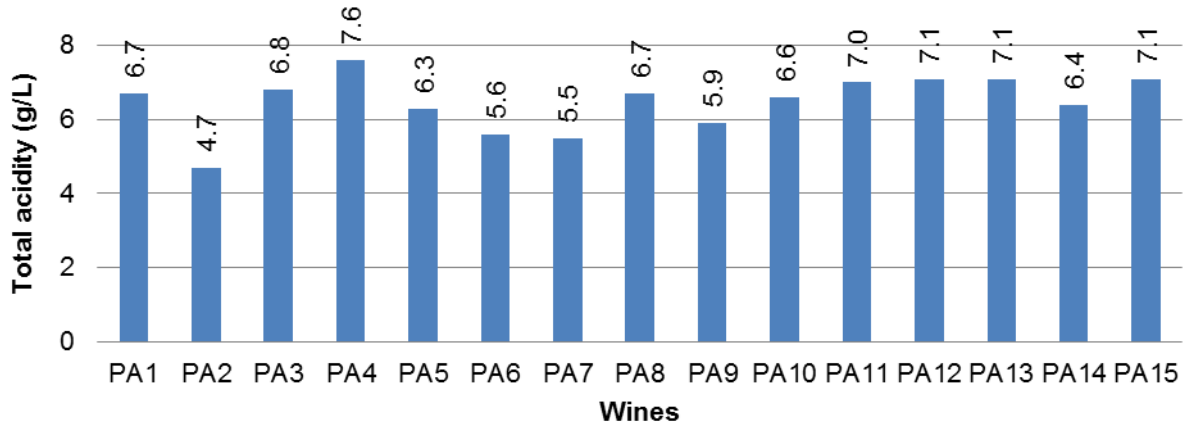


Figure 3-129: Total acidity of the 15 commercial wines from the Swiss variety Petite Arvine.

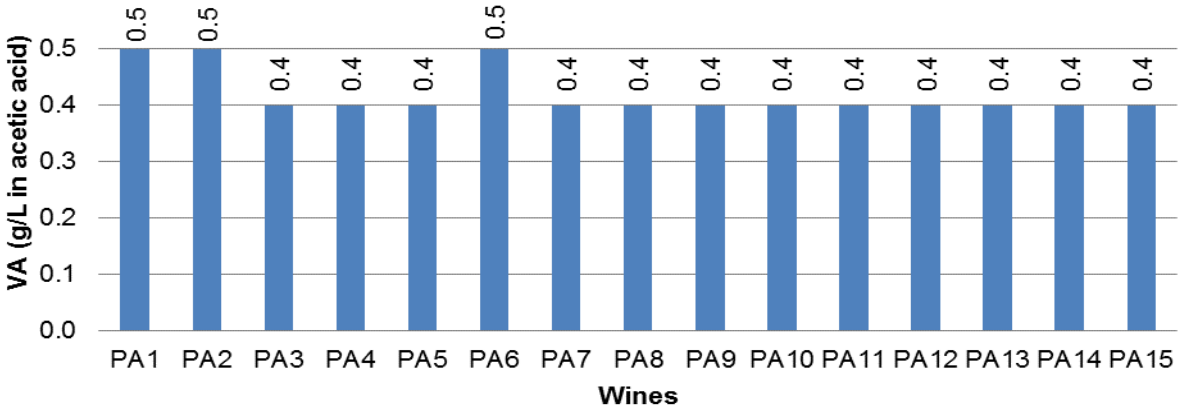


Figure 3-130: Volatile acidity (VA) of the 15 commercial wines from the Swiss variety Petite Arvine.

Some of the wines showed lower malic acid values as was the case for wine PA2 (**Figure 3-132**). The values for lactic acid revealed that some of the wines had underwent a partial MLF (**Figure 3-133**). Lactic acid opposed to malic acid has one carboxyl group lowering the total acidity of the wine, which could explain the lower total acidity values in some of the samples. Such partial MLF was also the case for wines PA1, PA6 and PA14 and was also commented by the tasters. Furthermore, PA2 and PA14 were commented by the tasters as 'buttery', which is associated with MLF.

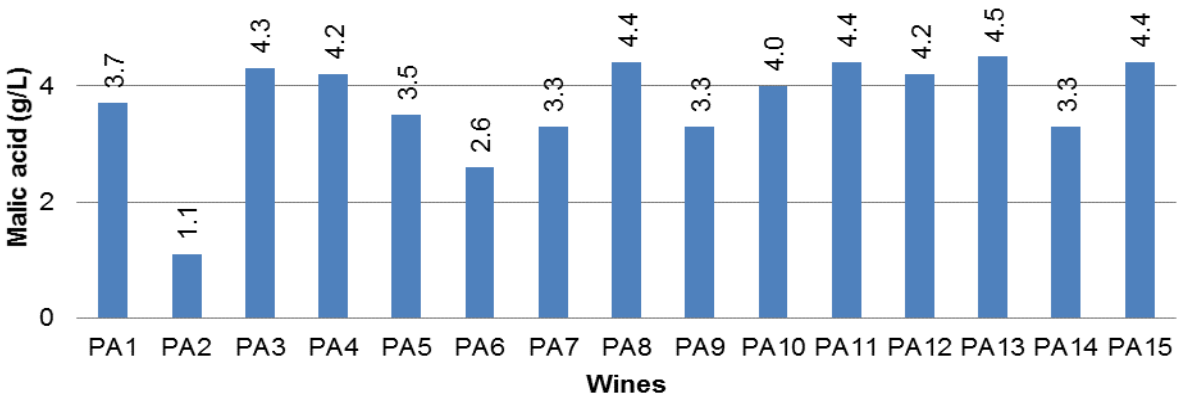


Figure 3-131: Malic acid concentration of the 15 commercial wines from the Swiss variety Petite Arvine.

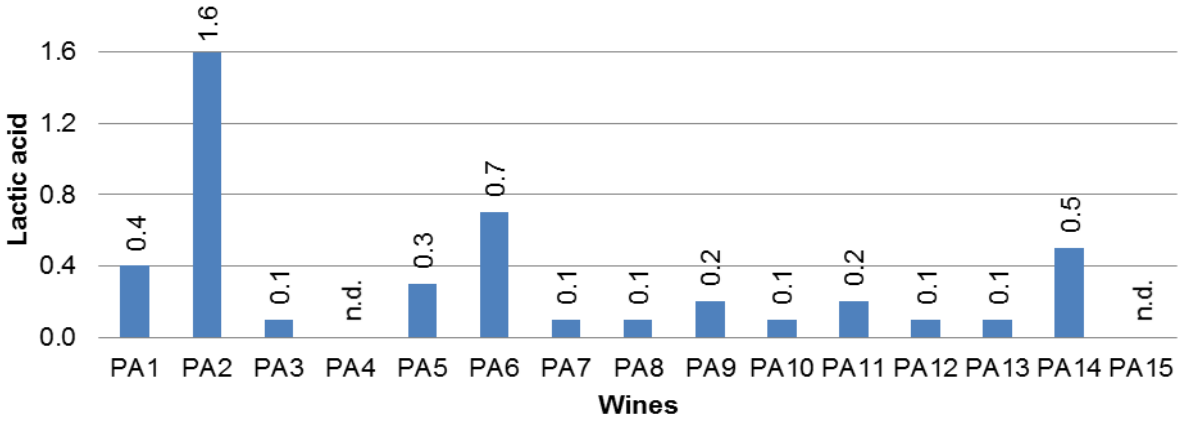


Figure 3-132: Lactic acid concentration of the 15 commercial wines from the Swiss variety Petite Arvine.

Data on total phenols for wines from Petite Arvine have not been published before. When compared with the concentrations measured for the wines from Malagousia (**Figure 3-101**) similar levels could be observed (refer to **Chapter 3.4.1.1**). The concentration of the total phenols were low to have an effect on the varietal thiols (Nikolantonaki *et al.*, 2010).

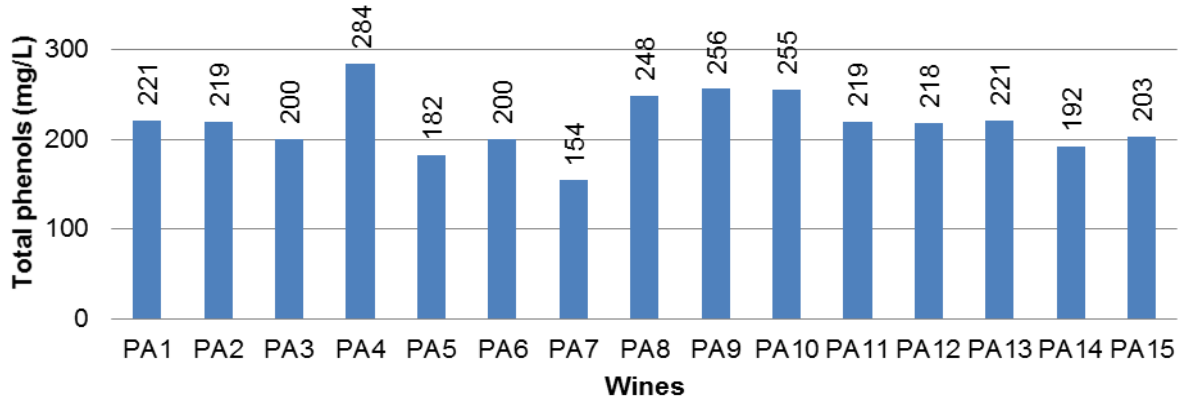


Figure 3-133: Total phenol concentration of the 15 commercial wines from the Swiss variety Petite Arvine.

3.4.2.2 Esters and higher alcohols

A summary of all the measured compounds with the 'Kaltron' method can be found in **Appendix 6-23.**

Esters: Acetic acid ethylester concentrations were low and could not contribute to the aromatic complexity of the wines (**Figure 3-134**). Significant variability was observed for acetic acid 3-methylbutylester ranging from 1.3 mg/L to 4.7 mg/L (**Figure 3-135**). Wine PA9 was commented by the tasters as "eisbonbon" (or reminiscent on the flavour of gummy bears), which is the description of this ester at high concentrations. Similar variations were also reported by Fretz *et al*. (2005a) although the minimum and maximum concentrations were lower. Acetic acid 2-phenylethylester has been reported by Fretz *et al.* (2005a) to be below the the odour threshold (250 µg/L; Guth 1997b). In the present study six of the wines were found to be above this threshold (**Figure 3-136**). Generally the esters are found in higher concentrations in young wines and during ageing they tend to decrease (Ribéreau-Gayon *et al.*, 1999; Fretz *et al.,* 2005a). The wines in this study were young so high amounts of esters were to be expected. Butyric acid ethylester also shows some significant concentrations (**Figure 3-137**) up to 36 times above its odour threshold (20 µg/L; Ferreira *et al.*, 2000). The concetrations reported by Fretz *et al.* (2005a) were much higher up to mg/L and could not be confirmed. The partial MLF was evident in the results of lactic acid ethylester (**Figure 3-138**). Both wines PA2 and PA6 showed increased values when compared with the other wines although the concentrations were below the odour threshold (155 mg/L; Etiévant, 1991). Succinic acid diethylester was below the odour threshold in all of the wines (**Figure 3-139**).

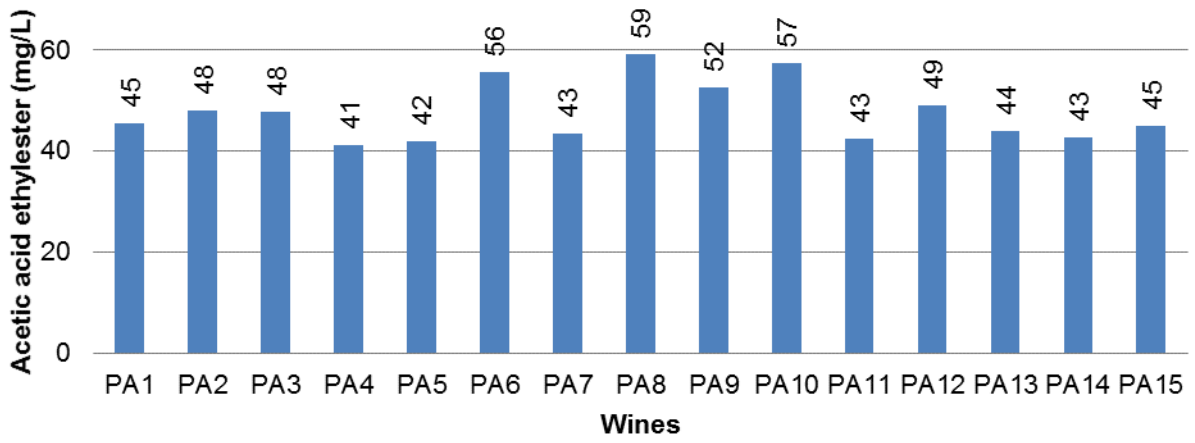


Figure 3-134: Acetic acid ethylester concentration of the 15 commercial wines from the Swiss variety Petite Arvine.

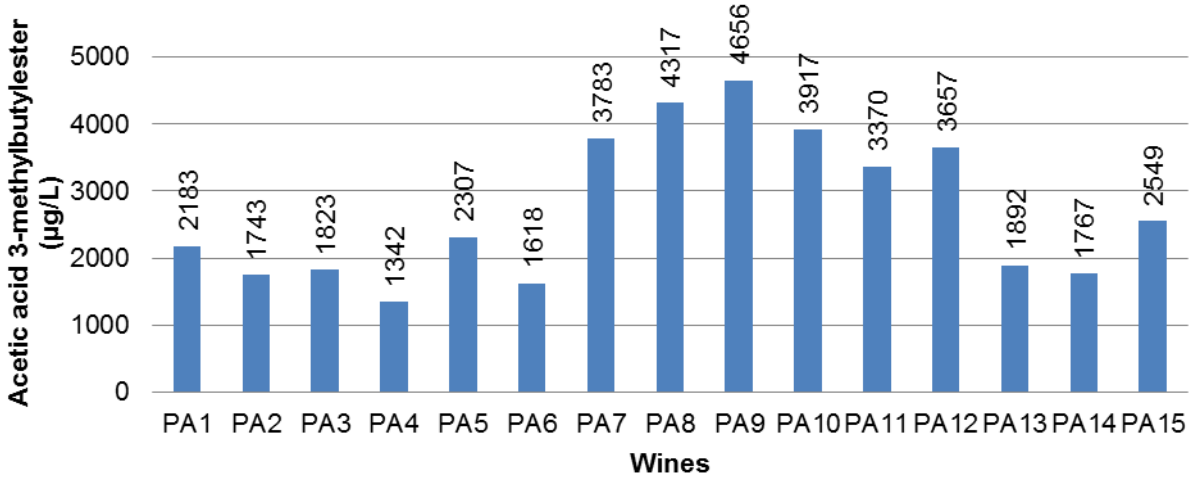


Figure 3-135: Acetic acid 3-methylbutylester concentration of the 15 commercial wines from the Swiss variety Petite Arvine.

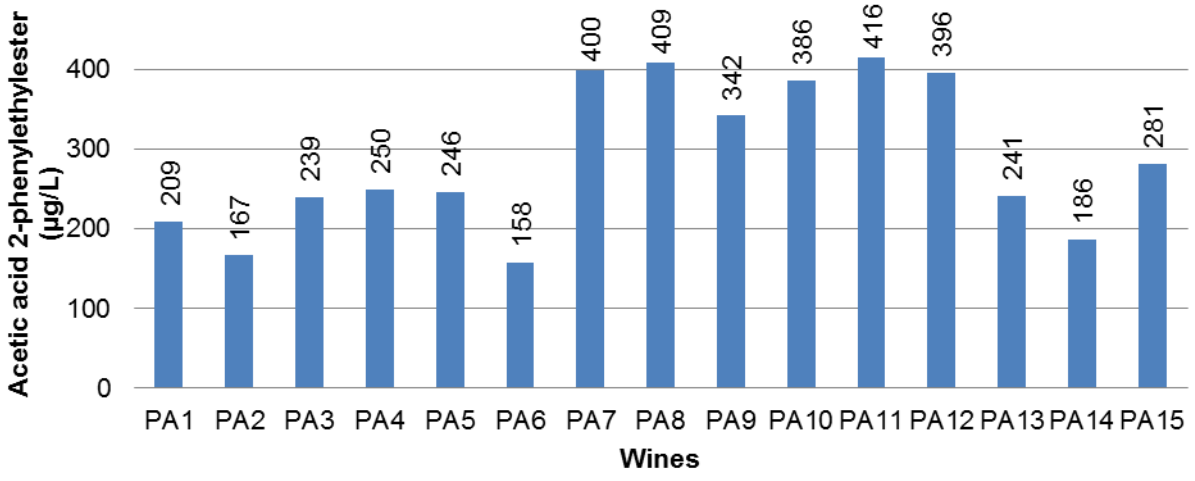


Figure 3-136: Acetic acid 2-phenylethylester concentration of the 15 commercial wines from the Swiss variety Petite Arvine.

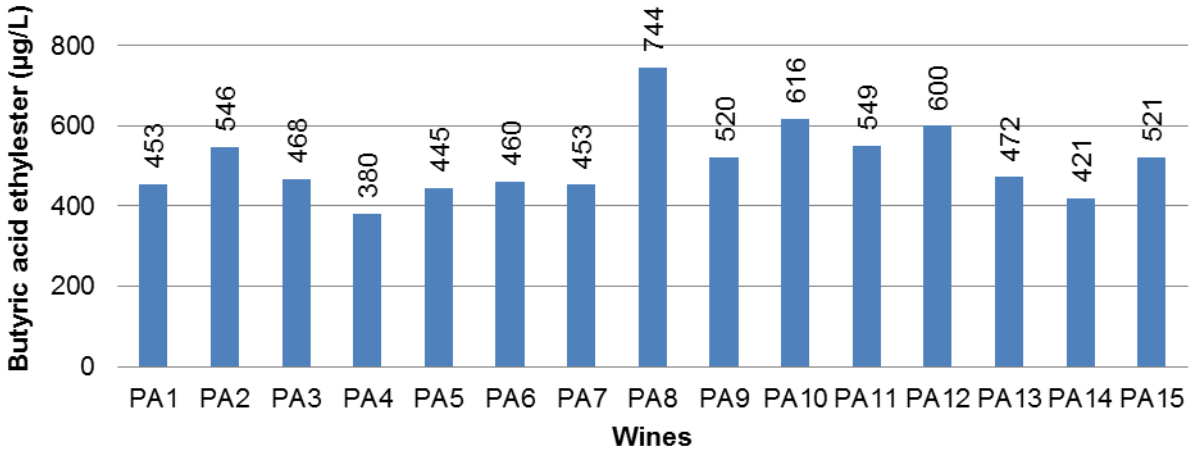


Figure 3-137: Butyric acid ethylester concentration of the 15 commercial wines from the Swiss variety Petite Arvine.

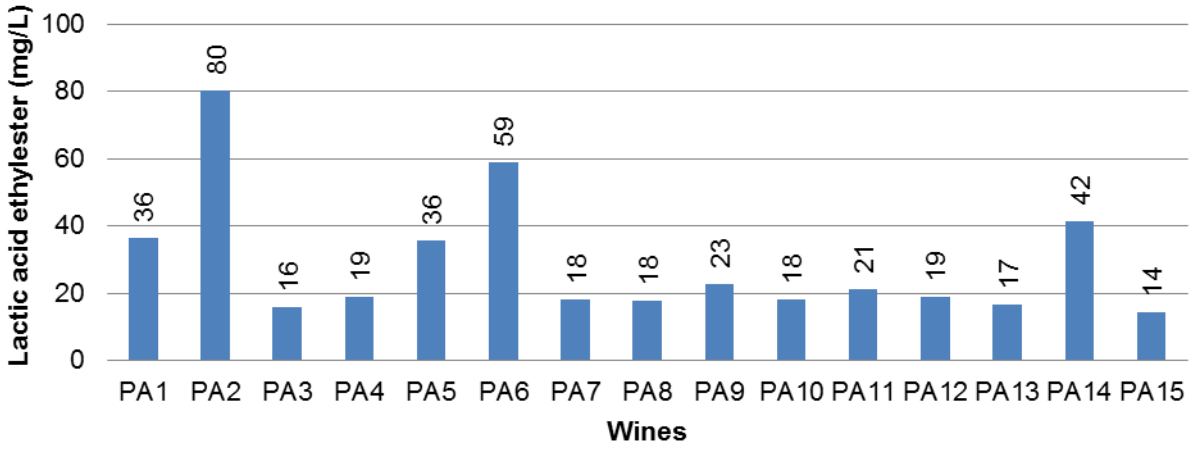


Figure 3-138: Lactic acid ethylester concentration of the 15 commercial wines from the Swiss variety Petite Arvine.

