JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Analysis of Volatiles in Pinotage Wines by Stir Bar Sorptive Extraction and Chemometric Profiling

BERHANE T. WELDEGERGIS AND ANDREW M. CROUCH*

Department of Chemistry and Polymer Science, University of Stellenbosch, Private Bag X1, Matieland 7602, Stellenbosch, South Africa

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

KEYWORDS: SBSE; headspace extraction; volatile compounds; Pinotage; wine; GC; MS

INTRODUCTION

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

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

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

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

Pawliszyn and co-workers developed a solvent-free extraction method in the early 1990s called solid phase microextraction (SPME) (*14*). It involves no solvent consumption, which has an important effect on analytical costs and the environment (*15*). SPME can be very selective and can result in the production of clear chromatograms from complex matrices such as wine,

* Corresponding author (telephone +27 21 808 3535; fax +27 21 808 3342; e-mail amc@sun.ac.za).

10.1021/jf8015706 CCC: $40.75
Published on Web 10/22/2008

depending on the type of fiber used. The application of SPME for wine analysis has increased tremendously since its invention (*7, 11*). However, due to the smaller sample capacity of SPME and the low concentrations of some of the volatile compounds in wine, a better enrichment probe is often desirable (*7*).

More recently, a new extraction procedure for aqueous samples, named stir bar sorptive extraction (SBSE), was developed by Baltussen and Sandra (*16*). The theory of SBSE is very similar to that of SPME, where the efficiency of analytes partitioning into the polydimethylsiloxane (PDMS) phase of the stir bar at the equilibrium can roughly be predicted by the octanol−water partition coefficients (*16*). SBSE offers higher sample capacity (50−250 times higher) due to the greater amounts of PDMS phase (24−126 μL) (*6, 15*) in which the amount of the analyte is extracted. The extraction efficiency is proportional to the coating thickness, resulting in lower detection limits. This can be very useful for trace and ultratrace analysis (*7*). SBSE extraction can be done either in the headspace mode (*17*) or by introducing the stir bar directly into the aqueous sample (*4*) and stirring for a given time. SBSE has been applied successfully for the analyses of aroma compounds in wine (*4, 6, 18*).

The concentration and type of flavoring compounds in wine are greatly influenced by many viticultural and enological factors (*19*). Despite the complexity of factors influencing the formation of volatiles in wine, a correlation between the concentration of wine volatiles and grape variety (*20*), winemaking practice (*21*), and aging (*9*) was evident. However, obtaining feasible information from wine analysis may result in a difficult task due to the multiple sources of variation stated above. As a result, the application of chemometrics to wine data has grown tremendously in the past few years because it provides fast and more precise assessment of composition. For example, Martí et al. (*22*) evaluated the classification and differentiation of wines based on grape varieties, origin, and aging using principal component analysis (PCA). Other authors applied discriminant analysis (DA) to classify wines according to grape variety (*23*). Similarly PCA (*10, 22, 23*), cluster analysis (*22*), and DA (*10, 21*) have been applied to characterize wines. By relating all of the components to the different factors that affect the quality of wine some control can be exercised on the conditions for producing a well-balanced good quality wine from one production year to the next.

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

MATERIAL AND METHODS

Standards, Reagents, and Equipment. Standards of ethyl acetate, ethyl butyrate, 1-propanol, isobutanol, *n*-butanol, hexyl acetate, acetoin, ethyl D-lactate, ethyl octanoate, furfural, diethyl succinate, 2-phenylethyl acetate, 2,6-dimethoxyphenol, eugenol, 5-(hydroxymethyl)furfural, propionic acid, *n*-butyric acid, isobutyric acid, *n*-valeric acid, isovaleric acid, and 4-methyl-2-pentanol (internal standard), as well as solvent acetone (pestanal grade) and NaCl were purchased from Fluka (Zwijndrecht, The Netherlands). Isoamyl acetate, isoamyl alcohol, 1-hexanol, 2-phenylethyl alcohol, 5-methylfurfural, ethyl hexanoate, *o*-cresol, *p*-cresol, whiskey lactone (4-hydroxy-3-methyloctanoic acid lactone, also called oak lactone), vanillin, hexanoic acid, octanoic acid, decanoic acid, ethyl decanoate, phenol, guaiacol, 4-ethylguaiacol, and solvents methanol and absolute ethanol (HPLC grade) were supplied by Aldrich (Steinheim, Germany). Acetic acid (Merck, Darmstadt, Germany), tartaric acid (Analar, the British drug Houses Ltd. England), and ultrapure water purified by a Milli-Q water purification system (Millipore, Bedford, MA) were used.

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

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

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

Instrumental Conditions. The instrumental conditions previously reported (*24*) were slightly modified as follows. The GC-MS analysis was carried out with an Agilent 6890 GC coupled to a 5973N MS (Agilent Technologies). A 30 m HP-INNOWax capillary column [0.250 mm i.d. × 0.5 μm film thickness (Agilent Technologies)] was used for

Table 1. Pinotage Wine Samples Analyzed (for Conditions, See Text)

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

[a] Number of samples from each region. [b] Codes given to the different regions from which the samples were collected: W, Worcester; S, Stellenbosch; P, Paarl; SW, Swartland; RO, Robertson; OR, Olifant River; KK, Klein Karoo. [c] Code given to each sample. [d] Code given to each wine producer (supplier).

