Authentication of Sauvignon blanc wine in terms of added flavourings

by

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Thesis presented in partial fulfilment of the requirements for the degree of
Master of Science

at
Stellenbosch University
Institute for Winebiotechnology, Faculty of AgriSciences

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March 2011

Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the owner of the copyright thereof (unless to the extent explicitly otherwise stated) and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: 15 December 2010

Summary

The varietal character of Sauvignon blanc wine is mostly defined by the balance between tropical and green vegetative flavour nuances. Grape derived methoxypyrazines are the main aroma contributors towards green vegetative flavours. Methoxypyrazines are heat and light sensitive. Due to warm climatic conditions in South Africa, methoxypyrazine levels decrease during grape ripening.

The addition of food flavourings to Sauvignon blanc wine, a practice known as spiking, has occurred in the past to improve the green character of the wines. Adding flavourings to wine and selling the wine as natural certified wine is illegal in South Africa. Currently, adulterated Sauvignon blanc wines are identified using gas chromatography–mass spectrometry (GC-MS) and liquid chromatography–mass spectrometry (LC-MS) methods to quantify methoxypyrazines and compare levels to an established database. Although of high sensitivity, GC-MS and LC-MS methods are costly and time consuming, therefore not optimal for routine screening of wines. Hence the need for the development of a fast and cost effective method for routine screening of large amounts of wines to identify adulteration.

Small scale vinification practices were used to prepare experimental Sauvignon blanc wine. Flavourings were added to Sauvignon blanc grape juice before fermentation, during the preparation of experimental spiked wines. Control wines, containing no flavouring, were also prepared. Commercial wines were spiked after fermentation and bottling. Each wine was only spiked with a single flavouring. The flavourings added were the juice of homogenised fresh green peppers and commercially available flavourings for wine. The following commercial flavourings were used: green pepper, asparagus, grassy and tropical.

The above mentioned wines were analyzed using Fourier transform infrared (FT-IR) spectroscopy, GC-MS, LC-MS and descriptive sensory analysis. The FT-IR techniques used were Fourier transform mid infrared (FT-MIR) transmission, FT-MIR attenuated reflection and Fourier transform near infrared (FT-NIR) reflection spectroscopy. The data was interpreted using the following multivariate statistical techniques: principal component analysis (PCA), partial least squares discrimination (PLS-D) and conformity testing.

Multivariate models constructed from FT-MIR and FT-NIR data were able to discriminate between spiked and control wines. Sensory analysis results clearly showed differences between non-spiked wines and spiked wines with 3-isobutyl-2-methoxypyrazine concentrations 10 times higher than naturally occurring in wine. Differences between control and spiked wines with concentrations of 3-isobutyl-2-methoxypyrazine similar to concentrations naturally occurring in wines could not be detected to prove adulteration conducting sensory analysis. However, differences between control and spiked wines with levels of 3-isobutyl-2-methoxypyrazine similar to levels naturally occurring in wines could be detected using FT-IR data in conjunction with multivariate statistics.

This study showed that, FT-IR spectroscopy in conjunction with multivariate statistical methods can be a possibility for the screening and identification of wines suspected of adulteration in terms of added flavourings. Descriptive sensory analysis also proved to be a potentially useful tool. However screening and training of potential panel members are time consuming.

Opsomming

Die variëteitskarakter van Sauvignon blanc wyn word grotendeels gedefinieer deur die balans tussen tropiese en groen vegetatiewe aromas. Metoksipirasiene is die hoof aroma verbindings wat verantwoordelik is vir groen vegetatiewe aromas. Metoksipirasien is hitte- en ligsensitief. Warm klimaatsomstandighede in Suid-Afrika het tot gevolg dat metoksipirasien konsentrasies daal tydens druif rypwording.

Sauvignon blanc wyne is in die verlede vervals deur middel van die byvoeging van vars groen soetrissies om die groen vegetatiewe karaktereienskappe van die wyne te bevorder. Die byvoeging van geurmiddels of plantekstrakte by wyn en verkoop van daardie wyn as gesertifiseerde natuurlike wyn is onwettig in Suid-Afrika. Tans word vervalsde wyne met behulp van gaschromatografie-massaspektrometrie (GC-MS) en vloeistofchromatografie-massa-spektrometrie (LC-MS) opgespoor. Kwantifisering van metoksiepirasien konsentrasies in wyne en druiwesappe word vergelyk met konsentrasies in ‘n bestaande databasis. Alhoewel GC-MS en LC-MS hoë sensitiwiteitsmetodes is, is dit duur en tydrowende metodes, dus nie optimaal vir roetine sifting nie. Dus word ‘n koste- en tydseffektiewe roetine metode benodig om vervalsing van wyne op te spoor.

Eksperimentele wyne is op klein skaal berei. Geurmiddels is voor fermentasie by die druiwesap gevoeg. Kontrole wyne waarby geen geurmiddels gevoeg is nie, is ook berei. Kommersiële wyne is gegeur na fermentasie en bottelering. Elke wyn is met ‘n enkele geurmiddel gegeur. Gehomogeniseerde vars groen soetrissie asook kommersieel beskikbare geursels vir wyn is gebruik. Die volgende kommersiële geursels is gebruik: groen soetrissie, aspersie, gras en tropiese geursel.

Die volgende analitiese tegnieke is gebruik vir analisering van bogenoemde wyne: Fourier transformasie infrarooi (FT-IR) spektroskopie, GC-MS, LC-MS en beskrywende sensoriese analise. Die spesifieke FT-IR tegnieke wat gebruik is, is: Fourier transformasie mid-infrarooi (FT-MIR) transmissie, FT-MIR verswakte weerskaatsing en Fourier transformasie naby-infrarooi (FT-NIR) reflektansie. Die volgende multiveranderlike statistiese tegnieke is gebruik ter interpretasie van data: hoof komponent analise (PCA), parsiële kleinste kwadraat diskriminant analise (PLS-D) en gelykvormigheidstoetsing.

Multiveranderlike modelle, bereken met behulp van FT-MIR en FT-NIR data, kon diskrimineer tussen gegeurde en kontrole wyne. Resultate wat verkry is tydens sensoriese analises het duidelike verskille uitgewys tussen gegeurde en kontrole wyne met betrekking tot 3-isobutiel-2-metoksipirasien konsetrasies waar 3-isobutiel-2-metoksipirasien konsentrasies 10 keer hoër was as wat natuurlik voorkom in wyn. Geen beduidende verskille kon waargeneem word in gevalle waar wyne vervals is met laer konsentrasies van geurmiddels deur sensoriese data te ontleed nie. Nietemin, statisitiese verskille tussen kontrole en vervalsde wyne kon waargeneem word vir lae-konsentrasie-geurmiddel vervalsde wyne deur FT-IR data met behulp van multiveranderlike statisitiese metodes te ontleed.

Hierdie studie het gewys dat FT-IR in kombinasie met multiveranderlike statistiese tegnieke spesifiek hoof komponent analise (PCA) en parsiële kleinste kwadraat diskriminant analise (PLS-D) asook gelykvormigheidstoetsing bruikbare tegnieke is om te onderskei tussen kontrole (egte natuurlike) en vervalsde wyne ten opsigte van die byvoeging van geurmiddels. Beskrywende sensoriese analise kan ook nuttig gebruik word, alhoewel keuring en opleiding van paneellede tydrowend is.

This thesis is dedicated to my family
and the reader

Hierdie tesis is opgedra aan my familie
en die leser

Biographical sketch

Jeanne Treurnicht was born in Pretoria, South Africa on 9 June 1980. She attended Stellenbosch Primary. After her parents moved to the Free State to farm and attending several schools in the area, she matriculated at Hopetown High in 1998. She obtained her BSc-degree at the University of Stellenbosch in 2003, majoring in Chemistry. She enrolled at the Institute for Wine Biotechnology in 2004 and obtained her HonsBSc-degree in Wine Bitoechnology in December 2004. Currently she is employed at Graham Beck Wines as quality coordinator.

Acknowledgements

I wish to express my sincere gratitude and appreciation to the following persons and institutions:

DR HH NIEUWOUDT, Institute for Wine Biotechnology, Department of Viticulture and Oenology, University of Stellenbosch, for guidance, encouragement and sharing her knowledge as well as invaluable suggestions.

PROF P VAN RENSBURG, Distell, Stellenbosch, for guidance and for sharing his knowledge especially with regards to winemaking.

MS MM MULLER, Department of Food Science, University of Stellenbosch, for introducing me to sensory science and guidance with the sensory analysis conducted in this study.

DR V WATTS, KWV Paarl (formerly), for his help with SPDE GC-MS analysis of wine.

PROF KH ESBENSEN, Extraordinary professor, Institute for Wine Biotechnology, Department of Viticulture and Oenology, University of Stellenbosch, for introducing me to multivariate statistical analysis and guidance with the strategies followed for this study.

DR AGJ TREDOUX, Institute for Wine Biotechnology, Department of Viticulture and Oenology, University of Stellenbosch for assisting with the logistics of GC-MS analysis of flavourings and wines.

EDMUND LAKEY, MARISSA, ANDY and **JUANITA JOUBERT** (formerly), Experimental Cellar, Department of Viticulture and Oenology, University of Stellenbosch, for their assistance in the cellar with the preparation of experimental wines.

THE NATIONAL RESEARCH FOUNDATION and **WINETECH** for financial support.

JACQUES VILJOEN, Zevenwacht Wine Estate, Stellenbosch, and **BOELA GERBER**, Groot Constantia, Cape Town for Sauvignon blanc grapes used for the preparation of experimental wines.

GARY BAUMGARTEN and **BRÜNHILDE LUYT,** Graham Beck Wines, Franschhoek, for the invaluable study leave granted to me at a very critical time to complete this study.

MY PARENTS, NICO and **MAGRIET TREURNICHT, SISTERS, PETRA** and **MIEKIE** and **BROTHER WILLEM** for encouragement, believing in me and helping me with this task.

JACQUES BRAND, my best friend, lifelong companion and husband for love and support and putting up with many evenings and weekends of neglect.

List of abbreviations and symbols

ANN	Artificial neural network
ANOVA	Analysis of variance
A3MH	3-Mercaptohexyl acetate
B	Boron
Ba	Barium
°B	Degrees Balling
Ca	Calcium
CA	Cluster analysis
CI	Conformity index
Cs	Cesium
CZE	Capillary zone electrophoresis
°C	Degrees Celsius
DA	Discriminant analysis
DFA	Discriminant function analysis
DF(E)	Degrees of freedom for SS(E)
DF(P)	Degrees of freedom for SS(P)
E	Error matrix
eg.	for example
FA	Factor analysis
FDA	Factorial discriminant analysis
Fe	Iron
FT-IR	Fourier transform infrared
FT-MIR	Fourier transform mid infrared
FT-NIR	Fourier transform near infrared
GC-MS	Gas chromatography – mass spectrometry
GC-NPD	Gas chromatography – nitrogen phosphorous detection
GC-FID	Gas chromatography – flame ionization detection
GCxGC	Gas chromatography – gas chromatography
GC-MS	Gas chromatography – mass spectrometry
HCA	Hierarchical cluster analysis
IBMP	3-Isobutyl-2-methoxypyrazine
IPMP	3-Isopropyl-2-methoxypyrazine
K	Potassium
K-ANN	Kohonen artificial neural networks
KNN	K-nearest neighbours
L	Litre
LC-MS	Liquid chromatography – mass spectrometry
LDA	Linear discriminant analysis
Li	Lithium
MIR	Mid infrared
mg/L	Milligrams per litre
Mg	Magnesium
mL	Millilitre
Mn	Manganese
MLR	Multiple linear regression
MP	Methoxypyrazine

MS	Mass spectrometry
3MH	3-Mercaptohexan-1-ol
3MMB	3-Mercapto-3-methylbutan-1-ol
4MMP	4-Methyl-4-mercaptopentan-2-one
4MMPOH	4-Mercapto-4-methylpentan-2-ol
n	Number of samples or objects
Na	Sodium
ng/L	Nanograms per litre
NIR	Near Infrared
NMR	Nuclear magnetic resonance
p	Probability used for ANOVA
p	Number of variables used for PCA
P-	Precursor of eg. P-3MH means precursor of 3MH
Pb	Lead
PC	Principal component
PCA	Principal component analysis
PCR	Principal component regression
PLS	Partial least squares regression
PLS-D	Partial least squares discrimination
$\mathbf{P}^T$	Loadings matrix transformed
RMSEC	Root mean square error of calibration
RMSEP	Root mean square error of prediction
R^2	Correlation coefficient
SAW	Surface acoustic wave
SBMP	3-*sec*-Butyl-2-methoxypyrazine
SEP	Standard error of prediction
SIMCA	Soft independent modelling of class analogy
SLDA	Step wise linear discriminant analysis
SPME	Solid phase micro extraction
Sr	Strontium
SS	Sum of squares
SS(A)	Sum of squares for assessors
SS(AP)	Sum of squares for assessor product interaction
SS(E)	Sum of squares for random error
SS(P)	Sum of squares for product
SS(T)	Total sum of squares
T	Scores matrix
TA	Titratable acidity
UV	Ultraviolet
UV-VIS	Ultraviolet-visible
W.O.	Wine of origin
X	Original data matrix
Y	Matrix containing all y values or variables
Zn	Zinc
% v/v	Percent volume per volume
%	Percent
$\propto_i$	Assessor effect for sample i
$\propto \beta_{ij}$	Assessor product interaction for product i and assessor j

β_j	Product effect for product j
e_i	Object residual for object i used in PCA
e_{ijk}	Random error for assessor i, product j and replicate k
μ	General mean
σ	Standard deviation
x_{ijk}	Sensory score for assessor i, product j and replicate k
y_i	y value measured for item i
$\hat{y}_i$	y value predicted for item i
$\hat{y}_{i,cal}$	y value predicted for item i for calibration
$\hat{y}_{i,cal\_ref}$	y reference value measured for item i for calibration
$\hat{y}_{i,val}$	y value predicted for item i for validation
$\hat{y}_{i,val\_ref}$	y reference value measured for item i for validation
$\sum$	Sum of

Preface

This thesis is presented as a compilation of 4 chapters. Each chapter is introduced separately.

Chapter 1 **General introduction and project aims**

Chapter 2 **Literature review**
Perspectives on Sauvignon blanc wine flavour characteristics and wine authentication

Chapter 3 **Research results**
Detection of Sauvignon blanc adulteration using infrared spectroscopy and chemometric techniques

Chapter 4 **General discussion and conclusions**

Contents

Chapter 1

General Introduction and Project Aims

Chapter 1: General Introduction and Project Aims

1.1 Introduction

Both New World Wine producing countries (Australia, Argentina, Chile, New Zealand, South Africa and the United States of America) and Old World wine producing countries (Italy, Spain and France amongst others) are competing for a share in the global wine market. World wine market studies report a positive trend in the value of wine export from the new world. Wine exporters increased their monetary value of exports by an average of 600% over the 1993 to 2003 period. This value increase is driven by increased quality and related price increase rather than per capita increase resulting in fierce competition (Rothfield and Wittwer 2005).

Sauvignon blanc is the second most distributed noble white wine variety in terms of hectares planted globally. Therefore it is one of the most important cultivar wines on the global wine market. Sauvignon blanc is the third largest export cultivar wine in terms of litres exported in 2008 from South Africa (SAWIS, 2008). France and New Zealand have cooler climates that are well suited for high quality Sauvignon blanc wines (Holter, 2009). South Africa's coastal region also has a cooler climate than the rest of the wine producing regions in the country making this the preferred region for Sauvignon blanc. The climate is however not as cool as that of France and New Zealand placing the South African producer at a competitive disadvantage. It therefore follows that an "easy" technique to achieve the same results as those of a cooler climate would be rather attractive to South African producers.

The quality of a Sauvignon blanc wine is highly dependent on the varietal character of the wine. The balance between tropical and vegetative flavour nuances mostly defines the varietal character of Sauvignon blanc wine. Tropical flavour compounds form during fermentation and can be influenced by the yeast strain used (Marais, 1994). The synthesis of the pre-cursor compounds that give rise to tropical flavour compounds take place in the grape berries during ripening (Allen *et al.*, 1991; Lacey *et al.*, 1991; Allen and Lacey, 1993; Marais, 1994). The synthesis of these compounds is favoured by warmer climatic conditions. The flavour compounds that give rise to the green vegetative aroma in Sauvignon blanc wines are methoxypyrazines originating from grape berries. Methoxypyrazines are also found in high concentrations in green peppers. The concentration of methoxypyrazines decreases during ripening as a result of their UV light and temperature sensitivity. Cooler climatic conditions are favourable for the preparation of wines having a green vegetative character. Due to warmer climatic conditions, South African Sauvignon blanc wines tend to have more tropical aromas than green vegetative aromas (Marais, 1994). Hence, the focus in the South African wine industry for distinguished high quality is to improve and preserve the green vegetative aroma nuances of Sauvignon blanc grapes during the winemaking process.

South African Sauvignon blanc wines have been adulterated by the addition of fresh green pepper to increase the green character of the wines due to the perception that more green tones can be associated with higher quality (Du Plessis, 2005; Marais, 2010).

Detection and authentication of Sauvignon blanc wines adulterated with methoxypyrazine-based flavourings are conducted using advanced analytical chemistry to quantify methoxypyrazine concentrations. Methoxypyrazines have a sensory threshold of 1-2 ng/l and can be found at levels between 5 – 35 ng/l in wine. Since the abundance is higher than the sensory threshold, it can be sensed by humans even though the concentration is too low to detect with most analytical instrumentation (Allen *et al.*, 1991; Lacey *et al.,* 1991; Allen and Lacey, 1993; Marais, 1994). The quantification of methoxypyrazines in wine can be done by gas

chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS). These methods involve sample preparation and are relatively costly, time consuming (Alberts *et al.*, 2009) and not fit for full screening of all wines produced to isolate possible adulteration. Flavourings used to adulterate wines that do not contain methoxypyrazines cannot be detected using GC-MS and LC-MS, since the method quantifies the amount of methoxypyrazines in the wine.

Fourier transform infrared (FT-IR) spectroscopy in conjunction with chemometrics, including principal component analysis (PCA) and partial least squares discrimination (PLS-D), is becoming a common technique used for authentication testing in food industries (Arvanitoyannis *et al.*, 1999; Roussel *et al.*, 2003; Osborne, 2007; Cozzolino *et al.*, 2009). The FOSS Winescan is a Fourier transform mid infrared (FT-MIR) spectrometer being used regularly in the wine industry for routine analyses (Nieuwoudt *et al.*, 2004; Louw *et al.,* 2009; Malherbe *et al.*, 2007; Swanepoel *et al.,* 2007). Analysis is relatively inexpensive and fast. FT-IR instruments developed by Bruker, Alpha (FT-MIR) and MPA Fourier transform near infrared (FT-NIR), also provide relatively inexpensive and fast analysis of wine samples. Sample preparation is not necessary when using a liquid probe with the MPA.

Therefore, it could be worthwhile to investigate the usage of FT-MIR and FT-NIR spectroscopy in combination with chemometrics, to screen for adulteration and identify suspect wines. Once suspect wines are identified, GC-MS analysis, the more expensive and time consuming method can be performed to confirm the adulteration of the wine.

1.2 Project Aims

The main aim of this project was to investigate the possibility of using FT-MIR and or FT-NIR to establish a system suitable for identification of adulterated Sauvignon blanc wines in terms of added flavourings. Since these instruments produce large amounts of data in terms of spectra, multivariate statistical analysis of data was proposed as analysis technique for constructing mathematical models to predict adulteration. The specific aims of the project were:

a) Constructing statistical models to predict adulteration using multivariate analysis techniques, specifically PCA and PLS-D, on FT-MIR transmission spectra obtained from spiked and control Sauvignon blanc wines using an instrument used in the wine industry for rapid analysis of wines.
b) Constructing statistical models to predict adulteration, performing conformity testing on FT-NIR reflection and FT-MIR attenuated reflection spectra of spiked and control Sauvignon blanc wines.
c) Conducting sensory analysis to investigate possible masking of the tropical flavour nuances by the addition of green vegetative flavourings.

This project was conducted as a pilot study under controlled small scale experimental cellar conditions. The control wines prepared were known not to be adulterated. In terms of authentication in the industry, control wines, known not to be adulterated will be used to establish a model against which future wines can be tested.

1.3 References

Alberts, P., Stander, M.A., Paul, S.O., De Villiers, A. (2009). Survey of 3-alkyl-2-methoxypyrazines content of South African Sauvignon blanc wines using a novel LC-APC1-MS/MS method. *J. Agric. Food Chem.* **57**, 9347-9355

Allen, M.S., Lacey, M.J., Harris, R.L.N., Brown, W.B. (1991). Contribution of methoxypyrazines to Sauvignon blanc wine aroma. *Am. J. Enol. Vitic.* **42**, 109-112

Allen, M.S., Lacey, M.J. (1993). Methoxypyrazine grape flavour: Influence of climate, cultivar and viticulture. *Vitic. Enol. Sci.* **48**, 211-213

Arvanitoyannis, I.S., Katsota, M.N., Psarra, E.P., Soufleros, E.H., Kallithraka, S. (1999). Application of quality control methods for assessing wine authenticity: Use of multivariate analysis (Chemometrics). *Trends Food Sci. Technol.* **10**, 321-336

Cozzolino, D., Holdstock, M., Dambergs, R.G., Cynkar, W.U., Smith, P.A. (2009). Mid infrared spectroscopy and multivariate analysis: A tool to discriminate between organic and non-organic wines grown in Australia. *Food Chem.* **116**, 761–765

Du Plessis, C. (2005). Sauvignon blanc – the dust hasn't settled. *Winelands* [URL. http://www.wineland.co.za/0204suvignon.php3]. Cited the 26th of August 2008

Holter, G. (2009). The dividing wine. *Meininger's WBI* **3**, 14-16

Lacey, M.J., Allen, M.S., Harris, R.L.N., Brown, W.V. (1991). Methoxypyrazines in Sauvignon blanc grapes and wine. *Am. J. Enol. Vitic.* **42**, 103-108

Louw, L., Roux, K., Tredoux, A., Oliver, T., Naes, T., Nieuwoudt, H., Van Rensburg, P. (2009). Characterization of selected South African young cultivar wines using FTMIR spectroscopy, gas chromatography, and multivariate data analysis. *J. Agric. Food Chem*. **57**, 2623-2632

Marais, J. (1994). Sauvignon blanc cultivar aroma – a review. *S. Afr. J. Enol. Vitic.* **15**, 41-45

Marais, J. (2010). Introduction to publications from the investigation into the aroma databases of different South African Cultivars. *Winelands* [URL. http://www.wineland.co.za/index.php?option=com_zine &view=article&id=81:introduction-to-publications-from-the-investigation-into-the-aroma-databases-of-different-south-african-cultivars&q=sauvignon]. Cited the 3rd of December 2010

Malherbe, S., Bauer, F.F., Du Toit, M. (2007). Understanding problem fermentations - A review. *S. Afr. J. Enol. Vitic.* **28**, 169-186.

Nieuwoudt, H.H., Prior, B.A., Pretorius, I.S., Manley, M., Bauer, F.F. (2004). Principal component analysis applied to Fourier Transform Infrared Spectroscopy for the design of calibration sets for glycerol prediction models in wine and for the detection and classification of outlier samples. *J. Agric. Food Chem.* **52**, 3726-3735

Osborne, C.D. (2007). Discriminating wine yeast strain and their fermented wines: an integrated approach., M.Sc(Wine Biotechnology), Stellenbosch University

Rothfield, J., Wittwer, G. (2005). Projecting the world wine market from 2003 – 2010. *Australasian Agribusiness Review*, **13** paper 21

Roussel, S., Bellon-Maurel, V., Roger, J.M., Grenier, P. (2003). Authenticating white grape must variety with classification models based on aroma sensors, FT-IR and UV spectrometry, *Chemom. Intell. Lab. Syst.* **65**, 209-219

SAWIS. (2008). South African wine industry information and systems. [URL. http://www.sawis.co.za/info/statistics.php]. Cited the 10th of December 2009

Swanepoel, M., Du Toit M., Nieuwoudt, H.H. (2007). Optimisation of the quantification of total soluble solids, pH and titrable acidity in South African must using Fourier transform mid-infrared spectroscopy. *S. Afr. J. Enol. Vitic.* **28**, 140-149

Chapter 2

Literature review

Perspectives on Sauvignon blanc wine flavour characteristics and wine authentication

Chapter 2: Literature Review

2.1 Introduction

Flavour evaluation is one of the most important assessments in the wine industry. All aroma nuances that contribute to the flavour of a wine, together with the basic tastes, sweet, sour, bitter and salty, as well as sensory perceptions such as astringency and mouthfeel, influence consumers' degree of product liking and preference. The minimum concentration at which a chemical compound is detected by the senses is called the sensory threshold and in the case of aromas, odour thresholds (Jackson, 2002; Meilgaard ***et al.***, 2007). The odour threshold values of the different aroma compounds show large variation and can influence the wine style (Guth, 1997; Francis and Newton, 2005). In other words, if a compound is present in a wine above its odour threshold it will most probably contribute to the aroma, except if its flavour is masked by other wine compounds. Therefore, some impact compounds can have an indisputable contribution to the flavour of a wine, even when present at a very low concentration. Other compounds will not contribute at all even though present at higher concentrations. The varietal character of a wine can be described as combinations of flavours typically associated with the specific cultivar. Sauvignon blanc wine has unique varietal characters, ranging from distinctive green to distinctive tropical aromas (Marais, 1998; Swiegers ***et al.,*** 2006). The delicate balance between these typical Sauvignon blanc flavour nuances can determine the class, style, popularity and pricing of this genre. Unusual intense aroma perceptions or ratios and amounts of certain impact compounds in Sauvignon blanc, can indicate the addition of flavourings to the wine, also referred to as adulteration, in an attempt to achieve better aroma complexity and to meet consumer expectations and perceptions of quality wine.

Food and wine adulteration in terms of adding substances illegally and classifying wines falsely in terms of origin is not a current issue. Early adulteration practices have been reported in trades since the existence of the Roman Empire. Adulteration is the illegal addition of a substance to wine, for example illegal addition of sugar, alcohol, glycerol and flavourings. Adulteration in terms of varietal, origin and vintage can be conducted not adhering to legal requirements in terms of the amount of wine in the blend not prepared from grape from the specific variety, origin or vintage (Schlesier ***et al.***, 2009).

Information on geographical origin, variety, vintage and chemical additions in terms of authenticity became increasingly important due to the more competitive global wine market as well as higher consumer demands in general lately. The demand for such information to be verified or determined with scientific methods also partly originated from higher demands set by regulatory bodies. In this context discrimination of wines according to geographical origin and varietal or cultivar has been the two most researched fields.

Chemical analytical techniques including spectroscopy, chromatography mostly coupled with mass spectrometric detection, nuclear magnetic resonance and isotope analysis amongst others have been developed (Sun, 2008). Authentication strategies can be targeted for detection of a specific marker compound or alternatively, for broad non-targeted comparison of metabolic profiles of samples. Consequently, large amounts of data have to be interpreted. Multivariate data analysis techniques provided a platform from which to organise data and extract important information related to wine authentication, without compromising variability within data of this nature. The combination of modern analytical techniques and multivariate statistics is a powerful tool that is used extensively to discriminate between authentic and adulterated products, as will be illustrated in the following sections.

2.2 Sauvignon blanc wine

Sauvignon blanc wine is one of the world's most popular white wine cultivars. Sauvignon blanc is classified as a noble white wine variety (Holter, 2009). Sauvignon blanc wines of renowned quality are produced in cooler climate regions, including areas in France, New Zealand and the coastal region of South Africa.

2.2.1 Production statistics

Sauvignon blanc wines play a tremendously important role in the economy of the South African wine industry, both on the local and export markets. South Africa produces 3.6% of the global wine production. France produces the most, followed by Italy, Spain, USA, Argentina, Australia and then South Africa (SAWIS, 2008; SAWIS, 2010).

In 2008, 31% (representing 2 838 ha) of Sauvignon blanc cultivated in South Africa was cultivated in the coastal region and 69% (6 317 ha) in the warmer regions. During the same period, a total of 78 741 tons Sauvignon blanc grapes, which represented 5.5% of all varieties, were harvested, consisting of 20.9% in the coastal region and 79.1% in the warmer regions such as the Robertson and Olifants River region. In the coastal region, Sauvignon blanc represented 43% of white varietals and 16% of all varietals cultivated. In terms of grape volumes crushed in 2008, Sauvignon blanc represented 40% of all white varietals and 13.6% of all varietals (red and white) crushed. Of the total area under vines in South Africa, Sauvignon blanc represented 6.1% in 2001, 8.2% in 2008 and 9% in 2009.

On the export market Sauvignon blanc was sold for an average of 593 cents per litre in 2010, leading in terms of price. The average price for South African Sauvignon blanc bulk wine sold on the domestic market in 2008 was 519 cents per litre and 539 cents per litre in 2010, being the highest for white wine in 2008 and 2010, and second highest for all wines in 2008. The price for Pinot noir was the highest of all wine at 523 cents per litre in 2008. In 2010 Shiraz, Cabernet Sauvignon and Merlot were sold for higher prices. Average prices, in cents per litre, for some other white varieties sold as bulk wine were Chardonnay, 482 in 2008, 530 in 2010, Semillon, 366 in 2008, 409 in 2010, Chenin blanc, 321 in 2008, 367 in 2010, Cape Riesling, 326 in 2008 and 360 in 2010.

In terms of prices for grapes delivered to wholesalers by members, Sauvignon blanc grapes cost wholesalers R 2 566 per ton on average in 2008 and R 2 569 in 2009. All other varieties were less expensive with Chardonnay at R 2 489 in 2008, R 2 396 in 2009 and Pinot Noir at R 2 291 in 2008, R 2 649 in 2009 and the other white cultivars were below R 2 000 per ton. Sauvignon blanc grapes sold by members to other than wholesalers retailed at an average price of R 4 668 per ton and Chardonnay at R 3 739 per ton in 2008. During 2003 4 474 621 L Sauvignon blanc and 36 331 833 L natural wine were sold in 750 mL glass bottles and 8 756 066 L Sauvignon blanc and 41 459 124 L natural wine in 2008. Both bulk and packaged wines were exported. During 2008, 15 744 622 L of Sauvignon blanc were exported as packaged wine and 4 157 748 L as bulk wine. These volumes were exceeded by those of Chenin blanc, Chardonnay, Cabernet Sauvignon and Shiraz. Chenin blanc took the lead in export quantities at 18 557 705 L packaged and 24 540 187 L exported as bulk wine (SAWIS, 2008; SAWIS, 2010).

2.2.2 Varietal Character

Sensory and chemical analyses of volatile compounds in wine are employed to better understand the contribution of specific chemical compounds to the aroma and varietal character of wines (Francis and Newton, 2005; Vilanova and Sieiro, 2006; Hernández-Orte *et al*., 2008). These compounds can originate from the grapes as free volatile compounds and stay unaltered during fermentation, as is the case with methoxypyrazines (Marais 1994; Swiegers *et al.,* 2006). Other flavour compounds occur as flavourless glycoconjugates in grape juice and can be liberated by mild chemical or enzymatic hydrolysis. During vinification, mild acid hydrolysis and/or enzymatic hydrolysis takes place that results in cleaving of these chemical bonds and liberation of flavour compounds such as monoterpenes (Sefton *et al*., 1994). During yeast-mediated alcoholic fermentation flavour compounds such as volatile thiols, higher alcohols and esters are formed that contribute to the aroma character of wine (Murat *et al*., 2001; Hernández-Orte *et al.*, 2008).

The characteristic aroma nuances of Sauvignon blanc wine can be classed in two broad categories, namely green and tropical. Sauvignon blanc wines from cooler regions have a more distinctive green vegetative characteristics, whereas those produced in the warmer regions of South Africa tend to have a stronger tropical and fruity character (Marais, 1994; Marais 1998). Warmer climatic conditions are favourable for the formation of the pre-cursor compounds that give rise to tropical flavour compounds such as thiols, but facilitate the breakdown of flavour compounds responsible for green flavour aroma nuances, such as the methoxypyrazines. Therefore making cooler climatic conditions favourable for the production of Sauvignon blanc wines with green, vegetative and herbaceous aroma characteristics.