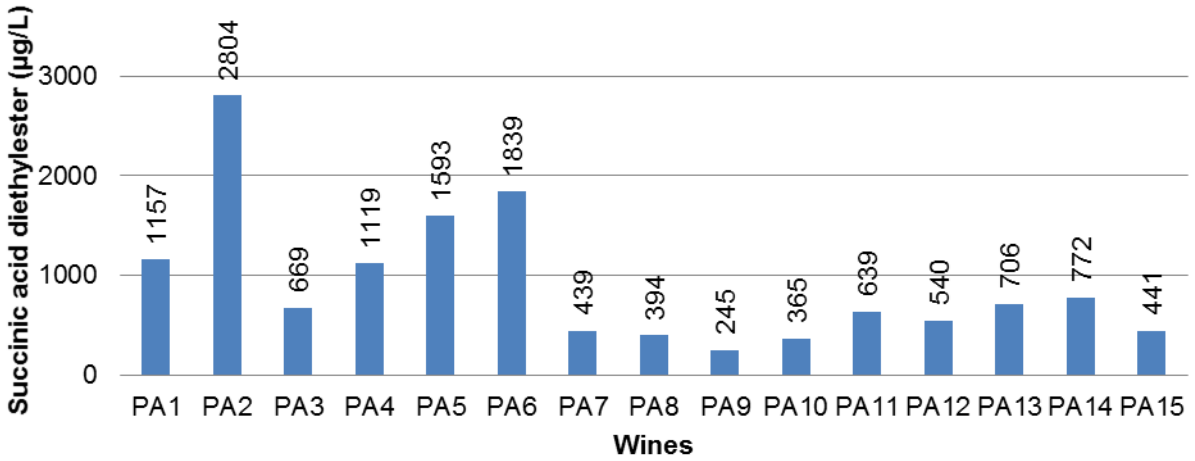


Figure 3-139: Succinic acid diethylester concentration of the 15 commercial wines from the Swiss variety Petite Arvine.

Higher alcohols: From the higher alcohols measured in the wines from Petite Arvine only 3-methyl butanol and 2-phenylethanol were present in significant concentrations to have an effect on

the aroma of the wines (**Figure 3-141** & **3-143**). 3-methyl butanol was found in amounts ranging form 3.3 to 6.1 times above the odour threshold in all the Petite Arvine wines (30 mg/L; Guth, 1997b). In a study by Fretz *et al.* (2005a) these concentrations did not exceed 75 mg/L (OAV = 2.5). 2-Phenylethanol was in all of the samples above the odour threshold of 14 mg/L (Ferreira *et al.*; 2000) and possibly contributing to the 'floral' character of the wines. i-Butanol and hexan-1-ol were found in such concentrations that could not contribute to the aroma of the wines (**Figure 3-140** & **3-142**).

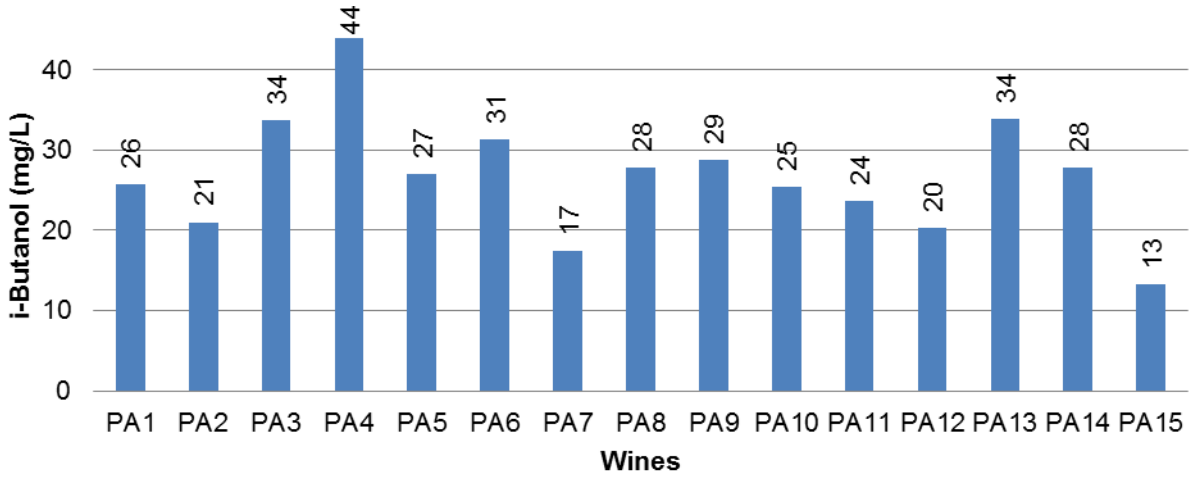


Figure 3-140: i-Butanol concentration of the 15 commercial wines from the Swiss variety Petite Arvine.

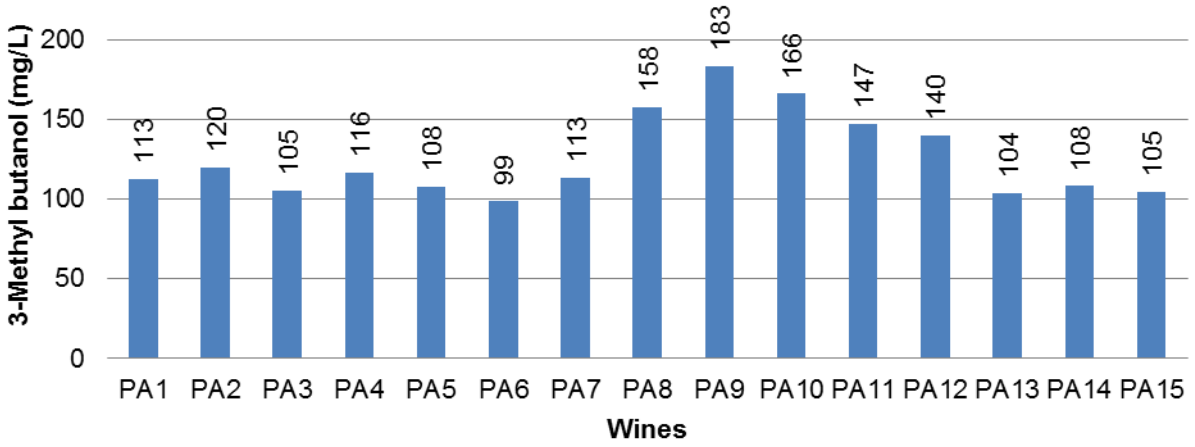


Figure 3-141: 3-Methyl butanol concentration of the 15 commercial wines from the Swiss variety Petite Arvine.

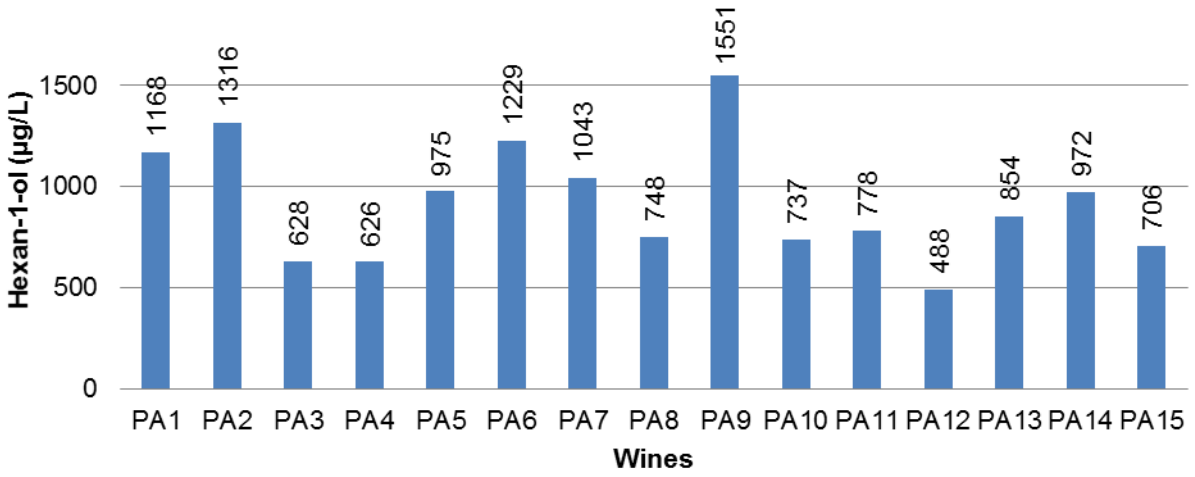


Figure 3-142: Hexan-1-ol concentration of the 15 commercial wines from the Swiss variety Petite Arvine.

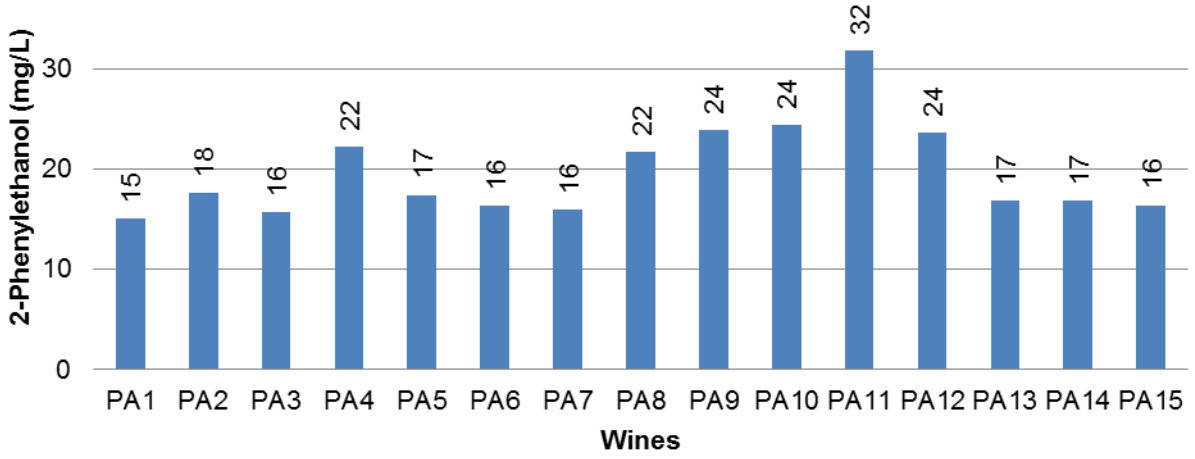


Figure 3-143: 2-Phenylethanol concentration of the 15 commercial wines from the Swiss variety Petite Arvine.

3.4.2.3 Terpenes

Wines from Petite Arvine showed low concentrations in terpenic compounds when compared to the wines from Malagousia (**Chapter 3.4.1.3)**. Only linalool and β-damascenone were found in concentrations that could have an effect on the aroma of the wines. For the case of linalool this did not apply for all the wines as only three (PA5, PA6 & PA7) had concentrations above the odour threshold (**Figure 3-144**) (25 µg/L; Ferreira *et al.*, 2000). β-Damascenone played a role in the aromatics of the Petite arvine wines as it was the strongest aromatically terpenic compound that was measured for this study. The concentrations of β-Damascenone varied considerably between 16 and 49 times above the odour threshold (**Figure 3-148**) (0.14 µg/L; Pineau *et al.*, 2007). These results would probably categorise Petite Arvine as a neutral variety not dependent upon monoterpenes for its flavour (Mateo & Jiménez, 2000). Hotrienol, α-terpineol, nerol and geraniol were below the odour threshold in all of the wines (**Figure 3-145, 3-146, 3-147** & **3-149**). A summary of all the measured terpenes can be found in **Appendix 6-24.**

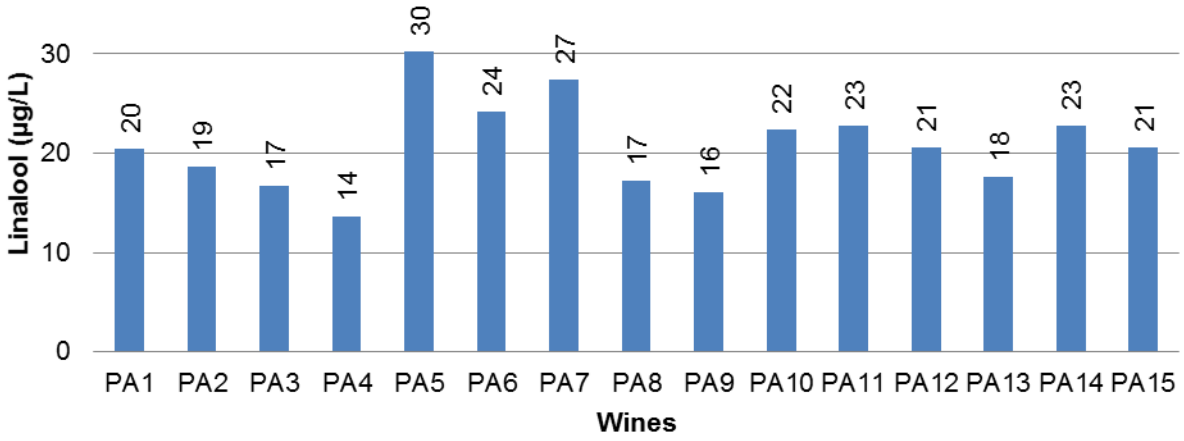


Figure 3-144: Linalool concentration of the 15 commercial wines from the Swiss variety Petite Arvine.

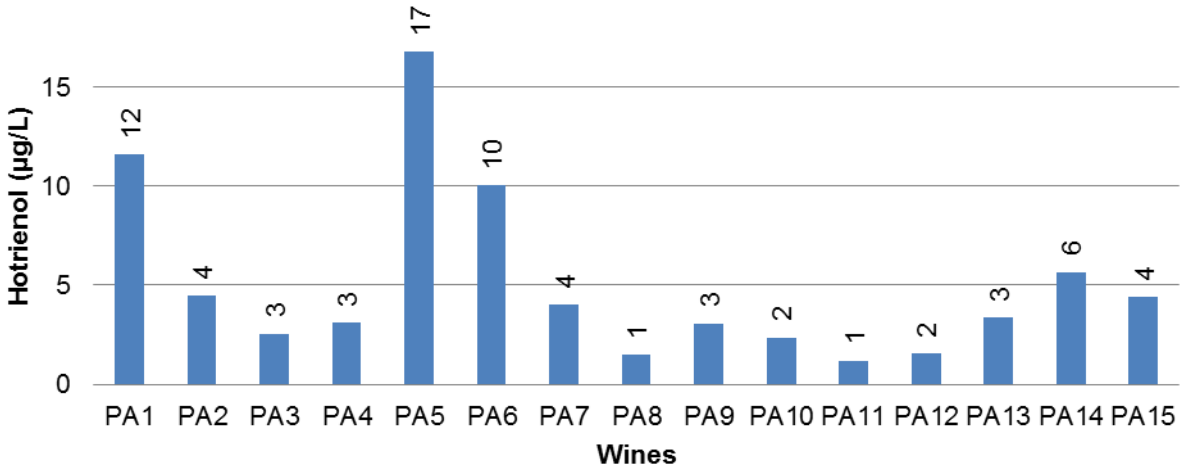


Figure 3-145: Hotrienol concentration of the 15 commercial wines from the Swiss variety Petite Arvine.

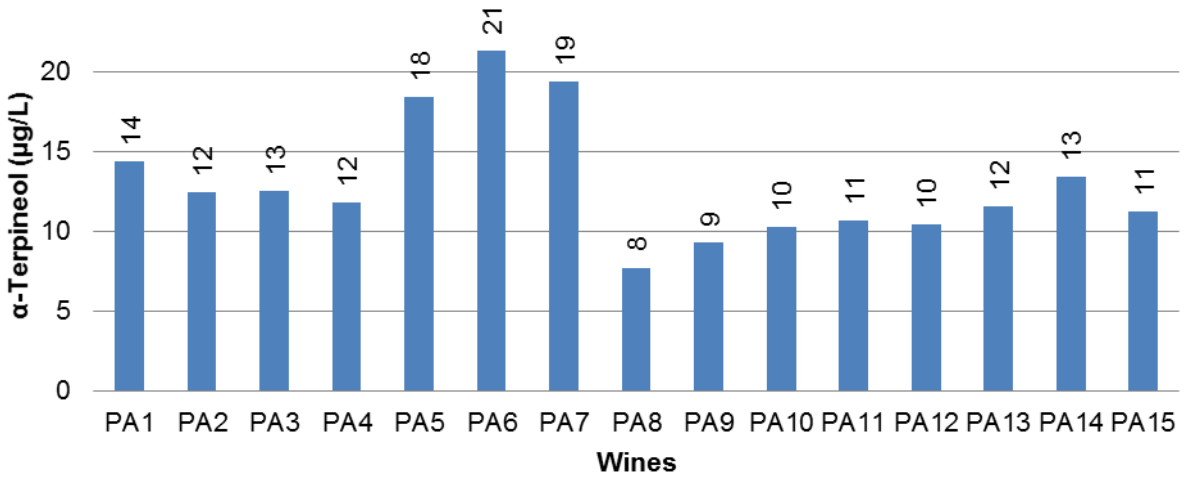


Figure 3-146: α-Terpineol concentration of the 15 commercial wines from the Swiss variety Petite Arvine.

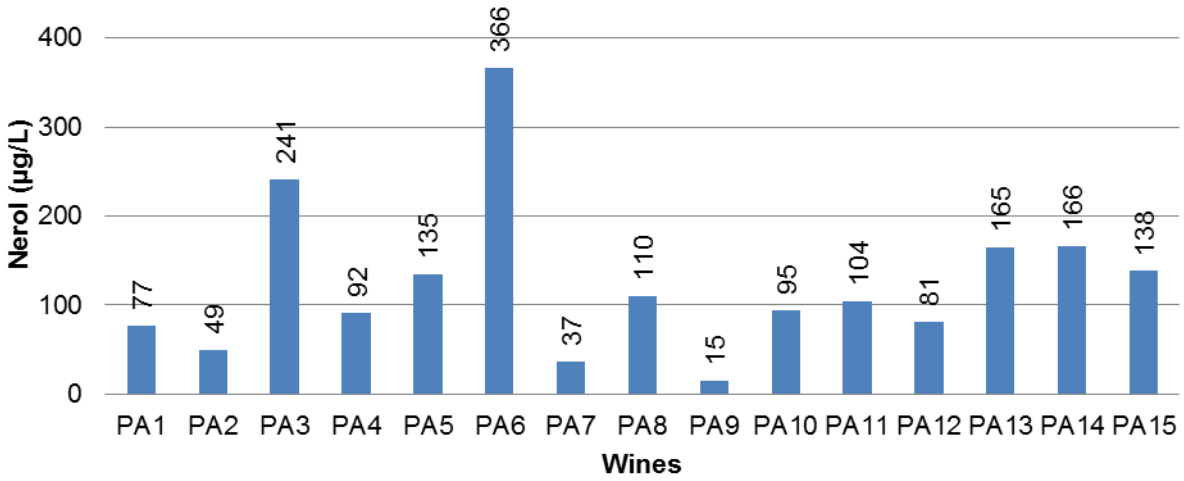


Figure 3-147: Nerol concentration of the 15 commercial wines from the Swiss variety Petite Arvine.

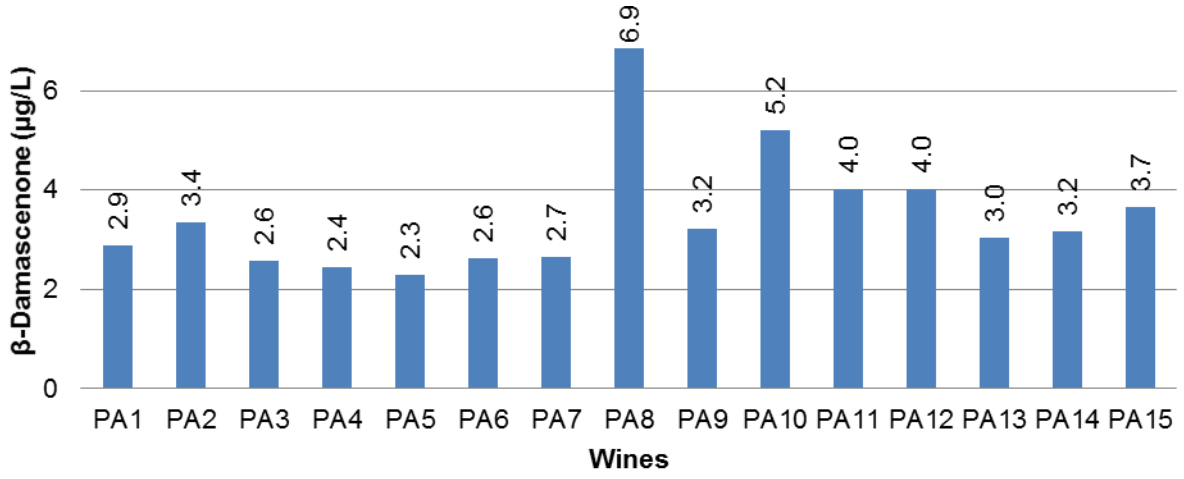


Figure 3-148: β-Damascenone concentration of the 15 commercial wines from the Swiss variety Petite Arvine.