Table 2. Selected Ions for SIM Mode and Method Linearity Data ($n = 3$) Obtained by Headspace SBSE-TD-GC-MS (for Conditions, See Text)

no.	compound	selected ions	*Y*-intercept	slope	R^2
1	ethyl acetate	61, 70, 88	0.0007	0.0092	0.9983
2	ethyl butyrate	72, 101, 116	−0.0004	0.1387	0.9997
3	1-propanol	31, 33, 34	−0.0002	0.0035	0.9985
4	isobutanol	31, 33, 40	0.0046	0.0035	0.9991
5	isoamyl acetate	69, 71, 87	−0.0006	0.532	0.9999
6	*n*-butanol	31, 33, 45	0.0118	0.0107	0.9985
7	isoamyl alcohol	31, 39, 69	0.0009	0.0204	1.0000
8	ethyl hexanoate	100, 101, 116	0.0004	3.4658	1.0000
9	hexyl acetate	56, 61, 84	−0.0016	6.389	0.9998
10	acetoin	45, 46, 88	0.0029	0.0005	0.9921
11	ethyl D-lactate	45, 47, 75	0.0043	0.0053	0.9994
12	1-hexanol	68, 69, 84	0.0078	0.0898	0.9991
13	ethyl octanoate	83, 127, 172	−0.003	23.996	0.9992
14	acetic acid	47, 60, 61	0.0828	0.0007	0.992
15	furfural	95, 96, 97	0.057	0.0271	0.9954
16	propionic acid	30, 31, 74	0.0065	0.0025	0.9956
17	isobutyric acid	41, 60, 88	0.00008	0.0006	0.9998
18	5-methylfurfural	81, 109, 110	0.0027	0.0438	0.9999
19	*n*-butyric acid	37, 38, 60	0.0019	0.0043	0.9999
20	ethyl decanoate	155, 157, 200	0.0032	3.6248	0.999
21	isovaleric acid	60, 87, 100	−0.0008	0.0126	0.9991
22	diethyl succinate	128, 130, 174	−0.0038	0.0116	0.998
23	*n*-valeric acid	60, 74, 87	−0.0007	0.0086	0.9987
24	2-phenethyl acetate	78, 104, 105	0.0395	0.1754	0.9985
25	hexanoic acid	60, 74, 87	−0.0012	0.0127	0.9967
26	guaiacol	81, 109, 124	0.0005	0.0358	0.9992
27	*trans*-oak lactone	96, 99, 100	−0.0024	0.028	0.9976
28	2-phenylethyl alcohol	92, 122, 123	−0.0009	0.0069	0.9994
29	*cis*-oak lactone	99, 100, 114	−0.0009	0.0112	0.9977
30	*o*-cresol	90, 107, 108	−0.0025	0.0441	0.9978
31	phenol	66, 93, 94	0.0015	0.0197	0.9994
32	4-ethylguaiacol	121, 137, 152	−0.0028	0.1016	0.997
33	octanoic acid	60, 84, 115	−0.0009	0.0191	0.9935
34	*p*-cresol	77, 107, 109	−0.0002	0.0169	0.9986
35	eugenol	121, 131, 164	−0.0003	0.0078	0.9963
36	decanoic acid	60, 143, 172	−0.0005	0.0115	0.9967
37	2,6-dimethoxyphenol	93, 96, 140	0.00007	0.00005	0.9945
38	5-(hydroxymethyl)furfural	97, 109, 126	0.0051	0.0044	0.9946
39	vanillin	81, 151, 152	0.0031	0.0004	0.9949

[a] Underscoring indicates the quantitative (target) ion.

separating the volatile compounds. The GC oven was held at 30 °C for 2 min and increased to 130 °C at a rate of 4 °C/min and then at 8 °C/min to 250 °C, at which it was kept for 5 min. Helium was used as the carrier gas with a flow of 1 mL/min in the constant pressure mode. The MS was operated in a scan mode with a scan range of 30−350 amu at 4.45 scans/s, for peak identification, ion selection, and locating the compounds in the TIC plot. However, for quantitation purposes the MS was operated in the selected ion monitoring (SIM) mode. Three ions with a dwell time of 50 ms for each compound (one quantitative or target ion and two qualitative ions) were selected (**Table 2**). Spectra were recorded in the electron impact mode (EI) at 70 eV. The MS transfer line, source, and quadrupole were at 250, 230, and 150 °C, respectively. Identification was based on comparison of mass spectra with Wiley 275 and NIST 98 libraries as well as retention times of known standards in synthetic wine for all compounds. As a complementary identification, linear retention indices (LRI) were experimentally determined using a mixture of *n*-alkanes and compared with literature values (**Table 3**).

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

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

Statistical Analysis. The quantitative chemical data obtained were used as variables for object description. The objects were young Pinotage wines of two vintages produced by different winemakers from seven regions (**Table 1**). The measured amount of the 39 analytes obtained from each wine was used for computerized multivariate analysis of data, as exploratory factor analysis (FA), principal component analysis (PCA), and ANOVA by the software package Statistica 8 (2007) from StatSoft, Inc. (Tulsa, OK). A 5% significance level ($p < 0.05$) was used as a guideline for determining significant differences.

RESULTS AND DISCUSSION

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

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

The precision (repeatability) of the method was evaluated with a synthetic wine of the same batch using different stir bars, presuming all PDMS-coated stir bars are the same and following the previously mentioned HS-SBSE procedure and TD-GC-MS analysis. It was estimated as percent relative standard deviation (%RSD) of the relative peak areas for seven replicates ($n = 7$) and varied between 6 and 20% (**Table 3**), with an average of 13%. The intermediate precision (intermediate repeatability) was examined by analyzing five replicates ($n = 5$) of different batches using different stir bars and calculated in terms of %RSD

Table 3. Method Validation Data Obtained Using Headspace SBSE-TD-GC-MS (for Conditions, See Text)