The correlation between the sensory perception of more intense green aromas and higher concentrations of methoxypyrazines was verified in a study of New Zealand Sauvignon blanc wines (Parr *et al.,* 2007). This study also showed that the perceptions of “ripe” associated with tropical aromas and “green” were mutually exclusive and that “ripe” was negatively correlated with high levels of methoxypyrazines. The quality ratings of the wine showed that there was a strong positive correlation between high scores for “high quality” and high intensities of green flavours. The latter in turn, was again positively correlated with the higher concentrations of methoxypyrazines found in the wines tested in this study.

2.2.2.1 Vegetative flavours

Vegetative aroma nuances such as green pepper, asparagus and grass are used to describe the green flavours. Methoxypyrazines are mainly responsible for these flavours (Augustyn *et al*., 1982; Allen *et al*., 1991; Marais, 1994, Howell *et al.,* 2004; Swiegers *et al.,* 2006).

Methoxypyrazines are organic, nitrogen containing, aromatic ring structure compounds formed as secondary products during amino acid catabolism in grape berry development (Cheng *et al*, 1991; Marais, 1994). The exact pathway of analysis is not known, although valine, glycine and methionine are considered to be the precursors (Dunlevy *et al*., 2010). The most important methoxypyrazines from the perspective of having an impact on aroma found in Sauvignon blanc are 3-isobutyl-2-methoxypyrazine (IBMP), 3-*sec*-butyl-2-methoxypyrazine (SBMP), 3-isopropyl-2-methoxypyrazine (IPMP). Although all three methoxypyrazines mentioned contribute to the vegetative aroma, they differ slightly with regards to the exact aromas they give rise to. It was shown that IBMP is associated with green pepper like aromas and IPMP with asparagus flavours (Lacey *et al.*, 1991; Swiegers *et al.,* 2006).

The average concentration of IBMP was found to be higher than those for IPMP and SBMP in Sauvignon blanc wine (Table 2.1). Therefore IBMP is believed to be the main contributor to green aromas (Lacey *et al*., 1991; Marais, 1994). The sensory threshold of these compounds in water as well as in wine is 1-2 ng/L. Therefore, only trace amounts have an impact on the perceived flavour of a wine (Allen *et al.,* 1991). Other pyrazines that occur in Sauvignon blanc, 3-methoxy-2-ethylpyrazine and 2–methoxy-3-methylpyrazine have also been studied (Augustyn *et al.,* 1982; Lacey *et al*., 1991). The odour thresholds of these compounds are much higher than their levels of abundance in wine. It was concluded that these compounds individually do not have an effect on Sauvignon blanc wine aroma (Lacey *et al*., 1991). However, in combination it is possible that they might have an impact on the aroma of Sauvignon blanc wines.

Higher alcohols such as C_6-alcohols (*n*-hexanol, *cis*-3-hexenol and *trans*-2-hexenol) together with C_6-aldehydes (*n*-hexenal, *cis*-3-hexenal, *trans*-2-hexenal) are also known to contribute to grassy, herbaceous and leafy aromas in wines (Augustyn *et al.*, 1982). These compounds have odour thresholds in the range of mg/L and occur in grape juice of all grape cultivars. They are formed as a result the oxidation of linoleic acid during grape crushing and resultant breaking of cell walls (Marais, 1994). Aldehydes are reduced to alcohols during alcoholic fermentation. Furthermore aldehydes bind to sulphur dioxide that is frequently used as preservative during winemaking, and occur as bisulphite compounds that do not contribute to wine aroma (Augustyn *et al*., 1982; Marais, 1994). For these reasons, it is believed that these aldehydes do not contribute to the distinctive green flavours of Sauvignon blanc wine (Marais, 1994).

A study conducted by Francis *et al*. (1992) demonstrated that compounds liberated from glycoconjugates did not contribute to the green aromas such as green pepper and asparagus of Sauvignon blanc wines. Wine aroma was enhanced with enzyme released hydrolysates in terms of floral, lime and grassy nuances. The floral nuances were suggested to be caused by the liberation of monoterpenes. Hydrolysates released by acid hydrolysis enhanced floral, lime, pineapple, honey, oaky and tea-like aromas, where honey and tea-like aromas are caused by C_{13}-norisoprenoids and oaky aromas by phenolic compounds (Marais, 1994).

2.2.2.2 Tropical and fruity flavours

Tropical and fruity aromas are believed to be caused by monoterpenes, norisoprenoids, esters and higher alcohols (Marais, 1998). Typical tropical flavour nuances associated with Sauvignon blanc wines are passion fruit (granadilla), pineapple and guava. The importance of the contribution of volatile thiols to aroma nuances such as passion fruit and citrus has been shown (Darriet *et al.,* 1993; Darriet *et al.,* 1995; Tominaga *et al.,* 1998a; Murat *et al*., 2001; Swiegers *et al*., 2006; King *et al.*, 2008).

Citrus fruity nuances described as citrus zest and grapefruit, as well as tropical nuances described as passion fruit and guava, are commonly reported in the sensory evaluation of Sauvignon blanc wine. The volatile thiols 4-methyl-4-mercaptopentan-2-one (4MMP), 4-mercapto-4-methylpentan-2-ol (4MMPOH), 3-mercaptohexyl acetate (A3MH) and 3-mercaptohexan-1-ol (3MH) are formed during yeast-mediated alcoholic fermentation and have been shown to be responsible for guava, citrus zest, grapefruit and passion fruit nuances (Tominaga *et al*., 1998a; Swiegers *et al.*, 2006; King *et al.*, 2008). However in another study conducted by Tominaga *et al.* (1998b) it has been shown that 4MMPOH occurred below its odour threshold in Sauvignon blanc wines and therefore had no impact on the flavour of the wines. For this particular study wines from France for vintages from 1992 – 1996 were analysed and this conclusion is not necessarily representative for all Sauvignon blanc wines and should

be investigated in further research. The thiols A3MH, 3MH as well as the precursor for 3MH are also found in passion fruit juice (Engel and Tressl, 1991; Tominaga *et al.*, 2000b).

Volatile thiols form during alcoholic fermentation from cysteinylated precursors in grape must due to the action of *Saccharomyces cerevisiae* yeast (Murat *et al.,* 2001). The formation of A3MH is mediated by a yeast ester-forming alcohol acetyltransferase converting 3MH to A3MH (Swiegers *et al*., 2005). The ability of different yeast strains to release 4MMP and 3MH from their precursors as well as the ability to convert 3MH to A3MH differs (Dubourdieu *et al.,* 2006, Swiegers *et al*., 2006, King *et al.,* 2008). Sauvignon blanc also have positive varietal aromas such as tomato leaf, box tree, floral and smoky (Augustyn *et al.,* 1982; Darriet *et al*., 1993). Smoky and cooked leak aromas can be negative if the nuances are overwhelming. The box tree flavour is associated with 4-methyl-4-mercaptopentan-2-one (4MMP) and 3-mercaptohexyl acetate (A3MH) (Darriet *et al.*, 1993; Tominaga *et al*., 1998a; Tominaga *et al*., 2000a). Cooked leek aroma nuances are present in some Sauvignon blanc wines. The chemical compound responsible for the cooked leak flavour is 3-mercapto-3-methylbutan-1-ol (3MMB). It was concluded by Tominaga *et al*., (1998b) that 3MMB mostly occurred in Sauvignon blanc wine below its odour threshold. The specific correlations between thiols and their olfactory description with regards to Sauvignon blanc wine is summarized in Table 2.1.

Table 2.1 Flavour compounds associated with Sauvignon blanc varietal character.

Flavour compound Chemical name	Precursor in grape juice	Olfactory description	Concentration range in wine (ng/L)	Odour threshold (ng/L)	Reference
3-isobutyl-2-methoxypyrazine (IBMP)	Already present	Green pepper	0-40	1-2[a,b]	Allen *et al.* (1991) Lacey *et al.* (1991)
3-isopropyl-2-methoxypyrazine (IPMP)	Already present	Asparagus	0-5	1-2[a,b]	Allen *et al.* (1991) Lacey *et al.* (1991)
3-*sec*-butyl-2-methoxypyrazine (SBMP)	Already present	Grassy	0-3	1-2[a,b]	Lacey *et al.* (1991) Alberts *et al.* (2009)
3-mercaptohexan-1-ol (3MH)	S-3-(hexan-1-ol)-L-cysteine (P-3MH)	Passion fruit Grape fruit	700-8400	17[a] 60[c]	Tominaga *et al*. (1998a,b,c) Peyrot des Gachons *et al.* (2002b)
4-mercapto-4-methylpentan-2-ol (4MMPOH)	S-4-(4-methylpentan-2-one)-L-cysteine (P-4MMPOH)	Grape fruit Citrus zest	Mostly<55	20[a] 55[c]	Tominaga *et al*. (1998a,b,c)
4-methyl-4-mercaptopentan-2-one (4MMP)	S-4-(4-methylpentan-2-ol)-L-cysteine (P-4MMP)	Box tree Broom Guava Cat urine	0-40	0.1[a] 0.8[c]	Tominaga *et al*. (1998a,b,c) Tominaga *et al*. (2000a) Mestres *et al.* (2000) Schneider *et al.* (2003)
3-mercaptohexyl acetate (A3MH)		Box tree Passion fruit	200-800	2.3[a] 4.2[c]	Tominaga *et al*. (1998a,b,c) Tominaga *et al*. (2000a) Darriet *et al.* (1995) Mestres *et al.* (2000)
3-mercapto-3-methylbutan-1-ol (3MMB)		Cooked leeks	<1500	1300[a] 1500[c]	Tominaga *et al*. (1998a,b,c)

Odour threshold in: [a]water, [b]wine, [c]aqueous alcohol solution (12% v/v)

2.2.3 Factors infuencing varietal character

The belief that tropical and fruity flavours should be well balanced with green flavours is the main driving forces behind the improvement of Sauvignon blanc varietal character. South African Sauvignon blanc wines tend to have more tropical flavour notes than green flavour nuances due to the warmer climate. Thus the focus in terms of varietal improvement in South Africa is to improve the green flavours or take special care that green flavours are preserved during ripening, harvest and winemaking procedures (Marais, 1998).

Methoxypyrazines, responsible for the green aroma nuances in Sauvignon blanc wine, are found as free volatile compounds in grape juice. Methoxypyrazines break down at high temperatures (Lacey *et al.,* 1991*)* and are also light sensitive (Heymann *et al.,* 1986). Therefore, Sauvignon blanc varietal character is dependent on temperature and light exposure of the grape berries during ripening. This result in the decrease of methoxypyrazines during ripening as well as lower levels for wines produced during warmer vintages than cooler vintages (Lacey *et al.*, 1991; Allen and Lacey, 1993). New Zealand and France have cooler climates than South Africa and Australia. This explains the fact that Sauvignon blanc wines from New Zealand and France contain higher levels of methoxypyrazines than wines from South Africa and Australia. For French wines the methoxypyrazine levels vary from 5 to 40 ng/L and for New Zealand from 10 to 35 ng/L (Alllen *et al.,* 1991). In the case of South African wines the levels vary from 1 to 14 ng/L and for Australia from 2 to 15 ng/L (Alllen *et al.,* 1991; Marais, 1994; Marais, 1998). Cooler regions in South Africa produced wines containing higher levels of methoxypyrazines than warmer regions. Marais (1998) showed that Sauvignon blanc wines from Elgin had higher concentrations of methoxypyrazines at 8 – 14 ng/L than wines from Stellenbosch at 2 – 4ng/L as a result of Stellenbosch having a warmer climate than Elgin.

Monoterpene and C_{13}-norisoprenoid concentrations generally increase during berry ripening. Therefore, harvesting grapes earlier will result in unbalanced aroma nuances in wines as well as low sugar content. It has been shown by Peyrot des Gachons *et al.* (2005) that the water and nitrogen content in the soil during ripening have effects on the formation of the precursors that give rise to 4MMP and 3MH. Therefore water and nitrogen of the soil if not managed carefully can lead to the production of wines with lower thiol concentrations and less tropical aromas. However, specific viticultural practices can be applied to maximize the formation of thiol precursors and minimize the loss of methoxypyrazines during ripening while allowing grape berries to ripen with regards to sugars and other aroma compounds.

2.2.3.1 Viticultural practices

Viticultural practices such as canopy management are frequently applied to manage UV exposure and to facilitate cooler ripening temperatures of grape berries. Therefore, the loss of methoxypyrazines is minimised. Allen and Lacey (1993) showed that different pruning techniques resulted in significantly different levels of IBMP in Sauvignon blanc wines. Cultivating Sauvignon blanc grape in cooler areas or against cooler slopes in warmer areas have been suggested by Marais (1994).

Severe water stress and nitrogen deficiency have been shown to lead to lower production of 4MMP and 3MH in Sauvignon blanc wines by Peyrot des Gachons *et al.,* (2005). However, moderate water stress lead to enhanced production of 4MMP and 3MH. Therefore nitrogen content in the soil should be tested and adjusted to ensure that enough nitrogen is available to the vine during ripening. Water content should only be adjusted if the vine suffers severe water stress. These practices should be applied to ensure the adequate syntheses of the precursors

of 4MMP and 3MH in the grape berries in order to produce wines with full tropical flavours (Swiegers *et al*., 2006).

2.2.3.2 Harvesting

Early morning, night and pre-dawn harvesting, as well as storing of grapes in dark cooled containers, have been shown to keep grapes cool during harvest and post-harvest processing until the start of fermentation (Rankine, 1998).

2.2.3.3 Vinification and bottling

Skin contact applied to Sauvignon blanc grape juice can extract more flavour compounds from grape skins. Roujou de Boubee *et al*. (2002) reported that methoxypyrazines concentrations in skins are higher than in grape flesh. The location of cysteine conjugates, precursors of 4MMP (P-4MMP), 4MMPOH (P-4MMPOH) and 3MH (P-3MH) was studied (Tominaga *et al.,* 1998b; Peyrot des Gachons *et al.,* 2000; Peyrot des Gachons *et al.,* 2002b). The concentrations of the precursors of 4MMP and 4MMPOH were found to be equivalent in juice and skins. However the concentration of the precursor for 3MH was almost eight times higher in skins. Modest increases in the concentrations of P-4MMP and P-4MMPOH and a considerable increase of the concentration of P-3MH were established by 18 hour skin contact at 10°C and 18°C (Peyrot des Gachons *et al*, 2002a). Skin contact is applied, leaving grape juice pulp after destemming and crushing, for up to 15 hours at low temperatures before pressing and discarding the skins (Marais, 1998). Though the increase of skin contact temperatures have been studied, more research must be conducted in that field in order to verify that higher temperatures during skin contact indeed increase the concentrations of P-4MMP and P-3MH in juice (Howell *et al*., 2004; Swiegers *et al*., 2006).

Yeast cultures, used for fermentation, can influence the production of volatile thiols, higher alcohols and esters formed during fermentation. Using specific yeast cultures or combinations of cultures for Sauvignon blanc wine production can influence the fruity and tropical nuances of the wine. It was shown that different *S. cerevisiae* yeast strains gave rise to different concentrations of 4MMP and 4MMPOH in Sauvignon blanc wine (Howell *et al*., 2004). The wines prepared with the strain VL3 had, on average, 2 – 3 ng/L higher concentrations of 4MMP and MMPOH than wines prepared with the EG8 and VL1 strains (Murat *et al.,* 2001; Dubourdieu *et al.*, 2006). King *et al*. (2008) showed that VIN7 and QA23 yeast strains gave rise to even higher concentration of 4MMP and 3MH than the VL3 strain. VIN7 has been shown to be mediating the best release of 3MH from its flavour precursors, whereas QA23 had better abilities to convert 3MH to A3MH. Co-inoculation of VIN7 and QA23 gave rise to higher concentrations of A3MH in wines, than inoculation with either VIN7 or QA23. The high volatile acidity that is typically associated with VIN7-mediated fermentations was also eliminated using co-inoculation (King *et al*., 2008; Swiegers *et al*., 2009).

Fermentation controls typically include temperature control of no higher than 15°C, and the use of dry ice (solid phase CO_2) to fill the space above the fermenting grape must in fermentation tanks (Rankine, 1998). Marais (1998) showed that reductive conditions during Sauvignon blanc preparation resulted in the highest quality wines. Lower temperatures and inert conditions preserve aroma compounds and prevent oxidation.

Sulphur dioxide is commonly used during and after the winemaking process to prevent spoilage. However, legal considerations must be adhered to that limit the amount of sulphur dioxide that can be used for preservation. Ascorbic acid also has the ability to preserve wine and is commonly used, in addition to sulphur dioxide, to preserve Sauvignon blanc wines in the South African wine industry (Rankine, 1998). Bottling Sauvignon blanc wines in dark coloured

bottles and storing wines in cool dark rooms have been suggested by Marias (1994) to prevent breakdown of methoxypyrazines after bottling.

2.3 Wine Authentication

In modern times, there is a greater awareness of food safety and quality amongst the consumer. Producers are also under pressure to reassure the public of the authenticity of the content and origin of the foodstuffs they consume (Sun, 2008). Wine legislation specifically state that flavourings may not be added to natural wine and be sold as wine. Legislation also requires that vintage, cultivar and origin of wines should be stated correctly on labels. Authentication of and discrimination between wines according to the following criteria has been studied using various analytical techniques in conjunction with a multivariate data analysis approach.

Since many factors contribute to the quality of a Sauvignon blanc wine throughout the whole winemaking process, adulteration by adding flavourings to the finished wine may be a tempting alternative to demanding and precise viticultural and winemaking practices, to some.

2.3.1 Added flavourings to Sauvignon blanc wine

It has been confirmed that adulteration of Sauvignon blanc wine with natural extracts of vegetables containing methoxypyrazines, specifically green pepper, has been conducted in South Africa during the 2004 vintage (Du Plessis, 2005; Marais, 2010). The practise of the addition of commercial food flavourings, although not necessarily containing methoxypyrazines, cannot be ruled out in wine production, worldwide, as illustrated by several reports. The addition of methoxypyrazine based artificial aroma enhancers have also been studied in the Czech Republic (Rajchl *et al.,* 2009). Methoxypyrazines does not only occur in grape juice and wine, but also in vegetables such as beans, spinach, beetroot, carrots, potatoes, peas, cucumber, asparagus and green pepper (Buttery *et al.,* 1969; Murray and Whitfield, 1975; Marais, 1994). In general, it has been speculated that the improvement of Sauvignon blanc wine flavour is the driving force behind adulteration with artificial flavourants.

In a large-scale survey of Sauvignon blanc juices and wines from different geographic origins, the ratios of IPMP to IBMP and of SBMP to IBMP, were 5% in each case (Alberts *et al.*, 2009). The analysis of methoxypyraxines in green peppers extracts, a source of confirmed adulteration in Sauvignon blanc wine (Du Plessis, 2005; Marais, 2010), also showed that IBMP is the predominant pyrazine; however, the relative abundance of IPMP and SBMP are much smaller than those reported for Sauvignon blanc wine. The addition of green pepper extract to wine will therefore distort the relative ratios of methoxypyrazines in grape juice or wine, and aid detection of this practice.

Due to the low concentrations of methoxypyrazines in Sauvignon blanc wines, it was difficult to quantify these compounds effectively, until recently. High cost, time-consuming methods have been developed such as capillary gas chromatography – mass spectrometry (GC-MS), capillary gas chromatography – nitrogen phosphorous detection (GC-NPD) and liquid chromatography – mass spectrometry (LC-MS) for quantitative analysis. Sample preparation varies from method to method and can be rather complex due to the low levels of methoxypyrazines. Methods used include liquid-liquid extraction, distillation and solid phase extraction (Kotseridis *et al.*, 1998; Sala *et al.*, 2002; Wampfler and Howell, 2004).

In South Africa, GC-MS (Marais, 1994; Kotseridis *et al.*, 1998) and LC-MS (Alberts *et al.*, 2009) analyses are used to quantify methoxypyrazines. These methods are currently also being used to detect adulteration in terms of the addition of external sources of methoxypyrazines to

Sauvignon blanc wines. Due to the time consuming and expensive nature of these methods, only random samples from different regions are tested. The levels of methoxypyrazines in wines suspected of adulteration are compared to a South African Sauvignon blanc juice and wine database of possible levels and ratios of the different methoxypyrazines relative to each other. Levels in grape juice samples are also tested and levels in the finished wines should not be higher than those in the grape juice from that specific wine producing region (Alberts *et al.*, 2009).

Currently no method exits to detect adulteration of Sauvignon blanc wines of non-methoxypyrazine substances. A fast screening method for all Sauvignon blanc wines could be beneficial for the authorities as well as the industry.

2.3.2 Cultivar

Classifying a wine as monovarietal has legal requirements. According to South African wine law, a wine can be certified as originating from one cultivar if at least 85% of that wine was made from that specific cultivar (Liquor products act, 1989).

The aroma characteristics of a wine are defined by the varietal character. Aroma compounds found in wine are mostly volatile compounds such as alcohols, esters, terpenes, sulphur compounds amongst others (Santos *et al.*, 2004). Due to the volatile nature of these compounds, gas chromatography (GC) is the most widely used analytical method for the quantification of these compounds. Numerous types of volatile compounds contribute to the varietal character of a wine. Typically GC analysis generates data pertaining to a relatively large number of compounds. In order to interpret the volatile profiles, GC data are often combined with multivariate data techniques in order to discriminate between wines of different cultivars, as illustrated in the following examples from literature.

Arozarena *et al*. (2000) used gas chromatography-flame ionization detection (GC-FID) combined with stepwise linear discriminant analysis (SLDA) to distinguish between different varieties and 85% of samples were correctly classified. Falqué *et al*., (2001) calculated an aromatic index to determine the contribution of each chemical measured to the aroma of a wine. The aromatic index was calculated using the concentration, determined by gas chromatography-mass spectrometry (GC-MS), and divided by the odour threshold. Principal component analysis (PCA) based on the aromatic index data, clearly discriminated between different Galician white wine varieties, Albarino, Loureira, Treixadura and Dona Branca. Santos *et al*., (2004) showed that results obtained from GC-MS analysis combined with discriminant function analysis (DFA) and results obtained using a sensor array in combination with PCA and radial base neural networks, could successfully distinguish between different cultivar wines. A surface acoustic wave (SAW) sensor array, electronic nose, in combination with linear discriminant analysis (LDA) and a probabilistic neural network were used in another study to discriminate between cultivars (Santos *et al*., 2005). Louw *et al.* (2009) used GC and Fourier transform mid infrared (FT-MIR) in combination with PCA, analysis of variance (ANOVA) and LDA to discriminate between different cultivars. The model discriminating between cultivars of different white wines classified 98.3% correctly and a model with combined data form GC and FT-MIR classified 86.8% correctly.

Wine is chemically complex and successful discrimination between different cultivars depends on various chemical compounds, not only volatile compounds. Thin-film multisensors array based on electronic nose technology have been investigated in combination with artificial neural networks (ANN) by Penza *et al.,* (2004a). Wines have been successfully classified as white, red or rosé wines using PCA and ANN as data analysis methods (Penza *et al.,* 2004b).

Red wines contain organic acids formed during sugar oxidation or during the winemaking procedures. Mardones *et al*. (2005) quantified shikimic acid to verify the varietal authenticity of red wines using capillary zone electrophoresis (CZE) in combination with analysis of variance (ANOVA). Capillary electrophoresis has also been used by Nunez *et al*., (2000). Trace metals such as Ca, K, Li, Mg, Mn and Na were analysed and various multivariate analysis techniques, K-nearest neighbours (KNN), LDA and soft independent modelling of class analogy (SIMCA) were used, to successfully discriminate between wines.

Spectroscopic methods such as Fourier transform infrared (FT-IR), near infrared (NIR), ultraviolet (UV) spectroscopy, as well as the electronic nose (GC-MS based) are rapid methods of analysis. Roussel *et al*. (2003) used FT-IR, UV spectroscopy and an electronic nose to discriminate between different varietals analysing grape must. The best results were obtained using FT-IR combined with partial least squares discrimination (PLS-D).

Cozzolino *et al*. (2003) conducted a feasibility study using visible and near-infrared spectroscopy to discriminate between Chardonnay and Riesling wines. PCA was used as initial data analysis step to investigate the differences between wines and the possibility of using multivariate data analysis to discriminate between wines of different variety. PLS-D models classified 100% of the Riesling wines and 96% of the Chardonnay wines correctly. In a subsequent study electronic nose technology and mass spectrometry were also used to discriminate between Riesling and Chardonnay wines with 90% accuracy (Cozzolino *et al*., 2005a).

2.3.3 Vintage and aging

Vintage and aging potential of wines are indicators of the quality of wines. Flavour nuances in white wines generally become less prominent and unpleasant flavours tend to develop if the wines are over-aged. Red wine, depending on the wine style, generally benefits from more extended periods of aging. The vintage of wines are indicated on wine labels as general practice in many countries, including South Africa. The South African wine and spirit board requires that if the vintage year is indicated on the label, at least 85 % of the wine consist of wine produced from grapes harvested during the year indicated (Liquor products act, 1989).

Carbon dating is a commonly applied technique to determine the age of substances. Jones *et al*., (2001) used carbon dating to detect adulteration of red wines with older materials. Marengo *et al.* (2001) classified Nebbiolo-based Italian wines according to vintage. Esters, terpenes and lower alcohols were quantified using solid phase micro extraction (SPME) coupled with GC-MS and GC-FID. PCA, and a variety of hierarchical cluster analysis techniques, including SIMCA and SLDA, was used to build classification models to distinguish between the wines from different vintages.

Polyphenols are secondary metabolites that differ in their respective ratios and incidence of occurrence in wines of different varieties, vintages and aging procedures (Dufour *et al*., 2006; Masoum *et al*., 2006). Two dimensional nuclear magnetic resonance (NMR) of polyphenols in combination with PLS-D and neural networks, was used to discriminate between red wines of different vintages (Masoum *et al*., 2006). Correct classification using neural networks (85%) was obtained. Polyphenols are also fluorescent molecules that can be detected using front-face fluorescence spectroscopy (Brossaud *et al*., 1999) and Dufour *et al*. (2006) used this technique in combination with PCA and factorial discriminant analysis (FDA) to observe vintage-related trends.

2.3.4 *Terroir* and geographical origin

Authentication of *terroir* and the geographical origin of wines are monitored and controlled worldwide. Strict regulations must be adhered to in Europe. Labelling of wines in South Africa, and elsewhere, are bound by legal technicalities as to specific words that may be used with regards to *terroir* and origin. Specific wine origins, abbreviated as W.O., for South African wines have been defined (Liquor products act, 1989) and only wine produced from grapes from that specific origin, may be declared as W.O. on the label. Similar legal requirement apply to wines from a specific farm as well as wines from a single vineyard (Liquor products act, 1989).

Many studies investigated discrimination between wines from different origins and wine made from grapes originating from different *terroir*. Multi-element analysis combined with multivariate statistics is often used for this purpose (Kallithraka *et al., 2001;* Perez-Magarino, *et al*., 2002; Del Mar Castineira Gómez *et al*., 2004; Kment, *et al*., 2005). The element content in wines originates from natural and anthropogenic sources. The natural source of these elements is soil, whose composition is determined by the parent rocks and weathering. Viticultural practices such as fertilization, pollution of the environment, application of food additives, machinery and operations performed during winemaking, are known anthropogenic sources (Kment *et al.*, 2005).The latter study used multi-element analysis of Mg, Mn, Cs, Ba, Sr and Pb and PCA to investigate correlations between vineyard soil and the element composition of wines. The concentration of Mg in the wines had strong correlations with the concentration of Mg in the soil. Therefore, if the concentration of Mg in the soil differs significantly from that in the wine, the origin of the wine stated on the label might be false. In another study, German white wines was classified 83% accurately according to origin using multi-element analysis of Li, B, Mg, Fe, Zn, Sr, Cs and Pb in combination with quadratic discriminant analysis (Del Mar Castineira Gómez *et al*., 2004).

Kallithraka *et al*. (2001) successfully discriminated between wines from different origins using sensory analysis, quantification of total phenols, anthocyanins and minerals performing PCA on the data. Wines were classified as Northern Greek and Southern Greek. Total phenol and mineral analysis alone, did not result in classification of wines.

Differentiation of Riesling, Chardonnay and Bordeaux-style red wine from Canada according to specific *terriors*, namely north facing slopes of the Niagara escarpment, the level plains area between the escarpment and Lake Ontario and the area immediately adjacent to the lake, has been studied (Douglas *et al*., 2001; Kontkanen *et al*., 2005; Schlosser et al, 2005) Sensory descriptive analysis and chemical analysis such as pH, titratable acidity (TA) and alcohol content in combination with ANOVA, PCA and SLDA, were used to successfully classify the wines according to *terroir*.

NMR spectroscopy is another method used for authentication of wine origin. A great number of chemical compounds can be simultaneously detected by NMR (Brescia *et al.*, 2002). ^{1}H NMR analysis, as well as anion, cation and organic acid analysis by chromatographic techniques in combination with PCA, DA and hierarchical cluster analysis (HCA) as statistical analysis tools, were used to determine the origin of Italian wines (Brescia *et al.*, 2002). Wines from the south, north and Apulia region could be distinguished from each other. ^{1}H NMR in combination with PCA and PLS was also used by Pereira *et al*. (2005) to discriminate between geographical origin of Merlot Noir, Cabernet franc and Cabernet Sauvignon from Bordeaux, Italy. Kosir *et al.* (2002) used one dimensional (1D) and two dimensional (2D) NMR looking at both ^{1}H and ^{13}C resonances of amino acids, succinic acid and butylene glycol. Cluster analysis successfully discriminated between the coastal and continental regions of Slovenian red wines. 2D NMR was also used in a study conducted by Masoum *et al.* (2006). PLS-D and neural

networks as data analysis techniques showed clear clustering of wines according to clay, sand and gravel *terrior*.

Oliveira *et al*. (2006) showed that the quantification, using GC-MS, of C6-alcohols 1-hexanol, (*E*)-3-hexenol and (*Z*)-3-hexenol can be used as varietal markers for assessment of wine origin. Discrimination between different origins could be achieved looking at respectively the ratios between (*E*)-3-hexenol and (*Z*)-3-hexenol and the ratios between 1-hexenol and (*Z*)-3-hexenol.

2.3.5 Food authentication: the challenge remains

Several advances have been made towards food authentication, particularly in the field of developments in instrumentation and chemometric techniques required for the extraction of information from the captured data. However, the challenge remains and will be a serious issue in future. The detection of several minor compounds, in many instances chemically yet undefined, must still be developed and it is foreseen that hyphenated instruments such as liquid chromatography-nuclear magnetic resonance (LC-NMR) could play an important role (Sun, 2008). Irrespective of the analytical strategy used, the availability of reference standards in the gram quantities also remains a challenge. Finally, the importance of establishing a comprehensive, searchable compositional and instrumental signal database, for authentication purposes, cannot be overemphasised.

2.4 Analytical techniques used in this study for the authentication of Sauvignon blanc wine

2.4.1 Chromatographic analysis

Chromatographic analysis is used as an analytical technique for authentication in the food industry and particularly in the wine industry. Chromatographic techniques are able to detect substances at lower levels than possible with most other conventional analytical instrumentation. The principal behind all chromatography techniques is the separation of chemical compounds in a mixture using two immiscible phases, a mobile and stationary phase. Chemical compounds are separated according to their different affinities for the stationary phase and mobile phase, which is gas for gas chromatography and liquid for liquid chromatography. The stationary phase can be a solid or a liquid. Different techniques for detection of the compounds being separated during the chromatographic procedure are used (Willard *et al.*, 1988). Detection techniques include mass spectrometry (MS), flame ionisation detection (FID) and ultraviolet-visible spectrometers, (UV-VIS and UV). Identification of compounds is usually done by external standards and spectral libraries.