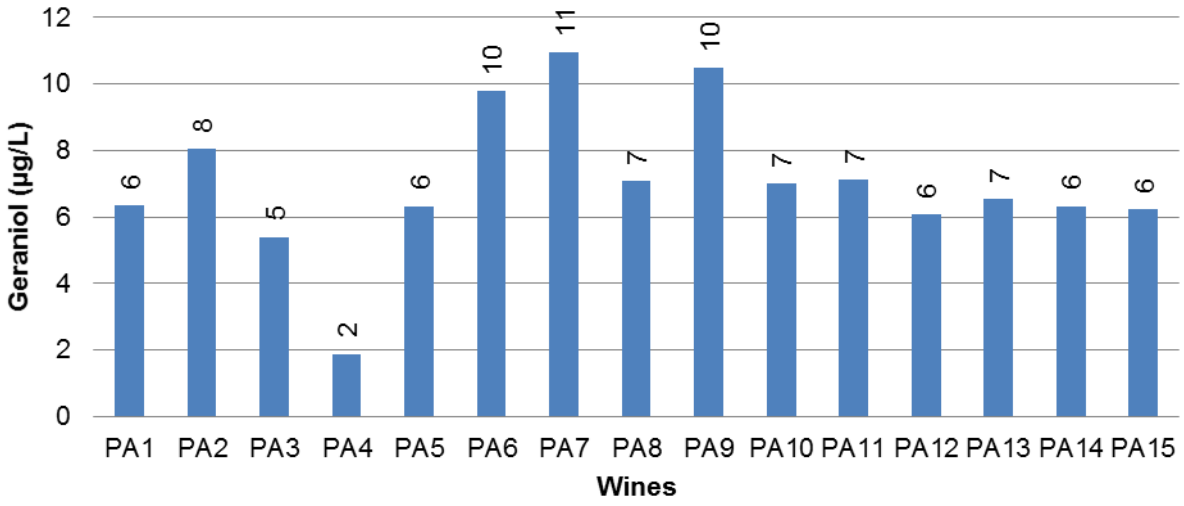


Figure 3-149: Geraniol concentration of the 15 commercial wines from the Swiss variety Petite Arvine.

3.4.2.4 Low boiling point sulphur compounds

For the wines from Petite Arvine of the vintage 2013 low boiling point suphur compounds did not seem to play a role in the aromatics. H_2S concentrations were mostly below the odour threshold except for wines PA1, PA2 and PA15 were these were marginally above (**Figure 3-150**) (10 µg/L; Dittrich & Grossmann, 2011). MeSH was in all of the wines below the odour threshold (**Figure 3-151**) (5 µg/L; Solomon *et al.*, 2010). None of the wines was characterised of having reductive notes by the tasters (**Figure 3-155**). Unusual though was the content of DMS for PA2 (**Figure 3-152**), which was above the odour threshold of 25 µg/L (Goniak & Noble, 1987). It is uncommon to have such high concentrations in young wines (Marais, 1979). One explanation for this could be a long contact of the wine with the lees that is known to increase the concentrations of DMS (Niefind, 1969). A summary of the results for the low boiling point sulphur compounds can be found in **Appendix 6-25**.

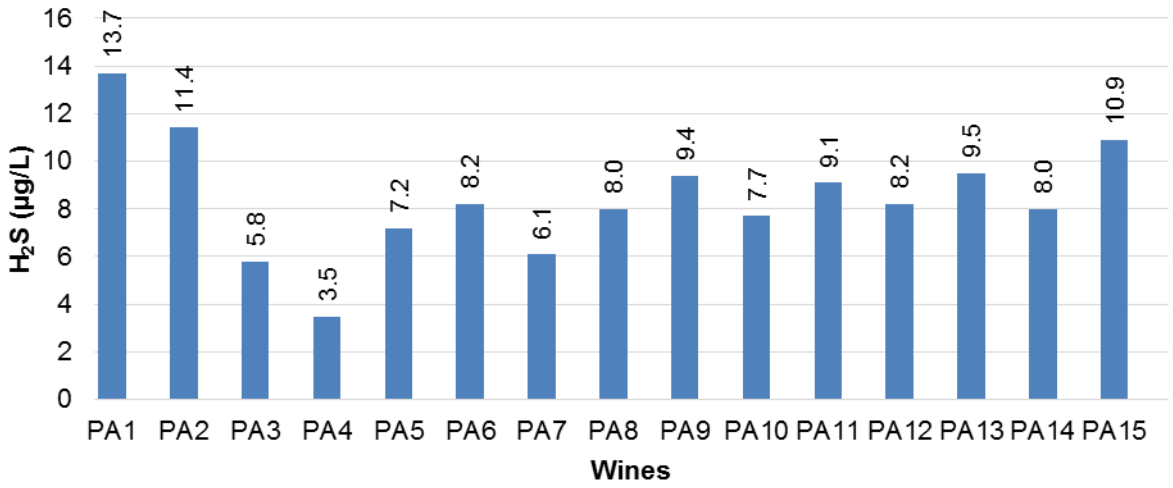


Figure 3-150: Hydrogen sulphide (H_2S) concentration of the 15 commercial wines from the Swiss variety Petite Arvine.

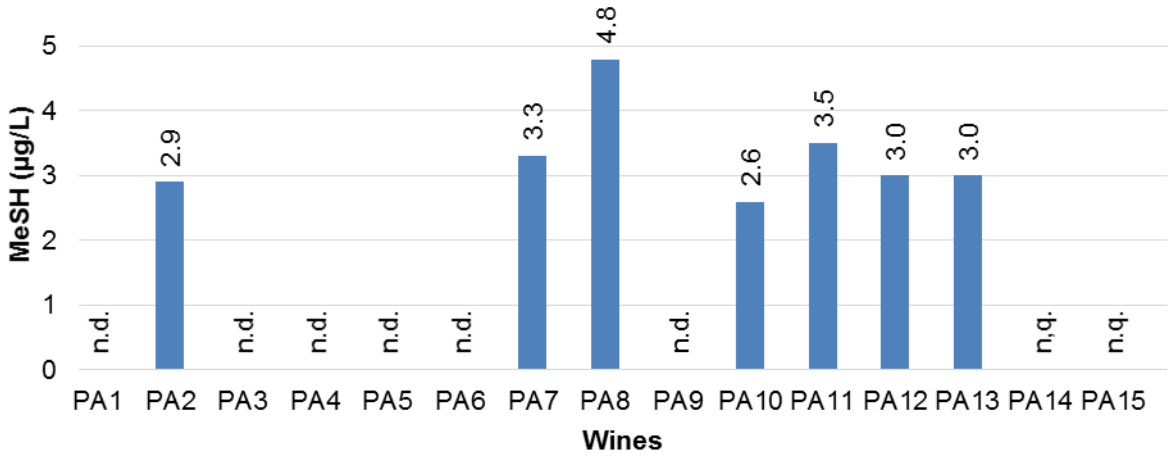


Figure 3-151: Methanethiol (MeSH) concentration of the 15 commercial wines from the Swiss variety Petite Arvine.

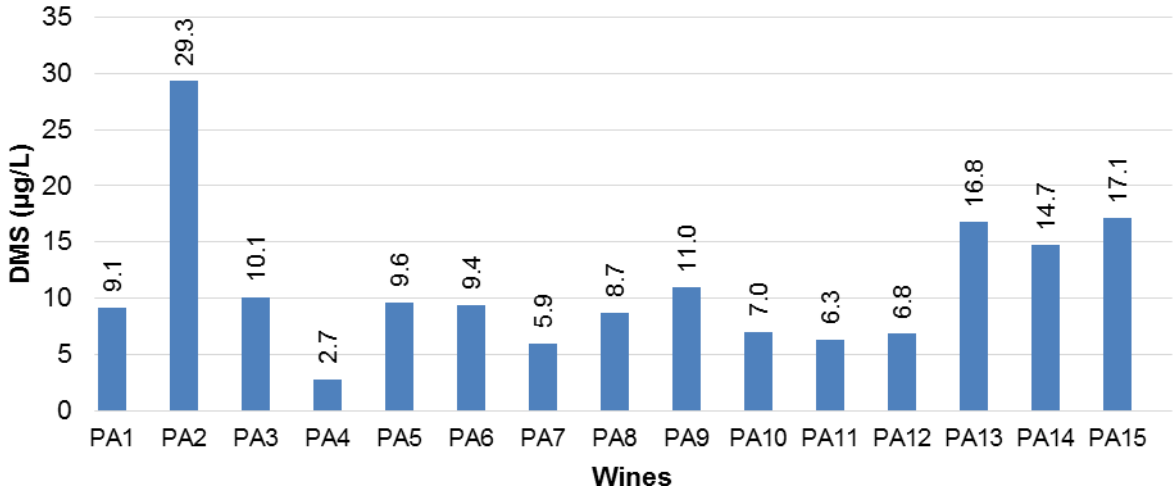


Figure 3-152: Dimethylsulphide (DMS) concentration of the 15 commercial wines from the Swiss variety Petite Arvine.

3.4.2.5 Varietal thiols

Petite Arvine wines has been studied only for 3SH by Fretz *et al.* (2005a; 2005b; 2005c). Data considering 4MSP in wines from this variety are presented for the first time. Eleven out of the fifteen wines studied showed concentrations of 4MSP above the odour threshold (**Figure 3-153**) (3 ng/L; Darriet *et al.*, 1995). 3SH concentrations for the Petite Arvine wines were similar to the amount found by Fretz *et al.* (2005b) (**Figure 3-154**). This enabeled to validatite the method and the measured concentrations found in wines from Malagousia, as the values for Petite Arvine were found within the ranges published (Fretz *et al.*, 2005b; 2005c). No 3SHA cound be measured probably for the same reasons as mentioned in **Chapter 3.4.1.5**.

Many of the wines from Petite Arvine in the canton of Valais are not being vinificated reductive but tend to be to the more oxidative (*pers. comm*. Hansueli Pfenninger, 2014). Besides that some of the wines contained high amounts of cooper (data not shown) that has an effect on these compounds. Varietal thiols are highly reactive with cooper (Nikolantonaki *et al.*, 2010). Nevertheless the concentrations found had an impact in the aromatics of the wines as in some cases 3SH was 28 times above the odour threshold (60 ng/L; Dubourdieu & Tominaga, 2009) and for 4MSP 2 times above (3 ng/L; Darriet *et al.*, 1995). A summary of the results for the varietal thiols can be found in **Appendix 6-25**.

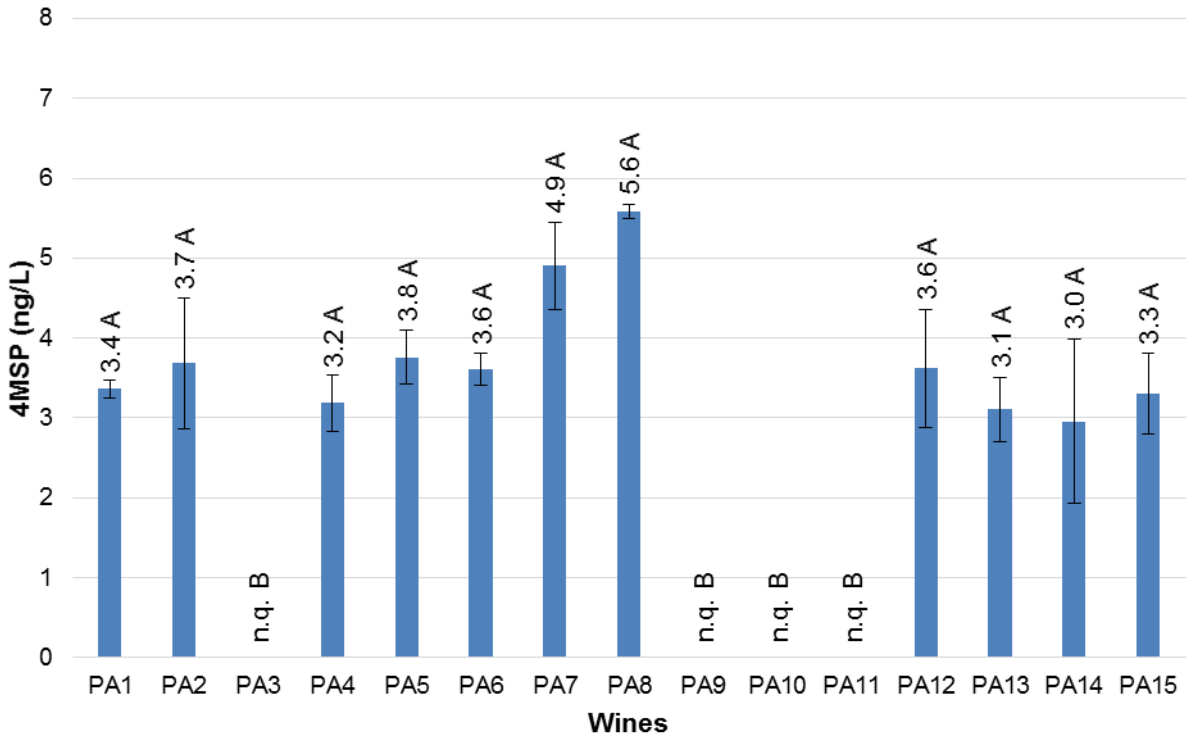


Figure 3-153: 4-methyl-4-sulfanylpentan-2-one (4MSP) c concentration of the 15 commercial wines from the Swiss variety Petite Arvine. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

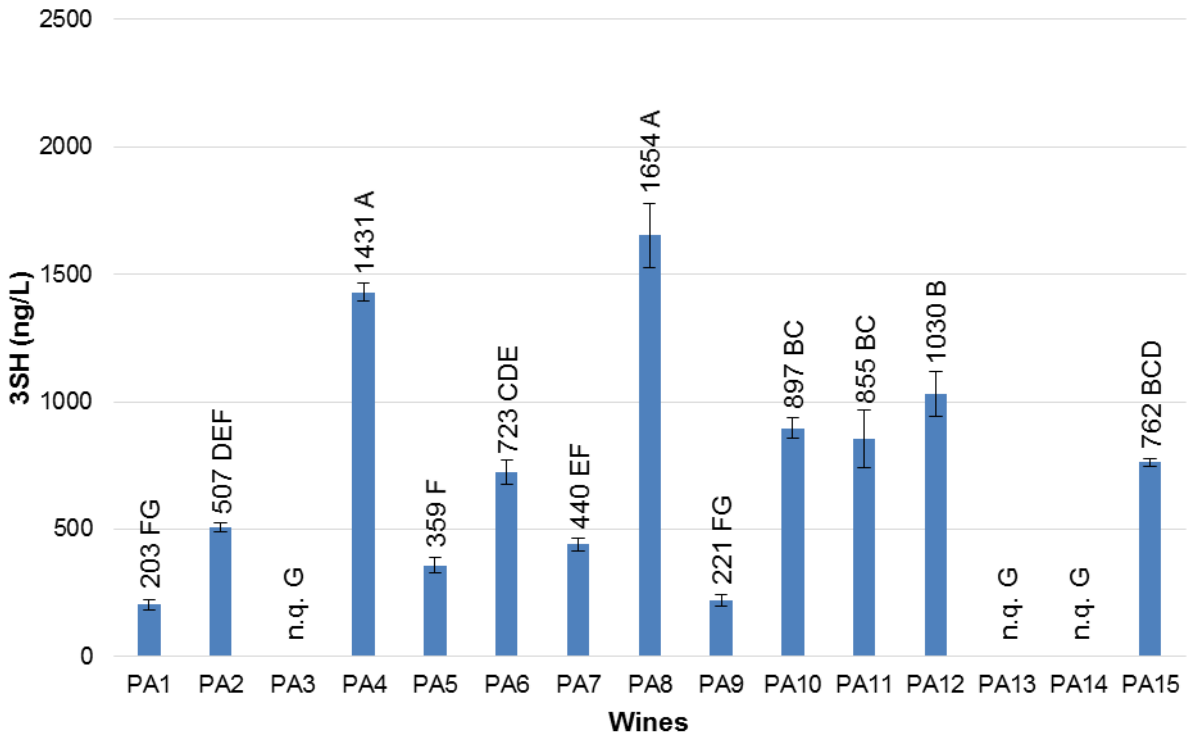


Figure 3-154: 3-sulfanylhexan-1-ol (3SH) concentration of the 15 commercial wines from the Swiss variety Petite Arvine. Different capital letters after the data label show significant difference between the samples ($p<0.05$, Tukey comparison test).

3.4.2.6 Sensory analysis

Because of the large number of wines tasted it was decided to plot the samples with the highest and lowest 'impression' score. A summary of the tasting scores for all the wines can be found in **Appendix 6-26**.

The wine with the lowest 'impression' score (PA2) was also the wine that had a partial MLF (**Figure 3-155**). It was characterised by the tasters as 'buttery' (x3), 'creamy' (x1) and 'oxidised' (x1). Due to these facts and the analytical results presented for the wine it could possibly be characterised as faulty.

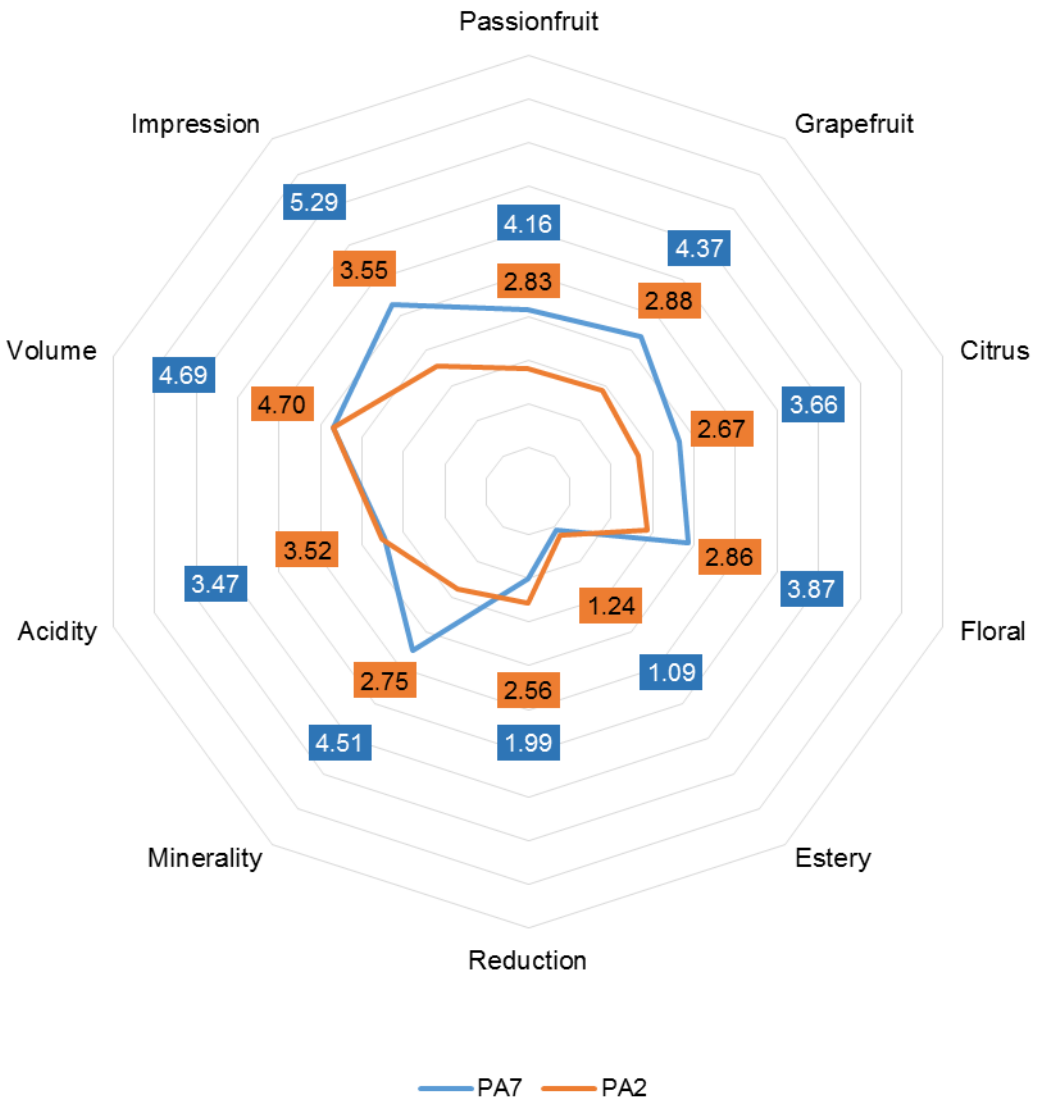


Figure 3-155: Spider plot for the sensory attributes of the 15 commercial wines from the Swiss variety Petite Arvine. Only the wines with the highest and lowest 'impression' score were plotted. The blue line shows the wine with the highest 'impression' score and its values are in the blue boxes, whereas the wine with the lowest 'impression' score is represented with orange line.