compound	LOD[a] (ng/ L)	LOQ[b] (ng/L)	precision[c]	intermediate precision[d]	rel % recovery	LRI_{calcd}[e]	$LRI_{lit.}$[f]	ΔLRI[g]
ethyl acetate	24.7	82.4	8	6	69	900	899 (*25*)	1
ethyl butyrate	210[h]	710[h]	6	6	42	1044	1046 (*25*)	2
1-propanol	281	938	10	17	27	1046	1051 (*25*)	5
isobutanol	2.74	9.14	13	13	93	1103	1105 (*25*)	2
isoamyl acetate	21.4	71.2	7	18	46	1128	1127 (*25*)	1
n-butanol	530[h]	1.75	16	12	24	1155	1155 (*25*)	0
isoamyl alcohol	104	347	6	14	80	1220	1221 (*25*)	1
ethyl hexanoate	1.06	3.55	7	16	66	1245	1242 (*25*)	3
hexyl acetate	810[h]	2.70	7	16	52	1285	1269 (*25*)	16
acetoin	18.3	61.1	16	20	68	1302	1291 (*3*)	11
ethyl D-lactate	38.2	128	18	14	73	1357	1353 (*3*)	4
1-hexanol	8.97	29.9	6	14	112	1365	1362 (*25*)	3
ethyl octanoate	60.0[h]	190[h]	10	14	53	1448	1444 (*5*)	4
acetic acid	460[h]	1.53	16	15	47	1463	1461 (*5*)	2
furfural	50.0[h]	180[h]	15	11	101	1483	1474 (*25*)	9
propionic acid	380[h]	1.25	15	20	24	1554	1554 (*25*)	0
isobutyric acid	1.41	4.69	17	10	43	1582	1584 (*3*)	2
5-methylfurfural	60.0[h]	200[h]	11	3	97	1597	1591 (*25*)	6
n-butyric acid	2.19	7.29	17	9	79	1643	1646 (*5*)	3
ethyl decanoate	1.81	6.04	11	15	83	1653	1647 (*25*)	6
isovaleric acid	2.77	9.22	16	9	77	1690	1687 (*5*)	3
diethyl succinate	46.2	154	17	9	74	1701	1690 (*3*)	11
n-valeric acid	4.03	13.4	20	5	71	1755	1755 (*26*)	0
2-phenethyl acetate	1.65	5.49	12	11	98	1845	1830 (*5*)	15
hexanoic acid	2.63	8.76	19	14	68	1857	1857 (*27*)	0
guaiacol	360[h]	1.20	12	18	92	1899	1880 (*5*)	19
trans-oak lactone	14.1	47.1	19	7	77	1925	1933 (*28*)	8
2-phenylethyl alcohol	6.82	22.7	18	17	105	1944	1942 (*5*)	2
cis-oak lactone	10.1	33.6	17	16	87	2006	1993 (*27*)	13
o-cresol	2.39	7.96	11	13	70	2030	2017 (*28*)	13
phenol	3.80	12.7	15	16	52	2035	2039 (*29*)	4
4-ethylguaiacol	1.75	5.83	12	2	69	2068	2055 (*27*)	13
octanoic acid	3.68	12.3	10	17	61	2077	2072 (*26*)	5
p-cresol	2.95	9.82	10	11	99	2112	2103 (*26*)	9
eugenol	10.9	36.2	18	18	68	2211	2215 (*27*)	4
decanoic acid	13.3	44.3	13	18	79	2281	2294 (*26*)	13
2,6-dimethoxyphenol	7.68	25.6	7	13	40	2298	2307 (*5*)	9
5-(hydroxymethyl)furfural	410[h]	1.36	11	9	63	2527	2526 (*30*)	1
vanillin	720[h]	2.41	18	18	49	2598	2581(*27*)	17

[a] Limit of detection. [b] Limit of quantitation. [c] Precision ($n = 7$). [d] Intermediate precision ($n = 5$). [e] Calculated linear retention indices using *n*-alkanes on HP-INNOWax column. [f] Linear retention indices obtained from the literature. [g] Difference between the calculated and literature values of the linear retention indices. [h] LODs and LOQs expressed in pg/L.

(**Table 3**). The results indicated fluctuations between 2 and 20% with a mean %RSD of 13%.

In the sorptive extraction procedure recovery should be expressed as the ratio of the extracted amount of solute into the PDMS phase (m_{PDMS}) over the original amount of solute in the water phase ($m_o = m_w + m_{PDMS}$), which depends on the partition coefficient (*15*). However, headspace SBSE involves three phases (liquid, gas, and PDMS), and analytes experience different distribution properties among the different phases (*6*). As a result, it was not practical to calculate the absolute recovery because the original concentration of the analytes was dispersed among the three phases. Even so, the relative recovery (**Table 3**) was carried out from a spiked wine at different concentrations and was varied between 24 and 112% for all of the analytes.

Wine Analysis. To the best of our knowledge this is the largest survey of South African Pinotage wine to date, which includes large numbers of major volatiles classified under different classes. The survey was done for 87 young wines from 2005 and 2006 vintages. Moreover, the wines were from seven different regions (districts) and produced by different wine-makers (**Table 1**). This paper indicates a large number of compounds, and it correlates the concentrations obtained among the different classes of volatiles as well as to their respective year and area of production.

Figure 1 is an example of an ion monitoring chromatogram of a typical aroma profile of a Pinotage wine from the vintage 2006 obtained by headspace SBSE in combination with TD-GC-MS. Identification of analytes was carried out using mass spectra from Wiley 275 and NIST 98 libraries, retention times of known standards in synthetic wine, and linear retention indices (LRI) (**Table 3**).