2.4.2 Spectroscopic analysis

Spectroscopy is the measurement and interpretation of electromagnetic radiation absorbed, scattered or emitted by atoms, molecules or other chemical species. Absorption or emission is associated with changes in energy levels or states of the interacting chemical species. Species have characteristic energy states; therefore spectroscopy can be used for identification of chemical species by looking at energy states (Willard *et al.*, 1988). Spectroscopic methods are often used for authentication in the food and beverage industry. Methods such as NMR and FT-IR spectroscopy, including reflection and absorption are used. NMR is based on the principal that nuclei with nonzero magnetic moments will absorb electromagnetic radiation at precise

frequencies when placed in an external magnetic field. The electrons in a molecule shield the nucleus from the external magnetic field causing different atoms to absorb energy at slightly different frequencies, the effect is known as chemical shifts. Chemical shifts are used to identify and calculate the concentration of certain compounds in a sample (Willard *et al.*, 1988; Hore, 1995).

FT-IR spectroscopy is based on the principle that molecular vibrations occur in the infrared region of the electromagnetic spectrum due to the absorption of a portion of radiation at a certain wavelength. The multiplicity of vibrations occurring simultaneously, produce a complex spectrum of absorption frequencies. Different chemical functional groups have different characteristic absorption frequency spectra. Therefore functional groups can be identified from spectra and hence molecules containing certain functional groups can be identified (Willard *et al.*, 1988). Instruments used, frequently use a scanning Michelson interferometer to construct a time domain interferogram. The latter is mathematically transformed to a frequency domain spectrum by means of a Fourier transformation. FT-IR spectroscopy is mostly used to achieve fast computation and processing of data and solve the problem of overlapping spectra due to a complex matrix.

Fourier transform mid-Infrared (FT-MIR) transmission, FT-MIR attenuated reflection as well as Fourier transform near-infrared (FT-NIR) reflection spectroscopic instruments, have been developed specifically for application in the analysis of food stuffs and beverages. These instruments are more accessible and more cost effective to operate than, for example chromatographic instrumentation. The level of expertise and training needed to operate these instruments are also at a lower level than needed to operate chromatographic instrumentation. Therefore, FT-IR analysis is an ideal method for authentication in terms of bulk screening, particularly due to the faster rate of analysis and low unit analysis costs. These instruments are commonly used for bulk screening and quality control purposes (Roussel *et al.,* 2003; Blanco *et al*., 2004; Cozzolino *et al*., 2004; Patz *et al*., 2004; Swanepoel *et al*., 2007; Bauer *et al.*, 2008; Versari *et al.,* 2008). The development of calibration models require extensive validation and several techniques are used for this purpose, including independent test set validation and cross validation (Esbensen, 2002; Patz *et al*., 2004).

2.4.3 Sensory Evaluation

Sensory analyses are widely used to determine and assess the quality of wines. To obtain quality data, sensory evaluation should control and standardise the external variables, to minimise bias that can negatively influence the accuracy of assessments. Usually, only variables of interest are measured (Jackson, 2002; Meilgaard *et. al*., 2007). Variables that should be controlled and standardized include; the test environment, sample preparation and presentation, and the type and number of evaluations and interaction between panellists. This is minimized during the final sensory evaluation by providing effective lighting, separate judge cubicles and controlled temperature in a well ventilated room. External interfering odours must be eliminated. Serving size (approximately 20 - 30 mL), temperature (20 – 25°C), covering glasses to eliminated evaporation of aroma compounds and using random three digit codes for samples must be taken into consideration. Different types of panels are used for different types of tests (Jackson, 2002; Meilgaard *et. al*., 2007).

Training of panel members to evaluate specific aroma attribute is conducted when descriptive analysis is performed for example for the profiling of a wine (Bakker and Arnold*,* 1993; Mansfield and Vickers, 2009) and grape juice (Abbott *et. al.,* 1991). In this case screening of panellists, training of panellists and panellist evaluation is conducted. Statistical treatment of

the data can show the impact of external variables that could be eliminated by either changing the experimental design or excluding outliers from the dataset, or by identifying inconsistent performance of panellists. The correlation of sensory analysis with chemical measurements can assist with the interpretation of chemical data with regards to wine characteristics (Cozzolino *et. al.,* 2005b; Preston *et. al.,* 2008). Different methods such as discrimination tests (the triangle test, paired comparison and duo – trio test), affective tests (paired preference, ranking and hedonic test), descriptive analysis as well as screen tasting for faulty wines are used. The selection of the specific method is based on the type of information required (Zoecklein *et al.*, 1995; Jackson, 2002).

Descriptive analysis can provide information that cannot be tested otherwise, by monitoring complex perceptions of flavour intensities. Descriptive analysis requires the training of a panel, statistical treatment to evaluate the effectiveness of the training, and interpretation of the data. Univariate statistical methods such as ANOVA and multivariate techniques such as PCA are often used to monitor panel performance and for interpretation of the data (Hootman, 1992). Quantitative descriptive analysis using an unstructured line scale was used for this study.

2.5 Statistical and chemometric data analysis techniques used in this study

Chemometrics play an important role in authenticity testing (Arvanitoyannis *et. al.*, 1999) since several variables must frequently be considered simultaneously. The application of univariate statistics alone to analyse multivariate data can lead to misinterpretation as well as ignorance of variances and correlations between variables (Esbensen *et. al.*, 2002). Supervised and unsupervised techniques are used for authenticity testing. Multivariate calibration is conducted using supervised techniques, mostly PLS (Bauer *et. al*., 2008). Unsupervised classification is used to identify patterns within the data structure when limited or no information regarding the dataset is known, or to investigate relationships not hypothesized previously. PCA is often used for this purpose. Supervised classification is used to classify samples into pre-determined classes on the basis of known parameters or a set of pre-determined rules (Esbensen, 2002). In this study PLS-D with a dummy variable of -1 for spiked wine (to which flavouring was added) and 1 for control wines, was used to classify wines as either spiked / adulterated or control / unadulterated wines.

The focus of this study in terms of statistics fell on multivariate analysis. Large data sets containing multiple variables were generated. ANOVA was specifically used to interpret sensory data. Multivariate techniques were used on spectroscopic data as well as sensory evaluation data. The multivariate techniques used in this study included PCA, an unsupervised technique, as well as a supervised technique, PLS-D. SIMCA could be used in a further study to evaluate the adulteration status of other wines using data and existing PCA models constructed during this study.

The key assumption made during all multivariate analysis is that the “direction of maximum variance” is directly related to the “hidden phenomena” in the data set (Esbensen, 2002). In other words the data structure and variance in the data set is caused by or related to the “phenomenon” identified using PCA and not co-incidence. It is therefore necessary to do rigorous validation of calibration models, be it of quantitative or qualitative nature, to use effective controls in the experimental design, in order to rule out co-incidence leading to false conclusions.

2.5.1 Univariate statistics

2.5.1.1 Analysis of variance (ANOVA)

ANOVA is a statistical technique commonly used on sensory data. During this study two-way analysis of variance was used to determine whether significant differences between spiked and control wines existed and to test judge agreement on sensory analysis data, 3-way analysis of variance was used to determine the replicate effect as well.

The equation describing a two-way ANOVA model is (Eq 1)[1]:

$$X_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + e_{ijk} \quad (1)$$

Where x is the sample, α_i is the assessor effect, β_j the product effect, $\alpha\beta_{ij}$ the product assessor interaction and e_{ijk} the random error term. The variation of the model (SS) is described as the sum of all the variations of individual observations with regards to the mean (Eq. 2).

$$\mathrm{SS(T)} = \sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{K}\left(X_{ijk} - \overline{X}\right)^2 \quad (2)$$

$$SS(T) = SS(A) + SS(P) + SS(AP) + SS(E) \quad (3)$$

Where x bar is the mean of all the samples, SS is the sum of the squares, I the number of assessors, J the number of products and K the number of repeats. SS(A) measures how much each assessor deviates from the overall mean summed over all the assessors. SS(P) corresponds to the deviation between a product and the mean summed over all of the products. SS(AP) the interaction between the product and the assessor and SS(E) the residual sum of the squares indicating random error. For this study it was important to look at the differences between different products (wines to which different flavourings were added in this study) , and *F* distribution tables were used:

$$F = \frac{SS(P)/DF(P)}{SS(E)/DF(E)} \quad (4)$$

Where *F* is dependent on the degrees of freedom for the products, DF(P) = *J* - 1, and the degrees of freedom for the error DF(E) = *IJ(K* – 1*)*. If *F* is close to one there is a significant difference between the products.

A similar approach can be used to determine if the judges are scoring products significantly different. In this study it was important to have judge agreement; therefore, judges were tested to not score significantly different.

The possibility of falsely classify different products as significantly different, or not, is called the significant level of the test, (*p*) which can be determined from tables. A typical significance level (*α*) is 5% or *α* < 0.05 (Lea *et al.*, 1997).

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[1] ANOVA equations and notation adopted from Lea *et al.* (1997)

The ability of the model to describe the data, where R^2 is the correlation coefficient is mathematically expressed as:

$$R^2 = 1 - \frac{SS(E)}{SS(T)} \quad (5)$$

ANOVA can only treat one sensory attribute at a time (Lea *et. al.,* 1997). Samples are classified as different or similar with regards to a specific attribute. Performing analysis for all the attributes to decide whether samples differ significantly, are time consuming. ANOVA cannot be used to study correlations between different attributes. Multivariate statistical methods, PCA and PLS-D described below, were used in addition to ANOVA to investigate correlations between attributes.

2.5.2 Multivariate statistics

2.5.2.1 Principal Component Analysis (PCA)

PCA is an unsupervised statistical technique used for data reduction, summarizing correlation patterns between variables and identifying similarities and differences between samples with no or minimum loss of information. The data matrix is decomposed into a "structure" part and a "noise part' (Sharma, 1996; Esbensen, 2002; Timm, 2002) according to the following equation:

$$\boldsymbol{X} = \boldsymbol{T}\boldsymbol{P}^T + \boldsymbol{E} \quad (6)$$

Where the original data set (**X**) is decomposed into a structural part, the scores matrix (**T**) and the loadings matrix ($\mathbf{P}^T$), plus a noise part, the error matrix (**E**).

The original data set is transformed into a new set of variables, orthogonal to each other. The new variables, called principal components (PC's), are linear combinations of the original variables. The first principal component (PC1) describes the most of the variation, with the second component (PC2) describing the second most variation and the third component (PC3) describing the third most etc.

The data consisting of n objects and p variables that collectively characterize the n objects is called the **X**-matrix. Objects can be samples and variables measurements, for example absorbances at certain wavelengths.

PC1 is positioned along the direction that explains the maximum variation after plotting the data in a p-dimensional space (having n data points). PC1 is calculated by drawing a temporary arbitrary axis through the n points in the p-dimensional space. Each data point is projected perpendicularly down onto the axis. The distance between the point and the projection of the point on the axis is called the object residual (e_i). The same concept is valid for loadings, where loadings relate to the variables in the same way that PC's relate to the samples. Any PC is a variance-scaled vector that can mathematically be written as a linear combination of the p unit vectors. Therefore the equation describing the linear combination of p unit vectors for a PC will have p coefficients. The coefficients are called loadings. Loadings construct the directions of each PC relative to the original data. If an object (data point) is projected perpendicularly onto a PC the distance from the PC-origin to the projection of the object onto a PC is called a score and as mentioned above the distance between the original data point and projected point is the residual. Each object will have the same number of scores and residuals as the number of PC's. The matrix of all the combined scores is called the **T** matrix. The loadings as mentioned above

can be combined to form the loadings matrix. The loadings matrix, $\mathbf{P}^T$, is calculated by transposing the data set and therefore viewing the variables as samples and projecting the variables onto the PC plane.

A PCA model can be interpreted by plotting the scores of two PC's on a two dimensional score plot (refer to Figure 2.1). In Figure 2.1 each marker on the score plot represents the FT-MIR spectrum of an object (a wine sample) that has two co-ordinates (PC's). In this case PC1, along the horizontal axis, and PC2, along the vertical axis. The control wines located towards the left of PC1 and spiked wines are grouped together. It is clear from the plot that PCA analysis is useful to distinguish between the control wines and spiked wines and that related objects (wine samples) are grouped. The amount of variation described by PC1 is 74% and 14% is explained by PC2. Therefore 98% of the variation within the dataset is explained by the first two PC's. This is a clear indication that the information captured in the spectra is well explained by the PCA model. Independent test set validation or cross validation of the model is required to confirm that the separation between the groups is indeed linked to the addition, or not, of flavouring to some samples.

Interpretation of the loadings provides information to the relationship between the variables. Variables with similar properties will be located close to each other, while not uncorrelated variables will appear perpendicular to each other with respect to the origin. Variables with negative correlation will appear opposite each other, with respect to the origin on the loadings plot (Esbensen 2002). The same strategy can be followed for interpretation of the score plots, although these refer to the relationships between objects (spiked wine samples in this study).

Bi-plots, where the scores and loadings are shown on the same plot is often used in sensory science (refer to Figure 2.2). Bi-plots visualize the correlation between the samples (objects) and attributes (variables) simultaneously. Scores are represented by letters (A-L) in Figure 2.2 and loadings by descriptive terms. Loadings and scores close to each other are positively correlated. Loadings close to a score gives an indication of those variables that most strongly influences that sample by means of positive correlation; for example, in Figure 2.2 the attribute asparagus correlates strongly with wine L (the asparagus flavoured wine) and the guava attribute has negative correlation to wine L. Therefore guava (as a sensory attribute) was scored low for the asparagus flavoured wine (L) and asparagus (as a sensory attribute), was scored high. These findings corroborate the sensory evaluations of the trained sensory panel, as will be discussed in the following chapter.

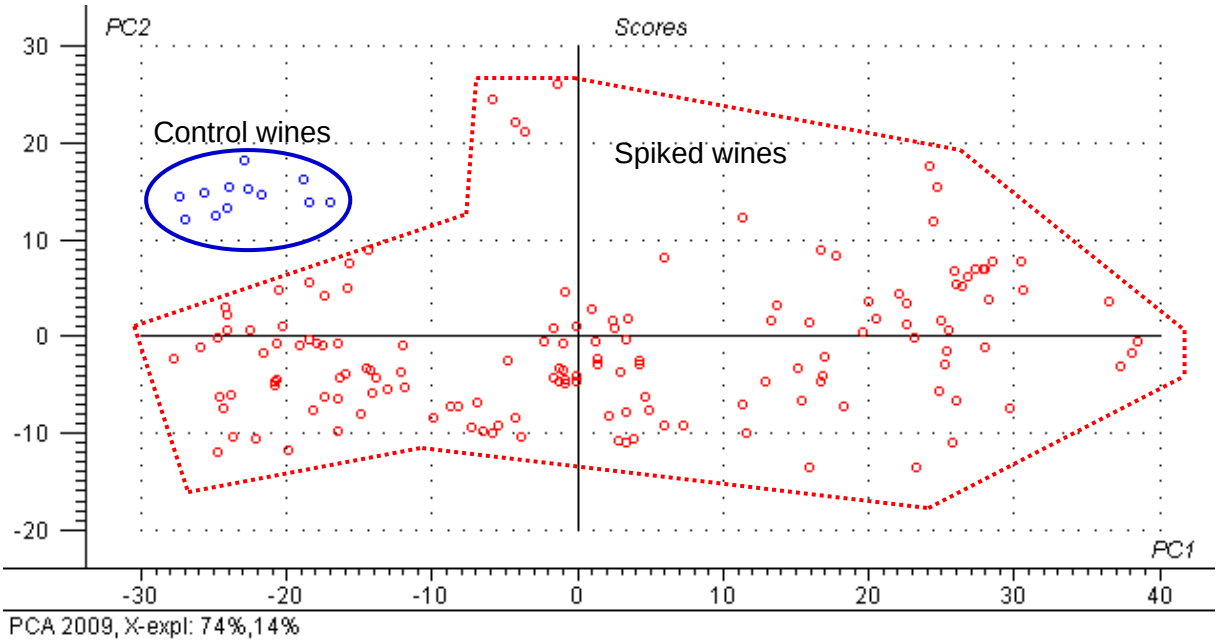

Figure 2.1 PCA score plot with the control wines located to the left top of the score plot. The spiked wines are outlined with a dotted line. It is clear from the plot that PCA analysis can be used to distinguish between the control wines and spiked wines. The amount of variation described by PC1 is 74% and by PC2, 14%. The explained variance is 98%. FT-IR data from this study was used to construct the score plot and test set validation was used to validate the model.

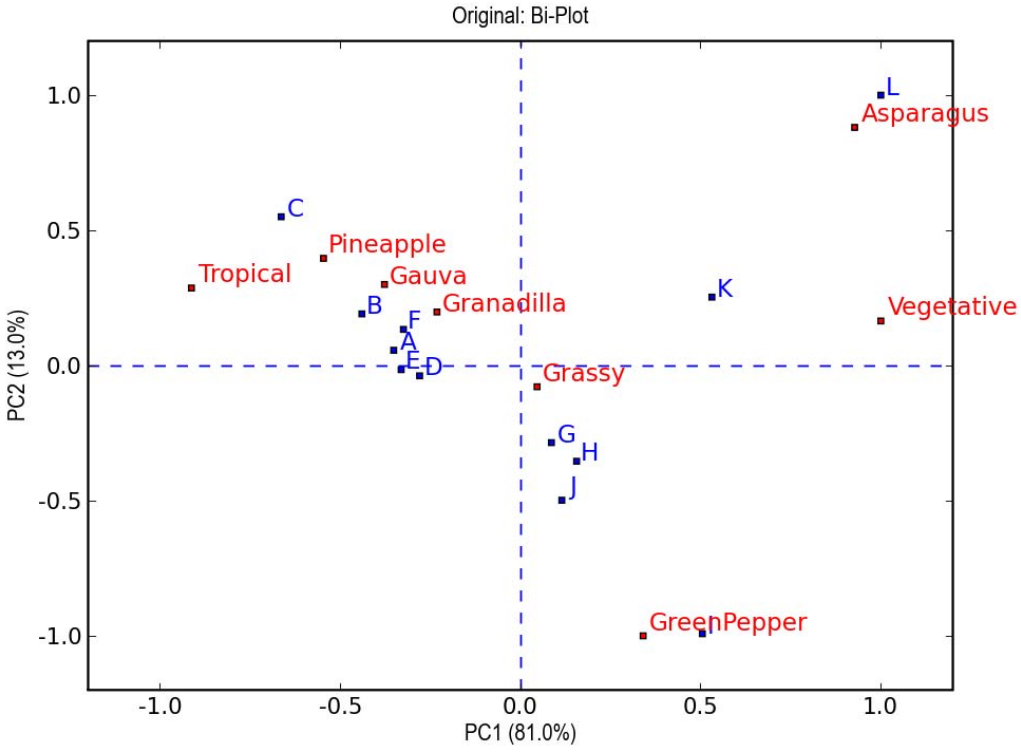

Figure 2.2 PCA bi-plot constructed from sensory data generated in this study. Scores are represented by letters (A-L) and loadings by descriptors. The asparagus attribute correlated positively with wine L (asparagus flavoured wine) and the guava attribute was negatively correlation to wine L. Therefore guava (as a sensory attribute) was scored low for the asparagus flavoured wine (L) and asparagus (as a sensory attribute) high.

2.5.2.2 Partial Least Squares Regression (PLS)

PLS is an unsupervised classification technique that can be used for multivariate calibration or classification. Two data matrixes **X** and **Y** are related by regression. **X** consists of the independent variables and **Y** consists of the dependent variables. Partial least squares regression can be divided into two groups according to the size of the **Y** matrix. If the **Y** matrix consists only of one variable, it is called PLS1 and if the **Y** matrix consists of more than one variable it is refered to as PLS2. PLS calibration involves establishing a model by means of a mathematical equation summarizing variations in the data set and form an understanding of structure of the data. The model describes the relation between **X** and a known set of **Y** values. The regression model established during calibration is then used for prediction, for example to predict unknown **Y** values for given **X** values (Esbensen, 2002; Naes, 2002).

Validation methods measure the difference between the measured (Y_{Ref}) and predicted values (Y_{Pred}). Accuracy and precision of the model is associated with Y_{Pred}. The smaller the difference between Y_{Ref} and Y_{Pred} the better the model can be considered to be. The optimal number of components is where the difference between Y_{Ref} and Y_{Pred} is a minimum. The difference between Y_{Ref} and Y_{Pred} can be expressed as the prediction variance (V_y_Val) or the RMSEP (root mean square error of prediction). The RMSEP is the square root of the prediction variance and a direct measure of the prediction error, expressed in the same units as the original measurements. Therefore being the average error to be expected for future predictions. (Eq. 7)[2]. The RMSEC (root mean square error of calibration) is the measure for the calibration fit of the model (Eq 8). The RMSEP can also be expressed as the square root of the sum of the SEP squared and Bias squared (Eq 9). Bias (Eq 10) is used to measure the accuracy of the prediction model. The SEP (Eq 11) is the standard error of prediction.

$$RMSEP = \sqrt{\frac{\sum_{i=1}^{n} \left(\hat{y}_{i,val} - y_{i,val\_ref}\right)^2}{n}} = \sqrt{V_v\_Val} \quad (7)$$

$$RMSEC = \sqrt{\frac{\sum_{i=1}^{n} \left(\hat{y}_{i,cal} - y_{i,cal\_ref}\right)^2}{n}} \quad (8)$$

$$RMSEP = \sqrt{SEP^2 + Bias^2} \quad (9)$$

$$Bias = \frac{\sum_{i=1}^{n} (\hat{y}_i - y_i)}{n} \quad (10)$$

$$SEP = \sqrt{\frac{\sum_{i=1}^{n} (\hat{y}_i - y_i - Bias)^2}{n-1}} \quad (11)$$

Where:

$\hat{\boldsymbol{y}}_{\boldsymbol{i,val}}$ is the y value predicted for item i for validation

$\hat{\boldsymbol{y}}_{\boldsymbol{i,val\_ref}}$ is the y reference value measured for item i for validation

$\boldsymbol{n}$ is the number of samples

$\hat{\boldsymbol{y}}_{\boldsymbol{i}}$ is the y value predicted for item i and $\boldsymbol{y}_{\boldsymbol{i}}$ is the y value measured for item i

$\hat{\boldsymbol{y}}_{\boldsymbol{i,cal}}$ is the y value predicted for item i for calibration

$\hat{\boldsymbol{y}}_{\boldsymbol{i,cal\_ref}}$ is the y reference value measured for item i for calibration

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[2] PLS equations and notation adopted from Esbensen (2002)

2.5.2.3 Partial Least Squares Discrimination (PLS-D)

PLS-D is a supervised classification technique similar to PLS. The PLS regression model is used to discriminate between two or more classes. The **Y** matrix consist of dummy variables -1 and 1 to discriminate between classes. In the case of two different classes -1 will be assigned as y-value for all objects (samples) falling into one class and 1 will be assigned to all objects falling into the other class. In the case where more than two classes exist the number of y-variables will be equal to the number of classes, assigning 1 to the variable corresponding with the class to which the object belongs and -1 to all other variables. The variable set represented as a vector can be written as (class 1; class 2; class 3; class 4), for example an object belonging to class one out of four classes will have a y-variable set (1;-1;-1;-1) (Esbensen, 2002). PLS-D was used in this study to discriminate between experimentally spiked and non-spiked Sauvignon blanc wines. Figure 2.4 is an example of a score plot of a PLS-D model constructed in this study. Each marker on the score plot represents an object (the FT-MIR spectrum of a wine sample). The control wines located towards the right hand side of PC1 and spiked wines are outlined with a dotted line (FT-MIR spectral data from this study). The amount of variation within the **X**-data matrix described by 1 PC (the first PC) is 55% and 24% by the second PC. Test set validation was used for this model. The predicted versus measured plot can be used to evaluate the performance of the model. The slope or R^2 should be as close to one, the exact value necessary to accept the model depends on the application (refer to Figure 2.3).

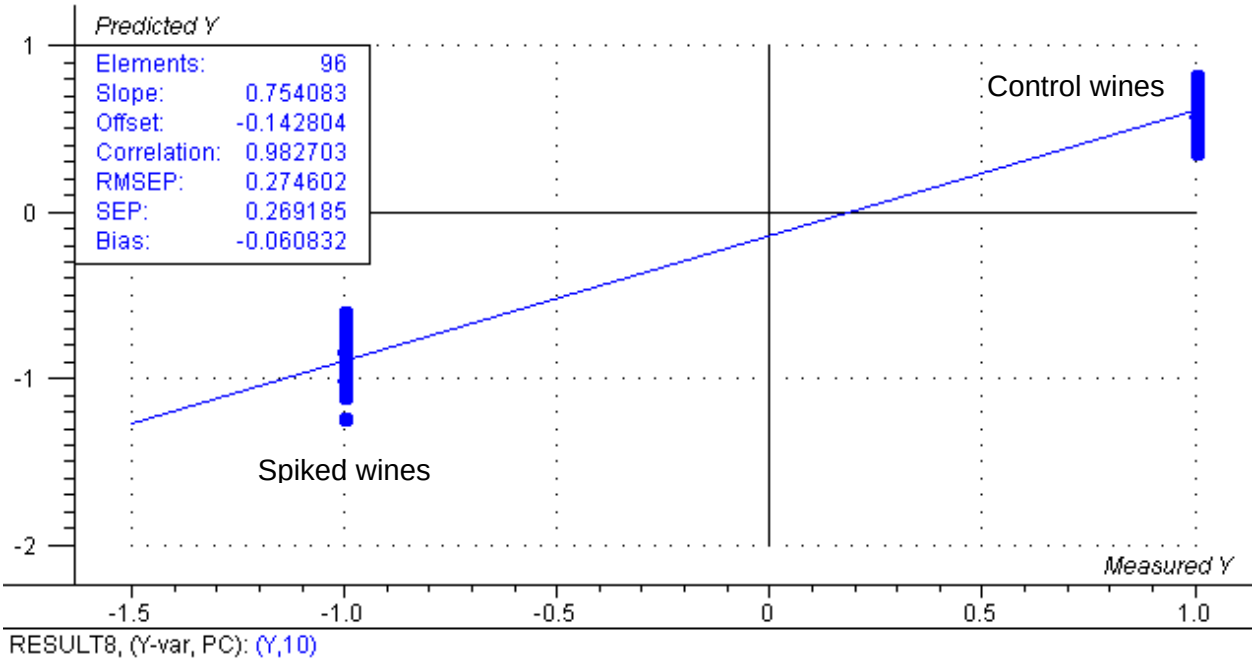

Figure 2.3 PLS-D predicted versus measured plot constructed from FT-MIR data from this study. Dummy variables -1 was used for spiked wines and 1 for control wines. Since the slope (R^2) is higher than 0.75 the correlation of 0.98 is relatively close to one and the bias of -0.06 is relatively close to zero the model is suitable for screening purposes between spiked and non-spiked wines.

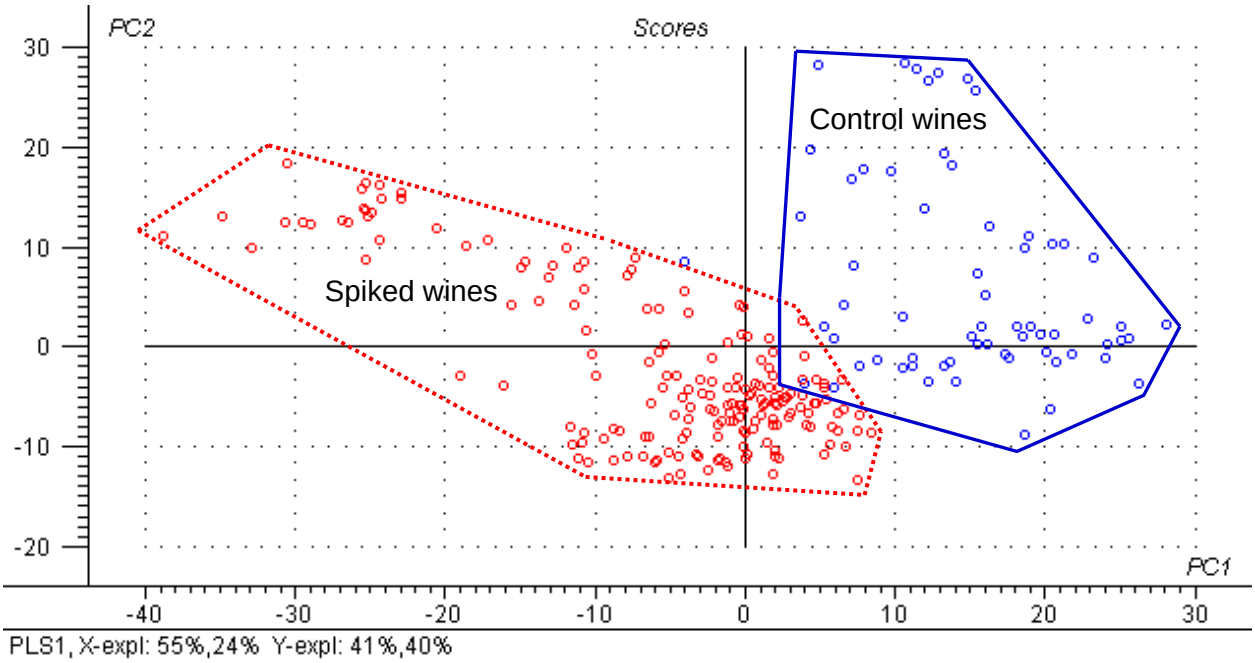

Figure 2.4 PLS-D score plot showing the control wines locating towards the right of PC1. Spiked wines are grouped together and outlined with a dotted line (FT-MIR spectral data form this study). The amount of variation within the X-data matrix described by PC1 is 55% and 24% by PC2. Test set validation was used. Discrimination between control and spiked wines can be observed.

2.5.2.4 Soft Independent Modeling of Class Analogy (SIMCA)

SIMCA is a supervised classification technique used to evaluate whether an object is similar to an already existing group of objects. PCA is used to construct these groups. Each group is described by its own PCA model. The first step concerning the construction of PCA models is called the training stage. During the second stage, classification, distances between the model and the new data can be calculated to decide to which group data belongs. Data can be classified as not belonging to any group in the model. Different distance measures can be used such as the distance between the object and the model and the object and the centre of the model. The distance between each data point and each group is calculated, the smallest of the distances between groups a specific data point will determine to which group the data point will belong. SIMCA can be used to detect objects or samples that are out of specification (Esbensen, 2002).

2.5.2.5 Conformity testing

Conformity testing is used on MIR and NIR spectra to measure the deviation of spectra within preset limits. Limits are set by reference spectra. The reference spectra form a confidence band. Spectra from (test samples) new samples pass the conformity test if within the confidence band and fail the conformity test if not within the confidence band.

The average and standard deviation (σ) of absorbance values for each wavelength (*i*) is calculated. The mean value plus / minus the standard deviation determine the confidence band within the spectral range. The difference between this sample $A_{Sample,1}$ and the average of the reference samples $A_{Reference,i}$ is calculated on each wavelength *i*. This absolute deviation is now weighted by the corresponding standard deviation σ on the respective wavelength, which results in a relative deviation referred to as the conformity index (*CI*). The maximum of all CI values is derived as test result (see Eq 12).

$$CI = (A_{Reference,i} - A_{Sample,i})/\sigma_{reference,i} \quad (12)$$

Vector normalization, first derivative and second derivative can be used as spectral pre-processing techniques. The optimum smoothing points must be chosen between 5 and 25. Vector normalisation re-scales each object in order to be of the same magnitude. First and second derivatives are used to eliminate noise often occurring in spectroscopy. The first derivative is often used to compensate for base-line shifts and the second derivative for scatter effects. Conformity index limits set, between three and four is recommended as a good starting point (OPUS, 2006).

Even though conformity testing is not used as commonly as PCA and PLS-D in research, it is a simple and useful tool to evaluate whether a sample is similar to a sample set or not. The advantage that the software purchased with some FT-MIR and FT-NIR instruments includes conformity testing can be of great value for routine screening purposes in the wine industry.