The spider plot for wine PA7 showed that it was characterised with a combination of attributes like 'passionfruit', 'grapefruit', 'floral' and also strong 'minerality' giving to the wine a complex character. 'Minerality' in Petite Arvine is very characteristic and in mostly being perceived as 'saltiness' (Robinson *et al.*, 2012). The tasters probably perceived the 'minerality' as such but due to the fact that this term is poorly defined (Ross, 2012) it should only be used as an orientation.

3.4.2.7 Statistical analysis

PCA plotting was used for analysing the data statistically. The OAVs were used for estimating the contribution of the aroma compounds to the aroma of the wines (Guth, 1997b; Ferreira *et al.*, 2000; Gómez-Míguez *et al.*, 2007). The data were mean centered and autoscaled before analysis (van den Berg *et al.*, 2006). A summary of the OAVs can be found in **Appendix 6-27.**

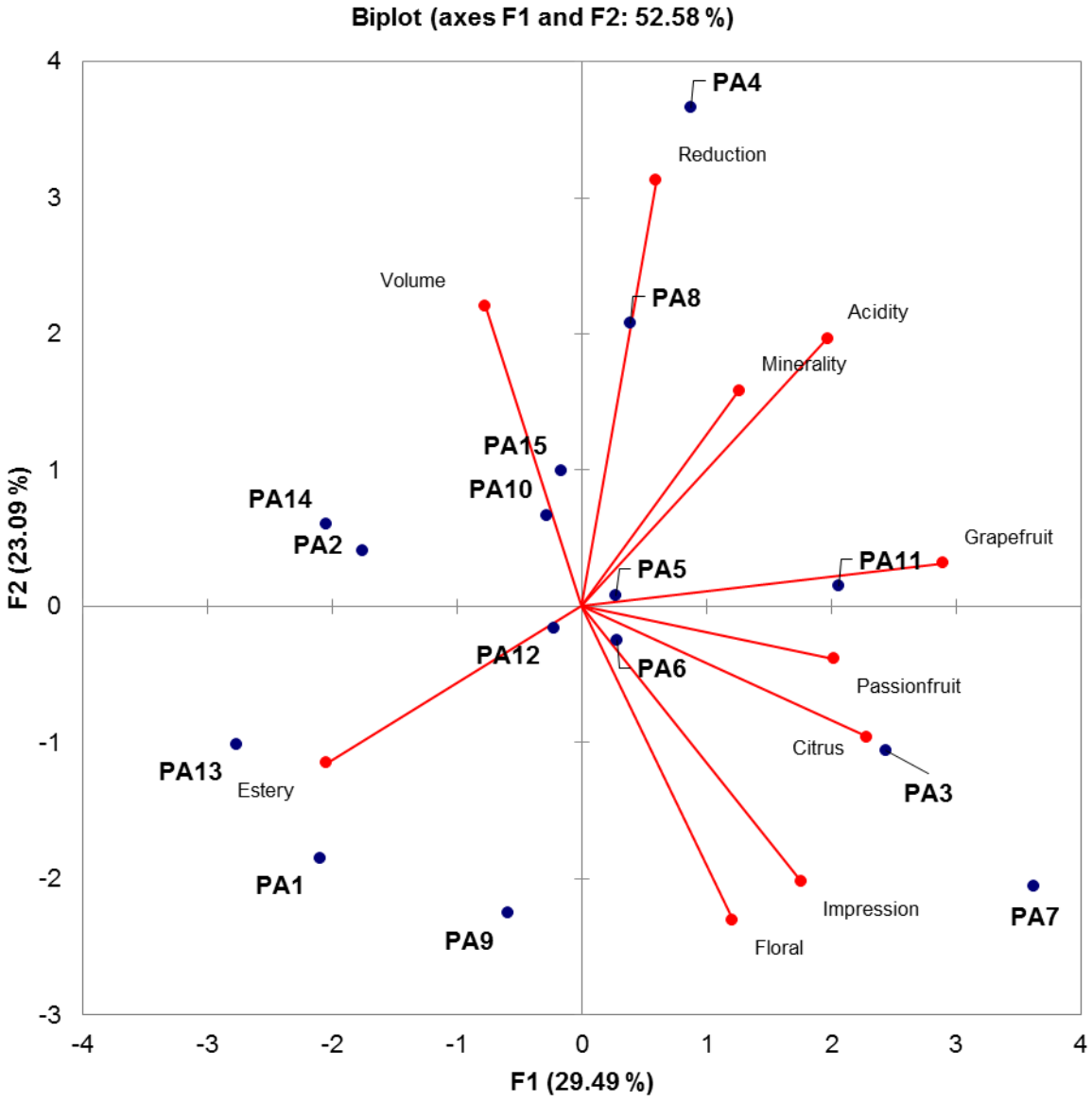


Figure 3-156: Principal Component Analysis (PCA) for the tasting results of the 18 commercial wines from the Swiss variety Petite Arvine.

The PCA for the tasting results accounted for 52.58 % of the variability in the data (**Figure 3-156**). F1 accounted for 29.49 % of the variability and was more heavily loaded with the descriptors 'grapefruit', 'passionfruit', 'citrus' and 'acidity' in the positive direction and 'estery' in the negative direction. F2 accounted for 23.09 % of the variability and was more heavily loaded with the descriptors 'reduction', 'volume' in the positive direction and 'floral', 'impression' in the negative direction. 'Minerality' was more heavily loaded in F3 (data not shown).

It can be seen that F1 is more responsible for the fruity and exotic character and F2 is responsible for the 'floral', the off-flavours, the palate and the 'impression' of the wines. 'Reduction' and 'volume' correlate negatively with the 'impression' of the wines. Wines PA11 and PA3 had a more fruity character and were described well by F1. PA3 did not show any concentration of varietal thiols (**Chapter 3.4.2.5**) but it is possible that β-damascenone was contributing to the tropical fruit flavour (Pineau *et al.*, 2007). Wine PA11 showed significant concentration of 3SH and it can be seen that is very close to the descriptor 'grapefruit'. Wines PA4 and PA8 are well explained by F2 but it is difficult to correlate the analytical results. Wine PA4 showed to have the lowest concentrations of off-flavours (**Chapter 3.4.2.4**). Wine PA8 had higher concentrations of reductive compounds when compared with the other Petite Arvine wines. The negative correlation of PA13 and PA14 with F1 can be explained by the trace concentrations of varietal thiols found. These two wines showed only 4MSP in concentrations marginally above the odour threshold (**Figure 3-153**).

For the PCA of the aromatic compounds and the tasting attributes it was chosen to plot only the compounds that showed OAV values above 1 in at least one of the variants. The PCA for F1 and F2 accounted for 45.87 % of the variability in the data (**Figure 3-157**). F1 accounted for 27.56 % of the variability and was more heavily loaded with 'β-damascenone', '3-methyl butanol', '2-phenyethanol', 'acetic acid 3-methylbutyl ester', 'acetic acid 2-phenylethylester', 'butyric acid ethylester', '3SH' and 'minerality' in the positive direction. F2 accounted for 18.33 % of the variability and was more heavily loaded with the descriptors 'passionfruit', 'grapefruit', 'citrus' in the positive direction and 'H_2S', 'DMS' in the negative direction.

From the plot of the aroma compounds and the descriptors it can be seen that the wines were widely scattered. It can be seen that 4MSP did not play a significant role in the descriptors 'passionfruit' and 'grapefruit'. 3SH seems to have a much more significant role. It could be attributed to the concentrations of 4MSP that were found in the wines from Petite Arvine (**Figure 3-155**) and probably these were not high enough to influence the aromatics. The 'floral' character is not a very strong characteristic in the wines except for wine PA7 although this particular sample did not show high concentrations in terpenic compounds. Wines PA13 and PA14 did not correlate with the fruity aromatics of the varietal thiols as almost none were measured.

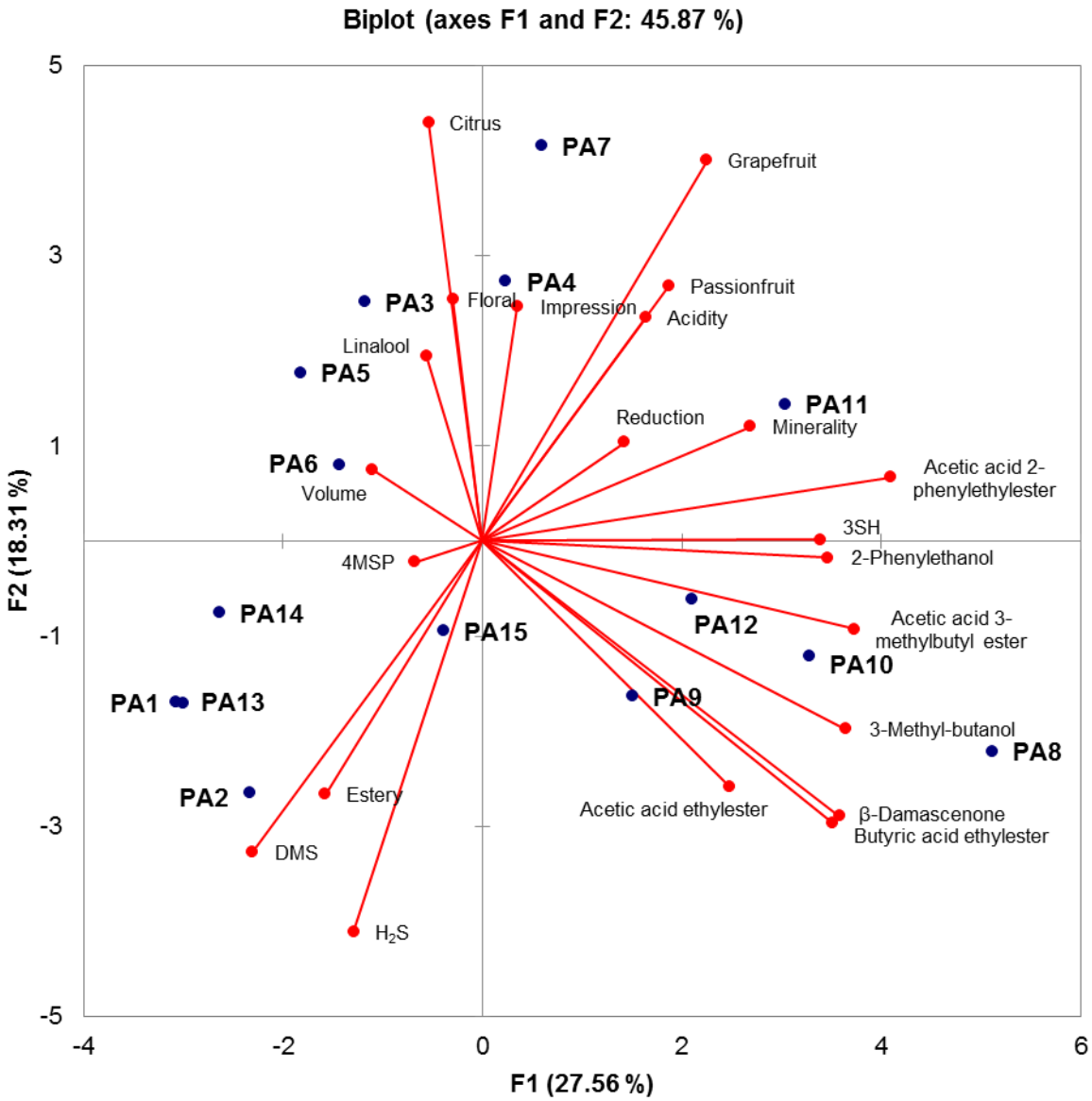


Figure 3-157: Principal Component Analysis (PCA) of the 18 commercial wines from the Swiss variety Petite Arvine with the aromatic compounds that had an OAV > 1 in at least one of the samples. The data were mean centered and autoscaled before analysis.

3.4.2.8 Discussion and conclusion

The goal of this work package was to study the wines from the variety Petite Arvine, autochthonous in the canton of Valais (Robinson *et al.*, 2012). Focus was set on the aromatic compounds and especially on varietal thiols. The results from the study on Petite Arvine were mainly used to validate the results of Malagousia as the variety has been partly characterised by Fretz *et al.* (2005a; 2005b). 15 commercial wines from the Swiss autochthonous variety Petite Arvine from the vintage 2013 were collected from specialised shops in Switzerland and directly from the wineries. Three of the wines were a kind donation of Robert Gilliard SA in Sion.

The wines showed a too high alcohol content for white wines, which is normal for the wines from Petite Arvine (Robinson *et al.*, 2012). Not all the wines were fermeted to dry, which was noted by the tasters

during sensory analysis. The highest concentration of residual sugars was found in the wine PA1 with 15.3 g/L. One of the wines (PA2) showed a partial MLF that could have originated from a contamination during the alcoholic fermentation. Although no data exist for the phenolic composition of Petite Arvine it can be assumed that the concentrations found (**Figure 3-134**) were normal as they were similar to the values found in Malagousia (**Figure 3-101**) and would not have an effect on the varietal thiols (Blanchard *et al.*, 2004; Lund *et al.*, 2009; Nikolantonaki *et al.*, 2010; Tourtoglou *et al.*, 2014).

High concentrations of acetic acid 3-methylbutylester were measured. They were up to 155 times above the odour threshold (30 µg/L; Guth 1997b). PA9 showed the higest concentration in that particular ester and was commented by the tasters with the attribute 'Eisbonbon' (or reminiscent on the flavour of gummy bears). This descriptor is very characteristic for high concentrations of acetic acid 3-methylbutylester. Acetic acid 2-phenylethylester has been reported by Fretz *et al.* (2005a) to be below the odour threshold (250 µg/L; Guth 1997b). In the present study six of the wines are above this threshold. It has been mentioned that the concentration of this ester is higher when the wines are young (Fretz *et al.,* 2005a), which is also the case for the present study. Butyric acid ethylester also showed some significant concentrations up to 36 times above its odour threshold (20 µg/L; Ferreira *et al.*, 2000). The concetrations reported by Fretz *et al.* (2005a) were up to mg/L but could not be confirmed. The partial MLF is evident if the concentration of lactic acid ethylester is increased. The wines PA2 and PA6 showed distinct higher values when compared with the other wines although the concentrations were below the odour threshold (155 mg/L; Etiévant, 1991). 3-Methyl butanol and 2-phenylethanol were found to be in such concentrations to be contributing to the aromatics of the wines. 3-methyl butanol was measured in concentrations up to 183 mg/L (odour threshold 30 mg/L; Guth, 1997b). The concentrations mentioned in the literature by Fretz *et al.* (2005a) did not exceed 75 mg/L. 2-phenylethanol was in all the wines above the odour threshold of 14 mg/L (Ferreira *et al.*; 2000) thus contributing to the 'floral' character. The concentrations of up to 58 mg/L that were found by Fretz *et al.* (2005a) could not be confirmed.

It is the first time that results were presented for terpenic compounds for wines from Petite Arvine. From the terpenic compounds measured only linalool and β-damascenone could play a role in the aromatics. The concentration of linalool was found to be in most of the wines around the odour threshold (25 µg/L; Ferreira *et al.*, 2000) and only the wines PA5 and PA7 exceeded this level. β-Damascenone was measured in concentrations 16-49 times of the odour threshold (0.14 µg/L; Pineau *et al.*, 2007) and can contribute to the fruity character of the wines.

Off-flavours did not seem to affect the wines from the vintage 2013 as all of the wines have concentrations around or below the odour threshold. Wine PA2 was an exception as a concentration of 29.3 µg/L of DMS (odour threshold 25 µg/L; Goniak & Noble, 1987) was measured and it is uncommon for such a young wine (Marais, 1979). One possible explanation would be a long contact of the wine with the lees during storage (Niefind, 1969) or warm storage either in the winery or in the shop (Marais, 1979).

For the first time 4MSP was identified in Petite Arvine wines although the concentrations that were found were low. Nevertheless 11 out of 15 wines had concentrations above the odour threshold (3 ng/L; Darriet *et al.*, 1995). 3SH concentrations were found to be similar to the published concentrations by Fretz *et al.* (2005b). They clearly show that the wines from Petite Arvine are rich in 3SH as concentrations up to 27 of times the odour threshold were measured (60 ng/L; Dubourdieu & Tominaga, 2009). The statistical results in **Figure 3-157** demonstrate that although small quantities of 4MSP are present they do not seem to play a dominant role in the exotic character of the wine. On the other hand 3SH is more closely correlated to the descriptors 'passionfruit' and 'grapefruit'. For wines PA3, PA13 and PA14 that showed no concentrations of 3SH a trace element analysis revealed (data not shown) that these have been treated with copper, which is known for its reactivity with varietal thiols (Nikolantonaki *et al.*, 2014).

The sensory analysis of the wines proved to be more difficult as probably the tasters were not familiar with the specific taste of Petite Arvine. Well-known descriptors like 'passionfruit' and 'grapefruit' were nonetheless essential for validating the data of the varietal thiols and others that need to be taken into consideration for better training of the tasters, like in the case of so-called 'reductive' notes. 'Reductive' notes in wines as mentioned in **Chapter 3.4.1** proved also to be troublesome with the wines from the variety Malagousia. Using PLS it was sought to find the descriptors that are correlating with the 'impression' of the wines. This was not possible because of the unfamiliarity of the tasting panel with Petite Arvine. A similar study on Petite Arvine that targeted on the typicality of the wines was carried out using a panel of local enologists of the Canton of Valais and a second panel with enology students for verifying the results (Fretz, 2005c), which was not possible in the presented study. Some of the wines exhibited a 'floral' character that could not be attributed to the terpenic compounds as their concentrations measured were low. Probably β-damascenone plays a role also in the 'floral' character of Petite Arvine. Pineau *et al.* (2007) described the compound having a 'flowery' character and a hint of 'tropical fruit'. The differences of the wines with the highest and lowest 'impression' score are evident from **Figure 3-157**, like in the case of Malagousia (**Figure 3-124**). The lowest score showed also a low score in all the 'fruity' and 'exotic' descriptors and the 'minerality' of the wine was low. The highest score showed the opposite characteristics in the 'fruitiness' and 'exotic' character but also a strong perception of 'minerality'.

4. General conclusion and perspectives

Varietal thiols have been proven to play a key role in wines from certain grape varieties (Guth, 1997a; 1997b; Dubourdieu & Tominaga, 2009; Roland *et al.*, 2011) so it is essential to have a reliable method for quantifying these compounds (4MSP, 3SHA and 3SH) and that can be carried out with relatively simple means and easy-to-find chemicals. This analytical method was a prerequisite for studying the hypothesis that varietal thiols were found in many more uncharacterised varieties and for exploring them analytically and sensorial. From the aims of the study presented in **Chapter 1.6** the final conclusions were the following:

1) A method for measuring varietal thiols was established and validated (**Chapter 3.1**). It showed excellent results for 4MSP with a LOQ around the odour threshold of the compound in wines (3 ng/L; Darriet *et al*., 1995) and very good results for 3SH with a LOQ of 104 ng/L, which was marginally above the odour threshold of 60 ng/L (Dubourdieu & Tominaga, 2009). Good results were achieved for 3SHA with a LOQ of 39 ng/L that is higher than the odour threshold but had excellent linearity and reproducibility. All the compounds showed a wide linear range, which makes easy to analyse samples with high concentrations of the compounds without dilution steps. Such high concentrations for 3SH were recently showed by Rigou *et al.* (2014). Although sample preparation is time consuming, it offers the advantage of using ready off-the-shelf chemicals and consumables. This makes the method usable almost in any laboratory that has a standard GC-MS system (MSD). The method developed was adapted from Ferreira *et al.* (2007) with some improvements like the optimised internal standard but also with less extraction steps, cutting down analysis time. Another improvement was the implementation of the 'Large-Volume-Injection' technique for the GC analysis which cuts down sample preparation time because the concentration step of the extract can be shortened.

Further improvements can be implemented in the method in the future. Testing the extraction for other matrixes (partially tested) demonstrated that these compounds play also a role in other fermented products as shown by Srisamatthakarn *et al.* (2012). More improvement work should be carried out for lowering the LOQ of both 3SHA and 3SH so that their quantification would be possible close to the odour threshold. The possibilities of different injection techniques that could enrich the sample could also be investigated so that a decrease in the LOQ can be achieved without increasing the sample volume or concentrating the extract to smaller volumes, which is more time consuming. The last point in the method procedure that could help to increase the throughput would be the partial automation of the method during extraction and concentration, cutting down analysis time. This would decrease labour costs and eliminate possible mistakes during sample preparation.