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

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

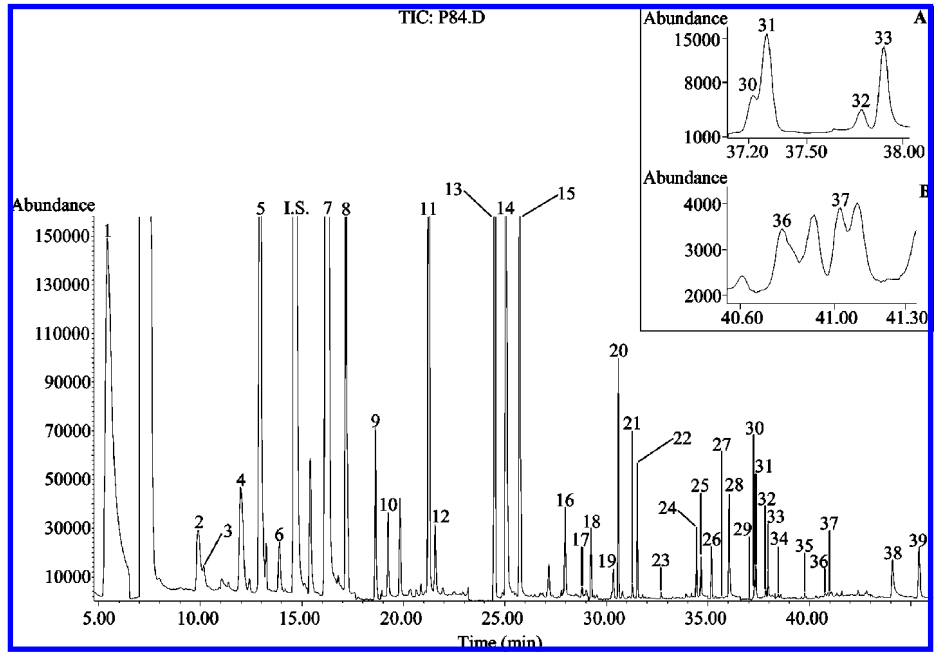

Figure 1. GC-MS ion monitoring chromatogram of young Pinotage wine from 2006 vintage: (**A**) inlay of peaks 30−33; (**B**) inlay of peaks 36 and 37. Concentration of I.S. was 1.7 mg/L. Peak identity is given in **Table 2** and quantitation in **Tables 4** and **5**. For conditions, see text.

Table 4. Average ± Standard Deviation (SD), Minimum, and Maximum Concentrations (Milligrams per Liter) of Volatiles in Pinotage Wines from Vintages 2005 and 2006 Obtained by Headspace SBSE-TD-GC-MS (for Conditions, See Text)

	Pinotage vintage 2005 ($n = 47$)[a]			Pinotage vintage 2006 ($n = 40$)[a]		
compound	av ± SD	max	min	av ± SD	max	min
ethyl acetate	142 ± 43	223	71.7	191 ± 35	277	124
ethyl butyrate	300 ± 90*	530*	130*	360 ± 90.0*	620*	210*
1-propanol	60.5 ± 39	211	15.7	50.4 ± 34	152	15.4
isobutanol	54.5 ± 23	118	230*	49.5 ± 16	86.3	7.63
isoamyl acetate	4.49 ± 2.3	9.59	420*	5.84 ± 2.9	10.9	400*
n-butanol	7.47 ± 3.5	11.8	30.0*	8.03 ± 4.0	29.7	10.0*
isoamyl alcohol	160 ± 21	201	122	152 ± 18	192	117
ethyl hexanoate	210 ± 90*	550*	60.0*	430 ± 120*	830*	280*
hexyl acetate	20.0 ± 10*	50.0*	10.0*	30.0 ± 20*	90.0*	4.00*
acetoin	57.4 ± 42	176	1.84	68.9 ± 52	219	3.15
ethyl D-lactate	294 ± 133	915	3.94	295 ± 97	486	1.04
1-hexanol	573 ± 318*	1.03	50.0*	610 ± 390*	1.62	500**
ethyl octanoate	30.0 ± 10*	90.0*	10.0*	120 ± 40*	220*	60.0*
acetic acid	847 ± 440	2.63×10^3	314	666 ± 470	2.64×10^3	188
furfural	15.7 ± 7.4	34.9	690*	10.0 ± 6.9	21.5	250*
propionic acid	19.4 ± 10	47.6	6.80	15.2 ± 9.4	39.3	1.36
isobutyric acid	1.73 ± 0.89	5.36	400*	2.52 ± 1.2	7.14	1.25
5-methylfurfural	430 ± 270*	840*	10.0*	320 ± 210*	830*	30.0*
n-butyric acid	3.13 ± 1.71	5.14	40.0*	2.20 ± 1.8	5.27	80.0*
ethyl decanoate	10.0 ± 2.0*	10.0*	70.0**	40.0 ± 30*	110*	4.00*
isovaleric acid	1.65 ± 0.45	4.46	1.27	1.60 ± 0.13	2.00	1.35
diethyl succinate	9.63 ± 2.4	15.4	5.30	9.33 ± 3.0	17.1	4.60
n-valeric acid	1.59 ± 0.35	3.80	1.30	1.63 ± 0.14	1.90	1.38
2-phenethyl acetate	200 ± 130*	560*	40.0*	300 ± 200*	1.04	30.0*
hexanoic acid	3.50 ± 0.62	5.66	2.43	4.13 ± 0.78	6.38	2.89
guaiacol	450 ± 260*	1.14	40.0*	470 ± 220*	1.26	90.0*
trans-oak lactone	1.04 ± 0.01	1.05	1.03	1.04 ± 0.004	1.04	1.03
2-phenylethyl alcohol	16.4 ± 6.5	36.8	8.47	13.4 ± 4.3	24.3	6.76
cis-oak lactone	1.00 ± 0.02	1.04	980*	980 ± 10*	1.00	970*
o-cresol	850 ± 60*	1.00	770*	830 ± 30*	910*	740*
phenol	1.02 ± 0.72	3.27	190*	740 ± 350*	1.55	200*
4-ethylguaiacol	360 ± 10*	390*	340*	370 ± 50*	700*	340*
octanoic acid	1.62 ± 0.42	3.33	1.04	1.90 ± 0.37	3.08	1.28
p-cresol	290 ± 50*	430*	220*	280 ± 20*	350*	250*
eugenol	650 ± 100*	950*	510*	635 ± 85*	952*	496*
decanoic acid	730 ± 190*	1.91	600*	780 ± 80*	1.02	690*
2,6-dimethoxyphenol	12.5 ± 12	53.7	3.40	9.69 ± 6.9	37.0	1.72
5-(hydroxymethyl)furfural	7.05 ± 6.8	27.8	590*	2.33 ± 3.0	12.9	70.0*
vanillin	40.9 ± 25	141	14.9	43.7 ± 50	237	3.98

[a] *n*, number of samples. *, measured in μg/L; **, measured in ng/L.