2.6 References

Abbott, N.A., Coombe, B.G., Williams, P.J. (1991). The contribution of hydrolysed flavour precursors to quality differences in Shiraz juice and wines: An investigation by sensory descriptive analysis. *Am. J. Enol. Vitic.* **42**, 167-174

Alberts, P., Stander, M.A., Paul, S.O., De Villiers, A. (2009). Survey of 3-alkyl-2-methoxypyrazines content of South African Sauvignon blanc wines using a novel LC-APC1-MS/MS method. *J. Agric. Food Chem.* **57**, 9347-9355

Allen, M.S., Lacey, M.J., Harris, R.L.N., Brown, W.B. (1991). Contribution of methoxypyrazines to Sauvignon blanc wine aroma. *Am. J. Enol. Vitic.* **42**, 109-112

Allen, M.S., Lacey, M.J. (1993). Methoxypyrazine grape flavour: Influence of climate, cultivar and viticulture. *Vitic. Enol. Sci.* **48**, 211-213

Arozarena, I., Casp, A., Marin, R., Navarro, M. (2000). Multivariate differentiation of Spanish red wines according to region and variety. *J. Sci. Food Agric.* **80** (13), 1909 – 1917

Arvanitoyannis, I.S., Katsota, M.N., Psarra, E.P., Soufleros, E.H., Kallithraka, S. (1999). Application of quality control methods for assessing wine authenticity: Use of multivariate analysis (Chemometrics). Trends *Food Sci. Technol.* **10**, 321-336

Augustyn, O.P.H., Rapp, A., Van Wyk, C.J., (1982). Some volatile aroma components of *Vitis Vinefera* L. cv. Sauvignon blanc. *S. Afr. J. Enol. Vitic.* **3**, 53-60

Bakker, J., Arnold, G.M. (1993). Analysis of sensory and chemical data for color evaluation of a range of red port wines. *Am. J. Vitic.* **44**, 27-34

Bauer, R., Nieuwoudt, H., Bauer, F., Kossmann, J., Koch, K.R., Esbensen, K.H. (2008). FT-IR spectroscopy for grape and wine analysis. *Anal. Chem.* **80**, 1371-1379

Blanco, M., Peinado, A.C., Mas, J. (2004). Analytical monitoring of alcolholic fermentation using NIR spectroscopy. *Biotechnol. and Bioeng.* **88**, 536-542

Brescia, M.A., Caldarola, V., De Giglio, A., Benedetti, D., Fanizzi, F.P., Sacco, A. (2002). Characterization of the geographical origin of Italian red wines based on traditional and nuclear magnetic resonance spectrometric determinations. *Anal. Chim. Ac.* **458**, 177-186

Brossaud, F., Cheynier, V., Asselin, C., Moutonet, M. (1999). Flavonoid Compositional Differences of Grapes Among Site Test Plantings of Cabernet franc. *Am. J. Enol. Viticulture*. **50**, 277-284

Buttery, R.G., Seifect, R.M., Guadagni, D. G., Ling, L.C. (1969). Characterization of some volatile constituents of bell peppers. *J. Agric. Food Chem.* **17**, 1322-1327

Cheng, T.B., Reineccius, G.A., Bjorklund, J.A., Leete, E. (1991). Biosynthesis of 2-methoxy-3-isopropylpyrazine in *Pseudomonas perolens. J. Agric. Food Chem.* **39**, 1009-1012

Cozzolino, D., Smyth H.E., Gishen, M. (2003). Feasibility study on the use of visible and near-infrared spectroscopy together with chemometrics to discriminate between commercial white wines of different varietal origins. *J. Agric. Food Chem.* **51**, 7703-7708

Cozzolino, D., Kwiatkowski, M.J., Parker, M., Cynkar, W.U., Dambergs, R.G., Gishen, M., Herderich, M.J. (2004). Prediction of phenolic compounds in red wine fermentations by visible and near infrared spectroscopy. *Anal. Chim. Ac.* **513**, 73-80

Cozzolino, D., Smyth H.E., Cynkar, W., Dambergs, R.G., Gishen, M. (2005a). Usefullness of chemometrics and mass spectroscopy-based electronic nose to classify Australian white wines by their varietal origin. *Talanta* **68**, 382-387

Cozzolino, D., Smyth, H.E., Lattey, K.A., Cynkar, W., Janik, L., Dambergs, R.G., Francis, I.L., Gishen, M. (2005b). Realtionship between sensory analysis and near infrared spectroscopy in Australian Riesling and Chadonnay wines. *Anal. Chim.* **539**, 341-348

Darriet, P., Tominaga, T., Demole, E., Dubourdieu, D. (1993). Evidence of the presence of a 4-mercapto-4-methylpentan-2-one precursor in *Vitis vinefera* Sauvignon blanc grape variety. *C.R. Acad. Sci. Paris.* **316**, 1332-1335

Darriet, P., Tominaga, T., Lavigne, V., Boidron, J.N., Dubourdieu, D. (1995). Identification of a powerful aromatic component of *Vitis vinefera* L. var. Sauvignon blanc wines: 4-mercapto-4-methylpentan-2-one. *Flavour Frag. J.* **10**, 385-392

Del Mar Castineira Gómez, M., Feldmann, I., Jakubowski, N., Andersson, J.T. (2004). Classification of German white wines with certified brand of origin by multielement quatitation and patter recognition techniques. *J Agric.Food Chem.* **52**, 2962 - 2974

Douglas, D., Cliff, M.A., Reynolds, A.G. (2001). Canadian terroir: characterization of Riesling wines from the Niagara Peninsula. *Food Res. Int.* **34**, 559-563

Dubourdieu, D., Tominaga, T., Masneuf, I., Peyrot de Gachons, C., murat, M.L. (2006). The role of yeasts in grape flavour development during fermentation: The example of Sauvignon blanc. *Am. J. Enol. Vitic.* **57**, 81-88

Dufour, É., Letort, A., Laguet, A., Lebecque, A., Serra, J.N. (2006). Investigation of variety, typicality and vintage of French and German wines using front-face fluorescence spectroscopy. *Anal. Chim. Ac.* **536***, 292-299*

Du Plessis, C. (2005). Sauvignon blanc – the dust hasn't settled. *Winelands* [URL. http://www.wineland.co.za/0204suvignon.php3]. Cited the 26th of August 2008

Dunley, J.D., Soole, K.L., Perkins, M.V., Dennis, E.G., Keyzers, R.A., Kalua, C.M., Boss, P.K. (2010). Two *O*-methyltransferases involved in the biosynthesis of mehoxypyrazines: grape-derived aroma compounds important to wine flavour. *Plant Mol. Biol.* **74**, 77-89

Engel, K.H., Tressl, R. (1991). Identification of new sulphur-containing volatiles in yellow passion fruits (*passiflora edulis f. flavicarpa). J Agric. Food Chem.* **39**, 2249-2252

Esbensen, K.H. (2002). (5th ed) Multivariate Data Analysis in practice – an introduction to multivariate data analysis and experimental design CAMO Process AS, Oslo

Falqué, E. Fernández, E., Dubourdieu, D. (2001). Differentiation of white wines by their aromatic index. *Talanta* **54**, 271-281

Francis, I.L., Sefton, M.A., Williams, P.J. (1992). Sensory descriptive analysis of the aroma of hydrolysed precursor fractions from Semillon, Chardonnay and Sauvignon blanc grape juices. *J. Sci. Food Agric.* **59**, 511-520

Francis, I.L., Newton, J.L. (2005). Determining wine aroma from compositional data. *Aust. J. Grape Wine Res.* **11**, 114-126

Guth, H. (1997). Quantitation and sensory studies of character impact odourants of different white wine varieties. *J. Agric. Food Chem.* **45**, 3027−3032

Hartmann, P.J., McNair, H.M., Zoecklein, B.W. (2002). Measurement of 3-alkyl-2-metoxypyrazines by headspace solid-phase microextraction in spiked model wines. *Am. J. Enol. Vitic.* **53**, 285-288

Heymann, H., Noble, A.C., Boulton, R.B. (1986). Analysis of methoxypyrazines in wines. I. Development of quantitative procedure. *J. Agric. food Chem.* **34**, 268-271

Hernández-Orte, P., Cersosimo, M., Loscos, N., Cacho, J., Garcia-Moruno, E., Ferreira, V. (2008). The development of varietal aroma from non-floral grapes by yeasts of different genera. *Food Chem.* **107**, 1064-1077

Holter, G. (2009). The dividing wine. *Meininger's WBI* **3**, 14-16

Hootman, R.C. (1992). Manual on descriptive analysis testing for sensory evaluation. ASTM, Baltimore

Hore, P.J. (1995). Nuclear magnetic resonance. Oxford University Press, Oxford

Howell, K.S., Swiegers, J.H., Elsey, G.M., Siebert, T.E., Bartowsky, E.J., Fleet, G.H., Pretorius, I.S., De Barros Lopes, M.A. (2004). Variation in 4-mercapto-4-methyl-pentan-2-one release by *Saccharomyces cerevisiae* commercial wine strains. *FEMS Microbiol. Lett.* **240,** 125–129

Jackson, R.S. (2002). Wine tasting: A professional handbook. Academic Press, London

Jones, G., Lawson, E., Tuniz, C. (2001). Carbon dating to authenticate the vintage of wines, *Australian Wine Industry Journal*. **16**, 14-15

Kallithraka, S., Arvanitoyannis, I.S., Kefalas, P., El-Zajouli, A., Soufleros, E., Psarra, E. (2001). Instrumental and sensory analysis of Greek wines; implementation of principal component analysis (PCA) for classification according to geographical origin. *Food Chem.* **73**, 501-514

King, E.S., Swiegers, J.H., Travis, B., Francis, I.L., Bastian, S.E.P., Pretorius, I.S. (2008) Coinoculated fermentations using *Saccharomyces* yeasts affect the volatile composition and sensory properties of *Vitis vinifera* L. cv. Sauvignon blanc wines. *J. Agric. Food Chem.* **56**, 10829–10837

Kontkanen, D., Reynolds, A.G., Cliff, M.A., King, M. (2005). Canadian terroir: sensory characterization of Bordeaux-style red wine varieties in the Niagara Peninsula. *Food Res. Int.* **38**, 417-425

Kosir, I.J., Kidric, J. (2002). Use of modern nuclear magnetic resonance spectroscopy in wine analysis: determination of minor compounds. *Anal. Chim. Ac.* **458**, 77-84

Kotseridis, Y., Anocibar Beloqui, A., Doazan, J.P. (1998). An analytical method for studying the volatile compounds of Merlot noir clone wines. *Am. J. Enol. Vitic.* **49**, 44-48

Kment, P., Milhaljevic, M., Ettler, V., Sebek, O., Strnad, L., Rohlová, L. (2005). Differentiation of Czech wines using multielement composition – A comparison with vineyard soil. *Food Chem.* **91**, 157- 165

Lacey, M.J., Allen, M.S., Harris, R.L.N., Brown, W.V. (1991). Methoxypyrazines in Sauvignon blanc grapes and wine. *Am. J. Enol. Vitic.* **42**, 103-108

Lea, P., Næs, T., Rødbotten, M. (1997). Analysis of variance for sensory data, John Wiley & Sons Inc., New York.

Liquor products act, Act 60 (1989). Government gazette of the Republic of South Africa. [URL: http://www.sawis.co.za/winelaw/southafrica.php]. Cited the 15th of October 2009

Louw, L., Roux, K., Tredoux, A., Oliver, T., Naes, T., Nieuwoudt, H., Van Rensburg, P. (2009). Characterization of selected South African young cultivar wines using FTMIR spectroscopy, gas chromatography, and multivariate data analysis. *J. Agric. Food Chem*. **57**, 2623-2632

Mansfield, A.K., Vickers, Z.M., (2009). Characterisation of the aroma of red Frontenac table wines by descriptive analysis. *Am. J. Enol. Vitic.* **60**, 435-441

Marais, J. (1994). Sauvignon blanc cultivar aroma – a review. *S. Afr. J. Enol. Vitic.* **15**, 41-45

Marais, J. (1998). Effect of grape temperature, oxidation and skin contact on Sauvignon blanc juice and wine composition and wine quality. *S. Afr. J. Enol. Vitic.* **19**, 10-16

Marais, J. (2010). Introduction to publications from the investigation into the aroma databases of different South African Cultivars. *Winelands* [URL. http://www.wineland.co.za/index.php?option=com_zine&view=article&id=81:introduction-to-publications-from-the-investigation-into-the-aroma-databases-of-different-south-african-cultivars&q=sauvignon]. Cited the 3rd of December 2010

Mardones, C., Hitschfield, A., Contreras, A., Lepe, K., Gutiérrez, L., Von Baer, D. (2005). Comparison of shikimic acid determination by capillary zone electrophoresis with direct and indirect detection with liquid chromatography for varietal differentiation of red wines. *J. Chromatogr. A* **1085**, 285-292

Marengo, E., Aceto, M., Maurino, V. (2001). Classification of Nebbiolo-based wines from Piedmont (Italy) by means of solid-phase microextraction-gas chromatography-mass spectrometry of volatile compounds. *J.* Chromatogr. A **943**, 123-137

Masoum, S., Bouveresse, D.J-R., Vercauteren, J., Jalali-Heravi, M., Ruthledge, D.N. (2006). Discrimination of wines based on 2D NMR spectra using learning vector quantization neural network and partial least squares discriminant analysis. *Anal. Chim. Ac.* **558**, 144-149

Meilgaard, M.C., Civille, G.V., Carr, B.T. (2007). (4th Ed.) Sensory evaluation techniques, CRC Press, New York

Mestres, M., Busto, O., Guash, J. (2000). Analysis of organic sulphur compounds in wine aroma. *J. Chromatogr. A* **881**, 569-581

Murat, M-L., Masneuf, I., Darriet, P., Lavigne, V., Tominaga, T., Dubourdieu, D. (2001). Effect of *Saccharomyces cerevisiae* yeast strains on the liberation of volatile thiols in Sauvignon blanc wine. A*m. J. Enol. Vitic.* **52**, 136-139

Murray, K.E., Whitfield, F.B. (1975). The occurrence of 3-alkyl-2-methoxypyrazines in raw vegetables. *J. Sci. Food. Agric.* **26**, 973-986

Næs, T. Isaksson, T., Fearn, T., Davies, T. (2002). A User-friendly Guide to Multivariate Calibration and Classification. NIR Publications, Chichester, UK

Nunez, M., Pena, R.M., Herrero, C., Garcia-Martín, S. (2000). Analysis of some metals in wine by means of capillary electrophoresis. Application to the differentiation of Ribeira Sacra Spanish red wines. *ANLSCY* **28**,97-101

Oliveira, J.M, Faria, M., Sá, F., Barros, F., Araújo, I.M. (2006). C6-alcohols as varietal markers for assessment of wine origin. *Anal. Chim. Ac.* **563**, 300-309

OPUS (2006). OPUS Spectroscopy software User Manual version 6, Bruker OPTIK, Ettlingen, Germany [URL. http://www.brukeroptics.com]. Cited the 3rd of October 2010

Parr, W.V., Green, J.A., White, K.G., Sherlock, R.R. (2007). The distinctive flavour of New Zealand Sauvignon blanc sensory characterisation by wine professionals. *Food Qual. Prefer.* **18**, 849-861

Patz, C.D., Blieke, A., Ristow, R., Dietrich, H. (2004). Application of FT-MIR spectroscopy in wine analysis. *Anal. Chim.* **513**, 81-89

Penza, M., Cassano, G. (2004a). Chemometric characterization of Italian wines by thin-film multisensors array and artificial neural networks. *Food Chem.* **86**, 283-296

Penza, M., Cassano, G. (2004b). Recognition of adulteration of Italian wines by thin-film multisensor array and artificial neural networks. *Anal. Chim. Ac.* **509**, 159-177

Pereira,G., Gaudillére, J.P., Van Leeuwen, C., Hilbert, G., Lavialle, O., Maucourt, M., Deborde, C., Moing, A., Rolin, D. (2005). ^{1}H NMR and chemometrics to characterize mature grape berries in four wine-growing areas in Bordeaux-France. *J. Agric. Food Chem.* **53**, 6382-6389

Pérez-Magarino, S., Ortega-Heras, M., González-San José, M.L. (2002). Multivariate classification of rosé wines from different Spanish protected designations of origin. *Anal. Chim. Ac.* **458**, 187-190

Peyrot des Gachons, C., Tominaga, T., Dubourdieu, D. (2000). Measuring the aromatic potential of *Vitis vinefera* L. cv. Sauvignon blanc grapes by assaying S-cysteine conjugates, precursors of the volatile thiols responsible for their varietal aroma. *J. Agric. Food Chem.* **48**, 3387-3391

Peyrot des Gachons, C., Tominaga, T., Dubourdieu, D. (2002a). Localization of S-cysteine conjugates in the berry: Effect of skin contact on aromatic potencial of *Vitis vinefera* L. cv. Sauvignon blanc must. *Am. J. Enol. Vitic.* **53**, 144-146

Peyrot des Gachons, C., Tominaga, T., Dubourdieu, D. (2002b). Sulphur aroma precursor present in S-gluthathione conjugate form: identification of S-3-(Hexan-1-ol)-gluthione in must from *Vitis vinefera* L. cv. Sauvignon blanc. *J. Agric. Food Chem.* **50**, 4076-4079

Peyrot des Gachons, C., Van Leeuwin, C., Tominaga, T., Soyer, J-P., Gaudillère, J-P, Dubourdieu, D. (2005). Influence of water and nitrogen deficit on fruit ripening and aroma potential of *Vitis vinefera* L. cv. Sauvignon blanc in field conditions. *J.Sci. Food Agric.* **85**, 73-85

Preston, L.D., Block, D.E., Heymann, H., Soleas, G., Noble, A.C., Ebeler, S.E. (2008). Defining vegetal aromas in Cabernet Sauvignon using sensory and chemical evaluations. *Am. J. Enol. Vitic.* **59**, 137-145

Rajchl, A., Cizkova, H., Voldrich, M.,m Lukesova, D., Panovska, Z. (2009). Methoxypyrazines in Sauvignon blanc wines, detection of addition of artificial aroma. *Czech J. Food Sci.* **27**, 259-266

Rankine, B.C. (1998). Making good wine: a manual of winemaking practice for Australia and New Zealand. Pan Macmillan Australia Pty Ltd, Sydney, Australia

Roujou de Boubee, D.,Cumsille, A.M., Pons, M., Dubourdieu, D. (2002). Location of 2-methoxy-3-isobutylpyarzine in Cabernet Sauvignon grape bunches and its extractability during vinification. *Am. J. Enol. Vitic.* **53**, 1-5

Roussel, S., Bellon-Maurel, V., Roger, J.M., Grenier, P. (2003). Authenticating white grape must variety with classification models based on aroma sensors, FT-IR and UV spectrometry, *Chemom. Intell. Lab. Syst.* **65**, 209-219

Sala, C., Mestres, M., Martí, M.P., Busto, O., Guasch, J. (2002). Headspace solid-phase microextraction analysis of 3-alkyl-2-methoxypyrazines in wines. J. Chromatogr. A. **953**, 1-6

Santos, J.P., Arroyo, T., Aleiandre, M., Lozano, J.,Sayago, I., García, M., Fernández, M.J., Arés, L., Gutiérrez, J., Cabellos, J.M., Gil, M., Horrillo, M.C. (2004). A comparative study of sensor array and GC-MS: application to Madrid wines characterization, *Sens. Actuators B. Chem.* **102**, 299 – 307

Santos, J.P., Fernández, M.J., Fontecha, J.L., Lozano, J., Aleiandre, M., García, M., Gutierrez, J., Horrillo, M.C. (2005). SAW sensor array for wine discrimination, *Sens. Actuators B. Chem.* **107**, 291 – 295

Schneider, R., Kotseridis, Y., Ray, J-L., Augier, C., Baumes, R. (2003). Quantitative determination of sulphur-containing wine odorants at sub parts per billion levels. 2. Development and application of a stable isotope dilution assay. *J. Agric. Food Chem.* **51**, 3243-3248

SAWIS. (2008). South African wine industry information and systems. [URL. http://www.sawis.co.za/info/statistics.php]. Cited the 10th of December 2009

SAWIS. (2010). South African wine industry information and systems. [URL. http://www.sawis.co.za/info/statistics.php]. Cited the 05th of December 2010

Schlesier, K., Fauhl-Hassek, C., Forina, M., Cotea, V., Kocsi, E., Schoula, R., Van Jaarsveld, F., Wittkowski, R. (2009). Characterisation and determination of the geographical origin of wines. Part I: Overview. *Eur. Food Res. Technol.* **230,** 1-13

Schlosser, J., Reynolds, A.G., King, M., Cliff, M. (2005). Canadian terroir: sensory characterization of Chardonnay in the Niagara Peninsula. *Food Res. Int.* **38**, 11-18

Sefton, M.A., Francis, I.L., Williams, P.J. (1994). Free and bound volatile secondary metabolites of Vitis Vinefera grape cv. Sauvignon blanc. *J. Food Sci.* **59**, 142-147

Sharma, S., Applied multivariate techniques (1996). Wiley & Sons, Inc., New York, 58-82

Sun, D.W. (2008). Modern Techniques for Food Authentication. Elsevier, Dublin, Ireland, 1-26

Swanepoel, M., Du Toit M., Nieuwoudt, H.H. (2007). Optimisation of the quantification of total soluble solids, pH and titrable acidity in South African must using Fourier transform mid-infrared spectroscopy. *S. Afr. J. Enol. Vitic.* **28**, 140-149

Swiegers, J.H., Bartowsky, E.J., Henschke, P.A., Pretorius, I.S. (2005). Yeast and bacterial modulation of wine aroma and flavour. *Aust. J. Grape Wine Res.* **11**, 139-173

Swiegers, J.H., Francis, I.L., Herderich, M.J., Pretorius, I.S. (2006). Meeting consumer expectations through management in the vineyard and winery: the choice of yeast for fermentation offers great potential to adjust the aroma of Sauvignon Blanc wine. *Aust. N.Z. Wine Ind. J.* **21,** 34–42

Swiegers, J. H., Kievit, R. L., Siebert, T., Lattey, K. A., Bramley, B. R., Francis, I. L., King, E. S., Pretorius, I. S. (2009) The influence of yeast on the aroma of Sauvignon Blanc wine. *Food Microbiol*. **26**, 204-211

Tominaga, T., Furrer, A., Henry, R., Dubourdieu, D. (1998a). Identification of new volatile thiols in the aroma of *Vitis vinifera* L. var. Sauvignon blanc wines. *Flavour Frag. J*. **13**, 159-162

Tominaga, T., Peyrot des Gachons, C., Dubourdieu, D. (1998b). A new type of flavour pre-cursors in *Vitis vinefera* L. cv. Sauvignon blanc: S-cysteine conjugates. *J. Agric. Food Chem.* **46**, 5215-5219

Tominaga, T. Murat, M-L., Dubourdieu, D. (1998c). Development of a method for analysing the volatile thiols involved in the characteristic aroma of wines made from *Vitis vineferal* L. cv. Sauvignon blanc. *J. Agric. Food Chem.* **46**, 1044-1048

Tominaga, T., Baltenweck-Guyot, R., Peyrot des Gachons, C., Dubourdieu, D. (2000a). Contribution of volatile thiols to the aromas of white wines made from several *Vitis vinefera* grape varieties. *Am. J. Enol. Vitic.* **51**, 178-181

Tominaga, T., Dubourdieu, D. (2000b). Identification of cysteinylated aroma precursors of certain volatile thiols in passion fruit juice. *J. Agric. Food Chem.* **48**, 2874-2876

Timm, N. H. (2002). Applied Multivariate Analysis. Springer-Verlag, New York, 445-477

Versari, A., Parpinello, G.P., Mattioli, A.U., Galassi, S. (2008). Determination of grape quality at harvest using Fourier-Transform mid-infrared spectroscopy and multivariate analysis. *Am. J. Enol. Vitic.* **59**, 317-322

Vilanova, M., Sieiro, C. (2006). Determination of free and bound terpene compounds in Albariño wine. *J. Food Compos. Anal.* **19**, 694-697

Wampfler D.J., Howell, G.S. (2004). Simplified Method for Detection and Quantification of 2-Methoxy-3-isobutylpyrazine in wine. *Am. J. Enol. Vitic.* **55,** 276-278

Willard, H.H., Merritt, L.L. Jr., Dean, J.A., Settle, F.A. Jr. (1988). Instrumental methods of analysis, 7th Ed. Wadsworth Publishing Company, California. 580-610

Zoecklein, B.W., Fugelsang, K.C., Gump, B.H., Nury, F.S. (1995). Wine analysis and production, Chapman and Hall, New York

Chapter 3

Research results

Detection of Sauvignon blanc adulteration using Infrared spectroscopy and chemometric techniques

Chapter 3: Research Results

Detection of Sauvignon blanc adulteration using Infrared spectroscopy and chemometric techniques

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Abstract

The potential of three different Fourier transform infrared (FT-IR) spectroscopy instruments, used in the South African wine industry, to discriminate between adulterated and control Sauvignon blanc wines were evaluated. The results showed that spectra obtained from a mid infrared (MIR) transmission, a MIR attenuated reflection and a near infrared (NIR) reflection instrument are able to predict statistical differences between flavoured and control wines, made from grapes harvested from the same vineyard, during the same vintage using multivariate data analysis. A liquid probe was used with a NIR reflection instrument, no degassing was performed prior to analysis. Degassing was performed on wine samples prior to analysis with MIR instrumentation. The main compounds in flavourings contributing to the desired aroma could be identified using gas chromatography–mass spectrometry (GC-MS). Discrimination between wines subjected to skin contact for 24 hours and wines made from juice directly pressed after destemming could be observed using FT-MIR transmission spectroscopy. Differences between control wines and wines spiked at three different levels (high, medium, low concentrations of the main aroma compound) not subjected to skin contact could be observed with FT-MIR and NIR models even though discrimination between control and low level spiked wines could not be observed with descriptive sensory analysis data. This study demonstrated the potential of FT-IR analysis to be developed further as a technique for bulk screening for adulterated wines.

3.1 Introduction

Authenticity of wines is an important matter with regards to fair trade, legislation and consumer satisfaction, both locally and globally, for example adding flavourings to wine and selling it as a certified cultivar pure wine is illegal (Liquor products act, 1989). Authentication of wines include authentication in terms of labelling information as well as the chemical composition in terms of chemicals added to the wine. Information on the label of a wine is subjected to legal requirements and connected to a certain expectation by the customer in terms of quality and sensory characteristics of that wine. Cultivar, vintage, origin, the addition of flavourings and the non-authorised addition of sugars, water, certain preservatives and chemicals require authentication testing. Wine authentication is the confirmation by means of analysis that the information on the label is correct and legal practices were followed during winemaking with regards to the chemicals added to the wine. Analyses associated with authenticity testing frequently give rise to more than one variable. Multivariate statistics have lately been applied to authenticity testing data sets in order to analyse more than one variable simultaneously,

investigating the correlation between the variables as well as the relationship between the samples analysed and the variables obtained during analysis (Schlesier *et al*., 2009).

In the South African wine industry Sauvignon blanc wines have been adulterated by adding methoxypyrazine-containing flavourings to the wines or juice before fermentation to increase the green character of the wines (Du Plessis, 2005; Rajchl *et al.*, 2009; Marais, 2010). Methoxypyrazines (MP) are important compounds in Sauvignon blanc wines contributing to green flavours described as green pepper, asparagus and green grass. It is known that these compounds have odour threshold values of 1 to 2 ng/L. Therefore the odour caused by these compounds can be detected in wines at concentration above 1 to 2 ng/L. Globally methoxypyrazines occur in Sauvignon blanc wines on average between 5 to 35 ng/L. In South African and Australian wines, levels of 5 to 15 ng/L have been recorded (Allen *et al.,* 1991; Lacey *et al.*, 1991; Marais *et al.*, 1994; Alberts *et al.*, 2009).

GC-MS analysis is commonly used to quantify the concentrations of methoxypyrazines in wines (Lacey *et al.,* 1991; Allen *et al.*, 1994, Kotseridis *et al.,* 1998; Hashizume and Samuta*,* 1999; Kotseridis *et al.*, 1999; Roujou De Boubee *et al.*, 2000; Sala *et al.,* 2002; Hartmann *et al.*, 2003; Ryan *et al.,* 2005). A liquid chromatography-mass spectrometry (LC-MS) method for the quantification of methoxypyrazines in wines has been developed by Alberts *et al.* (2009). Currently the detection of Sauvignon blanc adulteration in South Africa is conducted by measuring methoxypyrazine concentrations in the wines and grape juice. Methoxypyrazine levels are evaluated to be realistic in terms of individual quantities of 3-isobutyl-2-methoxypyrazine, 3-isopropyl-2-methoxypyrazine and 3-*sec*-2-methoxypyrazine as well as the ratio's between them by comparing analysis to an existing database (Marais, 2010). The analytical techniques used for the quantification of the methoxypyrazines are methods developed by Kotseridis *et al.* (1998) and Alberts *et al.* (2009). Adulteration of these wines by the addition of flavourings containing methoxypyrazines to wine in much higher quantities than those occurring naturally in wine can be identified by GC-MS and LC-MS analysis of the wine. Three possible drawbacks using GC-MS and LC-MS to identify adulterated wines are: GC-MS and LC-MS are relatively expensive methods and it is not viable to perform these experiments as routine analysis on hundreds of wines. Flavourings other than methoxypyrazines added will not be detected, since only the methoxypyrazine are analysed for.

The use of FT-IR analysis for wine authentication is expanding as a research field (Arvanitoyannis, 1999; Roussel *et al.*, 2003; Cozzolino *et al.,* 2009). FT-IR analysis on wine for the determination of chemical parameters have been shown to be an accurate method for the quantification of various analytes (Manley *et al.,* 2001; Roussel *et al.*, 2003; Blanco *et al.,* 2004; Patz *et al.*, 2004; Versari *et al.*, 2008). Analytical instrumentation commonly used for routine analysis of juice and wine are: the FOSS WineScan, Bruker Alpha, mid infrared spectrometers, and Bruker MPA, near infrared spectrometer. These instruments are specially designed for industrial applications of IR analysis (Swanepoel, 2007). Large amounts of data with more than one variable per sample (wavenumbers) are generated performing FT-IR analysis.

Principal component analysis (PCA), a multivariate statistical technique, can be used to identify correlation and variation between different objects (samples) by looking at numerous variables simultaneously. Correlation and variation between different samples can be observed by dimensionality reduction of the variable space in such a way that a new set of variables is generated that only describe the most significant variance between samples. This new set of variables is called principal components (PC's). The effect of this technique is that variance due to numerous variables can be expressed in terms of only a few variables and can be visualized and interpreted easily (Esbensen, 2002; Naes *et al.,* 2002).

The objective of this study was to investigate the possibility to discriminate between flavoured Sauvignon blanc wines and non-flavoured Sauvignon blanc wines using FT-MIR and FT-NIR spectroscopy in conjunction with multivariate statistical analysis techniques, specifically PCA, partial least squares discrimination (PLS-D) and conformity testing. The effect of flavouring on the sensory characteristics of Sauvignon blanc wine using descriptive sensory analysis was also investigated.

3.2 Materials and Methods

3.2.1 Chemicals

Chemicals used for winemaking: pectolytic enzyme (Laffort Oenologies, Cape Town, South Africa), Sodium metabisulfite (SO_2), Diammonium phosphate (DAP), Bentonite (Protea Chemicals, Cape Town, South Africa), clinitest (Link Pharmacies, Stellenbosch, South Africa).

Chemicals used for CG-MS analysis: NaCl (Sodium chloride from Saarchem, Merck, Johannesburg, South Africa), 2-octanol (Sigma-Aldrich, Munich, Germany) at a final concentration of 300 ng/L was used. Helium (Afrox, Cape Town, South Africa).