2) Fermentation experiments for two consecutive years and six commercial yeast strains were conducted with an international grape variety (Sauvignon blanc) and a local variety (Scheurebe), which are known for their varietal character. This had the aim of establishing the methodology of characterisation, adapt the analysis and induce high concentrations of volatile thiols in order to explore the limitations of the measuring methods. Furthermore these fermentations were used to optimise the tasting scheme that would help to characterise wines from unknown grape varieties.

The experiment was able to induce concentrations of varietal thiols in a very broad spectrum (4MSP 0-33.3 ng/L; 3SHA 0-300 ng/L; 3SH 378-6372 ng/L) that certainly were appropriate for testing the method but also help determine the descriptors for the tastings. Since this scheme would be used for the next step for varieties that have not been characterised before, a variety rich in thiols (Sauvignon blanc) and a variety rich in thiols and other aromatic compounds like monoterpenes (Scheurebe) were chosen. The fermentations also showed that with relatively simple winemaking practice (reductive winemaking using N_2 and wine protection with a simple SO_2 management) it is possible to produce and preserve high concentrations of thiols. The results make clear that the decision for the yeast strain is important for the varietal thiols as already demonstrated by Murat *et al.* (2001b). It was shown that some of the yeast strains (e.g. ALII) can be used with good results and minimum risk for off-flavours.

The main issue addressed was an appropriate tasting scheme and training of the tasters so that the results of the sensory analysis would be reliable. The training of the tasters with a neutral wine spiked with the aromatic compounds in question proved to be an efficient approach as a correlation/tendency of the sensory results with the analytical results could be established. One of the attributes that was decided not to be used in further experiments was ‘typicity’. The question of ‘typicity’ was easy amongst professionals for Scheurebe but for Sauvignon blanc wines this descriptor proved to be troublesome. Nevertheless when using unknown varieties it could be risky to ask for ‘typicity’ so the descriptor ‘impression’ would be used instead. The ‘typicity’ of the wines from Scheurebe was not only attributed to varietal thiols, although this variety has been proven to contain high concentrations (Guth, 1997b). High concentrations of varietal thiols does not mean a higher ‘typicity’ perception by the tasters. In the Scheurebe experiment, ‘typicity’ appeared to be a balance between ‘floral’, ‘fruity’ and ‘exotic’ character. The descriptor of ‘minerality’ was treated with caution during the preparations for the tasting and was thoroughly discussed with the sensory panel. For these two grape varieties, this descriptor would not be of high importance but could be used for future studies as some varieties that had already been selected were known for their mineral character. The descriptor ‘catpiss’ was employed for high concentrations of 4MSP although it is not highly probable that the wines that would be used for further studies would show this character. Last but not least the tasting scheme should be simple and easy to fill out. It should not contain any descriptor overlappings as these would be tiring for the tasters and make reliable results difficult (Prof. Dr. Rainer Jung, *Pers. Comm.,* 2009). One such overlapping that would be eliminated for future tastings were the descriptors ‘estery’ and ‘solvent’ as the expert panel tended to confuse them. In the future only the descriptor ‘estery’ would be used.

3) For the Greek varieties pre-selection, wines from three autochthonous varieties were studied in order to select the richest in thiols and the most complex in the tasting and continue further studies. The three varieties studied were Malagousia, Asyrtiko and Roditis. Additionally a mature Malagousia from the vintage 2006 was obtained, a blend Asyrtiko/Malagousia and a Sauvignon blanc as a benchmark wine.

For the first time varietal thiols were measured in wines from Greek autochthonous varieties. The concentrations found were significant (4MSP 0-10.5 ng/L; 3SHA 0-84 ng/L; 3SH 416-1942 ng/L) and had an impact on the sensory properties of the wines. The varietal thiols seem not to oxidize as fast as

described by Guth *et al.* (1995) and Roland *et al.* (2011a). A significant concentration of 3SH (1346 ng/L) was found in a four year old Malagousia wine (vintage 2006), which is more that 22 times above the odour threshold. Tominaga *et al.* (2006b) showed that 3SH could be found in three year old wines but this was the first time that 3SH was measured in a four year old wine. Which mechanism protect these compounds from oxidizing is not fully understood. Some aspects were discussed by Roland *et al.* (2011a). Glutathione plays an important role in protecting varietal volatile thiols from oxidation (Dubourdieu *et al.*, 2001). In the studies by Roussis *et al.* (2009) and Kontogeorgos & Roussis (2014) it has also been suggested that compounds with a free sulphydryl group can protect the aroma of a wine and these three varieties showed a significant concentration in these studies. Although the varieties Asyrtiko and Roditis showed high concentrations of 3SH it was not possible to detect any 4MSP, suggesting that probably the precursor for this compound was not present. Since nothing is known about the precursors in these Greek varieties 3SH could be synthesised with the alternative pathway suggested by Schneider *et al.* (2006).

The wines from Malagousia showed high concentrations of monoterpenes. This gave the wines a complex character when combined with the varietal thiols and also improved the preference of the tasters. This was the case for wine MA2 that had high concentration of linalool, α-terpineol, 3SH and had the highest 'impression' score among the wines from the same variety. On the other hand Asyrtiko and Roditis showed no significant concentrations of monterpenes.

The acidic- and mineral character for Asyrtiko were described by the sensory panel as it has also been described by the literature (Lazarakis, 2006). The description of the variety helped validating the model for sensory analysis developed in the previous experiment.

In conclusion, wines from Asyrtiko showed significant concentrations of varietal thiols although the reductive character and mineral character were not subject of this work. Furthermore, many Asyrtiko wines that are on the market are aged (Lazarakis, 2006) thus it would be difficult to collect many samples so it was decided not to study the variety further. The wines from Roditis showed difficulties in quality (faulty sample R3) and it was difficult to obtain monovarietal wines as this variety is mostly used for blending (Lazarakis, 2006). The wines from Malagousia fitted well in the tasting scheme developed and the description of the variety with its aromatics was sufficient. Due to the complex character that the wines showed and the high concentrations of both monoterpenes and varietal thiols it was decided to study the variety further as it was also easier to source young monovarietal wines from a lot of producers.

4) The aim of the study with Malagousia- and Petite Arvine commercial wines (all 2013 vintage), was mainly to characterise the wines from the first variety but also to detect the aromatics of the second one. As no results have been published up to now for Malagousia wines concerning varietal thiols it was important to have an autochthonous variety, which has been partially characterised (Fretz *et al.*, 2005a; 2005b) in order to validate the results.

Varietal thiols were measured for the wines from Malagousia in very high concentrations especially 4MSP, which was as high as 44.7 ng/L. No 3SHA was detected but this could be due to the high LOQ of the improved method. Malagousia wines showed high concentrations of 3SH up to 22 times the

odour threshold. Petite Arvine showed high concentrations of 3SH and some wines also had 4MSP in concentrations just above the odour threshold being shown for the first time.

The previous study showed high concentrations of terpenic compounds. It was decided to measure the monoterpenes and the C_{13} norisoprenoids for both varieties for a more detailed insight in their aromatics. It was again confirmed that the wines from Malagousia were very rich in monoterpenes and norisoprenoids with some reaching values many times above the odour threshold. This added complexity to the wines. It is the first time results are presented for commercial Malagousia wines although previously mentioned results by Lazarakis (2006) based only on empirical observations and measured in experimental wines by Metafa & Economou (2013).

The results of this study led to an initial characterisation for the wines of the variety Malagousia. Thiols and terpene content of the wines is high and shows a changing character in the wines from ‘fruity’ and ‘floral’ to ‘exotic’ and ‘catpiss’. Thus it would be of interest in the future to investigate the viticultural aspects of Malagousia in order to determine, which parameters and practices affect the character and promote or inhibit the production of aromatic compounds. A scheme for the sensory evaluation of the wines and analytical methods has been established and could be further developed for answering specific questions. All above mentioned aspects in combination with the increase of the popularity of this variety in Greece and also internationally (Lazarakis, 2006) would justify such a study. This would require the cooperation of Greek wineries as well as research institutes.

The validation of the results using of Petite Arvine wines proved also to be efficient as the results of many of the compounds measured could be correlated with the literature (Fretz *et al.*, 2005a; 2005b).

5. Literature

A

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6. Appendix

Appendix 6-1: Major components of the finished Scheurebe wines from the vintage 2008 & 2009.

Variant	X5		VL3		ALII		SAU		BOUQ		VIN13	
Vintage	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
Alcohol (% Vol.)	10.5 ± 0.1	12.4 ± 0.0	10.5 ± 0.1	12.2 ± 0.1	10.8 ± 0.2	12.3 ± 0.1	9.1 ± 0.3	10.3 ± 0.4	8.4 ± 0.0	12.5 ± 0.1	10.6 ± 0.0	12.3 ± 0.0
Residual sugars (g/L)	4.1 ± 0.5	12.8 ± 3.2	3.3 ± 0.9	10.9 ± 0.8	2.1 ± 0.3	10.1 ± 0.9	25.1 ± 5.0	45.6 ± 2.5	35.5 ± 0.0	7.5 ± 1.2	1.9 ± 0.0	10.5 ± 0.3
Volatile Acidity (g/L) in AA	0.4 ± 0.0	0.2 ± 0.1	0.5 ± 0.1	0.6 ± 0.0	0.6 ± 0.1	0.4 ± 0.1	0.5 ± 0.0	0.3 ± 0.1	0.6 ± 0.0	0.4 ± 0.1	0.3 ± 0.0	0.4 ± 0.0
Malic acid (g/L)	4.5 ± 0.0	3.5 ± 0.0	4.4 ± 0.0	3.4 ± 0.0	4.5 ± 0.0	3.5 ± 0.0	4.4 ± 0.0	3.6 ± 0.3	4.6 ± 0.1	3.4 ± 0.0	4.4 ± 0.0	3.5 ± 0.0
Lactic acid (g/L)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0

Appendix 6-2: Aroma compounds measured with the 'Kaltron' method in Scheurebe wines from the vintage 2008 & 2009.

Variant	X5		VL3		ALII		SAU		BOUQ		VIN13	
Vintage	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
Volatile acids												
Caproic acid (mg/L)	8 ± 0	7 ± 0	7 ± 0	6 ± 0	9 ± 1	7 ± 0	14 ± 2	14 ± 0	15 ± 0	7 ± 0	8 ± 0	7 ± 0
Caprylic acid (mg/L)	9 ± 1	6 ± 0	8 ± 1	6 ± 0	9 ± 0	7 ± 0	14 ± 0	12 ± 0	13 ± 0	6 ± 0	9 ± 1	7 ± 0
Capric acid (mg/L)	3 ± 0	3 ± 0	3 ± 0	3 ± 0	3 ± 0	3 ± 0	3 ± 0	3 ± 0	3 ± 0	3 ± 0	3 ± 1	3 ± 0
Higher alcohols												
i-Butanol (mg/L)	11 ± 1	16 ± 0	12 ± 2	18 ± 1	21 ± 1	16 ± 1	23 ± 5	23 ± 2	21 ± 1	30 ± 1	15 ± 1	18 ± 1
3-Methyl-butanol (mg/L)	84 ± 6	87 ± 2	77 ± 8	85 ± 3	130 ± 2	121 ± 3	106 ± 20	103 ± 4	108 ± 1	163 ± 7	118 ± 6	116 ± 4
2-Methyl butanol (mg/L)	15 ± 1	17 ± 0	14 ± 2	18 ± 0	24 ± 0	23 ± 1	22 ± 5	24 ± 1	22 ± 1	31 ± 2	24 ± 1	25 ± 1
Hexan-1-ol (µg/L)	1871 ± 54	2252 ± 40	1820 ± 89	2367 ± 64	1732 ± 52	2182 ± 23	1897 ± 180	2200 ± 57	2371 ± 32	2274 ± 64	1762 ± 66	2044 ± 8
2-Phenylethanol (mg/L)	14 ± 0	12 ± 0	17 ± 0	13 ± 0	19 ± 1	19 ± 1	16 ± 3	16 ± 0	20 ± 0	37 ± 1	13 ± 1	21 ± 1
Acetic acid esters												
Acetic acid ethylester (mg/L)	19 ± 4	109 ± 2	37 ± 23	116 ± 5	133 ± 3	129 ± 7	33 ± 27	63 ± 3	59 ± 4	72 ± 9	15 ± 6	50 ± 1
Acetic acid 3-methylbutylester (µg/L)	440 ± 135	136 ± 38	313 ± 100	77 ± 34	981 ± 66	638 ± 39	287 ± 25	214 ± 19	158 ± 26	1045 ± 43	1078 ± 19	588 ± 59
Acetic acid 2- methylbutylester (µg/L)	26 ± 4	11 ± 0	22 ± 4	11 ± 1	58 ± 3	32 ± 0	25 ± 2	27 ± 2	18 ± 2	51 ± 2	70 ± 3	32 ± 5
Acetic acid hexylester (µg/L)	145 ± 9	91 ± 7	120 ± 12	86 ± 2	175 ± 10	131 ± 6	90 ± 5	64 ± 1	93 ± 8	145 ± 6	166 ± 4	125 ± 13
Acetic acid phenylethylester (µg/L)	87 ± 11	47 ± 4	108 ± 16	48 ± 2	179 ± 7	87 ± 3	65 ± 4	43 ± 2	59 ± 4	167 ± 3	111 ± 3	85 ± 6
Ethylester												
Propionic acid ethylester (µg/L)	53 ± 2	97 ± 4	33 ± 6	61 ± 1	64 ± 2	107 ± 2	30 ± 6	39 ± 2	28 ± 0	60 ± 2	80 ± 3	86 ± 2
i-Butyric acid ethylester (µg/L)	30 ± 2	31 ± 2	46 ± 5	39 ± 2	40 ± 1	28 ± 2	36 ± 2	37 ± 2	31 ± 1	48 ± 4	24 ± 1	27 ± 0
Butyric acid ethylester (µg/L)	174 ± 15	165 ± 8	153 ± 13	155 ± 11	197 ± 13	175 ± 5	336 ± 17	338 ± 8	287 ± 12	162 ± 3	266 ± 2	195 ± 6
Lactic acid ethylester (mg/L)	13 ± 1	15 ± 0	11 ± 1	13 ± 0	15 ± 1	17 ± 0	13 ± 1	14 ± 0	12 ± 0	16 ± 0	14 ± 1	16 ± 0
Caproic acid ethylester (µg/L)	676 ± 36	499 ± 36	606 ± 16	469 ± 24	760 ± 41	553 ± 30	1204 ± 52	991 ± 32	1046 ± 12	472 ± 6	697 ± 11	612 ± 22
Succinic acid diethylester (µg/L)	883 ± 52	1737 ± 136	845 ± 20	1867 ± 139	411 ± 23	2156 ± 116	202 ± 109	513 ± 40	131 ± 3	1747 ± 79	1021 ± 26	2324 ± 148
Caprylic acid ethylester (µg/L)	1286 ± 54	892 ± 80	1229 ± 40	816 ± 36	1352 ± 27	1086 ± 61	1503 ± 201	1353 ± 41	1461 ± 189	968 ± 11	1222 ± 66	1192 ± 33
Capric acid ethylester (µg/L)	550 ± 36	409 ± 49	485 ± 28	387 ± 16	450 ± 11	486 ± 62	392 ± 8	353 ± 25	383 ± 70	410 ± 85	454 ± 115	478 ± 21
Monoterpenols												
Linalool (µg/L)	53 ± 1	84 ± 2	51 ± 2	94 ± 5	48 ± 0	93 ± 4	42 ± 1	78 ± 1	38 ± 3	96 ± 2	49 ± 1	88 ± 3
α-Terpineol (µg/L)	78 ± 1	95 ± 1	74 ± 3	95 ± 2	73 ± 1	98 ± 2	74 ± 1	105 ± 2	73 ± 2	99 ± 0	73 ± 1	95 ± 2
Monoterpenol oxides												
trans -linalool oxide (µg/L)	119 ± 1	83 ± 0	112 ± 3	84 ± 2	112 ± 1	88 ± 1	118 ± 6	100 ± 2	129 ± 2	89 ± 1	111 ± 4	84 ± 1
cis -linalool oxide (µg/L)	28 ± 0	26 ± 1	27 ± 1	26 ± 2	26 ± 0	27 ± 1	29 ± 1	30 ± 1	33 ± 1	29 ± 0	28 ± 1	27 ± 1

Appendix 6-3: Sulphur compound concentrations in Scheurebe wines from the vintage 2008 & 2009.

Variant	X5		VL3		ALII		SAU		BOUQ		VIN13	
Vintage	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
H_2S (µg/L)	n.q.	7.0 ± 1.9	n.q.	8.5 ± 3.2	n.d.	14.2 ± 1.7	n.d.	9.1 ± 0.5	n.d.	7.0 ± 2.0	n.q.	8.4 ± 1.3
MeSH (µg/L)	n.d.	n.d.	n.d.	n.d.	n.d.	4.7 ± 0.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
EtSH (µg/L)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DMS (µg/L)	11.3 ± 0.2	14.1 ± 0.4	11.1 ± 0.2	16.4 ± 2.1	15.5 ± 0.3	15.4 ± 0.7	12.8 ± 0.1	11.9 ± 0.8	13.6 ± 0.7	14.3 ± 0.4	11.6 ± 0.1	14.9 ± 0.7
CS_2 (µg/L)	3.7 ± 0.4	6.3 ± 2.0	2.6 ± 1.0	7.3 ± 3.6	3.1 ± 1.0	5.7 ± 0.2	2.3 ± 0.6	4.8 ± 0.4	2.7 ± 0.6	4.7 ± 1.8	3.0 ± 0.9	4.8 ± 1.4
MeSAc (µg/L)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DMDS (µg/L)	0.6 ± 0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
EtSAc (µg/L)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DEDS (µg/L)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DMTS (µg/L)	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.
4MSP (ng/L)	9.5 ± 2.9	4.4 ± 0.4	4.7 ± 0.8	n.q.	33.3 ± 0.7	5.4 ± 0.3	3.8 ± 0.0	n.q.	n.q.	n.d.	5.2 ± 0.1	4.9 ± 0.7
3SHA (ng/L)	226 ± 18	104 ± 3	212 ± 27	78 ± 8	300 ± 9	83 ± 3	284 ± 34	91 ± 16	235 ± 0	70 ± 0	176 ± 28	83 ± 4
3SH (ng/L)	3277 ± 605	4560 ± 339	3812 ± 766	4376 ± 1067	6372 ± 251	4513 ± 220	4328 ± 621	3455 ± 71	1869 ± 0	2041 ± 0	4157 ± 255	3219 ± 209

Appendix 6-4: Tasting scores of the finished Scheurebe wines from the vintage 2008 & 2009.

Variant	X5		VL3		ALII		SAU		BOUQ		VIN13	
Vintage	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
Passionfruit	4.66	2.98	6.18	3.2	4.85	1.99	-	4.29	-	3.28	5.03	4.42
Cassis	4.81	2.44	5.5	4.72	4.91	3.50	-	3.15	-	3.01	5.54	3.18
Grapefruit	4.63	3.50	3.39	3.49	4.78	4.51	-	3.06	-	3.13	5.18	5.06
Catpiss	4.33	3.93	3.46	4.21	5.7	3.72	-	4.03	-	3.44	3.36	4.93
Estery	5.64	4.38	5.68	3.13	2.66	1.88	-	5.05	-	3.53	3.65	4.45
Solvent	4.25	1.78	3.79	3.18	4.27	1.38	-	4.02	-	3.89	4.11	3.35
Reduction	3.37	2.63	4.87	3.36	4.15	3.72	-	3.52	-	3.11	5.19	4.10
Typicity	4.37	5.36	5.04	3.63	4.45	5.72	-	2.43	-	4.99	4.03	2.95

Appendix 6-5: OAV values for the Scheurebe wines from the vintage 2008 & 2009. OAV>1 values are bold.