Table 5. Mean ± Standard Deviations (SD) (Milligrams per Liter) of Volatiles in Pinotage 2005 and 2006 Vintages Collected from Various South African Regions Obtained Using Headspace SBSE-TD-GC-MS (for Conditions, See Text)

	Vintage 2005[a]					
compound	P ($n = 10$)	S ($n = 10$)	W ($n = 14$)	RO ($n = 4$)	OR ($n = 4$)	SW ($n = 5$)
ethyl acetate	161 ± 40	134 ± 43	134 ± 45	137 ± 38	116 ± 33	172 ± 47
ethyl butyrate	280 ± 80*	250 ± 40*	340 ± 110*	200 ± 60*	350 ± 73*	350 ± 80*
1-propanol	63.6 ± 42	64.9 ± 35	62.3 ± 24	43.1 ± 12	42.0 ± 20	78.3 ± 86
isobutanol	51.5 ± 12	57.9 ± 27	53.5 ± 23	81.9 ± 31	52.5 ± 8.3	36.1 ± 21
isoamyl acetate	4.45 ± 2.1	5.56 ± 2.3	4.64 ± 1.9	1.36 ± 0.60	6.27 ± 1.9	3.12 ± 2.9
n-butanol	7.83 ± 2.6	7.87 ± 3.6	8.36 ± 3.3	4.72 ± 3.5	5.61 ± 5.8	7.15 ± 3.6
isoamyl alcohol	145 ± 15	158 ± 26	164 ± 18	175 ± 23	173 ± 18	162 ± 16
ethyl hexanoate	190 ± 70*	150 ± 30*	250 ± 130*	150 ± 30*	270 ± 60*	230 ± 60*
hexyl acetate	10.0 ± 10*	20.0 ± 10*	20.0 ± 10*	10.0 ± 2.0*	20.0 ± 10*	10.0 ± 5.0*
acetoin	57.5 ± 57	55.9 ± 35	53.6 ± 41	81.8 ± 60	42.7 ± 27	62.9 ± 28
ethyl D-lactate	264 ± 110	337 ± 82	259 ± 93	241 ± 115	316 ± 90	389 ± 302
1-hexanol	655 ± 305*	570 ± 320*	570 ± 330*	340 ± 400*	650 ± 300*	560 ± 340*
ethyl octanoate	30.0 ± 10*	30.0 ± 10*	40.0 ± 20*	20.0 ± 10*	40.0 ± 10*	30.0 ± 10*
acetic acid	634 ± 232	973 ± 381	875 ± 657	842 ± 340	973 ± 161	848 ± 309
furfural	14.6 ± 7.5	18.7 ± 2.1	15.0 ± 10	13.3 ± 8.9	12.0 ± 8.0	18.4 ± 2.1
propionic acid	17.0 ± 7.3	23.2 ± 11	19.1 ± 13	20.8 ± 15	17.1 ± 5.1	18.7 ± 9.4
isobutyric acid	1.32 ± 0.56	2.11 ± 1.3	1.31 ± 0.49	2.13 ± 0.57	2.64 ± 0.82	1.96 ± 0.80
5-methylfurfural	470 ± 270*	490 ± 290*	370 ± 280*	370 ± 340*	490 ± 70*	400 ± 320*
n-butyric acid	2.60 ± 1.8	3.58 ± 1.5	3.45 ± 1.7	2.56 ± 1.6	3.03 ± 2.1	2.92 ± 2.3
ethyl decanoate	10.0 ± 2.0*	10.0 ± 3.0*	10.0 ± 2.0*	4.00 ± 2.0*	10.0 ± 3.0*	10.0 ± 2.0*
isovaleric acid	1.50 ± 0.14	1.92 ± 0.91	1.60 ± 0.19	1.54 ± 0.05	1.72 ± 0.17	1.61 ± 0.12
diethyl succinate	9.69 ± 1.7	9.59 ± 2.3	9.47 ± 2.7	7.07 ± 1.1	11.42 ± 1.83	10.6 ± 2.6
n-valeric acid	1.48 ± 0.09	1.76 ± 0.73	1.58 ± 0.13	1.55 ± 0.12	1.61 ± 0.10	1.53 ± 0.15
2-phenethyl acetate	150 ± 80*	320 ± 160*	170 ± 90*	110 ± 80*	300 ± 150*	160 ± 110*
hexanoic acid	3.19 ± 0.44	3.56 ± 0.87	3.52 ± 0.51	3.05 ± 0.52	4.06 ± 0.32	3.88 ± 0.46
guaiacol	420 ± 200*	500 ± 270*	450 ± 310*	460 ± 270*	370 ± 180*	460 ± 300*
trans-oak lactone	1.04 ± 0.01	1.04 ± 0.004	1.04 ± 0.01	1.04 ± 0.01	1.04 ± 0.01	1.04 ± 0.004
2-phenylethyl alcohol	13.3 ± 4.1	21.2 ± 9.1	14.2 ± 5.0	18.5 ± 4.6	17.7 ± 4.3	16.1 ± 6.3
cis-oak lactone	1.01 ± 0.02	990 ± 8.0*	1.00 ± 0.02	1.00 ± 0.01	990 ± 10*	990 ± 10*
o-cresol	840 ± 50*	850 ± 60*	850 ± 70*	870 ± 80*	830 ± 50*	840 ± 60*
phenol	910 ± 450*	830 ± 620*	1.09 ± 0.89	1.36 ± 0.55	1.31 ± 1.2	920 ± 620*
4-ethylguaiacol	360 ± 10*	360 ± 10*	360 ± 10*	360 ± 10*	360 ± 10*	360 ± 10*
octanoic acid	1.43 ± 0.27	1.86 ± 0.64	1.56 ± 0.30	1.36 ± 0.36	1.77 ± 0.36	1.79 ± 0.25
p-cresol	280 ± 50*	280 ± 50*	300 ± 60*	280 ± 40*	290 ± 26*	300 ± 40*
eugenol	620 ± 90*	670 ± 100*	660 ± 120*	650 ± 70*	620 ± 80*	650 ± 130*
decanoic acid	670 ± 50*	840 ± 390*	(690 ± 50)*	670 ± 60*	730 ± 70*	730 ± 80*
2,6-dimethoxyphenol	8.62 ± 6.1	6.68 ± 3.4	14.8 ± 17	11.3 ± 4.1	28.2 ± 10	13.8 ± 7.4
5-(hydroxymethyl)furfural	1.25 ± 0.88	13.0 ± 8.3	8.11 ± 6.2	6.34 ± 6.8	6.85 ± 4.1	4.48 ± 3.2
vanillin	32.2 ± 14	37.9 ± 24	42.5 ± 33	45.9 ± 12	45.9 ± 7.4	52.0 ± 36