Flavourings used for wine spiking: (1) fresh green pepper juice; (2) green grass flavouring for wine (E207047, VMF Mane, Cape Town, South Africa); (3) asparagus flavouring for wine (E201817, VMF Mane, Cape Town, South Africa); (4) green pepper flavouring for wine (E207044, VMF Mane, South Africa) and; (5) tropical flavouring for food and beverages (L-123910 Gooseberry Flavour, Givaudan, Johannesburg, South Africa). (1) Green peppers were homogenised and left for 30 minutes to settle. The supernatant was used as fresh green pepper flavouring.

3.2.2 Preparation of experimental wines

Sauvignon blanc grapes from the coastal region, specifically Cape Town and Stellenbosch, were used for the preparation of experimental wines. Grapes were harvested by hand at sugar levels of 22 - 24°B and titratable acid concentrations of 6.8 – 7 g/L. Grape juice was inoculated with the *Saccharomyces cerevisiae* VIN7 yeast strain (Anchor Wine Yeast, Cape Town, South Africa) using the standard rehydration procedure for dried yeast.

Experimental Sauvignon blanc wines were prepared using standard winemaking techniques for micro vinification (Figure 3.1). Grapes were destemmed and crushed. Pectolytic enzyme and SO_2 were added. Destemmed grapes were pressed, one hour after addition of SO_2 and pectolytic enzyme, twice in a basket press at 2 bar. Wines subjected to skin contact were prepared in addition to wines pressed directly after destemming to investigate the possibility of skin contact increasing methoxypyrazine concentrations in the grape juice by extraction of methoxypyarzines from skins. Grapes were left on the skins for 24 hours at 15°C before pressed. After pressing the juice was clarified overnight at -4°C, transferred to 20L buckets and flavourings were added (Table 3.1). The juice was divided into glass bottles (4.5 L) each containing 3 L of juice. DAP was added and juice was inoculated with rehydrated yeast. The final concentration of DAP added was 3 g/L.

Fermentation was carried out at 15°C. Fermentation progress of wines was monitored weighing fermentation vessels daily. After fermentation vessel weights stabilised for three days Clinitest™ tablets were used to estimate residual sugar concentrations of wines. Wines with estimated residual sugar concentrations below 5 g/L were regarded as completed since wines with residual sugar concentrations below 5 g/L are classified as dry wine. Fermentations took

3 to 4 weeks. Bentonite was added at a final concentration of 0.6 g/L as fining agent. The SO_2 levels of the wines were adjusted to 60 mg/L after fermentation ended. Wines were cold stabilized at -4°C for 2 weeks, filtered and bottled in dark green tinted 750 mL bottles (Consol XPRS, Bellville, South Africa) with screw caps (MCG industries, Cape Town, South Africa). Bottled wines were stored for no longer than three months at 15°C until analysed.

The whole winemaking process followed for the preparation of wines was repeated a day later with grapes from the same vineyard. All wines were prepared in triplicate (three fermentation repeats, A, B and C).

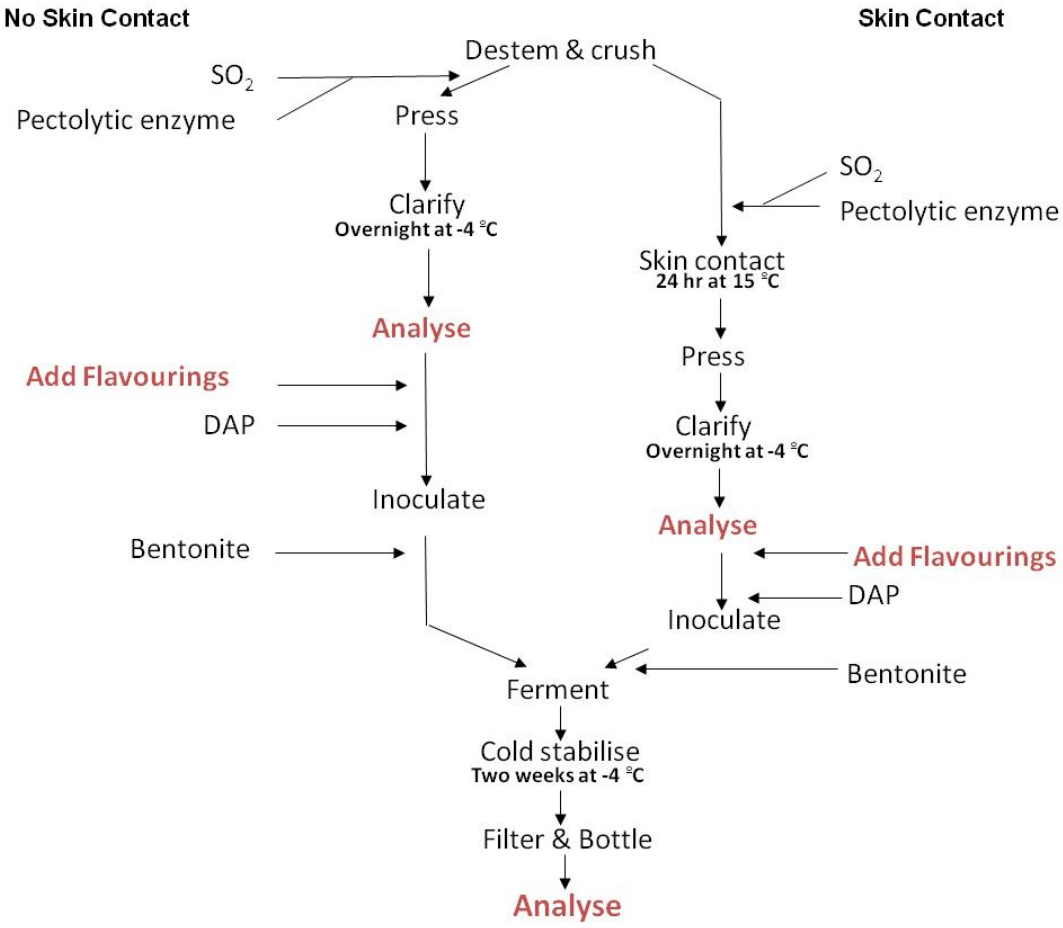

Figure 3.1 Flow diagram of standard micro vinification practices followed during the preparation of small scale wines for this study.

3.2.3 Characterisation of flavourings and addition to grape juice and wine

3.2.3.1 GC-MS analysis of flavourings

GC-MS analysis was performed on flavourings to determine the main aroma compound of the specific flavouring. The concentrations of the main aroma compounds of the different flavourings were estimated. Quantification of *Z*-3-hexenol and *E*-3-hexenol was performed on the green grass flavouring. The methoxypyrazines, 3-isopropyl-2-methoxypyrazine (IPMP), 3-sec-butyl-2-methoxypyrazine (SBMP) and 3-isobutyl-2-methoxypyrazine (IBMP) were quantified in all the above mentioned flavourings by an ISO17025 accredited laboratory (Vinlab, Stellenbosch, South Africa).

3.2.3.2 Spiking of experimental wines

Fresh green pepper was used for spiking of wines, since fresh green pepper was used for adulteration by a well known wine producer leading to the Sauvignon blanc adulteration scandal. Commercially available flavourings, indicating on the label that it can be used as a wine additive, were also used for spiking. The above mentioned flavourings are therefore believed to be representative of those possibly being used for adulteration of Sauvignon blanc wines produced in South Africa. Five different flavourings were used; fresh green pepper, green pepper flavouring, asparagus flavouring, grassy flavouring and tropical flavouring (Table 3.1). Experimental wines were spiked by adding a single flavouring to a wine.

Two experimental batches were prepared. 1. Flavourings were added to experimental wines subjected to skin contact, experimental wines not subjected to skin contact and commercial wines prepared from the same vineyard as experimental wines. Flavourings were only added at one concentration (see 3.2.3.3 for specific concentrations). Sensory analysis was conducted to determine spiking levels of flavourings. A wine with a strong green pepper aroma was compared to wines spiked with different concentrations of commercial green pepper flavouring, fresh green pepper extract, commercial asparagus flavouring and commercial green grass flavouring. The corresponding concentration was multiplied by 10 and used as spiking concentration. A single flavouring per wine was added to a fermentation vessel. A control wine, with no flavourings added, was also prepared. Commercial green pepper flavouring, fresh green pepper extract, commercial asparagus flavouring and commercial green grass flavouring were used, resulting in 12 different spiked wines as well as three different control wines.

2. Flavourings were added to experimental wines not subjected to skin contact at three different levels, low, medium and high. The estimated concentration of the main aroma compound, determined by GC-MS analysis, in comparison with the natural abundance of the aroma compound in wine was used to calculate the amount of flavouring substance added to the grape juice. Main aroma compounds were only identified for fresh green pepper (IBMP), commercial asparagus flavouring (IBMP) and commercial green grass flavouring (*Z*-3-hexenol). Sensory analysis was conducted to determine spiking levels of commercial tropical flavouring. A wine with a strong tropical aroma was compared to wines spiked with different concentrations of commercial tropical flavouring to determine low, medium and high spiking levels. Flavourings were added to grape juice of experimental wines before inoculation with the yeast culture (Table 3.1). Including the control wine, 13 different wines were prepared.

Table 3.1 Different treatments applied to Sauvignon blanc juice prior to inoculation with *S. cerevisiae* VIN7 for production of experimental wines.

Flavouring	Final Concentrations [mL/L]		
	Low	Medium	High
Control – none	N/A[a]	N/A	N/A
Green pepper (fresh, homogenised)	0.4	1.2	4.0
Asparagus[b]	0.2	0.8	4.0
Green grass[b]	2.0	10	20
Tropical[b]	0.2	1.0	4.0

[a]N/A = not applicable, [b]Brand name of commercial flavourings

3.2.3.3 Spiking of commercial wines

Commercial wines from the coastal region, specifically Groot Constantia, Cape Town, were spiked. A single flavouring per vessel at only one concentration was used, 6.7mL/L fresh homogenised green pepper, 5.0mL/L commercial green pepper flavouring, 4.0mL/L commercial asparagus flavouring and 5.0mL/L commercial green grass flavouring. Three bottles of each commercial wine were mixed and poured into two, one litre bottles. Wine was transferred to 250 mL volumetric flasks. Flavourings were added to the commercial wines, one flavouring per sample in duplicate.

3.2.4 Spectroscopic analysis of wines

3.2.4.1 Degassing of wine samples

The quality of spectra obtained by the MIR transmission spectroscopy using the FOSS WineScan and MIR attenuated reflection spectroscopy using the Bruker Alpha instruments is highly sensitive to the levels of CO_2. In Sauvignon blanc wines CO_2 levels above 1000 mg/L was observed. Reduction of the CO_2 levels below or close to 200 mg/L was required prior to analysis by degassing. Two methods for degassing were investigated: 1. Sonication; 2. Applying vacuum to 50 mL of wine in a 500 mL Erlenmeyer. Degassing by means of sonication were performed by shaking 50 mL of wine for 1 minute, incubating it in an ultrasonic water bath for 5 minutes and shaking it again for 1 minute. The success of reducing CO_2 in wine using a vacuum pump was investigated by connecting a vacuum pump to a 500 mL Erlenmeyer containing 50 mL of wine. Vacuum for 30 s, 1 min and 2 min were applied. The concentration of CO_2 was measured using the must under fermentation calibration (purchased with the instrument from FOSS Analytical, Hillerød, Denmark) on the FOSS WinesScan. Similar studies were conducted by Malherbe *et al.* (2007) and Osborne (2007). Wines were degassed using the method that reduced the levels of CO_2 effectively and consistently below 200 mg/L. No sample preparation was performed prior to NIR reflection spectroscopy analysis with the Bruker MPA NIR instrument with a liquid probe.

3.2.4.2 FT-MIR transmission spectroscopy

FT-MIR transmission spectra were obtained by a WineScan FT 120 with a Michelson interferometer (FOSS Analytical, Hillerød, Denmark). Duplicate spectra were acquired within the mid-infrared spectral range of 4992.25 to 929.778 cm^{-1} for each sample. Samples were degassed prior to analysis. Samples were heated to 40°C and passed through a 37 μm CaF_2-lined cuvette. FOSS S-6060 zeroing liquid was used as background (WineScan FT 120 Reference Manual, FOSS Analytical, Denmark, 2001).
The spectral data obtained from the FOSS WineScan analysis were analysed using Microsoft Office Excel and Unscrambler 9.2 software (Camo, 2005).

3.2.4.3 FT-MIR attenuated total reflection spectroscopy

The Bruker Alpha P-modul spectrometer with a diamond crystal, Michelson interferometer and tri-glycine sulphate (DTGS) detector was used to acquire attenuated total reflection spectra within the mid-infrared range, 373.1 – 3995 cm^{-1}. The interferometer consists of a gold plated cube corner mirror and KBr beamsplitter. A temperature control unit, operating at 40 ^{o}C was fitted to the 2 x 2 mm diamond crystal used to focus the IR light (Bruker Alpha P-modul Reference Manual, Bruker OPTIK, Ettlingen, Germany). The same degassing protocol as used

prior to analysis with the WineScan on samples was used. OPUS spectroscopy software (OPUS, 2006) was used to perform spectral pre-processing and conformity testing.

3.2.4.4 FT-NIR reflection spectroscopy

The Bruker MPA was used to generate NIR transmission spectra with a liquid Probe by inserting the probe directly into the opened wine bottle; no degassing was performed prior to analysis. The instrument consists of a Michelson interferometer with a gold plated cube corner mirror and CaF_2 beamsplitter (Bruker MPA Reference Manual, Bruker OPTIK, Ettlingen, Germany). The alignment of the instrument is established by means of a He-Neon red laser. Capturing of the light reflected by the sample and generation of the interferogram is conducted by means of a PbS gold plated integrated sphere and InGaAs semiconductor served as detector. The interferogram is converted from the time domain to the frequency domain by means of Fourier transform and reported as absorbances corresponding to wavenumbers between 3999.7 and 12498.4 cm^{-1}. OPUS spectroscopy software (OPUS, 2006) was used to perform spectral pre-processing and conformity testing.

3.2.5 Chromatographic analysis of wines

3.2.5.1 GC-MS analysis of wines

GC-MS analysis was conducted in order to quantify the concentrations of IBMP of control wines and experimental and commercial wines flavoured with a single concentration of flavouring. A single sample per fermentation repeat prepared was analysed. The average was calculated for the three fermentation repeats. An in-house method developed by KWV, Paarl, South Africa was used.

Headspace solid-phase dynamic extraction (HS-SPDE) was performed. Sample volumes of 10 mL wine in 20 mL glass vials with PTFE-teflon septa screw-caps (La-Pha Park, Langerwehe, Germany) were used. In order to decrease the water solubility and increase the amount of the volatile compounds in the headspace of the vile, 2.5 g NaCl and a small stir bar was added. An internal standard, 2-octanol at a final concentration of 300 ng/L was added to the sample after addition of the NaCl and wine.

A 10 minute pre-equilibration was performed to achieve equilibrium between the liquid phase of the sample and the headspace. Extraction was performed using a 74 mm, 90% polydimethylsiloxane / 10% activated carbon (PDMS/AC) coated needle (Chromsys, Alexandria VA, USA) connected to a 2.5 mL gas-tight syringe. A total of 50 aspiration repeats of 1000 μL at 70 μL/s, during bi-directional continuous stirring at 750 rpm, were performed.

Desorption was performed immediately after extraction by inserting the needle into the sample port (heated at 230°C, splitless mode) and pulling 500 μl helium (Afrox, Cape Town, South Africa) into the syringe, the helium was pumped through the needle into the inlet at 15 mL/s. Post desorption bake-out was applied at 270°C for 10 minutes.

GC-MS analysis was performed using a gas chromatograph (Agilent Technologies, model 6890N, Network GC system, USA) equipped with an autosampler (CombiPal, CTC Analytics, Switzerland) coupled to a mass spectrometer (Agilent Technologies, model 5973 inert, Network GC system, USA) was used. Chemstation version D.01.02.16 software (Agilent technologies, USA) was used for interpretation of the data.

Separation of volatile compounds was achieved by a 30 m x 0.25mm inside diameter x 0.5 μm film thickness J&W DB-WAX capillary column (Agilent Technologies, model 122-7033). Pulsed splitless injection was used. The split vent was closed for 2 minutes. The injector

temperature was held at 230°C and the transfer line at 240°C. The oven temperature was held at 35°C for 2 minutes, increased to 220°C at 5°C/min and kept at that temperature for 6 minutes. The oven was kept at 220°C for 2 min after every run. Helium at constant flow, 0.8 mL/min was used as carrier gas.

The mass spectrometer was set on electron-impact (EI) mode at 70eV. The ion source temperature was set at 230°C and the quadrupole temperature at 150°C. Selective ion monitoring (SIM) mode was used for peak identification. The 45 *m/z* ration, associated with the internal standard 2-octanol, was monitored until the retention time reached 22 minutes and the 124 *m/z* ratio, associated with 2-isobutyl-3-methoxypyrazine, was monitored from when the retention time reached 22 minutes and onwards.

Peak areas were normalised using the peak area of the internal standard. A calibration curve was constructed diluting IBMP in a 12%v/v aqueous alcohol solution. The final concentrations of IBMP used to construct the calibration curve obtaining $R^2 = 0.9998$ were, 3 ng/L, 30 ng/L and 300 ng/L.

3.2.5.2 LC-MS analysis of wines

Quantification of the levels of methoxypyrazines in experimental wines spiked at low, medium and high levels as well as control wines were measured using LC-MS. The LC-MS method for the quantification of methoxypyrazines in wine as describe by (Alberts *et al.*, 2009) was used.

3.2.6 Sensory analysis

Descriptive sensory analysis is commonly used in research concerning wine aroma (Andrews *et al.,* 1990; Bakker *et al.,* 1993; Cozzolino *et al.,* 2005; Lesschaeve*,* 2007). Descriptive sensory analysis was performed on experimental wines. One sample of each of three fermentation repeats prepared for each different spiked wine as well as the control wine were analysed. Commercial wines spiked after fermentation with the same flavourings were also analysed in triplicate.

Sensory analysis was conducted in a well ventilated sensory analysis lab with cubicles at an ambient temperature of 20 ± 2°C. Discussions took place at an open table and were limited to training sessions. No correspondence between judges was permitted during evaluation sessions. Tulip-shaped tasting glasses with 25 ± 5 ml of wine covered with lids to prevent evaporation of volatile compounds were presented randomly, coded with three-digit random numbers. Wines were evaluated orthonasally. Only smelling was permitted and no tasting. All tastings were conducted in triplicate using three different fermentation repeats. Samples were evaluated using sensory attributes decided on during training sessions. Attributes covered flavours generally associated with Sauvignon blanc wine. Each attribute was rated on a 10 cm unstructured line scale, anchored at the extremities with “none” and “intense”. Data acquisition was done by manually measuring the distances on the line scales.

3.2.6.1 Panel Training

Nine external assessors were recruited for this study. They were all frequent moderate white wine drinkers. A trial session showed that panel training was essential for the specific study. The training consisted of three 60 min sessions with a tea break after 30 min and two 30 min sessions. Training was scheduled once a day for a week. One control and six spiked wines were presented per session. Panel members were trained to become familiar with the specific vocabulary. Reference standards (Table 3.2) were presented for each attribute. All standards were freshly prepared before each session less than 30 minutes prior to analysis and

covered in air tight containers when not in use. Panel members had to detect and rank each attribute on a line scale in terms of intensity. During discussion, panel members had to agree on the intensity of each attribute for a specific wine. Attributes which caused confusion and did not contribute to better understanding of the aromatic character of the wines were eliminated. Panel performance was evaluated performing analysis of variance (ANOVA) and PCA on descriptive analysis data. No further training was required.

Table 3.2 Standards used for descriptive sensory profiling of Sauvignon blanc wines used in this study.

Sensory Attribute	Description of Standard
Asparagus (canned)	Canned asparagus salad cuts
	(Koo, Tiger Brands Ltd., Bryanston, South Africa)
Gooseberry	Canned gooseberries
	(Goldcrest, Johannesburg,South Africa)
Green beans (canned)	Canned green beans
	(Koo, Tiger Brands Ltd., Bryanston, South Africa)
Green grass / Grassy	Freshly cut green grass
Green pepper	Freshly cut green pepper
Guava	Fresh ripe guava
Pineapple	Canned pineapple pieces
	(Koo, Tiger Brands Ltd., Bryanston, South Africa)
Granadilla	Freshly cut granadilla (Local Supermarket)
Litchi	Freshly cut litchi (Local Supermarket)
Tropical	Tropical fruit juice (Ceres fruit juices, South Africa)
Vegetative	No standard used

3.2.7 Statistical analysis of data

Discrimination between spiked and non-spiked wines was investigated using multivariate data analysis techniques on different FT-IR spectral data sets. PCA and PLS-D was performed on FT-MIR transmission spectral data obtained from the FOSS WineScan. Dummy variables 1 for control samples and -1 for spiked samples were assigned for PLS-D. The following wavenumbers were excluded from the FT-IR variable sets, since absorbance at these wavenumbers are due to water, gas and instrumental artefacts (1543 – 1716, 2368 – 2299, 2970 – 5011 cm^{-1}). Auto-scaling was applied through mean centring and standardization. The data were standardized by dividing each absorbance associated with a certain wavenumber (variable) by the standard deviation of the absorbances for that wavenumber. Test set and leave one out cross validation was used.

Conformity tests were performed on the data sets from FT-MIR attenuated reflection spectroscopy and FT-NIR reflection spectroscopy instruments using various spectral pre-processing methods: vector normalisation, first derivative computations as well as second derivative computations. Conformity indexes were optimised to obtain the best results. Separate data sets were constructed for data from FT-MIR attenuated reflection spectroscopy and FT-NIR reflection spectroscopy instruments.

A data set containing GC-MS as well as FT-IR data was analysed using PLS-D. FT-IR data was used to construct the **X** data matrix and GC-MS to construct the **Y** data matrix. PLS-D was further used to analyse a data set containing sensory data combined with FT-IR data, FT-IR was used to construct the **X** data matrix and sensory data to construct the **Y** data matrix.
The discrimination ability of the sensory analysis panel and individual performance of assessors were evaluated using PCA and ANOVA. PCA was also used to further explore data. The software packages Panel check and The Unscrambler by CAMO were used.

3.3 Results and Discussion

3.3.1 Estimated levels of main aroma compounds in flavourings

Even though all flavourings, except the tropical flavouring, had a distinct green aroma character typically associated with methoxypyrazines, not all flavourings contained these compounds. Methoxypyrazines could not be detected in the commercially available green pepper flavouring (Table 3.3). Menthol was present as one of the main flavour compounds. This commercial flavouring was only used to flavour wines at one concentration level and was not included as a high, medium and low concentration flavouring due to its contribution to artificial aroma nuances not contributing to the varietal character of Sauvignon blanc wines as discussed in the section on sensory evaluation.

Table 3.3 Concentrations of IBMP, IPMP and SBMP in flavourings used for spiking of experimental wines.

	Concentration in flavouring		
Tested flavouring	**IBMP ng/L**	**IPMP ng/L**	**SBMP ng/L**
Grape juice	19	nd[a]	nd
Fresh Green Pepper juice	76 338	58	322
Tropical[b]	nd	nd	nd
Asparagus[b]	243 223	109	287
Green Grass[b]	18	26	19
Green Pepper[b]	nd	nd	nd

[a]nd = not detected, [b]Brand name of commercial flavourings

The main aroma compound found in the green grass flavouring was *Z*-3-hexenol (Table 3.4 and Figure 3.2). Methoxypyrazines were found to be present at concentrations within the natural abundance range found in wine, however further dilution would eliminate the presence of these compounds in wine above the odour threshold and detection limits of current analytical techniques.

Table 3.4 GC-MS results of the estimated quantification of *Z*-3-hexenol and *E*-3-hexenol in commercial Grassy flavouring used for spiking of experimental wines

Retention time	Compound	Peak area	Concentration in 10 mL (mg/L)	Concentration in flavouring (mg/L)
24.99	2,6-dimethyl-6-hepten-1-ol	19730243	1.00000	N/A[a]
23.75	*Z*-3-hexenol	5661071	0.28692	14.35
23.1	*E*-3-hexenol	71738	0.00364	0.18

[a]N/A = not applicable

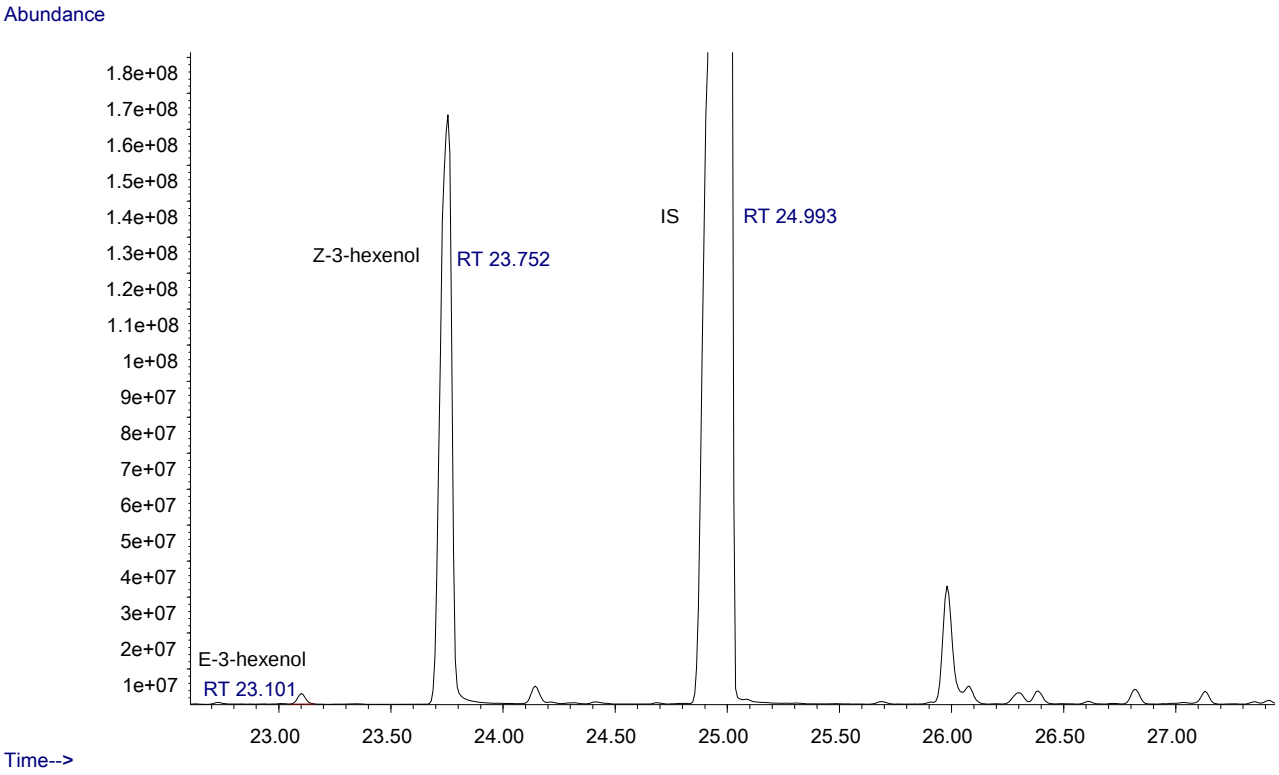

Figure 3.2 GC-MS chromatogram illustrating the presence of *Z*-3-hexenol and *E*-3-hexenol in the commercial green grass (grassy) flavouring used to spike experimentally prepared Sauvignon blanc wines. RT = retention time.

Methoxypyrazines were detected in commercially available asparagus flavouring as well as fresh green pepper extract at high enough concentrations to dilute the substances substantially in order to spike wine and still detect methoxypyrazines at concentrations 20-100 times higher than the natural abundance in wines (Table 3.3). The concentration of methoxypyrazines, IBMP, IPMP and SBMP, observed in the fresh green pepper juice were similar to levels measured in green pepper by Alberts *et al.* (2009).

3.3.2 Levels of methoxypyrazines in spiked and control wines

3.3.2.1 Levels of IBMP in spiked and control wine determined by GC-MS

The highest concentration of IBMP could be observed for wines flavoured with the commercial asparagus flavouring (Table 3.5). The natural abundance of IBMP in Sauvignon blanc wines are between 0 – 30 ng/L (Allen *et al*, 1991; Lacey *et al.,* 1991). Therefore the concentrations measured in wines spiked with commercially available asparagus flavouring during a trial experiment were ten times higher than the highest concentrations found in wines. The

concentrations of IBMP measured in wines spiked with natural green pepper extract fell within the natural abundance range of these compounds in wines. Wines spiked with commercial green pepper flavouring and green grass flavouring did not contain higher levels of IBMP than control wines (Table 3.5 and Figure 3.3). Marais, 1994 showed that skin contact extracted more methoxypyrazines, yielding wines with higher concentration of methoxypyrazines than wines made from juice not subjected to skin contact. In this study skin contact wines did not contain higher levels of IBMP than wine not subjected to skin contact. This could be due to small scale winemaking practises, making fermentation control harder (temperature control during skin contact, oxygen management and UV light management). The observations made that IBMP concentrations did not differ for green grass flavoured, commercial green pepper flavoured and control wines were due to a lack of IBMP in the commercial flavouring.

Table 3.5 Concentrations of IBMP in spiked and non-spiked experimental wines spiked with only one concentration per flavouring.

Flavouring	Average IBMP (ng/L)
Control wine	2.1
Green grass flavoured wine	2.7
Green pepper flavoured wine	2.8
Green pepper extract flavoured wine	27.7
Asparagus flavoured wine	312.1

Analysis were performed in triplicate, averages are shown.

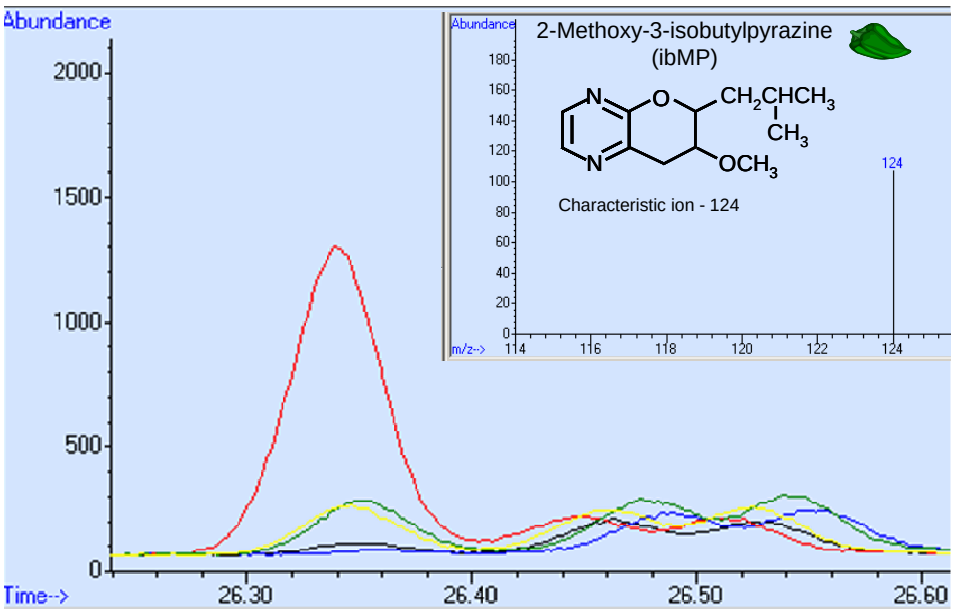

Figure 3.3 GC-MS peaks obtained using selective ion monitoring of the 124 ion for quantification of IBMP from different spiked and control experimental wines. The red line indicates asparagus flavoured wine, the green line and yellow lines natural green pepper extract flavoured wine, the black line green pepper flavoured wine and the blue line control wine.