Variant	X5		VL3		ALII		SAU	BOUQ	VIN13	
Vintage	2008	2009	2008	2009	2008	2009	2009	2009	2008	2009
Linalool	**2.10**	**3.37**	**2.05**	**3.77**	**1.92**	**3.71**	**3.13**	**3.83**	**1.97**	**3.53**
a-Terpineol	0.31	0.38	0.30	0.38	0.29	0.39	0.42	0.39	0.29	0.38
i-Butanol	0.07	0.11	0.08	0.12	0.14	0.11	0.15	0.20	0.10	0.12
3-Methyl-butanol	**2.80**	**2.89**	**2.58**	**2.82**	**4.33**	**4.03**	**3.42**	**5.42**	**3.93**	**3.87**
Hexan-1-ol	0.23	0.28	0.23	0.30	0.22	0.27	0.28	0.28	0.22	0.26
2-Phenylethanol	**1.00**	0.83	**1.24**	0.93	**1.39**	**1.36**	**1.12**	**2.64**	0.95	**1.48**
Acetic acid ethylester	0.12	0.68	0.23	0.73	0.83	0.81	0.40	0.45	0.09	0.31
Acetic acid 3-methylbutyl ester	**14.68**	**4.52**	**10.45**	**2.58**	**32.69**	**21.27**	**7.14**	**34.84**	**35.93**	**19.59**
Acetic acid 2-phenylethylester	0.35	0.19	0.43	0.19	0.72	0.35	0.17	0.67	0.44	0.34
Butyric acid ethylester	**8.71**	**8.27**	**7.67**	**7.75**	**9.84**	**8.77**	**16.92**	**8.10**	**13.31**	**9.77**
Lactic acid ethylester	0.09	0.09	0.07	0.09	0.10	0.11	0.09	0.10	0.09	0.10
Succinic acid diethylester	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.01	0.01
H2S	0.00	0.70	0.00	0.85	0.00	**1.42**	0.91	0.70	0.00	0.84
MeSH	0.00	0.00	0.00	0.00	0.00	0.93	0.00	0.86	0.00	0.00
DMS	**1.13**	**1.41**	**1.11**	**1.64**	**1.55**	**1.54**	**1.19**	**1.43**	**1.16**	**1.49**
4MSP	**3.18**	**1.48**	**1.56**	0.00	**11.08**	**1.80**	0.00	0.00	**1.73**	**1.63**
3SHA	**53.75**	**24.77**	**50.59**	**18.49**	**71.40**	**19.69**	**21.62**	**16.76**	**41.87**	**19.70**
3SH	**54.62**	**76.01**	**63.54**	**72.94**	**106.19**	**75.22**	**57.58**	**34.02**	**69.29**	**53.65**

Appendix 6-6: Major components of the finished Sauvignon blanc wines from the vintage 2008 & 2009.

Variant	X5		VL3		ALII		SAU		BOUQ		VIN13	
Vintage	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
Alcohol (% Vol.)	12.0 ± 0.1	12.9 ± 0.1	12.0 ± 0.2	13.1 ± 0.1	12.5 ± 0.0	13.3 ± 0.1	12.0 ± 0.1	12.7 ± 0.1	12.4 ± 0.0	13.4 ± 0.0	12.4 ± 0.0	13.4 ± 0.2
Residual sugars (g/L)	8.4 ± 0.9	10.4 ± 1.6	9.6 ± 1.7	8.8 ± 0.6	1.5 ± 0.2	5.9 ± 0.6	9.8 ± 1.6	14.4 ± 1.3	2.3 ± 0.2	3.0 ± 0.1	2.5 ± 0.2	3.3 ± 0.5
Volatile Acidity (g/L) in AA	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.5 ± 0.1	0.4 ± 0.0	0.4 ± 0.1	0.4 ± 0.0	0.6 ± 0.1	0.3 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.1
Malic acid (g/L)	3.3 ± 0.0	2.8 ± 0.0	3.1 ± 0.0	2.7 ± 0.0	3.4 ± 0.0	2.7 ± 0.0	3.4 ± 0.0	2.1 ± 0.3	3.0 ± 0.0	2.6 ± 0.0	3.0 ± 0.0	2.7 ± 0.0
Lactic acid (g/L)	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.9 ± 0.3	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.2

Appendix 6-7: Aroma compounds measured with the 'Kaltron' method in Sauvignon blanc wines from the vintage 2008 & 2009.

Variant	X5		VL3		ALII		SAU		BOUQ		VIN13	
Vintage	**2008**	**2009**	**2008**	**2009**	**2008**	**2009**	**2008**	**2009**	**2008**	**2009**	**2008**	**2009**
Volatile acids												
Caproic acid (mg/L)	8 ± 0	8 ± 0	7 ± 0	7 ± 0	7 ± 0	7 ± 0	7 ± 0	7 ± 0	7 ± 0	7 ± 0	7 ± 0	7 ± 0
Caprylic acid (mg/L)	9 ± 0	7 ± 0	7 ± 0	6 ± 0	9 ± 0	6 ± 0	8 ± 0	6 ± 0	8 ± 0	6 ± 0	8 ± 0	7 ± 0
Capric acid (mg/L)	3 ± 0	3 ± 0	2 ± 0	2 ± 0	3 ± 0	2 ± 0	2 ± 0	2 ± 0	3 ± 0	2 ± 0	3 ± 0	3 ± 0
Higher alcohols												
i-Butanol (mg/L)	24 ± 2	26 ± 0	33 ± 1	26 ± 1	43 ± 1	22 ± 1	44 ± 3	25 ± 1	48 ± 2	28 ± 1	33 ± 1	24 ± 1
3-Methyl-butanol (mg/L)	79 ± 5	97 ± 2	91 ± 1	100 ± 1	96 ± 1	123 ± 3	79 ± 4	96 ± 4	99 ± 4	144 ± 3	100 ± 2	125 ± 4
2-Methyl butanol (mg/L)	18 ± 1	22 ± 0	21 ± 0	21 ± 0	21 ± 0	23 ± 1	19 ± 1	23 ± 1	21 ± 1	27 ± 0	26 ± 0	25 ± 0
Hexan-1-ol (µg/L)	1612 ± 79	3104 ± 12	1677 ± 41	3028 ± 58	1368 ± 13	2781 ± 30	2063 ± 84	3448 ± 104	1600 ± 27	2858 ± 71	1513 ± 26	2711 ± 78
2-Phenylethanol (mg/L)	13 ± 0	12 ± 0	14 ± 0	12 ± 0	10 ± 0	15 ± 0	11 ± 0	12 ± 0	13 ± 0	18 ± 0	11 ± 0	15 ± 0
Acetic acid esters												
Acetic acid ethylester (mg/L)	37 ± 13	151 ± 9	84 ± 6	154 ± 11	81 ± 2	177 ± 0	53 ± 9	203 ± 5	51 ± 6	126 ± 20	84 ± 9	136 ± 8
Acetic acid 3-methylbutylester (µg/L)	291 ± 4	n.q.	207 ± 12	n.q.	874 ± 10	238 ± 16	n.q.	n.q.	917 ± 25	635 ± 30	885 ± 57	329 ± 13
Acetic acid 2- methylbutylester (µg/L)	28 ± 1	n.q.	21 ± 1	n.q.	58 ± 1	12 ± 0	n.q.	n.q.	59 ± 3	28 ± 2	75 ± 3	15 ± 1
Acetic acid hexylester (µg/L)	123 ± 3	71 ± 3	106 ± 2	63 ± 4	158 ± 5	95 ± 1	68 ± 2	35 ± 3	181 ± 4	132 ± 6	155 ± 11	101 ± 3
Acetic acid phenylethylester (µg/L)	90 ± 5	45 ± 1	90 ± 1	47 ± 3	119 ± 1	83 ± 5	41 ± 3	31 ± 1	137 ± 3	119 ± 5	112 ± 3	83 ± 1
Ethylester												
Propionic acid ethylester (µg/L)	75 ± 4	93 ± 2	57 ± 1	75 ± 2	94 ± 1	103 ± 5	54 ± 3	69 ± 2	65 ± 3	81 ± 2	124 ± 2	107 ± 6
i-Butyric acid ethylester (µg/L)	65 ± 2	56 ± 2	106 ± 1	72 ± 3	86 ± 2	51 ± 2	96 ± 1	76 ± 1	111 ± 5	65 ± 2	61 ± 3	53 ± 2
Butyric acid ethylester (µg/L)	223 ± 12	200 ± 9	209 ± 4	213 ± 5	248 ± 4	167 ± 2	262 ± 6	162 ± 2	219 ± 7	203 ± 8	292 ± 18	214 ± 5
Lactic acid ethylester (mg/L)	18 ± 1	22 ± 0	17 ± 0	18 ± 0	26 ± 1	19 ± 0	24 ± 1	91 ± 22	22 ± 0	21 ± 1	21 ± 1	20 ± 1
Caproic acid ethylester (µg/L)	828 ± 17	632 ± 28	718 ± 17	646 ± 15	808 ± 6	520 ± 2	706 ± 14	541 ± 10	704 ± 9	544 ± 22	764 ± 44	619 ± 24
Succinic acid diethylester (µg/L)	1489 ± 20	3105 ± 323	1377 ± 35	3359 ± 44	1468 ± 37	2519 ± 58	1270 ± 75	2412 ± 196	863 ± 15	2611 ± 200	1942 ± 56	3075 ± 140
Caprylic acid ethylester (µg/L)	1579 ± 36	1041 ± 52	1352 ± 47	1030 ± 35	1599 ± 2	884 ± 11	1340 ± 20	957 ± 8	1434 ± 26	928 ± 33	1457 ± 98	1096 ± 63
Capric acid ethylester (µg/L)	722 ± 21	440 ± 13	505 ± 8	383 ± 40	690 ± 17	373 ± 28	469 ± 16	327 ± 42	610 ± 11	403 ± 15	604 ± 51	438 ± 32
Monoterpenols												
Linalool (µg/L)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
α-Terpineol (µg/L)	9 ± 0	9 ± 0	9 ± 0	9 ± 0	10 ± 1	10 ± 0	9 ± 0	9 ± 0	9 ± 0	10 ± 0	9 ± 0	9 ± 0
Monoterpenol oxides												
***trans* -linalool oxide (µg/L)**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
***cis* -linalool oxide (µg/L)**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Appendix 6-8: Sulphur compound concentrations in Sauvignon blanc wines from the vintage 2008 & 2009.

Variant	X5		VL3		ALII		SAU		BOUQ		VIN13	
Vintage	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
H2S (µg/L)	n.d.	7.5 ± 0.4	n.d.	6.1 ± 0.9	n.d.	6.8 ± 0.2	n.d.	4.7 ± 1.1	n.d.	5.8 ± 0.3	n.d.	8.3 ± 0.4
MeSH (µg/L)	n.d.	5.9 ± 1.3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	4.0 ± 0.1	n.d.	4.4 ± 0.2
EtSH (µg/L)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DMS (µg/L)	11.0 ± 0.6	12.2 ± 0.2	10.0 ± 0.4	11.0 ± 0.2	23.9 ± 0.6	12.4 ± 0.3	13.9 ± 0.2	11.7 ± 0.7	11.4 ± 0.0	12.2 ± 0.5	20.6 ± 0.1	12.2 ± 0.9
CS2 (µg/L)	2.8 ± 0.4	6.8 ± 1.7	3.4 ± 0.2	7.2 ± 2.2	5.0 ± 0.3	7.0 ± 2.8	2.6 ± 0.7	3.9 ± 1.3	4.3 ± 1.1	5.0 ± 1.2	5.2 ± 1.4	6.6 ± 0.9
MeSAc (µg/L)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DMDS (µg/L)	n.d.	n.d.	n.d.	n.d.	0.5 ± 0.0	n.d.	n.d.	n.d.	0.6 ± 0.0	n.d.	n.d.	n.d.
EtSAc (µg/L)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DEDS (µg/L)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DMTS (µg/L)	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.
4MSP (ng/L)	6.7 ± 0.8	9.9 ± 0.8	3.4 ± 0.0	n.q.	8.5 ± 3.48	9.6 ± 0.8	n.q.	n.q.	n.d.	n.q.	4.1 ± 0.0	4.1 ± 0.8
3SHA (ng/L)	36 ± 0	88 ± 15	n.q.	87 ± 8	283 ± 15	98 ± 2	185 ± 21	61 ± 10	172 ± 14	99 ± 9	n.d.	118 ± 16
3SH (ng/L)	467 ± 70	679 ± 147	378 ± 0	575 ± 30	3199 ± 205	643 ± 71	4564 ± 354	724 ± 42	3558 ± 780	614 ± 23	473 ± 0	707 ± 82

Appendix 6-9: Tasting scores of the finished Sauvignon blanc wines from the vintage 2008 & 2009.

Variant	X5		VL3		ALII		SAU		BOUQ		VIN13	
Vintage	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
Passionfruit	2.91	4.05	2.24	3.88	2.45	3.12	2.44	3.41	3.43	3.89	1.25	3.59
Cassis	3.03	3.72	2.13	4.93	1.93	3.8	4.8	1.99	2.8	3.44	2.86	2.57
Grapefruit	1.62	2.04	2.11	4.54	0.72	2.48	0.88	1.39	0.99	3.67	3.95	2.85
Catpiss	1.8	3.94	0.89	3.74	0.96	1.8	2.09	3.66	0.49	4.16	1.51	3.00
Estery	2.37	3.14	2.89	3.13	2.43	2.77	3.31	5.09	2.78	5.38	0.44	2.41
Solvent	1.3	3.12	2.13	3.93	1.45	3.91	1.05	3.91	2.68	3.32	0.52	3.25
Reduction	1.28	3.68	1.52	4.36	1.57	2.34	1.71	3.57	1.36	4.38	2.32	3.22
Typicity	3.42	4.00	3.71	4.25	4.41	2.96	3.03	1.64	3.86	3.88	3.24	4.67

Appendix 6-10: OAV values for the Sauvignon blanc wines from the vintage 2008 & 2009. OAV>1 values are bold.

Variant	X5		VL3		ALII		SAU		BOUQ		VIN13	
Vintage	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
Linalool	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
a-Terpineol	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
i-Butanol	0.16	0.17	0.22	0.17	0.29	0.15	0.30	0.17	0.32	0.19	0.22	0.16
3-Methyl-butanol	**2.63**	**3.24**	**3.02**	**3.32**	**3.20**	**4.10**	**2.63**	**3.21**	**3.28**	**4.80**	**3.35**	**4.16**
Hexan-1-ol	0.20	0.39	0.21	0.38	0.17	0.35	0.26	0.43	0.20	0.36	0.19	0.34
2-Phenylethanol	0.90	0.83	**1.00**	0.86	0.71	**1.05**	0.75	0.88	0.94	**1.29**	0.78	**1.05**
Acetic acid ethylester	0.23	0.94	0.53	0.96	0.51	**1.11**	0.33	**1.27**	0.32	0.79	0.52	0.85
Acetic acid 3-methylbutyl ester	**9.69**	0.00	**6.89**	0.00	**29.15**	**7.92**	0.00	0.00	**30.58**	**21.18**	**29.49**	**10.97**
Acetic acid 2-phenylethylester	0.36	0.18	0.36	0.19	0.47	0.33	0.16	0.12	0.55	0.48	0.45	0.33
Butyric acid ethylester	**11.16**	**9.98**	**10.47**	**10.63**	**12.39**	**8.37**	**13.09**	**8.12**	**10.94**	**10.17**	**14.62**	**10.72**
Lactic acid ethylester	0.12	0.14	0.11	0.12	0.17	0.12	0.16	0.58	0.14	0.14	0.14	0.13
Succinic acid diethylester	0.01	0.02	0.01	0.02	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.02
H2S	0.00	0.75	0.00	0.61	0.00	0.68	0.00	0.47	0.00	0.58	0.00	0.83
MeSH	0.00	**1.17**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.80	0.00	0.89
DMS	**1.10**	**1.22**	**1.00**	**1.10**	**2.39**	**1.24**	**1.39**	**1.17**	**1.14**	**1.22**	**2.06**	**1.22**
CS2	**1.12**	**2.73**	**1.36**	**2.88**	**1.99**	**2.81**	**1.05**	**1.55**	**1.72**	**2.00**	**2.08**	**2.63**
4MSP	**2.22**	**3.31**	**1.14**	0.00	**2.82**	**3.20**	0.00	0.00	0.00	0.00	**1.36**	**1.36**
3SHA	**8.59**	**21.00**	0.00	**20.76**	**67.46**	**23.44**	**44.05**	**14.51**	**40.96**	**23.67**	0.00	**27.98**
3SH	**7.78**	**11.32**	**6.30**	**9.58**	**53.31**	**10.72**	**76.07**	**12.07**	**59.31**	**10.24**	**7.89**	**11.79**

Appendix 6-11: Major components of the commercial wines used for the Greek varieties pre-selection.

Wine	MA1	MA2	MA3	MA4	MA5	MA6	A1	A2	A3	A4	A5	R1	R2	R3	R4	AM1	SB1
Alcohol (% Vol.)	13.9	11.7	12.2	12.9	11.7	13.2	13.0	12.3	13.8	13.3	13.1	12.2	12.8	12.7	13.3	12.9	13.7
Residual sugars (g/L)	1.6	10.9	1.3	2.8	1.9	3.1	0.9	2.5	1.4	0.3	0.9	1.6	0.4	1.6	3.7	2.6	1.1
Volatile Acidity (g/L) in AA	0.2	0.3	0.5	0.6	0.5	0.8	0.3	0.5	0.6	0.4	0.5	0.5	0.1	0.9	0.7	0.6	0.5
Malic acid (g/L)	1.5	1.1	1.7	1.8	0.9	1.1	1.1	1.7	1.1	0.9	1.0	1.4	1.0	n.q.	1.2	1.8	0.7
Lactic acid (g/L)	0.2	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.2	1.1	n.q.	0.2	0.2

Appendix 6-12: Aroma compounds measured with the 'Kaltron' method of the commercial wines used for the Greek varieties pre-selection.

Wine	MA1	MA2	MA3	MA4	MA5	MA6	A1	A2	A3	A4	A5	R1	R2	R3	R4	AM1	SB1
Volatile acids																	
Caproic acid (mg/L)	5.3	8.3	8.5	7.6	6.8	8.2	8.3	7.5	6.5	7.3	8.9	7.3	6.4	6.7	7.8	7.4	7.8
Caprylic acid (mg/L)	4.7	7.2	7.6	7.2	6.6	6.8	8.2	6.8	5.5	5.7	6.9	6.3	4.8	6.2	6.9	7.0	7.0
Capric acid (mg/L)	0.9	1.4	1.1	1.8	1.9	1.5	1.9	1.2	1.5	0.9	1.4	1.2	0.7	1.6	1.7	1.8	1.5
Higher alcohols																	
i-Butanol (mg/L)	37	17	24	21	34	20	20	26	28	24	24	29	38	30	25	23	34
3-Methyl-butanol (mg/L)	233	117	129	128	192	110	129	162	141	181	155	136	200	167	135	137	177
2-Methyl butanol (mg/L)	41	27	26	23	40	21	24	30	28	30	27	26	45	38	27	25	37
Hexan-1-ol (µg/L)	1154	1015	1112	915	608	1198	817	1181	1116	2003	1715	814	1486	1073	905	940	1371
2-Phenylethanol (mg/L)	31	11	13	13	41	11	13	28	32	33	23	19	29	36	17	13	18
Acetic acid esters																	
Acetic acid ethylester (mg/L)	36	53	99	132	33	126	60	138	181	151	135	105	40	203	198	171	104
Acetic acid 3-methylbutylester (µg/L)	438	881	1529	1295	222	n.q.	1404	336	198	n.q.	216	1645	24	595	n.q.	1288	847
Acetic acid 2- methylbutylester (µg/L)	31	39	49	38	14	n.q.	40	14	13	n.q.	10	63	4	20	n.q.	31	27
Acetic acid hexylester (µg/L)	20	50	95	65	20	n.q.	63	27	31	25	46	64	15	17	14	63	53
Acetic acid 2-phenylethylester (µg/L)	67	65	112	110	74	n.d.	98	65	65	31	54	168	26	83	23	105	72
Ethylester																	
Propionic acid ethylester (µg/L)	194	69	82	90	75	90	70	107	109	185	102	73	109	118	148	94	121
i-Butyric acid ethylester (µg/L)	66	129	70	44	110	102	89	104	194	113	97	45	123	54	83	46	98
Butyric acid ethylester (µg/L)	301	338	340	308	197	280	370	254	266	332	344	342	218	295	277	306	327
Lactic acid ethylester (mg/L)	36	20	25	25	23	25	24	23	26	37	24	19	37	117	20	25	32
Caproic acid ethylester (µg/L)	366	597	764	657	473	835	819	573	565	661	754	577	420	537	724	644	764
Succinic acid diethylester (µg/L)	2626	1370	1631	2230	2430	7606	2176	4229	5014	6493	3888	1115	7045	10983	5568	1991	3591
Caprylic acid ethylester (µg/L)	635	820	1016	1141	834	1242	1219	904	872	760	994	798	555	1056	1190	1116	1129
Capric acid ethylester (µg/L)	138	172	174	307	264	245	323	159	277	119	219	175	96	262	298	312	245
Monoterpenols																	
Linalool (µg/L)	148	199	53	57	79	n.d.	n.d.	10	n.d.	n.d.	n.d.	9	3	8	9	55	n.d.
α-Terpineol (µg/L)	127	364	55	54	74	78	n.d.	35	8	11	10	14	12	13	50	59	12
Monoterpenol oxides																	
trans -linalool oxide (µg/L)	29	178	17	18	22	141	n.d.	27	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	37	24	n.d.
cis -linalool oxide (µg/L)	26	84	14	14	17	84	n.d.	19	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	25	16	n.d.