	Vintage 2006[a]					
compound	P ($n = 9$)	S ($n = 4$)	W ($n = 11$)	RO ($n = 7$)	KK ($n = 4$)	SW ($n = 5$)
ethyl acetate	215 ± 41	194 ± 48	190 ± 26	174 ± 34	177 ± 12	181 ± 28
ethyl butyrate	420 ± 110*	330 ± 70*	390 ± 80*	300 ± 60*	350 ± 50*	330 ± 70*
1-propanol	58.8 ± 28	61.8 ± 61	62.1 ± 43	45.7 ± 22	34.9 ± 7.5	35.0 ± 14
isobutanol	44.3 ± 19	52.5 ± 25	45.6 ± 11	57.6 ± 22	48.3 ± 6.7	55.1 ± 10
isoamyl acetate	6.06 ± 3.0	7.13 ± 4.1	6.53 ± 2.1	5.31 ± 3.3	5.12 ± 2.3	4.18 ± 3.5
n-butanol	7.01 ± 3.1	8.73 ± 1.8	9.66 ± 6.9	7.09 ± 1.1	7.28 ± 1.5	7.67 ± 2.0
isoamyl alcohol	149 ± 18	148 ± 14	148 ± 11	169 ± 21	149 ± 22	145 ± 21
ethyl hexanoate	500 ± 150*	390 ± 50*	430 ± 100*	380 ± 110*	350 ± 60*	510 ± 160*
hexyl acetate	30.0 ± 20*	40.0 ± 20*	40.0 ± 20*	30.0 ± 20*	20.0 ± 10*	40.0 ± 30*
acetoin	77.5 ± 66	77.8 ± 92	52.1 ± 25	76.8 ± 48	67.4 ± 74	73.1 ± 26
ethyl D-lactate	335 ± 89	319 ± 57	288 ± 82	275 ± 128	235 ± 160	296 ± 68
1-hexanol	640 ± 320*	500 ± 280*	620 ± 570*	780 ± 170*	510 ± 460*	450 ± 350*
ethyl octanoate	140 ± 40*	110 ± 40*	110 ± 30*	100 ± 30*	100 ± 30*	120 ± 40*
acetic acid	622 ± 294	(1.48 ± 1.1) × 10^3	494 ± 223	748 ± 229	452 ± 202	533 ± 228
furfural	9.68 ± 7.8	13.7 ± 7.6	10.9 ± 7.4	8.14 ± 5.4	10.9 ± 6.7	7.69 ± 7.5
propionic acid	16.4 ± 6.9	22.3 ± 17	12.8 ± 7.5	15.2 ± 11	12.5 ± 2.4	15.0 ± 12
isobutyric acid	2.60 ± 1.5	2.29 ± 0.60	1.87 ± 0.35	3.28 ± 2.0	2.59 ± 0.51	2.84 ± 0.88
5-methylfurfural	330 ± 220*	390 ± 310*	370 ± 260*	240 ± 190*	200 ± 150*	310 ± 80*
n-butyric acid	1.44 ± 1.3	3.43 ± 1.9	2.86 ± 1.7	1.66 ± 2.1	1.30 ± 1.1	2.62 ± 2.4
ethyl decanoate	60.0 ± 30*	40.0 ± 20*	30.0 ± 20*	30.0 ± 25*	20.0 ± 10*	41.2 ± 40*
isovaleric acid	1.58 ± 0.13	1.69 ± 0.10	1.55 ± 0.12	1.71 ± 0.15	1.61 ± 0.12	1.54 ± 0.11
diethyl succinate	8.30 ± 2.4	12.6 ± 3.4	8.79 ± 2.0	10.8 ± 4.0	8.54 ± 3.2	8.37 ± 2.2
n-valeric acid	1.63 ± 0.14	1.65 ± 0.21	1.60 ± 0.12	1.60 ± 0.12	1.67 ± 0.19	1.65 ± 0.19
2-phenethyl acetate	260 ± 130*	370 ± 240*	260 ± 90*	410 ± 340*	290 ± 190*	230 ± 190*
hexanoic acid	4.51 ± 0.98	3.98 ± 0.59	4.02 ± 0.63	4.18 ± 0.12	3.96 ± 0.70	3.88 ± 0.61
guaiacol	560 ± 220*	740 ± 360*	360 ± 140*	430 ± 190*	420 ± 150	430 ± 130*