3.3.2.2 Levels of IBMP, IPMP and SBMP in spiked and control wine determined by LC-MS

Different concentrations of IBMP originating from the same flavouring were studied at low, medium and high levels. The control wine had concentrations of methoxypyrazines typical for South African wines. The concentrations of IBMP in wines flavoured with green pepper extract and asparagus flavouring at a low level fell within the natural abundance levels of IBMP in wines. The medium level green pepper extract spiked wine could also be debated to fall within the natural abundance levels with regards to the concentration of IBMP or just outside. The wines flavoured with a high concentration of green pepper extract and medium concentration of asparagus flavouring had IBMP levels equivalent to ten times the concentration of IBMP in natural wine (Table 3.6). The wine flavoured with a high concentration of asparagus flavouring had a concentration of IBMP of 2 – 100 times the concentration of IBMP in natural wine. Concentrations of IPMP did not vary significantly for wines spiked with flavourings from control wines. Concentrations of SBMP slightly increased as the concentration of the adulterant increased for wines spiked with green pepper extract and asparagus flavouring (Table 3.6).

Table 3.6 LC-MS results of the quantification of IBMP, IPMP and SBMP of finished wines spiked with natural green pepper extract/juice and wines spiked with commercial asparagus flavouring.

Flavouring	IBMP (ng/L)	IPMP (ng/L)	SBMP (ng/L)
Control wine	11	0.53	0.63
Green pepper extract flavoured wine Low level	23	0.60	0.65
Green pepper extract flavoured wine Medium level	67	0.49	0.82
Green pepper extract flavoured wine High level	324	0.99	2.40
Asparagus flavoured wine Low level	42	0.62	0.50
Asparagus flavoured wine Medium level	208	0.60	0.59
Asparagus flavoured wine High level	1004	0.63	0.83

3.3.3 Sensory description of spiked and control wines

A trial session where potential panel members evaluated Sauvignon blanc wines without prior panel training demonstrated the necessity for panel training and can clearly be seen from the line plot (Figure 3.4a and Figure 3.4b). Panel members initially had different ideas about how to score certain perception intensities of specific aroma attributes, using different parts of the line scale. After three training sessions panel members used the unstructured line scale similarly to rate aroma attributes of wines.

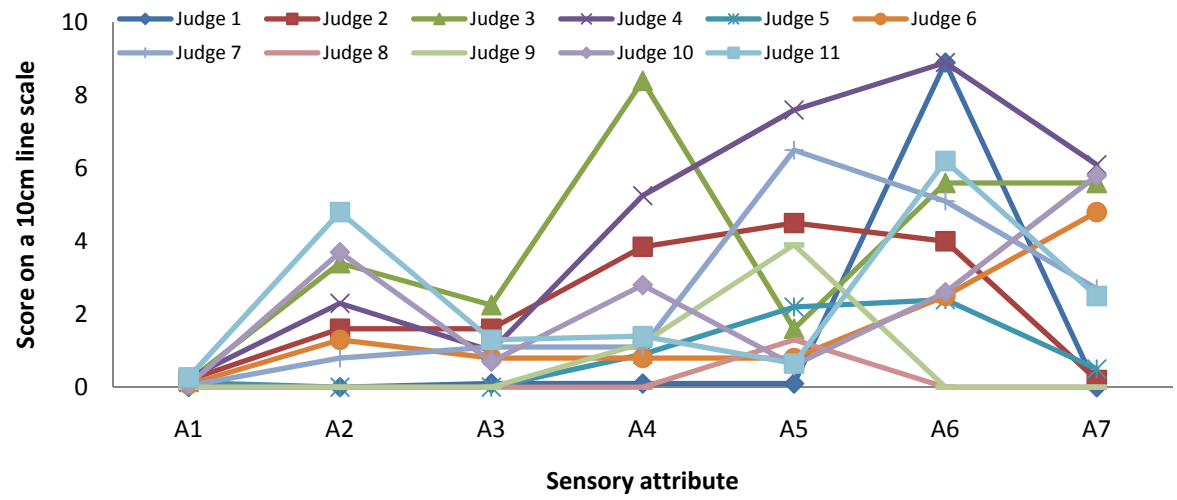

Figure 3.4 (a) Line plot of aroma attributes scored by different judges before and after training The abbreviations used for attributes are: A1, canned asparagus, A2, gooseberry, A3, canned green beans, A4, green grass, A5, green pepper, A6, guava and A7, pineapple.

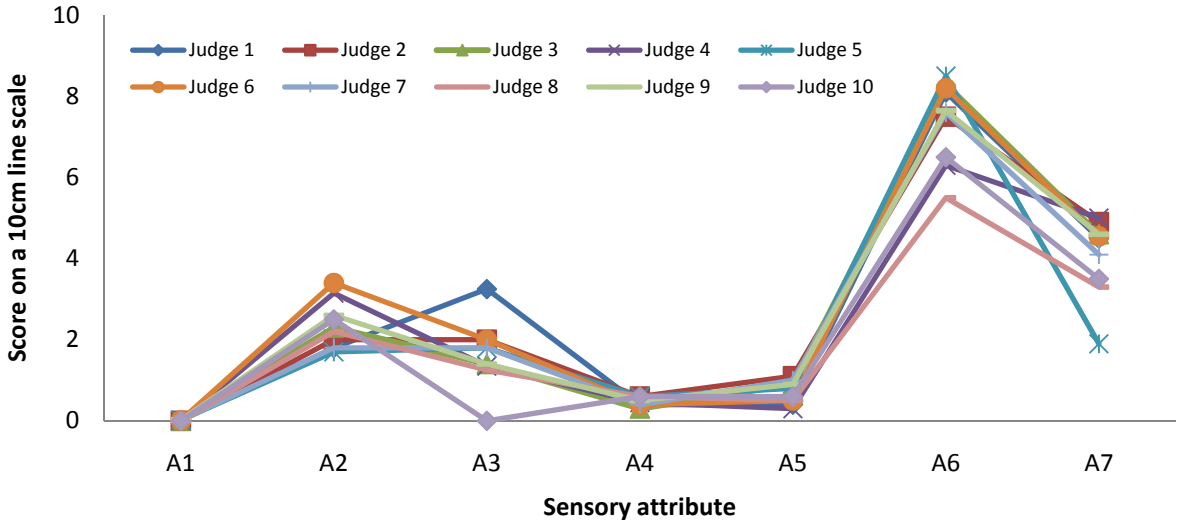

Figure 3.4 (b) Line plot of aroma attributes scored by different judges after training. After training the different panel members used the unstructured line scale similarly to score aroma attributes. The abbreviations used for attributes are: A1, canned asparagus, A2, gooseberry, A3, canned green beans, A4, green grass, A5, green pepper, A6, guava and A7, pineapple.

3.3.3.1 Panel performance

All attributes varied significantly for the wines spiked with different flavourings, $p< 0.001$ for all attributes accept grassy ($p<0.05$) using a 2-way ANOVA, product x assessor interaction (Figure 3.5b). The tucker plot of the tropical attribute is shown (Figure 3.5a). The red border indicates a p value<0.001. Since all judges are plotted close to each other on the tucker plot it can be concluded that judges scored the tropical aroma attribute similarly. Tucker plots for all attributes were constructed. Panel consistency were good except for the grassy attribute and the litchi attribute since $p<0.05$ for the product x assessor interaction using a two way ANOVA.

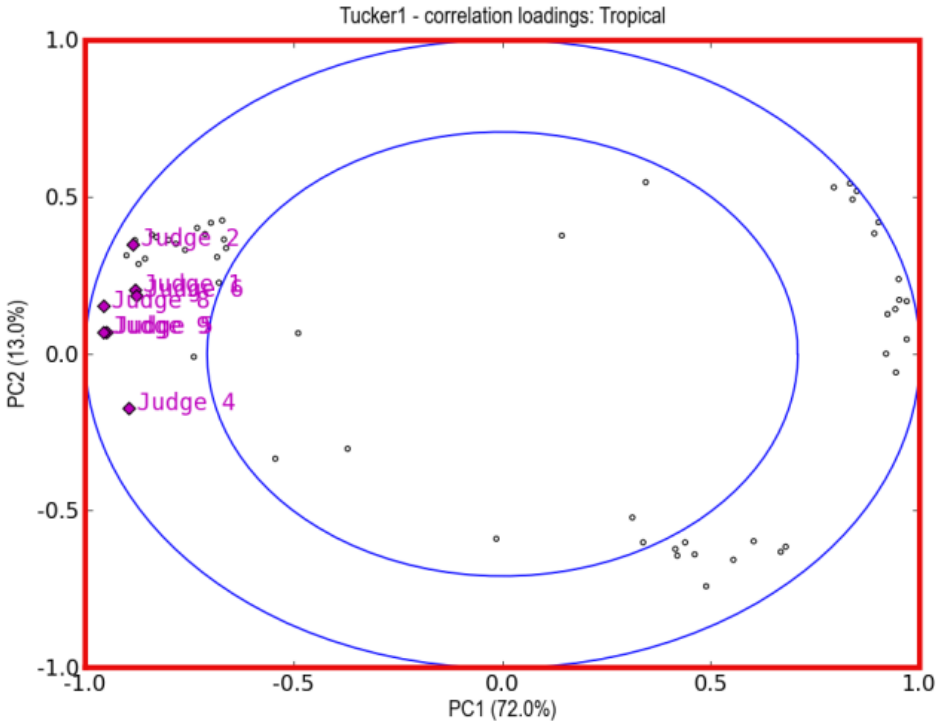

Figure 3.5 (a) Tucker plot illustrating good panel consistency with the score of the tropical attribute. PC1 explains 72% of the total variance and PC2 13%.

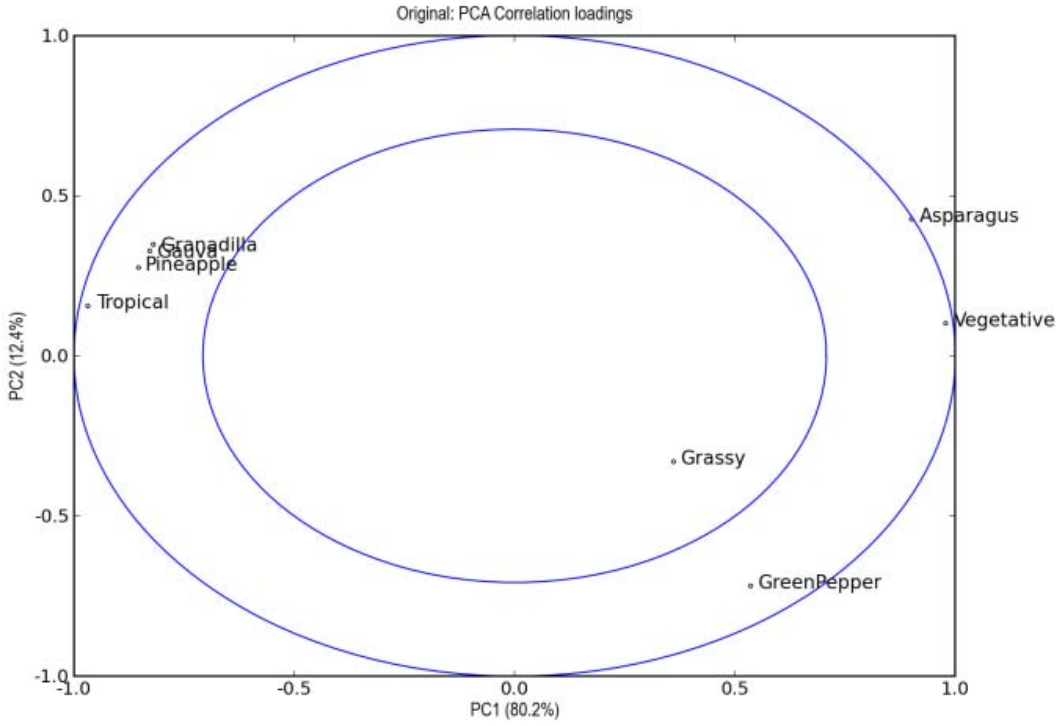

Figure 3.5 (b) PCA correlation loadings illustrating positive correlation between tropical attributes; tropical, granadilla, pineapple and guava, positive correlations between vegetative and asparagus and negative correlation between the tropical attributes and vegetative attributes (asparagus and vegetative). PanelCheck software used for analysis.

3.3.3.2 Multivariate discrimination between spiked and control wines using sensory analysis

Principal component analysis conducted on sensory data to investigate correlations between aroma attributes scored by the panel and flavourings added to the spiked wines indicated strong positive correlation between wines flavoured with asparagus flavouring and the asparagus sensory attribute where high levels of methoxypyrazines were present in the finished wine (Figure 3.7). This could also be observed for wines prepared during a trial run where high levels of methoxypyrazines were present in the finished wines for both experimental wines flavoured before fermentation and commercial wines flavoured after fermentation and bottling (Figure 3.6a and Figure 3.6b). Wines flavoured with medium levels of asparagus flavouring were also positively related to a certain extent to the asparagus attribute, but were strongly positively related to the vegetative aroma attribute. Wines flavoured with the low level asparagus flavouring did not show direct positive correlation with the asparagus aroma attribute, however it tended to be correlated to the green pepper aroma attribute (Figure 3.7).

Wines flavoured with fresh green pepper had strong correlations with the perception of green pepper aroma nuances in the wine. Aromas such as pineapple, litchi and granadilla (passion fruit) did not have a large impact on the variance between different wines. Wines spiked with green grass flavourings could not be correlated to green aroma attributes. Negative correlations between control wines and green pepper aromas can be observed (Figure 3.6a, Figure 3.6b and Figure 3.7). Flavourings added to commercial wines after fermentation had a more profound effect on the aroma attributes of the wine than flavourings added before fermentation to experimental wines (Figure 3.6b).

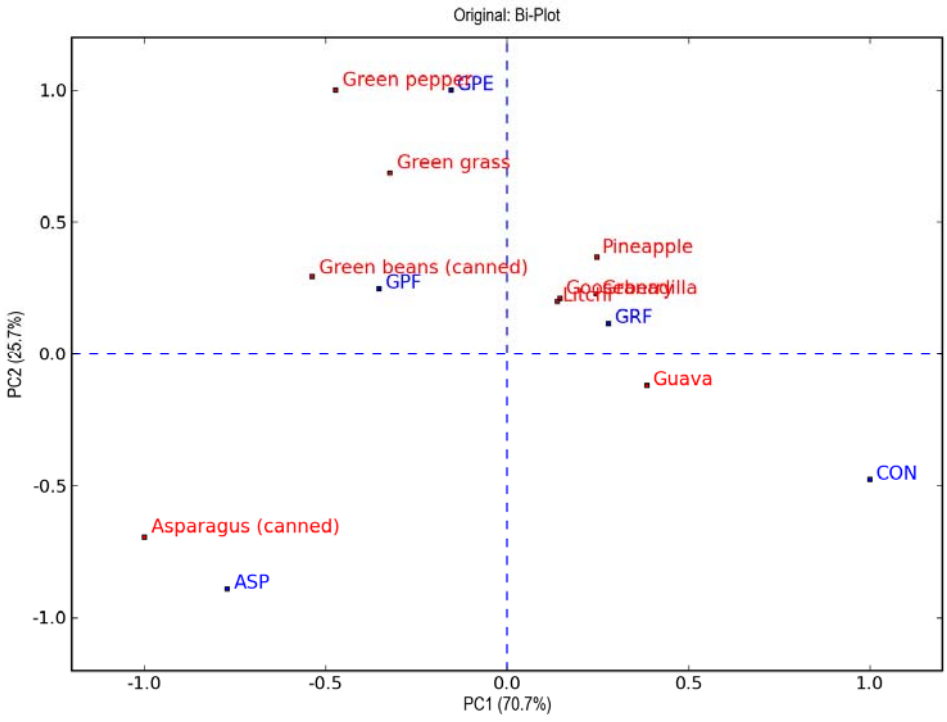

Figure 3.6 (a) Bi-plot of PCA scores and loadings of sensory analysis of experimental wines. Attributes are written out in full in red. Abbreviations used for wines, in blue, are: GPE, green pepper extract flavoured wine, GPF, commercial green pepper flavoured wine, GRF, commercial green grass flavoured wine, ASP, commercial asparagus flavoured wine, CON, control wines with no added flavourings.

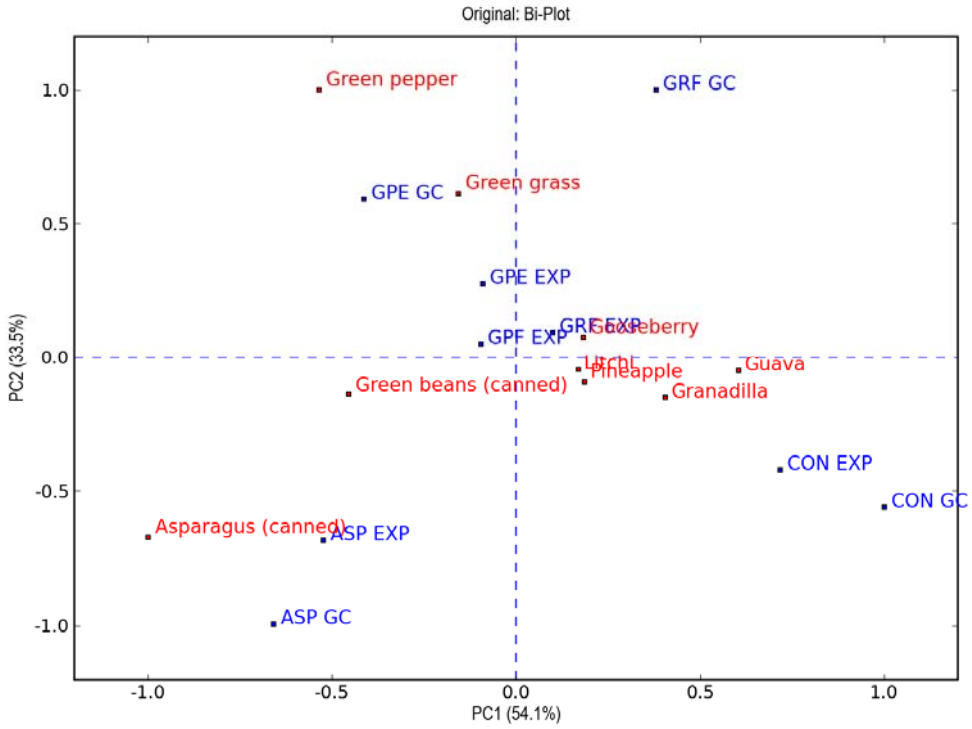

Figure 3.6 (b) Bi-plot of PCA scores and loadings of sensory analysis of experimental and commercial wines spiked. Attributes are written out in full in red. Abbreviations used for wines, in blue, are: GPE, green pepper extract flavoured wine, GPF, commercial green pepper flavoured wine, GRF, commercial green grass flavoured wine, ASP, commercial asparagus flavoured wine, CON, control wines. EXP indicates experimental wine spiked before fermentation. Groot Constantia commercial wines spiked after fermentation are indicated by GC.

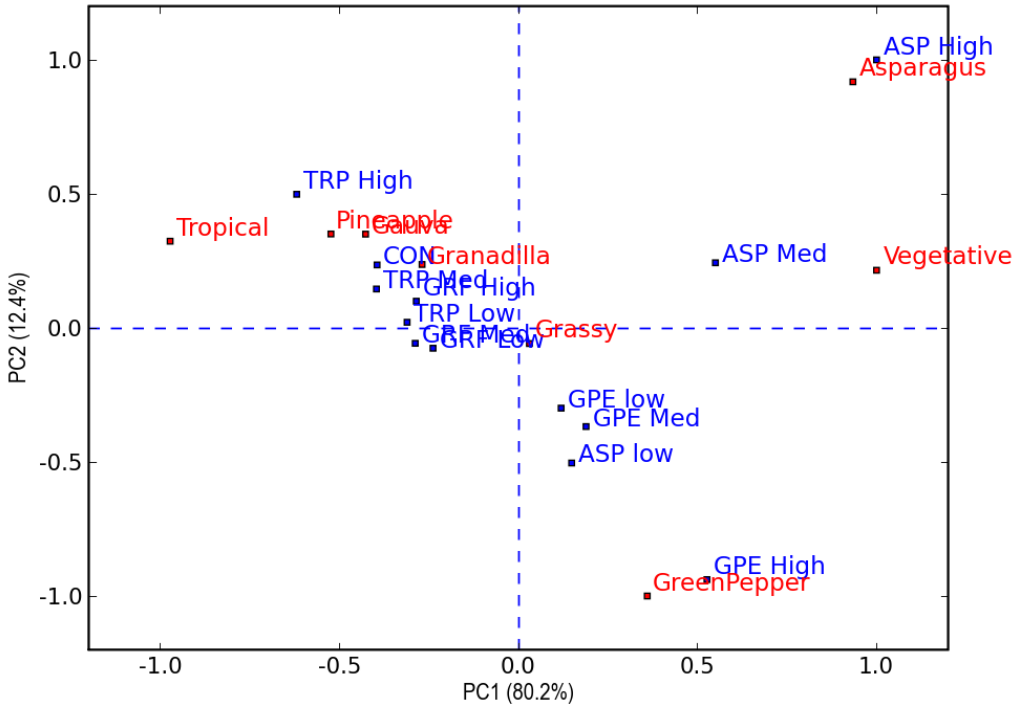

Figure 3.7 Bi-plot of PCA scores and loadings illustrating the correlations between different flavourings added to wines at different concentrations and the impact on the perception of sensory attributes scored by judges. Different attributes are written out in full in red. Abbreviations used for wines in blue are: GPE, green pepper extract flavoured wine, GPF, commercial green pepper flavouring added to wine, GRF, commercial green grass flavouring added to wine, ASP, commercial asparagus flavouring added to wine, TRP, commercial tropical flavouring added to wine, CON, control wines.

3.3.3.3 Sensory description of spiked and control wines

From the spider plots it can be concluded that a shift towards the green character or flavours of the wines occurred. The perception of the aroma intensity of tropical flavours was masked by the addition of green type flavourings to the wine (Figure 3.8a, Figure 3.8b and Figure 3.9a). The panel mean score for tropical flavours were less for wines spiked with green flavourings even though the concentrations of the flavour compounds were unaltered. This could be concluded for experimental as well as Constantia commercial wines spiked during the trial run with high concentrations of flavourings (Figure 3.8b). Even though wines spiked with the commercially available green pepper flavouring could be used to gain insightful information, artificial nuances not typical to Sauvignon blanc was observed. Therefore this flavouring would most probably not be used illegally as adulterant in the wine industry. It was excluded for the experimental design used to evaluate the effect of spiking wines with low, medium and high levels of flavourings with regards to the main aroma compounds in the flavourings as determined using GC-MS analysis.

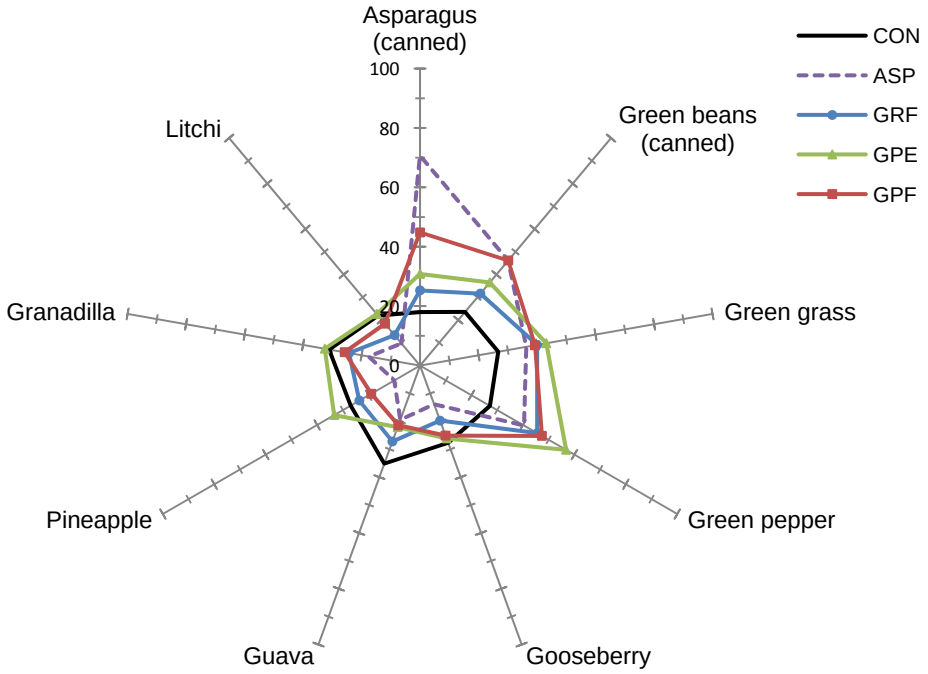

Figure 3.8 (a) Spider plot indicating the effect of adulteration by different flavourings on the aroma profile of experimental spiked wines. Abbreviations used for wines are: GPE, green pepper extract flavoured wine, GPF, commercial green pepper flavouring added to wine, GRF, commercial green grass flavouring added to wine, ASP, commercial asparagus flavouring added to wine, CON, control wines with no added flavourings.

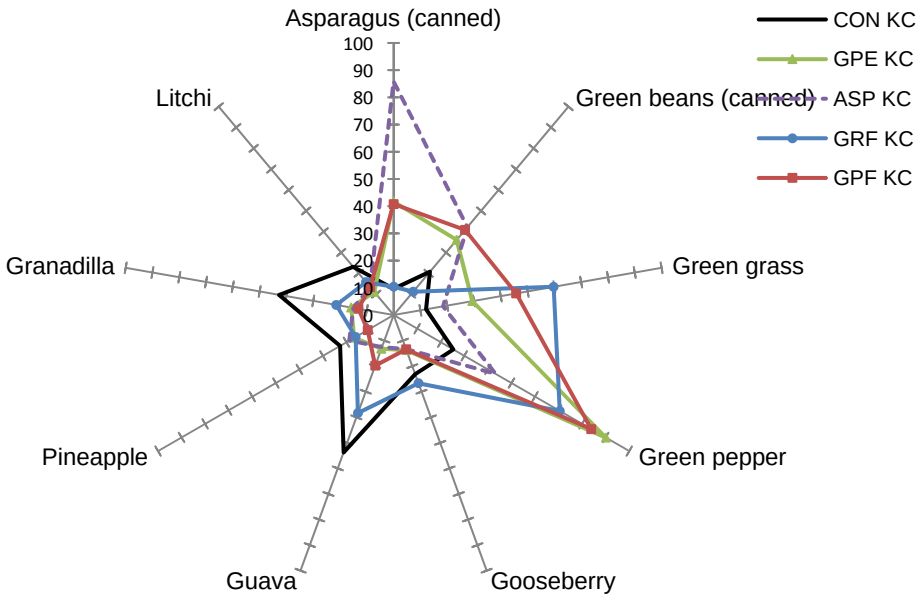

Figure 3.8 (b) Spider plot indicating the effect of adulteration by different flavourings on the aroma profile of commercial wines spiked after bottling. Abbreviations used for wines are: GPE, green pepper extract flavoured wine, GPF, commercial green pepper flavouring added to wine, GRF, commercial green grass flavouring added to wine, ASP, commercial asparagus flavouring added to wine, CON, control wines with no added flavourings. Klein Constantia commercial wines spiked after fermentation are indicated by KC.

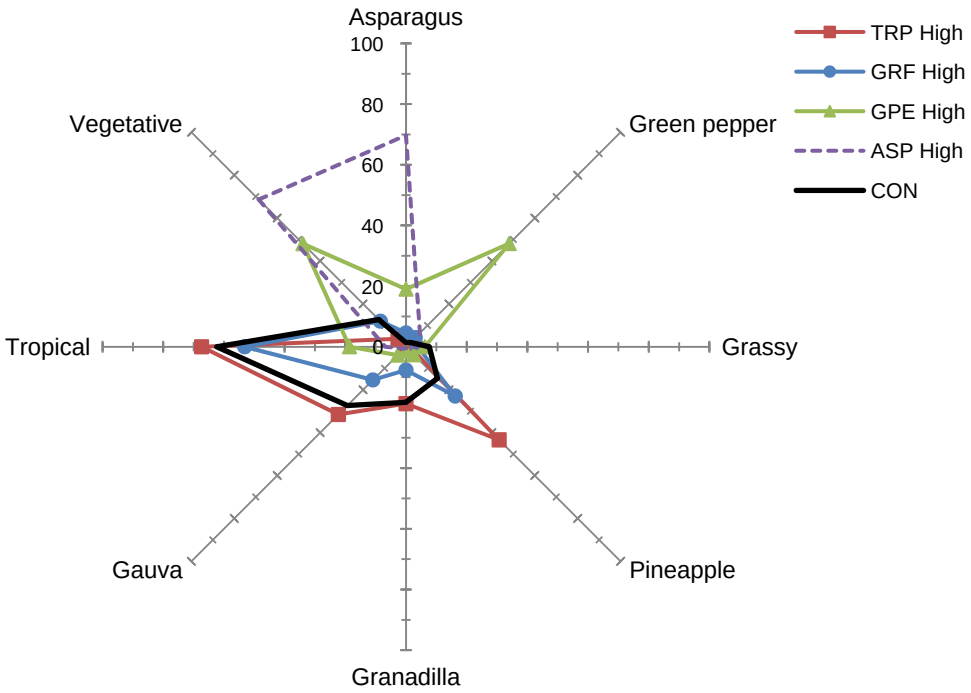

Figure 3.9 (a) Spider plot illustrating the effect of adulteration on the aroma profile of experimental wines using different flavourings at concentrations of the main flavour compound ten times or higher than the natural abundance of the main flavour compound in natural wine. Abbreviations used for wines are: GPE, green pepper extract flavoured wine, TRP, commercial tropical flavouring added to wine, GRF, commercial green grass flavouring added to wine, ASP, commercial asparagus flavouring added to wine, CON, control wines with no added flavourings.

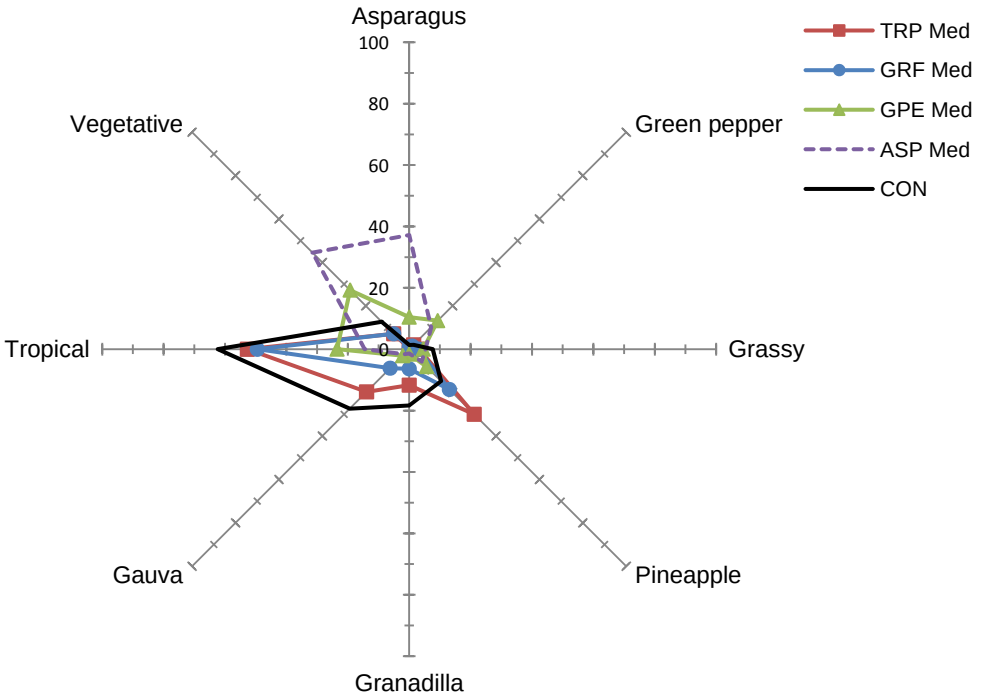

Figure 3.9 (b) Spider plot illustrating the effect of adulteration on the aroma profile of experimental wines using different flavourings at medium concentrations of the main flavour compound in comparison to the natural abundance of the main flavour compound in natural wine. Abbreviations used for wines are: GPE, green pepper extract flavoured wine, TRP, commercial tropical flavouring added to wine, GRF, commercial green grass flavouring added to wine, ASP, commercial asparagus flavouring added to wine, CON, control wines with no added flavourings.