Appendix 6-13: Sulphur compound concentrations in the commercial wines used for the Greek varieties pre-selection.

Wine	MA1	MA2	MA3	MA4	MA5	MA6	A1	A2	A3	A4	A5	R1	R2	R3	R4	AM1	SB1
H_2S (µg/L)	9.2	9.9	3.6	17.2	12.8	11.8	3.4	5.9	5.5	8.8	6	14.1	9.3	14.6	18.4	17.4	12.9
MeSH (µg/L)	6.5	6.5	6.3	10	4.4	6.6	5.9	n.d.	5	9.2	n.q.	6.5	11.4	10.4	6.2	9.9	4.9
EtSH (µg/L)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DMS (µg/L)	9.8	22.8	12.5	18	4.9	47.7	14.3	15	7.5	8.9	7.9	21.6	17.1	20.8	23.6	18.4	36.3
CS_2 (µg/L)	2.4	2.6	0	1.4	1.5	3.7	1.9	1.6	1.4	1.5	1.2	n.q.	1.2	1.8	1.6	1.6	1.4
MeSAc (µg/L)	n.d.	n.d.	n.d.	n.d.	n.q.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DMDS (µg/L)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
EtSAc (µg/L)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DEDS (µg/L)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DMTS (µg/L)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4MSP (ng/L)	6.3	n.q.	n.q.	5	10.5	n.d.	n.q.	n.q.	3.5	n.q.	n.d.	n.q.	n.d.	n.d.	n.d.	n.q.	4
3SHA (ng/L)	74	84	n.q.	n.q.	n.q.	n.q.	58	n.q.	n.q.	n.q.	n.q.	58	n.q.	n.q.	n.q.	n.q.	n.q.
3SH (ng/L)	426	1942	416	1394	875	1346	506	472	594	675	510	889	614	506	1161	1436	1489

Appendix 6-14: Tasting scores of the commercial wines used for the Greek varieties pre-selection.

Wine	MA1	MA2	MA3	MA4	MA5	MA6	A1	A2	A3	A4	A5	R1	R2	R3	R4	AM1	SB1
Passionfruit	3.44	5.48	4.11	4.64	5.79	2.28	3.34	3.20	2.26	2.31	2.18	3.95	1.24	1.79	3.00	3.10	4.56
Grapefruit	2.74	2.98	3.22	2.43	4.14	2.11	3.10	2.50	3.00	2.15	2.79	3.15	1.18	1.67	3.13	2.65	3.45
Citrus	2.45	1.96	2.71	3.46	2.75	0.79	2.13	2.19	2.71	2.31	2.51	2.66	1.11	1.11	1.97	2.03	2.29
Floral	3.11	5.73	3.14	3.54	4.07	2.07	1.79	3.49	2.48	2.45	2.68	3.89	1.56	2.33	3.07	2.74	3.54
Estery	2.78	3.23	2.51	3.99	5.04	3.00	1.86	2.81	1.74	2.51	2.27	3.69	1.72	3.24	3.22	2.73	3.23
Reduction	3.02	1.43	1.61	2.22	2.37	2.83	3.64	1.85	3.30	3.54	2.33	0.81	4.04	3.08	2.77	1.85	1.90
Minerality	3.16	2.10	2.80	2.83	2.19	2.00	3.58	2.02	3.71	3.48	3.13	1.64	3.39	2.78	3.30	3.37	2.61
Acidity	4.64	3.50	3.77	4.20	4.78	3.44	4.49	3.34	5.02	5.40	4.24	3.56	3.74	2.44	3.80	3.86	3.26
Volume	3.58	5.01	3.26	4.91	4.17	4.75	2.77	3.35	3.77	3.27	3.64	3.44	2.54	3.79	4.93	3.11	4.64
Impression	4.18	6.20	4.38	5.33	5.09	2.45	3.60	2.41	3.34	3.03	3.53	5.17	1.58	2.47	5.04	3.65	4.85

Appendix 6-15: OAV values for the commercial wine for the Greek variety pre-selection. OAV >1 values are bold.

Wine	MA1	MA2	MA3	MA4	MA5	MA6	A1	A2	A3	A4	A5	R1	R2	R3	R4	AM1	SB1
Linalool	**5.91**	**7.96**	**2.12**	**2.30**	**3.17**	0.00	0.00	0.39	0.00	0.00	0.00	0.37	0.13	0.34	0.35	**2.18**	0.00
a-Terpineol	0.51	**1.46**	0.22	0.22	0.30	0.31	0.00	0.14	0.03	0.04	0.04	0.05	0.05	0.05	0.20	0.24	0.05
i-Butanol	0.25	0.11	0.16	0.14	0.23	0.13	0.13	0.18	0.19	0.16	0.16	0.19	0.26	0.20	0.17	0.15	0.23
3-Methyl-butanol	**7.75**	**3.88**	**4.31**	**4.27**	**6.41**	**3.67**	**4.32**	**5.40**	**4.72**	**6.04**	**5.16**	**4.54**	**6.67**	**5.58**	**4.50**	**4.55**	**5.91**
Hexan-1-ol	0.14	0.13	0.14	0.11	0.08	0.15	0.10	0.15	0.14	0.25	0.21	0.10	0.19	0.13	0.11	0.12	0.17
2-Phenylethanol	**2.21**	0.78	0.92	0.94	**2.96**	0.82	0.92	**1.97**	**2.30**	**2.33**	**1.67**	**1.32**	**2.05**	**2.60**	**1.25**	0.94	**1.26**
Acetic acid ethylester	0.23	0.33	0.62	0.82	0.21	0.79	0.37	0.86	**1.13**	0.94	0.84	0.65	0.25	**1.27**	**1.24**	**1.07**	0.65
Acetic acid 3-methylbutyl ester	**14.60**	**29.35**	**50.96**	**43.18**	**7.41**	0.00	**46.81**	**11.21**	**6.61**	0.00	**7.18**	**54.83**	0.79	**19.83**	0.00	**42.94**	**28.22**
Acetic acid 2-phenylethylester	0.27	0.26	0.45	0.44	0.29	0.00	0.39	0.26	0.26	0.12	0.22	0.67	0.11	0.33	0.09	0.42	0.29
Butyric acid ethylester	**15.04**	**16.89**	**16.99**	**15.42**	**9.83**	**14.02**	**18.50**	**12.72**	**13.31**	**16.58**	**17.18**	**17.11**	**10.89**	**14.74**	**13.83**	**15.31**	**16.36**
Lactic acid ethylester	0.23	0.13	0.16	0.16	0.15	0.16	0.16	0.15	0.17	0.24	0.16	0.12	0.24	0.76	0.13	0.16	0.21
Succinic acid diethylester	0.01	0.01	0.01	0.01	0.01	0.04	0.01	0.02	0.03	0.03	0.02	0.01	0.04	0.05	0.03	0.01	0.02
H2S	0.92	0.99	0.36	**1.72**	**1.28**	**1.18**	0.34	0.59	0.55	0.88	0.60	**1.41**	0.93	**1.46**	**1.84**	**1.74**	**1.29**
MeSH	**1.30**	**1.30**	**1.26**	**2.00**	0.88	**1.32**	**1.18**	0.00	**1.00**	**1.84**	0.00	**1.30**	**2.28**	**2.08**	**1.24**	**1.98**	0.98
DMS	0.98	**2.28**	**1.25**	**1.80**	0.49	**4.77**	**1.43**	**1.50**	0.75	0.89	0.79	**2.16**	**1.71**	**2.08**	**2.36**	**1.84**	**3.63**
4MSP	**2.11**	0.00	0.00	**1.67**	**3.49**	0.00	0.00	0.00	**1.16**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**1.32**
3SHA	**17.63**	**20.09**	0.00	0.00	0.00	0.00	**13.83**	0.00	0.00	0.00	0.00	**13.69**	0.00	0.00	0.00	0.00	0.00
3SH	**7.10**	**32.36**	**6.93**	**23.23**	**14.58**	**22.43**	**8.44**	**7.87**	**9.89**	**11.24**	**8.50**	**14.82**	**10.23**	**8.43**	**19.35**	**23.93**	**24.81**

Appendix 6-16: Major components of the commercial wines from the Greek grape variety Malagousia.

Wine	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12	M13	M14	M15	M16	M17	M18
Alcohol (% Vol.)	12.8	13.3	13.2	12.7	13.4	13.3	12.2	13.7	12.3	12.8	13.3	12.7	13.7	12.4	13.1	12.6	11.9	12
Residual sugars (g/L)	1.6	0.3	0.3	n.d.	1.1	0.4	n.d.	0.2	n.d.	n.d.	0.2	0.1	0.1	2.8	0.6	1.2	n.d.	0.4
Total acidity (g/L)	4.9	4.2	4.8	5.1	5.1	4.8	5.1	5.6	5.1	5.1	4.6	4.4	5.2	5.0	5.5	4.9	5.2	5.4
Volatile Acidity (g/L) in AA	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Malic acid (g/L)	1.8	1.4	1.2	1.6	1.9	1.5	1.9	1.3	1.5	2.1	0.7	1.5	0.7	1.5	1.4	0.9	1.8	1.1
Lactic acid (g/L)	0.3	n.d.	n.d.	0.3	n.d.	n.d.	0.1	n.d.	0.1	n.d.	n.d.	0.2	0.1	0.4	0.1	0.1	0.4	0.1
Total phenols (mg/L)	201	164	182	245	241	230	184	266	215	204	206	170	146	223	201	173	208	210

Appendix 6-17: Aroma compounds measured with the 'Kaltron' method of the commercial wines from the Greek variety Malagousia.

Wine	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12	M13	M14	M15	M16	M17	M18
Volatile acids																		
Caproic acid (mg/L)	13.2	10.1	12.1	11.1	11.8	13.0	12.1	10.2	11.4	12.8	13.2	11.1	9.3	11.1	11.2	13.1	11.7	11.6
Caprylic acid (mg/L)	7.2	5.5	6.4	5.3	6.5	6.8	6.9	4.5	6.3	6.9	7.3	6	4.2	5.3	6.1	6.5	6.3	6.4
Capric acid (mg/L)	2.3	1.5	1.8	1.6	2.0	1.8	1.4	1.3	1.8	2.0	2.1	1.3	1.0	1.1	1.7	2.4	1.7	1.9
Higher alcohols																		
i-Butanol (mg/L)	19	53	17	36	20	17	10	23	22	15	16	22	30	13	20	27	19	20
3-Methyl-butanol (mg/L)	140	172	97	153	112	128	98	128	103	115	121	115	127	104	135	145	102	92
2-Methyl butanol (mg/L)	24	41	21	36	23	21	16	27	22	26	24	25	29	23	24	31	19	20
Hexan-1-ol (µg/L)	637	728	334	319	308	1279	688	3235	360	564	249	1089	1772	787	514	1661	915	512
2-Phenylethanol (mg/L)	25	28	11	32	15	14	10	19	11	15	32	15	15	18	21	19	10	11
Acetic acid esters																		
Acetic acid ethylester (mg/L)	53	42	50	43	52	51	45	33	33	41	41	34	46	34	56	41	23	55.01
Acetic acid 3-methylbutylester (µg/L)	2758	1893	2380	1044	2308	2375	2628	747	1940	2616	1681	2257	357	3023	2625	2653	2197	1460
Acetic acid 2- methylbutylester (µg/L)	130	110	138	42	116	105	106	41	91	152	76	110	24	153	121	123	96	80
Acetic acid hexylester (µg/L)	211	121	148	29	121	312	215	91	124	227	78	236	23	225	168	304	128	114
Acetic acid 2-phenylethylester (µg/L)	326	154	217	123	197	176	203	61	132	206	310	142	8	272	268	197	144	117
Ethylester																		
Propionic acid ethylester (µg/L)	114	97	124	189	109	132	102	184	100	167	128	122	125	97	141	87	67	116
i-Butyric acid ethylester (µg/L)	46	87	71	141	85	86	48	129	99	82	109	112	93	104	92	109	39	66
Butyric acid ethylester (µg/L)	586	537	572	462	432	613	526	442	490	598	504	389	478	600	565	544	580	445
Lactic acid ethylester (mg/L)	13	16	34	47	25	18	20	34	22	15	21	14	27	34	21	21	21	21
Caproic acid ethylester (µg/L)	1029	763	942	755	906	1054	934	733	897	1055	1076	845	784	883	924	924	766	846
Succinic acid diethylester (µg/L)	366	666	565	7127	1255	786	504	3223	836	580	1579	989	2906	1160	1095	1050	841	662
Caprylic acid ethylester (µg/L)	1090	910	1057	866	1115	1172	1070	798	1015	1135	1286	999	849	912	1077	1026	842	801
Capric acid ethylester (µg/L)	324	240	312	248	363	292	192	202	315	298	376	215	161	184	280	389	252	236
Monoterpenols																		
Linalool (µg/L)	31	304	487	379	349	184	293	230	231	189	122	255	291	525	168	139	218	219
α-Terpineol (µg/L)	15	143	246	384	180	87	154	286	136	94	87	115	369	229	108	100	95	161
Monoterpenol oxides																		
trans -linalool oxide (µg/L)	4	14	50	90	1	9	8	28	11	8	11	13	79	29	10	12	8	24
cis -linalool oxide (µg/L)	1	9	30	43	15	6	6	26	6	6	8	7	53	15	6	7	4	17

Appendix 6-18: Terpenes of the commercial wines from the Greek variety Malagousia.

Wine	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12	M13	M14	M15	M16	M17	M18
trans -linalool oxide (µg/L)	3	12	47	82	15	9	7	24	11	7	10	12	87	32	9	11	8	23
Neroloxide (µg/L)	17	25	33	56	26	22	21	31	23	22	22	24	52	31	22	24	21	30
cis -linalool oxide (µg/L)	3	6	12	17	8	4	4	10	5	4	5	5	21	8	5	5	4	8
Vitispirane (µg/L)	3	3	3	5	4	3	3	13	3	4	6	4	14	4	5	6	3	10
Linalool (µg/L)	24	258	464	350	322	161	254	208	214	167	107	223	258	540	144	128	195	191
Hotrienol (µg/L)	6	87	95	259	78	65	34	79	62	49	49	59	476	140	35	44	47	89
α-Terpineol (µg/L)	20	194	279	446	200	97	172	430	159	112	89	145	608	323	137	120	124	212
TDN (µg/L)	0.2	0.2	0.4	0.7	0.3	0.1	0.1	0.7	0.2	0.3	0.5	0.2	0.9	0.4	0.5	0.4	0.2	0.4
Nerol (µg/L)	93	157	103	40	55	92	43	43	15	56	8	144	54	105	25	20	19	21
β-Damascenone (µg/L)	5.4	5.1	7.0	1.4	7.0	2.7	1.6	6.1	5.6	2.7	5.4	7.3	5.7	7.3	4.9	3.6	4.4	6.2
Geraniol (µg/L)	9	76	85	78	62	33	40	53	38	33	16	61	68	252	28	21	46	47

Appendix 6-19: Sulphur compound concentrations of the commercial wines from the Greek variety Malagousia.

Wine	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12	M13	M14	M15	M16	M17	M18
H_2S (µg/L)	9.7	16.7	9.9	10.5	9.4	8.7	11.6	19.7	7.3	7.0	6.3	9.6	6.1	4.3	9.1	5.0	11.8	8.7
MeSH (µg/L)	3.0	n.q.	3.2	n.d.	3.1	3.0	3.2	n.d.	2.5	3.5	n.d.	n.d.	n.d.	3.0	n.q.	3.1	3.0	n.q.
EtSH (µg/L)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DMS (µg/L)	7.8	2.9	3.3	9	1.5	6.5	7.6	4.6	6.8	7.4	5.2	4.6	6.5	5.1	5.9	5.0	6.6	8.2
CS_2 (µg/L)	1.4	1.6	n.q.	2.5	n.q.	1.6	n.d.	2.9	1.2	n.d.	n.d.	1.6	n.d.	1.5	n.d.	n.q.	1.0	2.0
MeSAc (µg/L)	n.d.	n.q.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DMDS (µg/L)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
EtSAc (µg/L)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DEDS (µg/L)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DMTS (µg/L)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4MSP (ng/L)	4.0	3.5	5.2	44.7	6.5	4.2	3.1	7.5	n.q.	n.q.	15.9	n.q.	n.q.	3.7	n.q.	n.q.	n.q.	n.q.
3SHA (ng/L)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
3SH (ng/L)	388	n.q.	842	264	601	675	192	884	275	159	305	n.q.	177	1356	328	159	405	649

Appendix 6-20: Tasting scores of the commercial wines from the Greek variety Malagousia.

Wine	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12	M13	M14	M15	M16	M17	M18
Passionfruit	5.42	5.98	6.34	6.17	4.18	3.73	3.34	5.03	4.28	4.07	5.50	4.61	3.88	5.77	2.58	2.23	3.42	4.64
Grapefruit	4.70	4.40	5.89	5.13	4.11	4.02	3.67	4.14	4.02	3.39	4.39	2.97	4.13	4.39	3.47	2.96	3.06	3.87
Citrus	3.53	3.88	3.75	4.19	3.33	4.05	3.64	3.72	4.15	4.13	3.47	2.90	3.05	3.48	2.46	3.10	3.32	2.77
Floral	3.78	5.61	4.42	4.14	3.68	3.93	5.27	5.09	2.91	4.42	3.99	4.57	3.31	5.39	3.14	2.46	3.15	3.07
Estery	0.84	2.36	1.56	1.56	2.12	2.06	2.09	1.98	1.69	2.17	1.36	2.01	1.84	0.76	1.86	0.93	1.20	1.04
Reduction	3.63	3.46	3.98	3.45	3.98	4.60	4.17	4.49	5.87	3.46	5.06	2.78	2.90	2.27	3.05	3.87	3.33	4.01
Minerality	3.28	4.38	3.61	3.72	4.18	3.65	3.09	3.17	3.38	3.68	3.35	3.02	2.93	3.09	2.42	3.27	2.84	3.56
Acidity	3.72	3.58	3.48	4.01	3.41	4.62	3.71	4.34	3.86	3.99	4.58	2.71	4.25	3.36	2.50	3.46	3.22	4.29
Volume	4.23	4.43	4.39	4.46	5.40	4.23	3.85	5.17	4.08	5.14	5.57	4.23	4.99	4.59	4.07	3.28	4.25	4.20
Impression	4.89	5.62	4.50	3.98	4.80	3.74	3.73	3.74	3.51	4.82	4.97	3.56	4.54	4.71	3.14	2.55	3.64	4.12

Appendix 6-21: OAV values for the 18 Malagousia commercial wines. OAV>1 values are bold.