Table 5. Continued

	Vintage 2006[a]					
compound	P ($n = 9$)	S ($n = 4$)	W ($n = 11$)	RO ($n = 7$)	KK ($n = 4$)	SW ($n = 5$)
trans-oak lactone	1.03 ± 0.004	1.04**	1.04 ± 0.004	1.04 ± 0.0003	1.04**	1.04**
2-phenylethyl alcohol	11.6 ± 3.4	15.1 ± 4.3	11.4 ± 1.8	18.7 ± 4.7	12.1 ± 2.6	13.2 ± 4.7
cis-oak lactone	980 ± 10*	980 ± 20*	980 ± 10*	980 ± 10*	980 ± 10*	980 ± 10*
o-cresol	840 ± 30*	850 ± 40*	810 ± 40	830 ± 30*	820 ± 20*	840 ± 30*
phenol	830 ± 340*	1.33 ± 0.29	560 ± 250*	690 ± 330*	640 ± 200*	690 ± 240*
4-ethylguaiacol	360 ± 10*	370 ± 10*	350 ± 10*	360 ± 10*	360 ± 10*	420 ± 150*
octanoic acid	2.11 ± 0.50	1.80 ± 0.26	1.83 ± 0.30	1.90 ± 0.47	1.72 ± 0.18	1.90 ± 0.27
p-cresol	290 ± 20*	300 ± 40*	270 ± 20*	280 ± 20*	280 ± 20*	280 ± 30*
eugenol	670 ± 90*	2.59 ± 3.7	600 ± 50*	620 ± 80*	610 ± 70*	620 ± 20*
decanoic acid	820 ± 100*	780 ± 30*	760 ± 50*	780 ± 90	730 ± 60*	790 ± 100*
2,6-dimethoxyphenol	10.2 ± 4.8	22.7 ± 10	5.48 ± 3.0	9.00 ± 3.6	11.3 ± 6.3	7.39 ± 6.4
5-(hydroxymethyl)furfural	2.44 ± 4.0	6.27 ± 4.6	760 ± 430*	2.07 ± 1.1	390 ± 520*	4.38 ± 1.8
vanillin	58.5 ± 54	92.3 ± 99	27.3 ± 26	50.5 ± 50	25.3 ± 27	19.2 ± 17

[a] n = number of samples analyzed from each region. P, S, W, RO, OR, SW, and KK are the codes given to the different regions (for full descriptions of the regions, refer to the text and footnote of **Table 1**). *, measured in μg/L; **, identified in only one sample.

could result in losing sensitivity for some compounds, especially C_4-C_{10} acids and volatile phenols.

The mean, maximum, and minimum values for the volatile compounds determined in the Pinotage wines over the two vintages studied are presented in **Table 4**. Most of these compounds are major volatiles and have been identified in all of the wines.

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

Small variations in the values obtained were also observed among different regions (**Table 5**). For instance, isoamyl acetate content was highest in samples obtained from the OR region and lowest in region RO for the 2005 vintage. Ethyl lactate was highest in region SW in 2005. Ethyl acetate, diethyl succinate, and 2-phenylethyl acetate were comparatively higher in regions SW, OR, and S, respectively, in 2005. On the other hand, hexyl acetate levels were lowest in region RO. Ethyl acetate and 2-phenylethyl acetate were higher in regions P, OR, and RO, respectively. On the contrary, the level of diethyl succinate of the same vintage 2006 for the former two regions was lower. It should be noted that the use of nitrogen-containing fertilizers can have a significant effect on the amount of esters in the wine (*33*). The mean concentrations of C_6-C_{10} ethyl esters were significantly lower relative to the rest and were higher in the 2006 compared to 2005. Although slightly lower, these levels are in general agreement with the values found by Alves et al. (*6*) in Madeira wine.

Alcohols. The mean values of most of the alcohols investigated between the two vintages were comparable. The fusel alcohols (1-propanol, isobutanol, isoamyl alcohol, and 2-phenylethyl alcohol) were present at highest concentrations. These alcohols are believed to be formed as secondary products of metabolism by yeast (*13*). 1-Propanol and isobutanol levels appear to be slightly higher in 2005 compared to 2006. The average content of isoamyl alcohol was the highest of all the alcohols in all wines, whereas the average level of 1-hexanol was the lowest in both vintages, which seems to coincide with the previous result for Mencía wines (*12*). In a similar fashion as detailed for the esters, variations in the mean values among few regions were evident (**Table 5**). The highest mean concentration of 1-propanol was measured in region SW of 2005 vintage. 2-Phenylethyl alcohol, which has an aromatic description of "rose" (*3*) and may contribute to the floral nuance of the wines (*20*), appeared to be present in high concentration next to isoamyl alcohol, 1-propanol, and isobutanol. These values show similarity to those reported by Selli et al. (*3*) and Calleja et al. (*12*) for other red cultivars. The above variations among the wines mentioned could be due to either their geographical origin or winemaking practice such as yeast strains used during fermentation (*34*). The rest of the alcohols among the wines of the different regions and vintages showed comparable concentrations.

Fatty Acids. Acids are normally derived from grape must and yeast fermentation. The mean concentration of isovaleric acid, valeric acid, and decanoic acid were balanced among the regions and vintages and showed no significant variations (**Table 5**). Similar circumstances were observed for hexanoic acid (vintage 2006) and octanoic acid (vintage 2005). Acetic acid, commonly known by its vinegar odor (*27*), was present at the highest concentrations of all acids. The mean concentration of acetic acid in vintage 2005 was higher, whereas no significant variation was observed among the regions. For region S (vintage 2006) the highest level of all wines amounting to a mean value of 1.48×10^3 mg/L was recorded, which is also higher than the mean value of vintage 2005 of the same region. This was as a result of the higher concentration of acetic acid (2.64×10^3 and 2.13×10^3 mg/L, respectively) obtained from samples P85 and P87, which were supplied by cellars C85 and C87, respectively. Moreover, a value of 2.63×10^3 mg/L was measured in sample P7 supplied by cellar C7 from region W (2005). This variation could be due to relatively higher oxidation of esters and alcohols (*35*). Despite their contribution to volatile acidity, higher amounts of acids could also indicate bacterial spoilage (*5*). Reynolds et al. (*36*) have indicated that the use of yeasts with lower ethanol formation can result in a higher concentration of acetic acid. Similar results of acetic acid for cultivars of Cabernet Sauvignon and Merlot were reported (*8*).

The next highest level of acid recorded was propanoic acid, with a mean concentration ranging between 12.5 and 23.2 mg/L for regions KK and S of vintages 2006 and 2005, respectively. This is similar to values obtained by Lilly et al. (*37*) for white wines. Generally speaking, the mean concentrations obtained were comparable among the different regions of the two vintages. Isobutyric and butyric acids are characterized by a fatty and cheesy smell (*10*). The mean concentrations of isobutyric acid from vintage 2005 were very similar to butyric acid in vintage 2006 samples. A similar trend was observed for isobutyric acid of 2006 and butyric acid of 2005. Octanoic acid, described as being responsible for a fatty and unpleasant odor (*5*), showed slightly higher concentrations in region P of 2006 in comparison to the rest of wine samples. Very similar contents of C_6, C_8, and C_{10} fatty acids were reported by Falqué et al. (*20*). On average, the values for all the acids among the different regions of the vintages were similar.