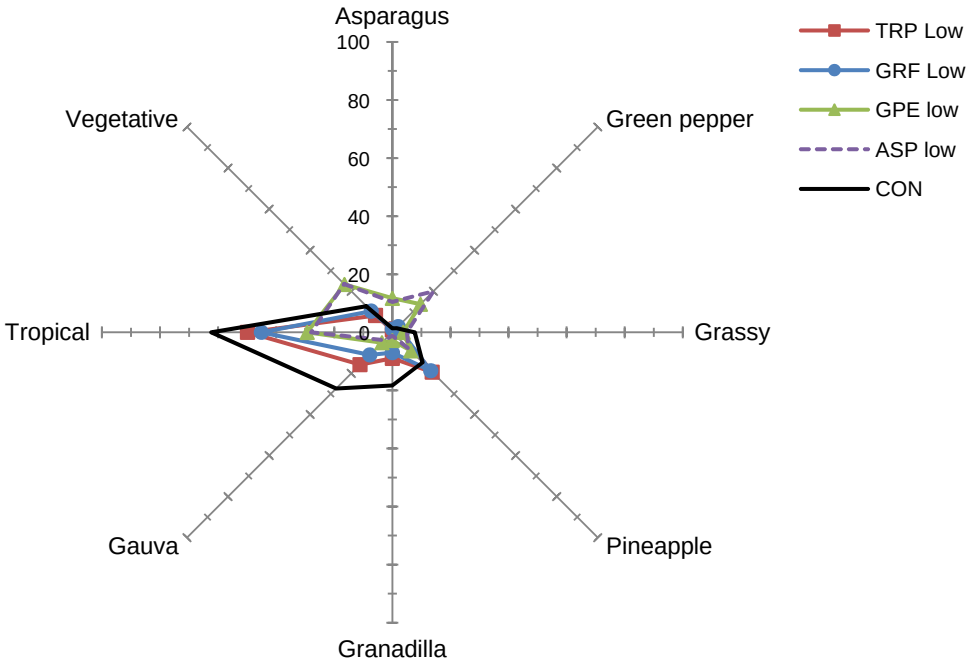

Figure 3.9 (c) Spider plot illustrating the effect of adulteration on the aroma profile of experimental wines using different flavourings at low concentrations (commonly found in wine) of the main flavour compound in comparison to the natural abundance of the main flavour compound in natural wine. Abbreviations used for wines are: GPE, green pepper extract flavoured wine, TRP, commercial tropical flavouring added to wine, GRF, commercial green grass flavouring added to wine, ASP, commercial asparagus flavouring added to wine, CON, control wines with no added flavourings.

From the spider plots it is clear that the asparagus and fresh green pepper extract flavourings masked the tropical attributes at low, medium and high levels of adulteration even though the intensity of the attributes associated with green flavours decreased from high (Figure 3.9a) to medium (Figure 3.9b) to low (Figure 3.9c). The green grass could not be perceived in the wines analysed during the sensory analysis of wines spiked with high, medium and low levels of flavourings to the extent that differences with regards to the control wine could be observed. The addition of tropical flavouring to wine enhanced the tropical aromas but did not mask vegetative aromas.

Therefore it can be concluded that the addition of green flavourings to wine masked the tropical aroma characteristics of the wines even when added at levels similar to the natural abundance of the main aroma contributers in wines.

3.3.4 Degassing of wine samples prior to FT-MIR and FT-NIR analysis of wine samples

Degassing by means of sonication reduced CO_2 levels of Sauvignon blanc wine from above 1000 mg/L to 396 mg/L, 320 mg/L, 497 mg/L, 441 mg/L and 520 mg/L. The method had to be repeated for some wines to obtain levels of CO_2 below 200 mg/L. The vacuum system reduced the CO_2 levels from above 1000 mg/L to 340 mg/L, 315 mg/L, 281 mg/L, 310 mg/L and 352 mg/L in 30 seconds, 175 mg/L, 222 mg/L, 205 mg/L, 213 mg/L and 183 mg/L after one minute. It was concluded that the vacuum system was the most effective in terms of reducing the concentration of CO_2 and keeping sample preparation time to a minimum. Therefore the vacuum system was used for the sample preparation prior to FT-MIR analysis of the wine using the FOSS WineScan and Bruker Alpha instruments.

3.3.5 Multivariate models discriminating between spiked and control wines using FT-MIR and FT-NIR spectroscopy

PCA and PLS-D were used to build statistical models to distinguish between control and spiked wines. PCA was used as the first multivariate analysis method to explore the data structure. PLS-D was used to build test set and cross validated models to discriminate between spiked and control wines.

Extra information could not be obtained from models build from datasets consisting of a combined dataset of data collected from different analytical techniques, chromatography, spectroscopy and sensory analysis. The effect of the spectroscopic data on the models masked effects that chromatographic or sensory data might have had on the models. From the loadings plots it was clear that sensory and/or chromatographic data had very little or no effect on the statistical differences between spiked and control samples.

Models successfully discriminating between spiked and control wines were build from FT-MIR spectra. A PCA model constructed using data from commercial control wines and wines spiked after bottling as well as experimental spiked and control wines combined in one dataset could discriminate between adulterated and control wines (refer to Figure 3.10). A PLS-D model using test set validation on FT-MIR data could be used to discriminate between wines subjected to skin contact and wines not subjected to skin contact (Figure 3.11a). Discrimination between spiked and control wines was also clear (Figure 3.11b).

PCA models were used to investigate the possibility of FT-IR analysis to discriminate between spiked and control wines when lower levels of flavourings were used for spiking than used in the trial run with high levels of flavourings added to experimental and commercial wines. The following models showed discrimination between control and flavoured wines: A PCA consisting of spectra from all wines spiked with low levels of flavourings as well as control

wines. A PCA consisting of spectra from all wines spiked with medium levels of flavourings as well as control wines. A PCA consisting of spectra from all wines spiked with high levels of flavourings as well as control wines. A PCA model containing spectra obtained from low, medium, high level spiked wines as well as control wines. A PLS-D model using test set validation containing spectra obtained from low, medium and high level spiked wines as well as control wines also showed clear separation between control and spiked wines.

Conformity testing applied to MIR attenuated refection and NIR reflection data showed that spiked samples fell outside of the conformity range.

3.3.5.1 Discrimination between spiked and control wines prepared experimentally and commercially

A PCA model constructed from FT-MIR data of experimental and commercial spiked wines prepared from grapes from the same vineyard showed separation between spiked and control wines (Figure 3.10). Of the x-variance 53% is explained by PC1 and 31% by PC2. Therefore 2 PC's explain 84% of the x-variance. Although separation cannot be observed mainly along PC1 or PC2 looking at the score plot (Figure 3.10), separation between spiked and control wines can clearly be observed as indicated by the dotted line. Commercial control wines are located in between experimentally prepared control wines. Commercial wines spiked after fermentation clustered close to experimental wines prepared from grapes from the same vineyard, spiked before fermentation.

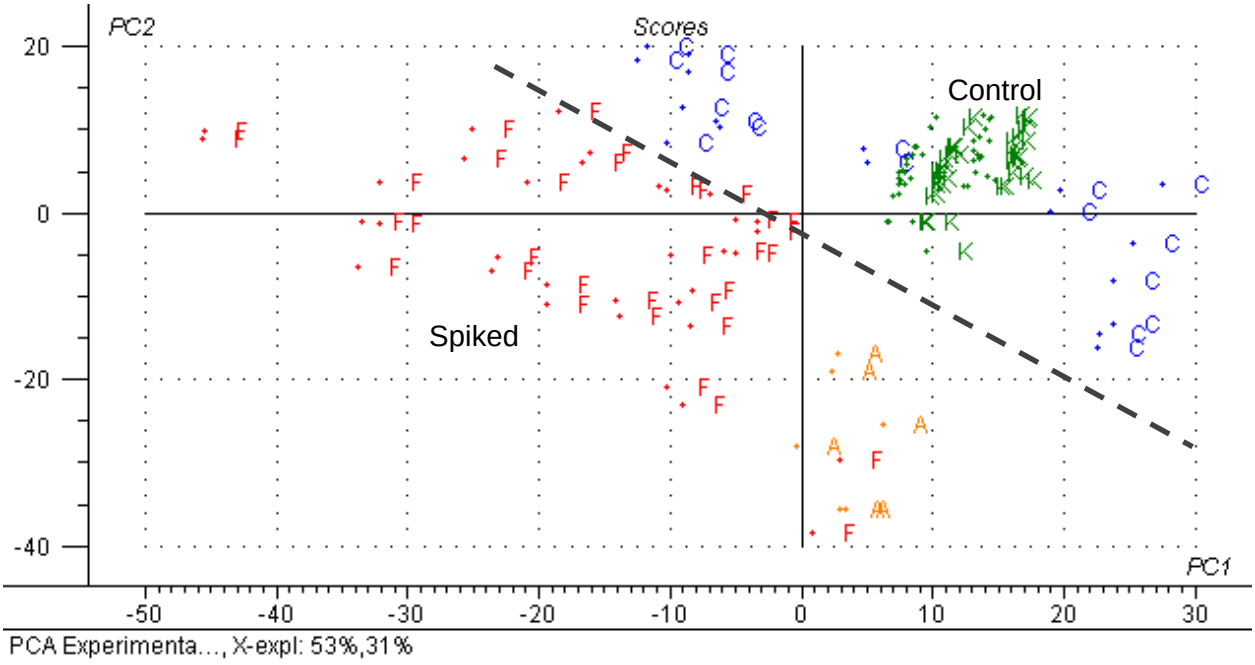

Figure 3.10 PCA score plot of spiked and control experimental and commercial wines. PC1 explains 53% of the variance and PC2 31%. **C** = experimentally prepared control wines, **K** = commercial control wines, **F** = flavoured experimental wines, **A** = flavoured commercial wines.

3.3.5.2 Differences between spiked and control wines subjected to skin contact and wines not subjected to skin contact by means of PLS-D

From the PLS-D discrimination model, built from MIR spectra where skin contact was applied to control as well as spiked wines, using dummy variables 1 for control, -1 for spiked wines and test set validation of experimental wines, discrimination can be observed. A trend in terms of discrimination between wines subjected to skin contact and wines pressed directly after destemming can be observed mainly along PC2 from the PLS-D score plot (Figure 3.11a). Discrimination between spiked and non-spiked wines can be observed mainly along PC1 (Figure 3.11b). Therefore, although difference between skin contact and non-skin contact wines are observed, it did not influence the discrimination between control and flavoured wines negatively. From the predicted versus measured plot in can be concluded that separation between control and flavoured wines occur looking at the gap between the control and flavoured wines on both axis. The correlation coefficient of 0.99 and bias close to zero indicates good prediction (Figure 3.11c).

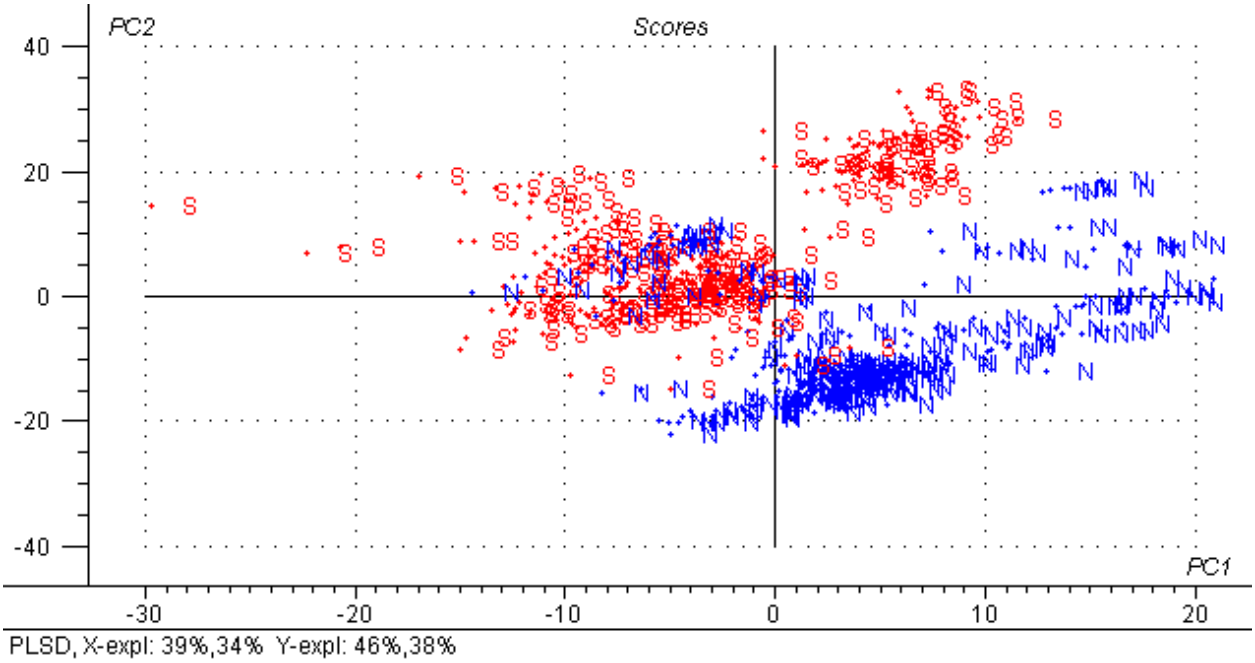

Figure 3.11 (a) PLS-D score plot demonstrating separation between wines subjected to skin contact and wines pressed directly after destemming. The validation method used was test set validation. Of the total x-variance explained, 39% is explained by PC1 and 32% by PC2. **S** = Wines subjected to skin contact, **N** = wines not subjected to skin contact.

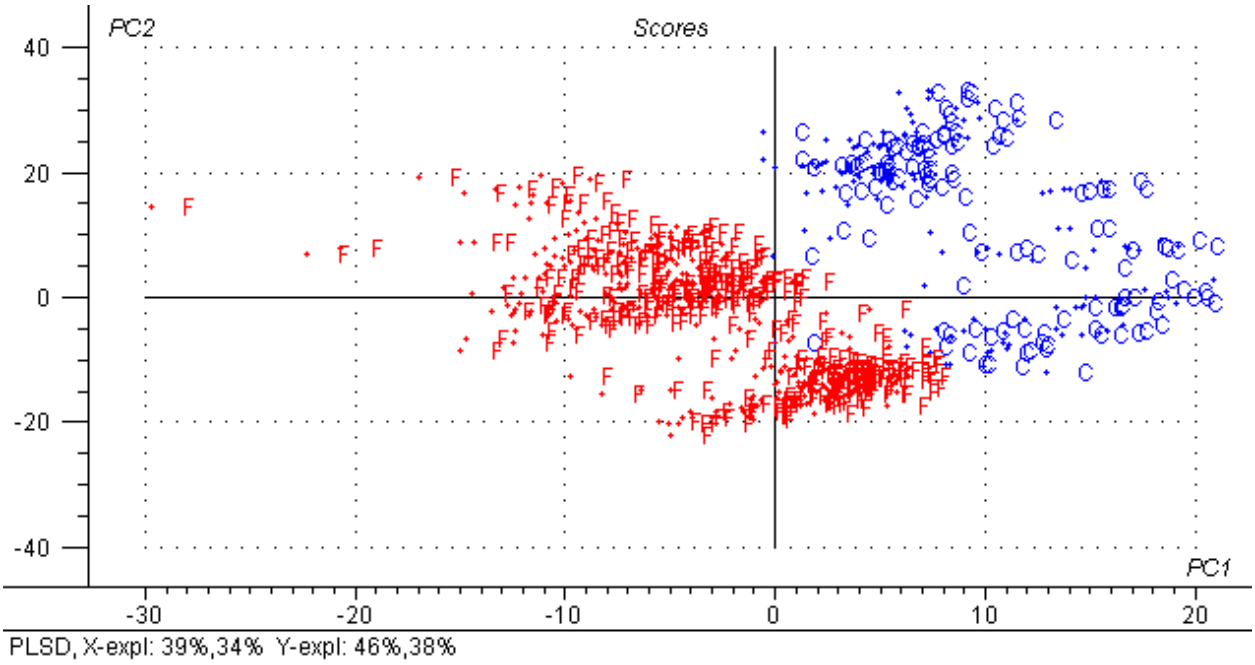

Figure 3.11 (b) PLS-D score plot demonstrating separation between (1) flavoured and (2) control wines subjected to skin contact and (3) wines pressed directly after destemming. The validation method used was test set validation. Of the total x-variance explained, 39% is explained by PC1 and 32% by PC2. **F** = Flavoured wines, **C** = control wines.

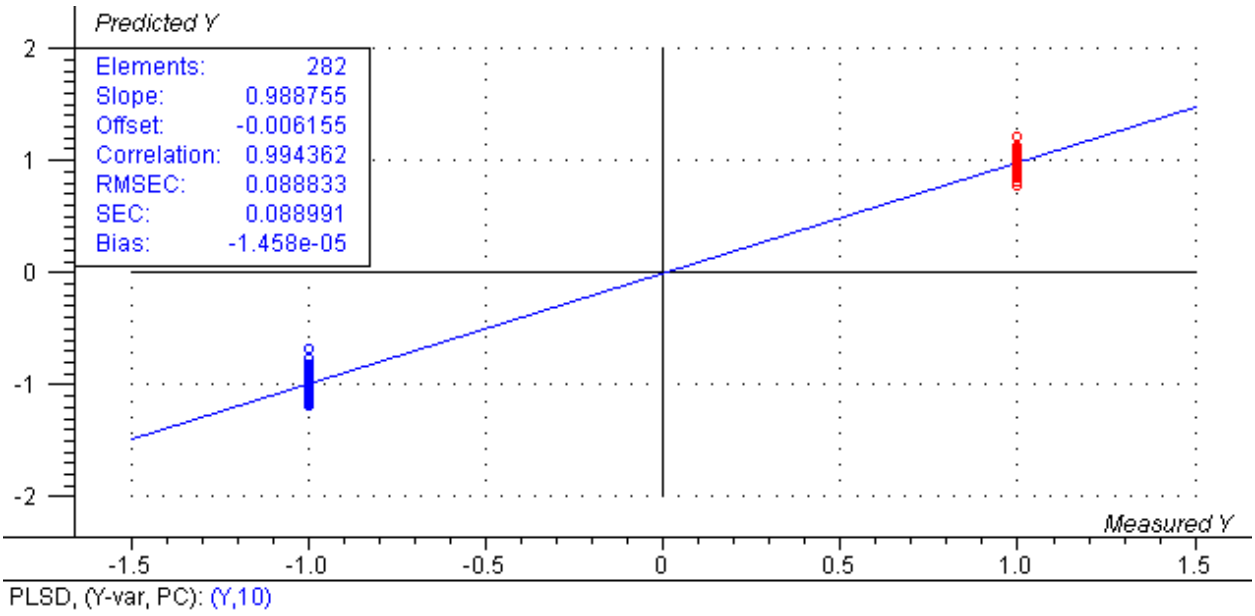

Figure 3.11 (c) PLS-D predicted versus measure plot for spiked and control wines using wines subjected to skin contact and wines not subjected to skin contact. **Blue** indicates control wines and **red** flavoured wines.

3.3.5.3 Discrimination between spiked and control experimental wines using different spiking concentrations

PCA analysis conducted on FT-MIR data discriminated well between spiked and control wines even using spectral data from wines spiked with different concentrations of flavourings (Figure 3.12). PC1 explained 74% of the variation within the data set and PC2 14%. Therefore, the first two PC's explain 88% of the variation. Clear separation between control wines and spiked wines can be observed. Separation between control wines and spiked wines can be observed

mostly along PC2 as well as slightly along PC1. Although clear separation can not be observed between wines spiked with different types of flavourings, a trend can be observed along PC1.

Better discrimination between the wines spiked with different flavourings was achieved by modelling low, medium and high levels of adulteration separately (Figure 3.13a, Figure 3.13b and Figure 3.13c). The amount of variation explained were higher for high and medium-level spiked wines, for low level spiked wine the variance explained by the first two PC's stayed the same.

The PCA of wines spiked with high levels of flavourings could explain 92% of the total variation, where PC1 described 76% and PC2 16% (Figure 3.13a). Separation between spiked and control wines can be observed along PC2 and separation between wines flavoured with different flavourings occur along PC1.

PC1 explained 75% and PC2 18% interpreting the PCA plot of wines spiked with medium levels of flavourings with a total of 93% variance explained by the first two PC's (Figure 3.13b) and PC1 70% and PC2 17% with a total of 87% explained variance for the first two PC's for wines spiked with low levels of flavourings (Figure 3.13c).

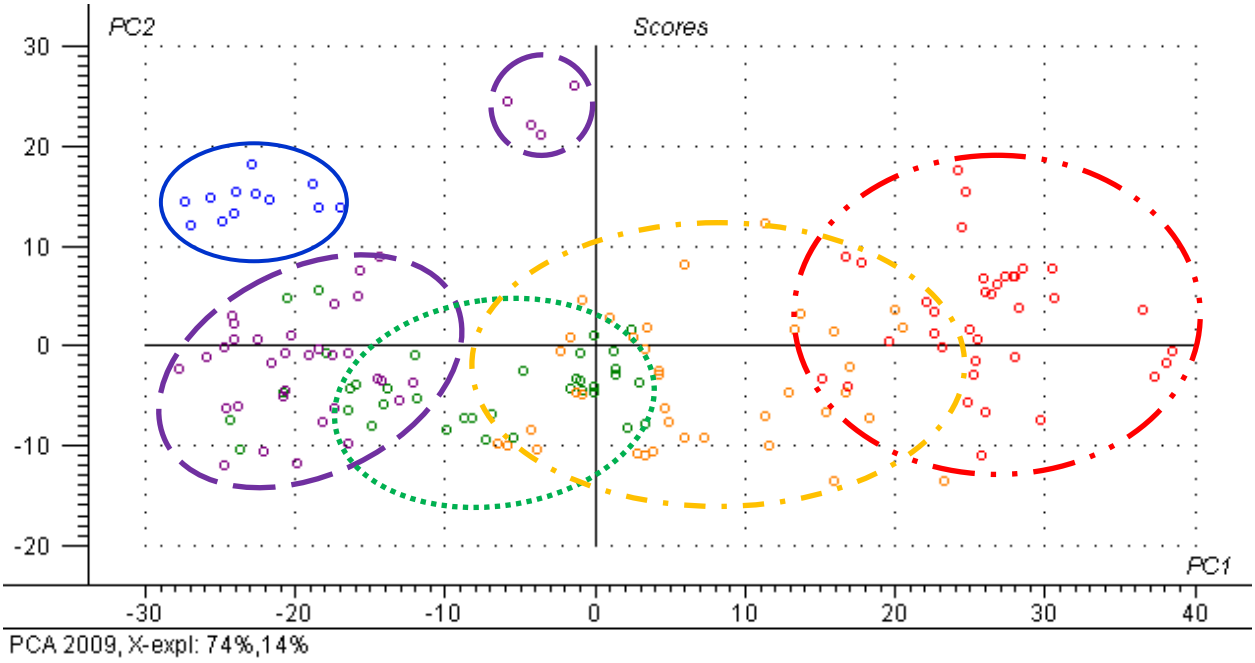

Figure 3.12 PCA score plot of flavoured and control experimental wines using wines with low, medium and high concentrations of flavourings in one model. PC1 explains 74% of the variance and PC2 14%. Control wines, wines flavoured with commercial asparagus flavouring, wines flavoured with natural fresh green pepper juice, wines flavoured with commercial green grass flavouring, wines flavoured with commercial tropical flavouring.

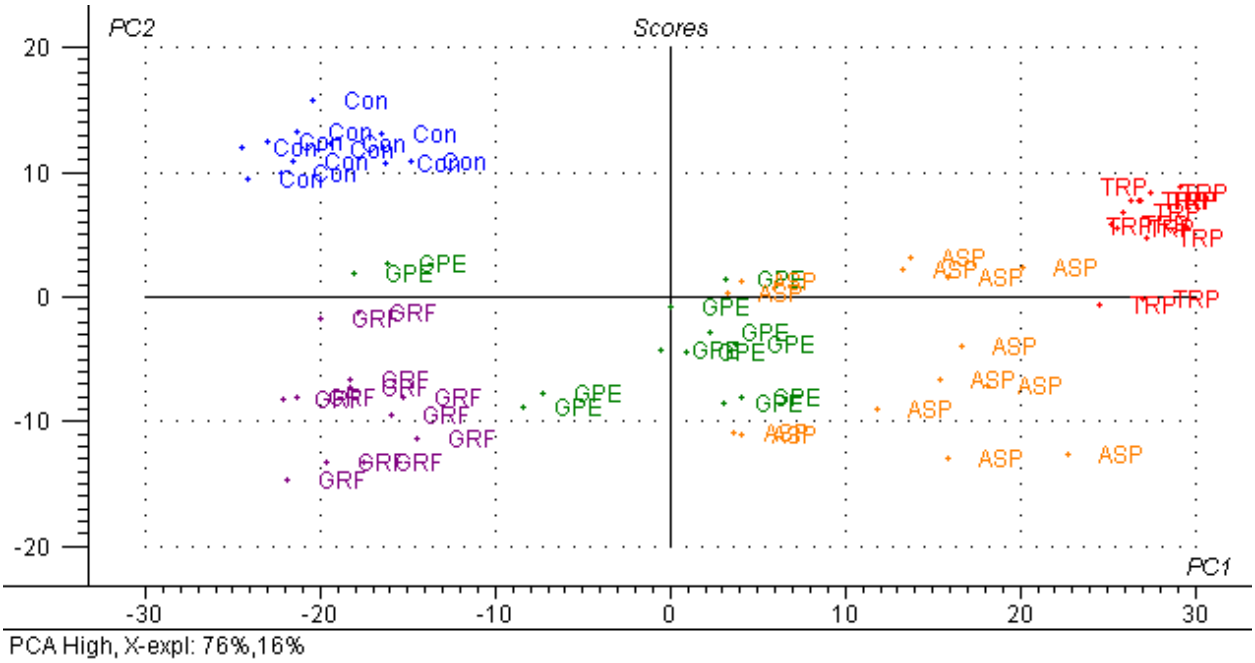

Figure 3.13 (a) PCA score plot of control wines and wines spiked at high levels of the main aroma compound in flavourings. PC1 explains 76% of the variance and PC2 16%. **Con** = Control wines, **ASP** = wines flavoured with commercial asparagus flavouring, **GPE** = wines flavoured with natural fresh green pepper juice, **GRF** = wines flavoured with commercial green grass flavouring, **TRP** = wines flavoured with commercial tropical flavouring.

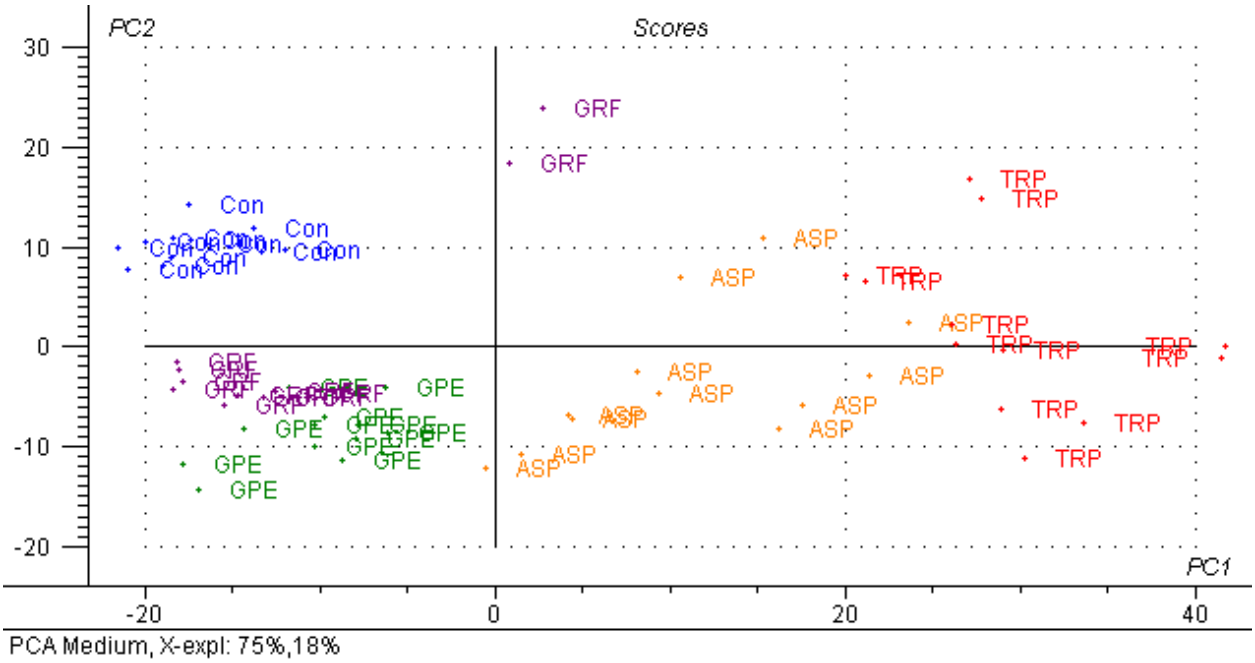

Figure 3.13 (b) PCA score plot of control wines and wines spiked at medium levels of the main aroma compound in flavourings. PC1 explains 75% of the variance and PC2 18%. **Con** = Control wines, **ASP** = wines flavoured with commercial asparagus flavouring, **GPE** = wines flavoured with natural fresh green pepper juice, **GRF** = wines flavoured with commercial green grass flavouring, **TRP** = wines flavoured with commercial tropical flavouring.

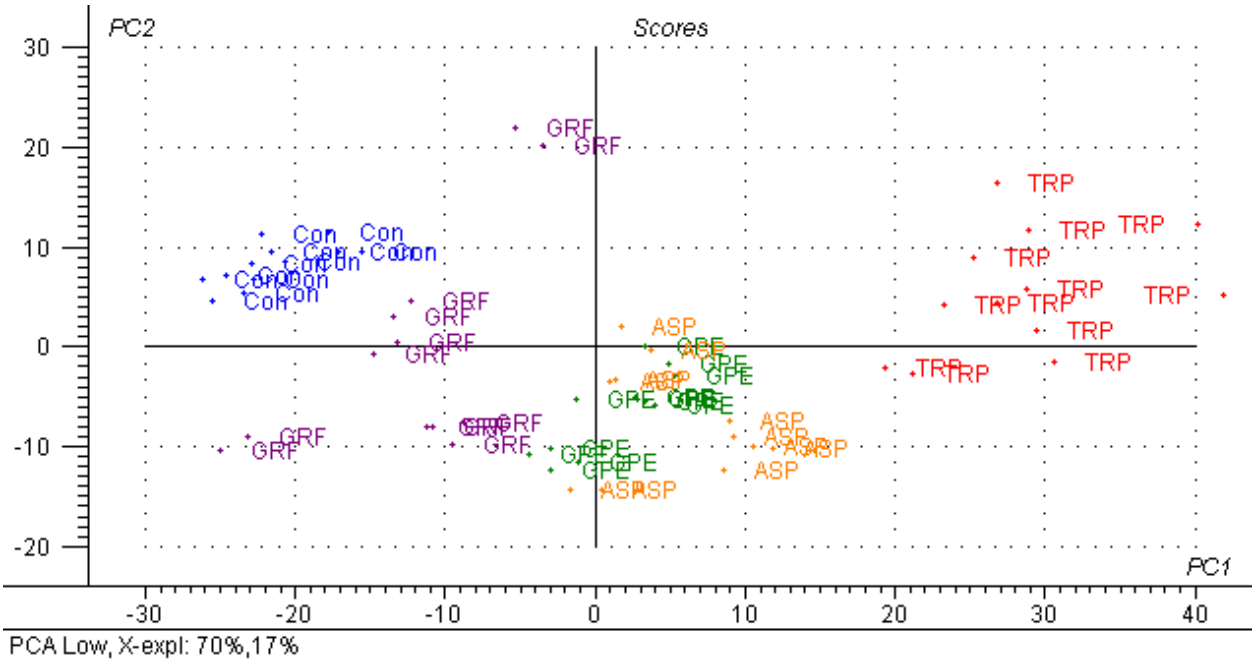

Figure 3.13 (c) PCA score plot of control wines and wines spiked at low levels of the main aroma compound in flavourings. PC1 explains 70% and PC2 17% of the variance. **Con** = Control wines, **ASP** = wines flavoured with commercial asparagus flavouring, **GPE** = wines flavoured with natural fresh green pepper juice, **GRF** = wines flavoured with commercial green grass flavouring, **TRP** = wines flavoured with commercial tropical flavouring (gooseberry).