Wine	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12	M13	M14	M15	M16	M17	M18
Acetic acid ethylester	0.33	0.26	0.31	0.27	0.32	0.32	0.28	0.21	0.21	0.26	0.25	0.21	0.29	0.21	0.35	0.26	0.14	0.34
Acetic acid 3-methylbutyl ester	**91.93**	**63.09**	**79.34**	**34.81**	**76.92**	**79.16**	**87.59**	**24.91**	**64.67**	**87.21**	**56.02**	**75.23**	**11.90**	**100.80**	**87.50**	**88.42**	**73.22**	**48.68**
Acetic acid 2-phenylethylester	**1.30**	0.61	0.87	0.49	0.79	0.70	0.81	0.25	0.53	0.82	**1.24**	0.57	0.03	**1.09**	**1.07**	0.79	0.58	0.47
Butyric acid ethylester	**29.30**	**26.87**	**28.59**	**23.09**	**21.61**	**30.65**	**26.29**	**22.10**	**24.51**	**29.91**	**25.22**	**19.44**	**23.92**	**30.01**	**28.25**	**27.19**	**29.00**	**22.23**
Lactic acid ethylester	0.09	0.10	0.22	0.31	0.16	0.11	0.13	0.22	0.14	0.10	0.13	0.09	0.18	0.22	0.13	0.13	0.13	0.13
Succinic acid diethylester	0.00	0.00	0.00	0.04	0.01	0.00	0.00	0.02	0.00	0.00	0.01	0.00	0.01	0.01	0.01	0.01	0.00	0.00
i-Butanol	0.13	0.35	0.12	0.24	0.14	0.11	0.07	0.15	0.15	0.10	0.11	0.15	0.20	0.08	0.14	0.18	0.12	0.13
3-Methyl-butanol	**4.66**	**5.75**	**3.23**	**5.12**	**3.73**	**4.28**	**3.28**	**4.26**	**3.45**	**3.84**	**4.05**	**3.83**	**4.22**	**3.48**	**4.48**	**4.85**	**3.40**	**3.06**
Hexan-1-ol	0.08	0.09	0.04	0.04	0.04	0.16	0.09	0.40	0.04	0.07	0.03	0.14	0.22	0.10	0.06	0.21	0.11	0.06
2-Phenylethanol	**1.77**	**1.98**	0.76	**2.30**	**1.06**	**1.00**	0.73	**1.35**	0.75	**1.10**	**2.27**	**1.08**	**1.08**	**1.30**	**1.49**	**1.36**	0.70	0.81
Linalool	0.97	**10.31**	**18.57**	**14.00**	**12.87**	**6.44**	**10.15**	**8.30**	**8.58**	**6.67**	**4.29**	**8.91**	**10.32**	**21.58**	**5.77**	**5.10**	**7.82**	**7.64**
Hotrienol	0.05	0.79	0.87	**2.36**	0.71	0.59	0.31	0.72	0.57	0.45	0.44	0.54	**4.33**	**1.27**	0.32	0.40	0.42	0.81
a-Terpineol	0.08	0.78	**1.12**	**1.79**	0.80	0.39	0.69	**1.72**	0.64	0.45	0.36	0.58	**2.43**	**1.29**	0.55	0.48	0.49	0.85
TDN	0.01	0.01	0.02	0.03	0.01	0.01	0.01	0.04	0.01	0.02	0.02	0.01	0.05	0.02	0.03	0.02	0.01	0.02
Nerol	0.23	0.39	0.26	0.10	0.14	0.23	0.11	0.11	0.04	0.14	0.02	0.36	0.14	0.26	0.06	0.05	0.05	0.05
β-Damascenone	**38.38**	**36.38**	**50.35**	**10.01**	**50.23**	**19.51**	**11.71**	**43.82**	**39.80**	**19.47**	**38.64**	**52.47**	**40.78**	**52.48**	**35.19**	**25.56**	**31.08**	**44.10**
Geraniol	0.07	0.59	0.65	0.60	0.48	0.26	0.31	0.41	0.29	0.25	0.12	0.47	0.52	**1.94**	0.21	0.16	0.35	0.36
H2S	0.97	**1.67**	0.99	**1.05**	0.94	0.87	**1.16**	**1.97**	0.73	0.70	0.63	0.96	0.61	0.43	0.91	0.50	**1.18**	0.87
MeSH	0.60	0.00	0.64	0.00	0.62	0.60	0.64	0.00	0.50	0.70	0.00	0.00	0.00	0.60	0.00	0.62	0.60	0.00
DMS	0.78	0.29	0.33	0.90	0.15	0.65	0.76	0.46	0.68	0.74	0.52	0.46	0.65	0.51	0.59	0.50	0.66	0.82
4MSP	**1.32**	**1.18**	**1.72**	**14.91**	**2.18**	**1.41**	**1.04**	**2.52**	0.00	0.00	**5.29**	0.00	0.00	**1.22**	0.00	0.00	0.00	0.00
3SH	**6.46**	0.00	**14.03**	**4.41**	**10.02**	**11.25**	**3.20**	**14.73**	**4.58**	**2.65**	**5.08**	0.00	**2.96**	**22.60**	**5.47**	**2.65**	**6.75**	**10.82**

Appendix 6-22: Major components of the commercial wines from the Swiss variety Petite Arvine.

Wine	PA1	PA2	PA3	PA4	PA5	PA6	PA7	PA8	PA9	PA10	PA11	PA12	PA13	PA14	PA15
Alcohol (% Vol.)	12.4	13	12.4	14	13.3	12.8	12.5	13.9	12.8	13.5	13.4	13.5	12.9	12.8	12.9
Residual sugars (g/L)	15.3	1.9	2.2	4.1	1.8	6.8	3.5	0.4	0.8	0.2	0.3	0.4	5.0	0.5	1.3
Total acidity (g/L)	6.7	4.7	6.8	7.6	6.3	5.6	5.5	6.7	5.9	6.6	7.0	7.1	7.1	6.4	7.1
Volatile Acidity (g/L) in AA	0.5	0.5	0.4	0.4	0.4	0.5	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Malic acid (g/L)	3.7	1.1	4.3	4.2	3.5	2.6	3.3	4.4	3.3	4.0	4.4	4.2	4.5	3.3	4.4
Lactic acid (g/L)	0.4	1.6	0.1	n.d.	0.3	0.7	0.1	0.1	0.2	0.1	0.2	0.1	0.1	0.5	n.d.
Total phenols (mg/L)	221	219	200	284	182	200	154	248	256	255	219	218	221	192	203

Appendix 6-23: Aroma compounds measured with the 'Kaltron' method of the commercial wines from the Swiss variety Petite Arvine.

Wine	PA1	PA2	PA3	PA4	PA5	PA6	PA7	PA8	PA9	PA10	PA11	PA12	PA13	PA14	PA15
Volatile acids															
Caproic acid (mg/L)	11.9	15.1	11.4	12.2	11.4	10.9	11.2	14.4	13	15.2	14.3	14.3	12.1	13.3	12.9
Caprylic acid (mg/L)	5.7	7.0	6.8	6.3	6.0	4.9	5.7	7.4	6.8	8.1	7.9	8.5	7.1	6.7	7.6
Capric acid (mg/L)	1.7	1.6	1.9	2.3	2.0	1.3	1.5	2.1	1.8	2.4	2.2	2.6	2.0	1.9	2.3
Higher alcohols															
i-Butanol (mg/L)	26	21	34	44	27	31	17	28	29	25	24	20	34	28	13
3-Methyl-butanol (mg/L)	113	120	105	116	108	99	113	158	183	166	147	140	104	108	105
2-Methyl butanol (mg/L)	22	23	20	25	21	23	22	33	31	26	25	22	20	18	16
Hexan-1-ol (µg/L)	1168	1316	628	626	975	1229	1043	748	1551	737	778	488	854	972	706
2-Phenylethanol (mg/L)	15	18	16	22	17	16	16	22	24	24	32	24	17	17	16
Acetic acid esters															
Acetic acid ethylester (mg/L)	45	48	48	41	42	56	43	59	52	57	43	49	44	43	45
Acetic acid 3-methylbutylester (µg/L)	2183	1743	1823	1342	2307	1618	3783	4317	4656	3917	3370	3657	1892	1767	2549
Acetic acid 2- methylbutylester (µg/L)	116	94	103	67	117	94	216	236	212	197	166	164	109	92	127
Acetic acid hexylester (µg/L)	278	171	282	184	276	218	559	349	363	340	305	344	313	264	396
Acetic acid 2-phenylethylester (µg/L)	209	167	239	250	246	158	400	409	342	386	416	396	241	186	281
Ethylester															
Propionic acid ethylester (µg/L)	96	90	172	76	104	98	60	144	82	136	139	144	166	150	109
i-Butyric acid ethylester (µg/L)	55	53	88	114	72	63	41	62	49	48	50	54	87	59	44
Butyric acid ethylester (µg/L)	453	546	468	380	445	460	453	744	520	616	549	600	472	421	521
Lactic acid ethylester (mg/L)	36	80	16	19	36	59	18	18	23	18	21	19	17	42	14
Caproic acid ethylester (µg/L)	872	1138	1001	918	931	789	925	1225	888	1212	1160	1305	1064	925	1145
Succinic acid diethylester (µg/L)	1157	2804	669	1119	1593	1839	439	394	245	365	639	540	706	772	441
Caprylic acid ethylester (µg/L)	901	1133	1236	1106	1029	807	1060	1550	1138	1560	1464	1674	1327	1105	1399
Capric acid ethylester (µg/L)	265	264	348	414	332	198	255	440	308	458	401	494	364	313	425
Monoterpenols															
Linalool (µg/L)	27	24	22	17	37	31	35	21	21	28	30	29	23	28	26
α-Terpineol (µg/L)	8	7	6	5	13	11	11	2	3	9	7	8	6	9	7
Monoterpenol oxides															
trans -linalool oxide (µg/L)	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.
cis -linalool oxide (µg/L)	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.

Appendix 6-24: Terpenes of the commercial wines from the Swiss variety Petite Arvine.

Wine	PA1	PA2	PA3	PA4	PA5	PA6	PA7	PA8	PA9	PA10	PA11	PA12	PA13	PA14	PA15
trans -linalool oxide (µg/L)	3.9	4.2	2.3	1.7	8.8	6.0	1.9	0.8	1.7	2.5	1.5	1.6	2.0	2.5	3.0
Neroloxide (µg/L)	18	17	17	17	18	18	17	17	16	17	17	17	16	17	17
cis -linalool oxide (µg/L)	2.8	3.1	2.3	2.3	3.3	2.9	2.5	2.4	2.3	2.3	2.3	2.3	2.3	2.4	2.5
Vitispirane (µg/L)	6	5	7	6	5	6	5	4	6	5	5	5	9	6	5
Linalool (µg/L)	20	19	17	14	30	24	27	17	16	22	23	21	18	23	21
Hotrienol (µg/L)	12	4	3	3	17	10	4	1	3	2	1	2	3	6	4
α-Terpineol (µg/L)	14	12	13	12	18	21	19	8	9	10	11	10	12	13	11
TDN (µg/L)	0.4	0.4	0.5	0.5	0.3	0.3	0.3	0.3	0.3	0.4	0.4	0.4	0.6	0.4	0.4
Nerol (µg/L)	77	49	241	92	135	366	37	110	15	95	104	81	165	166	138
β-Damascenone (µg/L)	2.9	3.4	2.6	2.4	2.3	2.6	2.7	6.9	3.2	5.2	4.0	4.0	3.0	3.2	3.7
Geraniol (µg/L)	6	8	5	2	6	10	11	7	10	7	7	6	7	6	6

Appendix 6-25: Sulphur compound concentrations of the commercial wines from the Swiss variety Petite Arvine.

Wine	PA1	PA2	PA3	PA4	PA5	PA6	PA7	PA8	PA9	PA10	PA11	PA12	PA13	PA14	PA15
H_2S (µg/L)	13.7	11.4	5.8	3.5	7.2	8.2	6.1	8.0	9.4	7.7	9.1	8.2	9.5	8.0	10.9
MeSH (µg/L)	n.d.	2.9	n.d.	n.d.	n.d.	n.d.	3.3	4.8	n.d.	2.6	3.5	3.0	3.0	n.q.	n.q.
EtSH (µg/L)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d	n.d.	n.d.
DMS (µg/L)	9.1	29.3	10.1	2.7	9.6	9.4	5.9	8.7	11.0	7.0	6.3	6.8	16.8	14.7	17.1
CS_2 (µg/L)	1.2	1.2	1.1	n.q.	n.q.	n.q.	n.q.	1.3	1.2	1.1	n.q.	n.q.	1.7	1.7	n.q.
MeSAc (µg/L)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d	n.d.	n.d.
DMDS (µg/L)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d	n.d.	n.d.
EtSAc (µg/L)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d	n.d.	n.d.
DEDS (µg/L)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d	n.d.	n.d.
DMTS (µg/L)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d	n.d.	n.d.
4MSP (ng/L)	3.4	3.7	n.q.	3.2	3.8	3.6	4.9	5.6	n.q.	n.q.	n.q.	3.6	3.1	3.0	3.3
3SHA (ng/L)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d	n.d.	n.d.
3SH (ng/L)	203	507	n.q.	1431	359	723	440	1654	221	897	855	1030	n.q.	n.q.	762

Appendix 6-26: Tasting scores of the commercial wines from the Swiss variety Petite Arvine.

Wine	PA1	PA2	PA3	PA4	PA5	PA6	PA7	PA8	PA9	PA10	PA11	PA12	PA13	PA14	PA15
Passionfruit	2.04	2.83	3.22	3.69	2.40	3.23	4.16	3.07	4.07	2.68	4.12	2.83	3.22	2.55	2.80
Grapefruit	3.18	2.88	4.30	4.02	3.17	3.94	4.37	3.85	3.26	3.76	3.68	3.54	2.69	2.94	3.07
Citrus	2.48	2.67	3.91	3.01	3.68	2.58	3.66	2.36	2.79	2.58	3.17	2.32	2.84	2.21	2.67
Floral	2.99	2.86	3.01	2.38	2.89	3.31	3.87	1.96	2.89	3.06	3.76	3.31	2.97	2.86	2.25
Estery	2.04	1.24	1.42	1.38	0.98	1.54	1.09	1.49	1.90	1.42	1.30	1.12	2.69	1.60	1.33
Reduction	1.77	2.56	1.78	3.88	2.58	2.60	1.99	2.76	1.68	2.62	3.36	2.31	2.36	2.03	3.04
Minerality	2.57	2.75	3.02	3.84	3.85	3.58	4.51	4.71	3.38	4.19	4.26	3.96	3.23	4.48	4.71
Acidity	3.38	3.52	4.80	4.45	3.53	3.44	3.47	4.38	3.03	3.55	3.97	3.47	2.92	3.27	3.70
Volume	4.66	4.70	4.48	5.26	4.71	4.89	4.69	4.74	4.35	4.77	4.48	4.65	4.89	5.02	4.48
Impression	4.63	3.55	4.48	3.80	4.29	4.54	5.29	4.41	4.33	4.02	4.16	4.19	4.07	4.01	4.54

Appendix 6-27: OAV values for the 15 Petite arvine commercial wines. OAV>1 values are bold.

Wine	PA1	PA2	PA3	PA4	PA5	PA6	PA7	PA8	PA9	PA10	PA11	PA12	PA13	PA14	PA15
Linalool	0.19	0.17	0.15	0.12	0.27	0.22	0.25	0.16	0.15	0.20	0.21	0.19	0.16	0.21	0.19
Hotrienol	0.11	0.04	0.02	0.03	0.15	0.09	0.04	0.01	0.03	0.02	0.01	0.01	0.03	0.05	0.04
a-Terpineol	0.06	0.05	0.05	0.05	0.07	0.09	0.08	0.03	0.04	0.04	0.04	0.04	0.05	0.05	0.04
TDN	0.02	0.02	0.03	0.02	0.02	0.02	0.01	0.01	0.01	0.02	0.02	0.02	0.03	0.02	0.02
Nerol	0.19	0.12	0.60	0.23	0.34	0.92	0.09	0.28	0.04	0.24	0.26	0.20	0.41	0.42	0.35
β-Damascenone	**20.64**	**23.97**	**18.34**	**17.49**	**16.41**	**18.72**	**18.98**	**48.96**	**22.97**	**37.08**	**28.76**	**28.61**	**21.68**	**22.64**	**26.14**
Geraniol	0.05	0.06	0.04	0.01	0.05	0.08	0.08	0.05	0.08	0.05	0.05	0.05	0.05	0.05	0.05
i-Butanol	0.17	0.14	0.23	0.29	0.18	0.21	0.12	0.19	0.19	0.17	0.16	0.14	0.23	0.19	0.09
3-Methyl-butanol	**3.76**	**3.99**	**3.50**	**3.88**	**3.60**	**3.28**	**3.77**	**5.26**	**6.11**	**5.53**	**4.91**	**4.66**	**3.46**	**3.60**	**3.49**
Hexan-1-ol	0.15	0.16	0.08	0.08	0.12	0.15	0.13	0.09	0.19	0.09	0.10	0.06	0.11	0.12	0.09
2-Phenylethanol	**1.08**	**1.26**	**1.12**	**1.59**	**1.25**	**1.17**	**1.14**	**1.56**	**1.71**	**1.74**	**2.27**	**1.69**	**1.21**	**1.21**	**1.17**
Acetic acid ethylester	0.28	0.30	0.30	0.26	0.26	0.35	0.27	0.37	0.33	0.36	0.27	0.31	0.28	0.27	0.28
Acetic acid 3-methylbutyl ester	**72.75**	**58.11**	**60.78**	**44.72**	**76.90**	**53.92**	**126.09**	**143.91**	**155.20**	**130.57**	**112.34**	**121.90**	**63.07**	**58.92**	**84.97**
Acetic acid 2-phenylethylester	0.84	0.67	0.96	1.00	0.98	0.63	**1.60**	**1.64**	**1.37**	**1.54**	**1.66**	**1.58**	0.96	0.74	**1.12**
Butyric acid ethylester	**22.66**	**27.31**	**23.42**	**19.02**	**22.25**	**23.02**	**22.64**	**37.22**	**26.02**	**30.81**	**27.45**	**30.00**	**23.61**	**21.03**	**26.05**
Lactic acid ethylester	0.24	0.52	0.10	0.12	0.23	0.38	0.12	0.11	0.15	0.12	0.14	0.12	0.11	0.27	0.09
Succinic acid diethylester	0.01	0.01	0.00	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
H2S	**1.37**	**1.14**	0.58	0.35	0.72	0.82	0.61	0.80	0.94	0.77	0.91	0.82	0.95	0.80	**1.09**
MeSH	0.00	0.58	0.00	0.00	0.00	0.00	0.66	0.96	0.00	0.52	0.70	0.60	0.60	0.00	0.00
DMS	0.91	**2.93**	**1.01**	0.27	0.96	0.94	0.59	0.87	**1.10**	0.70	0.63	0.68	**1.68**	**1.47**	**1.71**
4MSP	**1.12**	**1.23**	0.00	**1.06**	**1.25**	**1.20**	**1.63**	**1.86**	0.00	0.00	0.00	**1.21**	**1.04**	0.99	**1.10**
3SH	**3.39**	**8.45**	0.00	**23.84**	**5.98**	**12.05**	**7.33**	**27.56**	**3.68**	**14.95**	**14.25**	**17.17**	0.00	0.00	**12.70**

Appendix 6-28: Odour threholds of the compounds studied.

Compound	Descriptor	Odour Threshold	Reference
Acetic acid ethylester	Solvent, pineapple	160 mg/L	Ribéreau-Gayon *et al.* (1999)
Acetic acid 3-methylbutylester	Banana	30 µg/L	Guth (1997b)
Acetc acid 2-phenylethylester	Rose, honey, tobacco	250 µg/L	Guth (1997b)
Butyric acid ethylester	Sweet caramel	20 µg/L	Ferreira *et al.* (2000); Fretz *et al.* (2005a)
Lactic acid ethylester	Butter, Sour milk	155 mg/L	Etiévant, P.X. (1991); Ribéreau-Gayon *et al.* (1999)
Succinic acid diethylester	Faint pleasant odour	200 mg/L	Clarke & Bakker (2004)
i-Butanol	Wine, solvent, bitter	150 mg/L	Etiévant, P.X. (1991)
3-methyl-butanol	Whiskey, malt, burnt	30 mg/L	Guth (1997b)
Hexan-1-ol	Resin, flower, green	8 mg/L	Ferreira *et al.* (2000)
2-phenylethanol	Honey, spice, rose, lilac	14 mg/L	Ferreira *et al.* (2000)
Linalool	Rose	25 µg/L	Ferreira *et al.* (2000)
Hotrienol	Linden	110 µg/L	Ribéreau-Gayon *et al.* (1999)
α-Terpineol	Lilly of the valley	250 µg/L	Ferreira *et al.* (2000)
TDN	Petroleum	20 µg/L	Ribéreau-Gayon *et al.* (1999)
Nerol	Rose	400 µg/L	Ribéreau-Gayon *et al.* (1999)
β-Damascenone	Flower, Tropical fruit, steewed apple	0.14 µg/L	Pineau *et al.* (2007)
Geraniol	Rose	130 µg/L	Ribéreau-Gayon *et al.* (1999)
H_2S	Rotten eggs	10 µg/L	Dittrich & Grossmann (2011), *Pers. Comm*. Rauhut (2014)
MeSH	Rotten Eggs	5 µg/L	Solomon *et al.* (2010)
DMS	Cooked cabbage, asparagus	25 µg/L	Goniak & Noble (1987)
4MSP	Box-tree, passionfruit	3 ng/L	Darriet *et al*. (1995); Dubourdieu & Tominanga (2009)
3SHA	Cassis, box-tree	4.2 ng/L	Dubourdieu & Tominanga (2009)
3SH	Passionfruit, Grapefruit	60 ng/L	Dubourdieu & Tominanga (2009)

Appendix 6-29: The origin of the 18 Malagousia wines of the 2013 vintage that were used, marked with red dots (Adapted from Wikipedia).

7. Eidesstattliche Erklärung

Ich erkläre: Ich habe die vorgelegte Dissertation selbständig, ohne unerlaubte fremde Hilfe und nur mit den Hilfen angefertigt, die ich in der Dissertation angegeben habe. Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht. Bei den von mir durchgeführten und in der Dissertation erwähnten Untersuchungen habe ich die Grundsätze guter wissenschaftlicher Praxis, wie sie in der „Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis“ niedergelegt sind, eingehalten.

Geisenheim, den 29. Dezember 2014 Unterschrift: ……