PLS-D performed on the FT-MIR data set consisting of spectral data from wines spiked with different concentrations of flavourings using cross validation discriminated well between control and flavoured wines. Separation between spiked and control wines can be observed along PC1. Savitzky Golay first derivative spectral pre-processing was used. A correlation coefficient of 0.73 was obtained. The gap between the data point show that discrimination between control and flavoured wines can be observed (Figure 3.14b). The small bias is an indication that the prediction is good.

Sensory data (set as **Y**-variables) combined with FT-IR (set as **X**-variables) were used to perform PLS-D, using PLS2 analysis with the **Y** matrix consisting of more than one variable. No separation between spiked and non spiked wines was observed even though separation was observed when conducting a PCA on the sensory analysis data alone and PLS-D using PLS1 on the FT-IR data alone.

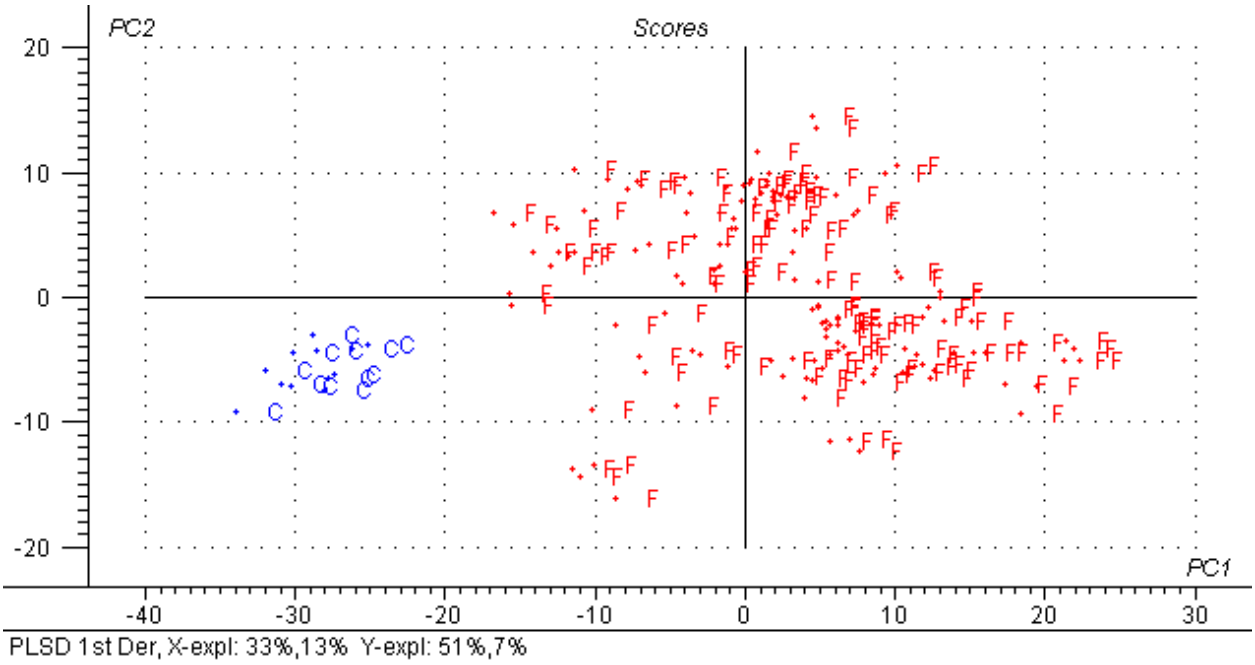

Figure 3.14 (a) PLS-D score plot demonstrating separation between control wines and wines spiked with different flavourings as well as at different concentration levels. Cross validation was used to build the model. Savitzky Golay first derivative spectral pre-processing was used.

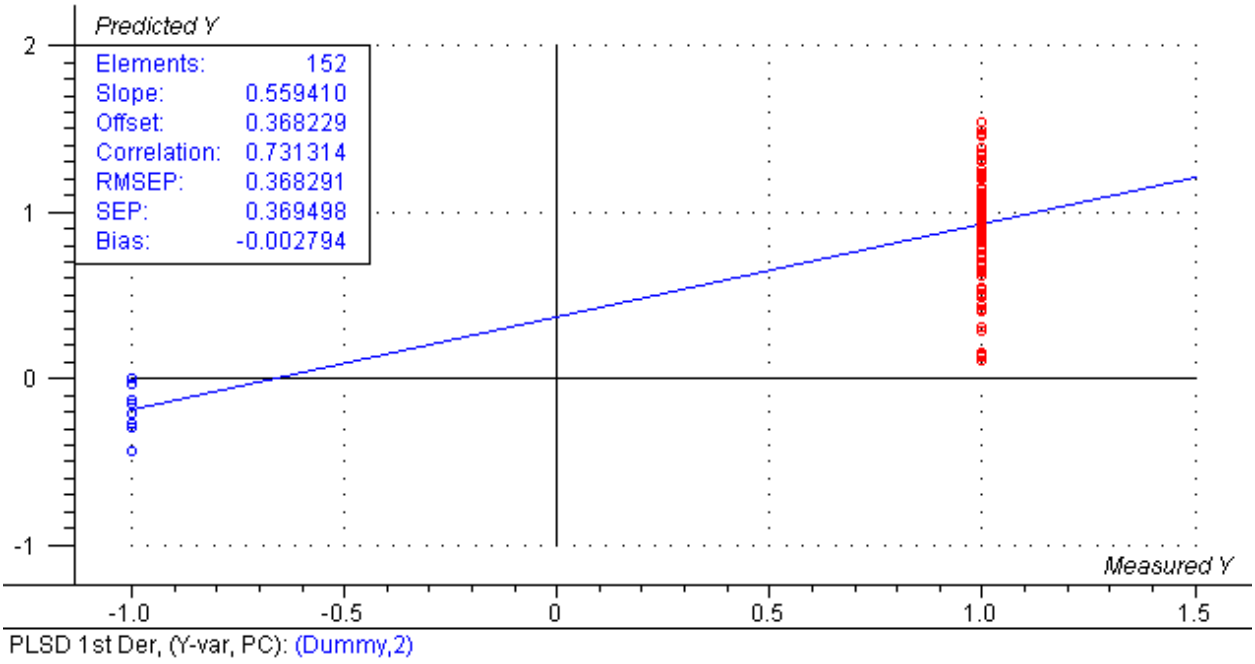

Figure 3.14 (b) PLS-D predicted versus measure plot for spiked and control wines using low, medium and high levels of flavourings. **Blue** indicates control wines and **red** flavoured wines.

PLS-D was performed on a data set consisting of FT-IR data as **X**-variables and GC-MS data (concentration of IBMP) as **Y**-variable. No discrimination between spiked and non-spiked wines could be observed. Discrimination between wines spiked with asparagus flavouring and all other wines could be observed (Data not shown)

3.3.5.4 Conformity testing

Conformity testing conducted on MIR and NIR spectra showed that all spiked samples fell outside of or on the border of the conformity range set up using unspiked control samples. Therefore it can be concluded that spiked wines failed the conformity test. Therefore spectra obtained from spiked wines differ significantly from spectra obtained from control samples in terms of the standard deviation of absorbances calculated for all wavelengths of the spectra (conformity testing). Conformity tests conducted on NIR spectra (wavenumbers 3999.7 – 12498.4 cm^{-1}) using a liquid probe showed slightly better discrimination between spiked and control wines than MIR attenuated reflection spectra (wavenumbers 373.1 – 3995 cm^{-1}) using 40°C temperature control.

NIR data showed that all spiked wines fell clearly outside of the conformity range or confidence band, failing the conformity test. Therefore it can be concluded that spiked samples differ significantly from control samples. The model can be used to identify spiked wines as wine falling outside of the conformity range. Although good results were obtained using various spectral pre-processing methods such as vector normalisation, first derivative and second derivative computations, the best results were obtained using second derivative computation with 17 smoothing points and a conformity index of 4 (Figure 3.16).

Spectra obtained from the Alpha subjected to a conformity test with conformity index 3.1 and vector normalisation applied as spectral pre-processing technique showed good separation between spiked and control samples although not all of the spiked samples fell completely out of the conformity range. Two spiked samples (low level flavoured green pepper wine and low level flavoured tropical wine) fell within and three samples (a low level asparagus flavoured and two low level flavoured tropical wines) fell on the border of the conformity range (Figure 3.15).

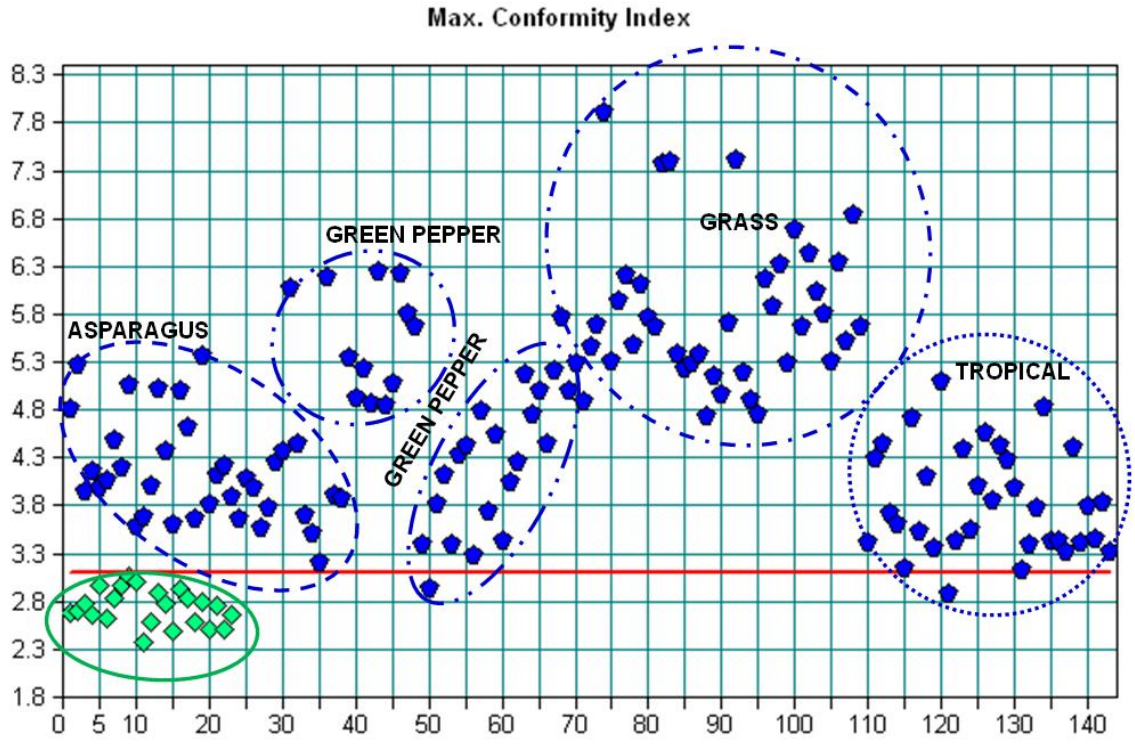

Figure 3.15 Conformity testing of MIR attenuated reflection spectra indicated clear distinction between control and spiked wines. Control wines were used to construct the conformity range. Spiked wines were plotted outside the conformity test range. Vector normalization and second derivative computations were applied as pre-processing. The conformity index limit used was 3.1 and wavenumbers 373.1 – 3995 cm^{-1}.

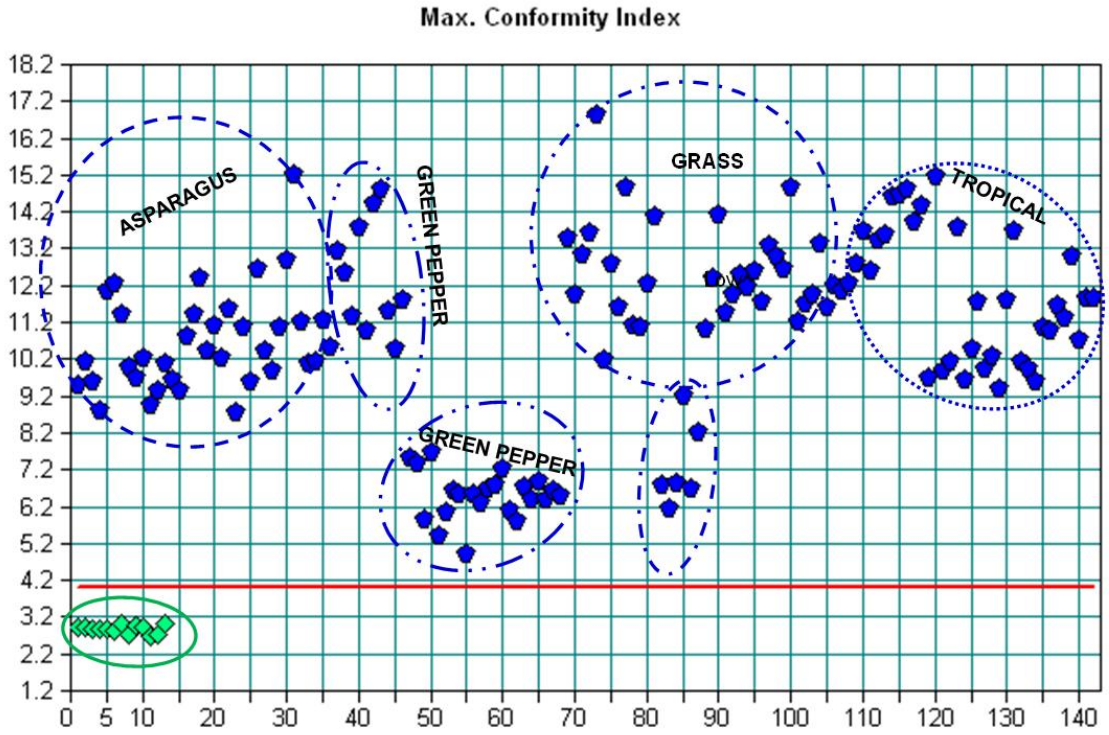

Figure 3.16 Conformity testing preformed on NIR spectra indicated clear distinction between control and spiked wines. Control wines were used to construct the conformity range. All spiked wines were plotted outside the conformity test range. Second derivative computations, using 17 smoothing points, were applied as pre-processing. The conformity index limit used was 4 and wavenumbers 3999.7 – 12498.4 cm^{-1}.

From the results it is clear that multivariate statistics, specifically PCA, PLS-D and conformity testing combined with FT-MIR and FT-NIR analysis as well as descriptive sensory analysis can discriminate between wine flavoured with commercial flavourings as well as fresh green pepper.

3.4 References

Alberts, P., Stander, M.A., Paul, S.O., De Villiers, A. (2009). Survey of 3-alkyl-2-methoxypyrazines content of South African Sauvignon blanc wines using a novel LC-APC1-MS/MS method. *J. Agric. Food Chem.* **57**, 9347-9355

Allen, M.S., Lacey, M.J., Harris, R.L.N., Brown, W.B. (1991). Contribution of methoxypyrazines to Sauvignon blanc wine aroma. *Am. J. Enol. Vitic.* **42**, 109-112.

Allen, M.S., Lacey, M.J., Boyd, S. (1994). Determination of methoxypyrazines in red wines by stable isotope dilution gas chromatography-mass spectrometry. *J. Agric. Food Chem.* **42**, 1734-1738.

Andrews, J., Heymann, H., Allersieck, M. (1990). Sensory and chemical analysis of Missouri Seyval blanc wines. *Am. J. Enol. Vitic.* **41**, 116-120

Arvanitoyannis, I.S., Katsota, M.N., Psarra, E.P., Soufleros, E.H., Kallithraka, S. (1999). Application of quality control methods for assessing wine authenticity: Use of multivariate analysis (Chemometrics). Trends *Food Sci. Technol.* **10**, 321-336

Bakker, J., Arnold, G.M. (1993). Analysis of sensory and chemical data for color evaluation of a range of red Port wines. *Am. J. Vitic.* **44**, 27-34

Blanco, M., Peinado, A.C., Mas, J. (2004). Analytical monitoring of alcolholic fermentation using NIR spectroscopy. *Biotechnol. and Bioeng.* **88**, 536-542

Bruker Alpha P-modul Reference Manual, Bruker OPTIK, Ettlingen, Germany. [URL. http://www.brukeroptics.com]. Cited the 3rd of October 2010

Bruker MPA Reference Manual, Bruker OPTIK, Ettlingen, Germany. [URL. http://www.brukeroptics.com]. Cited the 3rd of October 2010

Cozzolino, D., Smyth, H.E., Lattey, K.A., Cynkar, W., Janik, L., Dambergs, R.G., Francis, I.L., Gishen, M. (2005). Relationship between sensory analysis and near infrared spectroscopy in Australian Riesling and Chardonnay wines. *Anal. Chim. Acta* **539**, 341-348

Cozzolino, D., Holdstock, M., Dambergs, R.G., Cynkar, W.U., Smith, P.A. (2009). Mid infrared spectroscopy and multivariate analysis: A tool to discriminate between organic and non-organic wines grown in Australia. *Food Chem.* **116**, 761–765

Du Plessis, C. (2005). Sauvignon blanc – the dust hasn't settled. *Winelands* [URL. http://www.wineland.co.za/0204suvignon.php3]. Cited the 26th of August 2008

Esbensen, K.H. (2002). (5th ed) Multivariate Data Analysis in practice – an introduction to multivariate data analysis and experimental design CAMO Process AS, Oslo

Hashizume, K., Samuta, T. (1999). Grape maturity and light exposure affect berry methoxypyrazine concentration. *Am. J. Enol. Vitic.* **50**, 194-198.

Kotseridis, Y., Anocibar Beloqui, A., Bertrand, A., Doazan, J.P. (1998). An analytical method for studying the volatile components of Merlot noir clone wines. *Am. J. Enol. Vitic.* **49**, 44-48.

Kotseridis, Y., Baumes, R.L., Bertrand, A., Skouroumounis, G.K. (1999). Quantitative determination of 2-methoxy-3-isobutylpyrazine in red wines and grapes of Bordeauxusing a stable isotope dilution assay. *J. Chromatogr.* **841**, 229-237.

Lacey, M.J., Allen, M.S., Harris, R.L.N., Brown, W.V. (1991). Methoxypyrazines in Sauvignon blanc grapes and wine. *Am. J. Enol. Vitic.* **42**, 103-108.

Lesschaeve, I. (2007). Sensory evaluation of wine and commercial realities: Review of current practices and perspectives. *Am. J. Enol. Vitic.* **52**, 252-258

Liquor product act, Act 60 (1989). Government gazette of the Republic of South Africa. [URL: http://www.sawis.co.za/winelaw/southafrica.php]. Cited the 15th of October 2009

Malherbe, S., Bauer, F.F., Du Toit, M. (2007). Understanding problem fermentations - A review. *S. Afr. J. Enol. Vitic.* **28**, 169-186.

Manley, M., Van Zyl, A., Wolf, E.E.H. (2001). The evaluation of the applicability of Fourier Transform Near-Ifrared (FT-IR) spectroscopy in the measurement of analytical parameters in must and wine. *S. Afr. J. Enol. Vitic.* **22**, 93-100.

Marais, J. (1994). Sauvinon blanc cultivar aroma – a review. *S. Afr. J. Enol. Vitic.* **15**, 41-45

Marais, J. (2010). Introduction to publications from the investigation into the aroma databases of different South African Cultivars. *Winelands* [URL. http://www.wineland.co.za/index.php?option=com_zine &view=article&id=81:introduction-to-publications-from-the-investigation-into-the-aroma-databases-of-different-south-african-cultivars&q=sauvignon]. Cited the 3rd of December 2010

Naes, T. Isaksson, T., Fearn, T., Davies, T. (2002). A User-friendly Guide to Multivariate Calibration and Classification. NIR Publications, Chichester, UK

OPUS (2006). OPUS Spectroscopy software User Manual version 6, Bruker OPTIK, Ettlingen, Germany [URL. http://www.brukeroptics.com]. Cited the 3rd of October 2010

Osborne, C.D. (2007). Discriminating wine yeast strain and their fermented wines: an integrated approach., M.Sc.(Wine Biotechnology) thesis, Stellenbosch University

Patz, C.D., Blieke, A., Risow, R., Dietrich, H. (2004). Application of FT-MIR spectrometry in wine analysis. *Anal. Chim. Acta.* **513**, 81-89

Rajchl, A., Cizkova, H., Voldrich, M., Lukesova, D., Panovska, Z. (2009). Methoxypyrazines in Sauvignon blanc wines, detection of addition of artificial aroma. *Czech J. Food Sci.* **27**, 259-266

Rayn, D., Watkins, P., Smith, J., Allen, M., Marriott, P. (2005). Analysis of methoxypyrazines in wine using headspace solid phase microextraction with isotope dilution and comprehensive two-dimensional gas chromatography. *J. Sep. Sci.* **28**, 1075-1082.

Roujou De Boubee, D., Van Leeuwen, C., Dubourdieu, D. (2000). Organoleptic impact of 2-methoxy-3-isobutylpyrazine on red Bordeaux and Loire wines, effect of environmental conditions on concentrations in grapes during ripening. *J. Agric. Food Chem.,***48**, 4830-4834.

Roussel, S.,Bellon-Maurel, V., Roger, J-M.,Grenier, P. (2003). Authenticating white grape must variety with classification models based on aroma sensors, FT-IR and UV spectrometry. *J. Food Eng.***60**, 407-419

Sala, C., Mestres, M., Marti, M.P., Bustro, O., Guash, J. (2002). Headspace solid-phase microextraction of analysis of 3-alkyl-2-methoxypyrazines in wine. *J. Chromatogr. A.* **953**, 1-6.

Schlesier, K., Fauhl-Hassek, C., Forina, M., Cotea, V., Kocsi, E., Schoula, R., Van Jaarsveld, F., Wittkowski, R. (2009). Characterisation and determination of the geographical origin of wines. Part I: Overview. *Eur. Food Res. Technol.* **230,** 1-13

Swanepoel, M., Du Toit M., Nieuwoudt, H.H. (2007). Optimisation of the quantification of total soluble solids, pH and titrable acidity in South African must using Fourier transform mid-infrared spectroscopy. *S. Afr. J. Enol. Vitic.* **28**, 140-149

Versari, A., Parpinello, G.P., Mattioli, A.U., Galassi, S. (2008). Determination of grape quality at harvest using Fourier-Transform mid-infrared spectroscopy and multivariate analysis. *Am. J. Vitic.* **59**, 317-322.

WineScan FT 120 Reference manual, FOSS Analytical, Denmark, 2001. [URL. http://www.foss.dk]. Cited the 3rd of October 2010

Chapter 4

General discussion and conclusions

Chapter 4: General Discussion and Conclusion

4.1 Conclusions

Food and beverage authentication is becoming an increasingly important issue globally that could be seen as an ongoing process aimed at verification of the absence of listed substances, also referred to as adulterants, in foodstuffs. Export of South African wines plays an important part in the economy of the country. Sauvignon blanc is one of the most important single cultivar South African white wines in terms of export quantity and quality (SAWIS, 2010). Wines that are not acceptable for the global wine market, in terms of quality and authenticity can negatively influence the perception of the quality and good standing of South African wines. Tropical flavours, such as guava, litchi and pineapple, and the green varietal characters of green pepper, green grass and asparagus, determine the value and quality of Sauvignon blanc wines (Allen *et al.*, 1991; Lacey *et al.*, 1991; Marais, 1994).

The main aroma compounds responsible for the green aroma nuances in Sauvignon blanc wine break down when exposed to light and heat. South African Sauvignon blanc producers must therefore take extra care to control the ripening temperature of the grape berries in local warm climatic conditions. Viticultural as well as winemaking practices such as canopy management, early morning harvest and low fermentation temperatures should be adhered to, to produce wines with a good balance between tropical and green flavours. Sauvignon blanc wines have been adulterated using green aroma enhancing flavourings (Du Plessis, 2005; Marais, 2010). Testing methods for the identification of wine adulteration have been developed. Current methods such as GC-MS and LC-MS are used for quantification of methoxypyrazines (Alberts, 2009). The levels of methoxypyrazines in wines suspected of adulteration are compared to a South African Sauvignon blanc juice and wine database that contains information on the typical grape methoxypyrazine concentrations and ratios between the chemical species of IBMP, SBMP and IPMP.

GC-MS and LC-MS methods, although very sensitive, are costly and time consuming and are not fit for rapid screening of large numbers of samples. Furthermore, targeted methods such as these also require the presence of methoxypyrazines in the flavouring or adulterant.

This study explored the possibility of FT-IR technology, commonly used for routine analysis of wines and suitable for bulk screening of samples (Manley *et al.*, 2001; Blanco *et al.*, 2004; Patz *et al.*, 2004; Swanepoel *et al.*, 2007; Versari *et al.*, 2008) to identify Sauvignon blanc wines suspected of adulteration in terms of added flavourings.

Different flavourings as well as control and flavoured wines were analysed using GC-MS to identify and estimate the concentration of the methoxypyrazine compounds. Estimated concentrations were used to calculate spiking volumes that would a) fall within the lower end of the natural abundance range of the main aroma compound in Sauvignon blanc wine, b) fall within the medium to higher range of the natural abundance range of the main aroma compound in Sauvignon blanc wine, and c) fall outside of the natural abundance range of the main aroma compound in wine. Commercially available green grass flavour contained amounts of methoxypyrazines similar to the concentration of methoxypyrazines in wine. The dilution of this flavouring to spike wine would lead to the dilution of methoxypyrazines below the detection limit and odour threshold value in the spiked wine. However, hexenol was identified to be present at high concentrations and could be considered as an additional marker compound to detect the addition of this flavouring to the experimental wines produced in this study. The natural green pepper extract and commercial asparagus flavouring contained high

concentrations of methoxypyrazines. Wines adulterated with either of these two flavourings would be positively identified using the current GC-MS and LC-MS methods quantifying methoxypyrazines. Therefore, for wines adulterated with the four “green flavour” enhancing flavourings used in this study, only two groups would be positively identified. This finding highlights the importance of using complementary techniques of targeted nature (such as GC-MS and LC-MS) and broad range non-targeted profiling techniques, such as infrared spectroscopy.

Sensory analysis conducted on control and spiked wines showed that adulteration of the wine masked the tropical character of the wines. Addition of the asparagus flavouring to wine masked the tropical flavours even when added at a low concentration level. Therefore by adding flavourings to the wine the balance between tropical and green flavours could be disturbed. This would happen in such a way that the tropical flavours are negatively affected instead of creating a wine with well balanced tropical and green flavours. It is generally assumed that the tropical flavours are already present and only green flavours need to be enhanced.

Multivariate models that discriminated between adulterated and control wines could be constructed from FT-IR (MIR and NIR) spectral data obtained from wines spiked with the different flavourings, as well as different concentrations of flavourings. Spectra in both transmission mode and total attenuated reflection were used to establish the models. PCA, PLS-D and spectral conformity testing performed on FT-MIR data sets clearly showed discrimination between spiked and control wines, although of various degrees of success. Best discrimination results were obtained with near infrared spectra collected with a sampling probe that was inserted into freshly opened bottles of wine. The latter technique also had the advantage that no sample preparation was needed prior to collection of the spectra.

Thus from this pilot study, it can be concluded that screening Sauvignon blanc wines for adulteration in terms of added flavourings using Fourier transform infrared spectroscopy, both in the mid-infrared and near infrared region shows potential that can be further investigated in future work. The work also highlights the shortcomings of existing GC-MS and LC-MS methods, targeted at quantification of methoxypyrazines alone and emphasizes the usefulness of a rapid infrared-based screening method that can be used in conjunction to classical chromatography.

4.2 Future work

Although this study proved the potential of FT-IR as screening method for the identification of suspect Sauvignon blanc wines, this method must be optimised and tested on an industrial / commercial level with commercial wines. In addition, a method for the detection of adulteration that can be used for conclusive legal proof, still needs to be developed.

Recently 2D GC (GC x GC) has been increasingly applied as analytical technique. Results obtained from 2D GC can be plotted as a 3D graph or a colour image, where colour represents the third dimension of the image. Image processing applied to the GC X GC colour graph can be used as statistical tool for discrimination between spiked and control wines. Another possible approach is to search for or identify compounds in green pepper extract not occurring in wines that can still be detected in wines at low levels after adulteration of the wine with green pepper extract. The same strategy should also be followed then for the identification of such compound in artificial flavourings. The identified compounds foreign to wine can be tested for in wines suspected of adulteration after failing the screening process.

4.3 References

Alberts, P., Stander, M.A., Paul, S.O., De Villiers, A. (2009). Survey of 3-alkyl-2-methoxypyrazines content of South African Sauvignon blanc wines using a novel LC-APC1-MS/MS method. *J. Agric. Food Chem.* **57**, 9347-9355

Allen, M.S., Lacey, M.J., Harris, R.L.N., Brown, W.B. (1991). Contribution of methoxypyrazines to Sauvignon blanc wine aroma. *Am. J. Enol. Vitic.* **42**, 109-112.

Blanco, M., Peinado, A.C., Mas, J. (2004). Analytical monitoring of alcoholic fermentation using NIR spectroscopy. *Biotech. and Bioengineering.* **88**, 536-542.

Du Plessis, C. (2005). Sauvignon blanc – the dust hasn't settled. *Winelands* [URL. http://www.wineland.co.za/0204suvignon.php3]. Cited the 26th of August 2008

Lacey, M.J., Allen, M.S., Harris, R.L.N., Brown, W.V. (1991). Methoxypyrazines in Sauvignon blanc grapes and wine. *Am. J. Enol. Vitic.* **42**, 103-108.

Manley, M., Van Zyl, A., Wolf, E.E.H. (2001). The evaluation of the applicability of Fourier Transform Near-Infrared (FT-IR) spectroscopy in the measurement of analytical parameters in must and wine. *S. Afr. J. Enol. Vitic.* **22**, 93-100.

Marais, J. (1994). Sauvignon blanc cultivar aroma – a review. *S. Afr. J. Enol. Vitic.* **15**, 41-45

Marais, J. (2010). Introduction to publications from the investigation into the aroma databases of different South African Cultivars. *Winelands* [URL. http://www.wineland.co.za/index.php?option=com_zine &view=article&id=81:introduction-to-publications-from-the-investigation-into-the-aroma-databases-of-different-south-african-cultivars&q=sauvignon]. Cited the 3rd of December 2010

Patz, C.D., Blieke, A., Risow, R., Dietrich, H. (2004). Application of FT-MIR spectrometry in wine analysis. *Anal. Chim. Acta.* **513**, 81-89

SAWIS. (2010). South African wine industry information and systems. [URL. http://www.sawis.co.za/info/statistics.php]. Cited the 10th of December 2010

Swanepoel, M., Du Toit M., Nieuwoudt, H.H. (2007). Optimisation of the quantification of total soluble solids, pH and titrable acidity in South African must using Fourier transform mid-infrared spectroscopy. *S. Afr. J. Enol. Vitic.* **28**, 140-149

Versari, A., Parpinello, G.P., Mattioli, A.U., Galassi, S. (2008). Determination of grape quality at harvest using Fourier-Transform mid-infrared spectroscopy and multivariate analysis. *Am. J. Vitic.* **59**, 317-322.