Volatile Phenols. Volatile phenols originate from the thermal degradation of lignin from oak wood during the toasting of the staves, but some of them are also present in the wood itself (*38*). The mean concentrations of phenol compounds studied in this work (guaiacol, *o*-cresol, phenol, 4-ethylguaiacol, *p*-cresol, and eugenol) were between 0.110 and 1.36 mg/L, with similar values among all of the regions. Eugenol, with its clove-like odor, was reported as an important contributor to the aroma of wine (*38*). Contrary to the other volatile phenols, 2,6-dimethoxyphenol displayed a distinct result for most of the regions, which varied between 6.68 mg/L for region S, being the lowest, and 14.8 mg/L for region W of the 2005 vintage. The mean concentration 28.2 mg/L of 2,6-dimethoxyphenol for the sample obtained from region OR was slightly higher when compared to the other regions. In the 2006 vintage, however, the values were slightly different, ranging between 5.48 mg/L in region W and 22.7 mg/L for region S. 4-Ethylguaiacol, which is responsible for the spicy and clove-like aroma in a wine, was observed to have very similar values among all of the regions as well as the vintages. This compound results from enzymatic decarboxylation and reduction of ferulic acid (*39*).

Carbonyls. The carbonyl compounds dealt with in this study were acetoin, furfural, 5-methylfurfural, 5-(hydroxymethyl)furfural (5-HMF), and vanillin. The last four aldehydes are believed to be derived from wood cooperage (*40*). Acetoin was estimated at higher concentration values with slight differences among the regions. This is in agreement with the previously reported values for red and white wines (*41*). However, a significant gap between the lowest and highest mean concentration values in wines from vintage 2005 compared to 2006 was visible, which could be related to the winemaking practice (especially the yeast strain) (*42*). Vanillin, commonly associated with vanilla flavor (*3*), is related to the lignin of wood (*32*) and has the next highest mean concentration. Region S in 2006 showed the highest mean value of 92.31 mg/L. According to Morales et al. (*40*) the use of oak chips is a valuable alternative to oak barrels in order to increase the concentration of vanillin in wine. Comparable results among the different regions and vintages were obtained for furfural and 5-methylfurfural, the latter being the lowest mean concentration of all carbonyls. The mean concentration of 5-HMF for samples from the majority of the regions displayed between 1.25 mg/L (region P) and 13.0 mg/L (region S). However, some discrepancy in regions W and KK was evident, showing mean values of 0.760 and 0.390 mg/L, respectively.

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

Table 6. Results of FA Using 37 Volatile Components and 87 Samples

factor	eigenvalue	cumulative eigenvalue	% total variance	cumulative %
1	8.15	8.15	22.03	22.03
2	6.41	14.57	17.33	39.37
3	3.25	17.82	8.79	48.15
4	2.13	19.95	5.77	53.92
5	1.98	21.93	5.35	59.27

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

Factor Analysis. FA is a method of multivariate analysis that linearly transforms one set of variables into another set of fewer variables (factors) that conserve the information of the original set, searches for associations among the variables, and is able to detect natural groups present in the samples (unsupervised method) (*13*). FA was done using the independent variables (concentration of volatiles) with respect to the dependent variables (two vintages and seven regions). As mentioned above, trans- and cis-isomers of whiskey lactone were unidentified in some samples largely in the 2006 vintage wines. Hence, the two isomers of whiskey lactone were removed from the statistical analysis, reducing the number of variables to 37.

Even though selection of factors that can explain >75% of the total variability is preferable, this could only be achieved from 10 factors with eigenvalues >1. However, only the first five factors that cover 59.27% of the total variance (**Table 6**) were selected because it was evident from the analysis that increasing the number of factors adds only a very small percentage to the total variability, as well as reduces the number of components loaded to each factor.

Table 7 presents the loading of each variable to the selected factors. To simplify the presentation of the results, loading variables with absolute coefficient values of ≥0.30 were selected.

Factor 1 explained 22% of the total variance. The highest numbers of variables were associated with this factor. Lower acids of C_2 and C_3 showed positive correlation with factor 1. On the contrary, ethyl acetate was observed to have a high negative correlation. This behavior could be related to the oxidation of ethyl acetate into acetic acid (*35*). Most volatile phenols, which are believed to originate from thermal degrada-

Table 7. Loadings of the Variables to the Selected Factors

variable	factor 1	factor 2	factor 3	factor 4	factor 5
ethyl acetate	−0.42	0.52			−0.41
ethyl butyrate		0.70			
1-propanol				0.76	
isobutanol	−0.32				0.62
isoamyl acetate		0.76			
n-butanol				0.41	
isoamyl alcohol					0.76
ethyl hexanoate		0.81			
hexyl acetate		0.79			
acetoin	0.42			0.37	
ethyl D-lactate			0.40	0.42	
1-hexanol					
ethyl octanoate		0.83			
acetic acid	0.84			0.32	
furfural	0.32	−0.43			
propionic acid	0.86				
isobutyric acid			0.46	−0.30	
5-methylfurfural	0.49				
n-butyric acid				0.42	
ethyl decanoate		0.81			
isovaleric acid			0.84		
diethyl succinate					
n-valeric acid			0.87		
2-phenethyl acetate		0.64			0.41
hexanoic acid		0.54	0.65		
guaiacol	0.86				
2-phenylethyl alcohol	0.31		0.35		0.66
o-cresol	0.89				
phenol	0.85				
4-ethylguaiacol	0.31	0.32			
octanoic acid		0.46	0.73		
p-cresol	0.77				
eugenol				0.53	
decanoic acid			0.90		
2,6-dimethoxyphenol	0.77				
5-(hydroxymethyl)furfural	0.72				
vanillin	0.69				

tion of the lignin of oak wood during the toasting of the staves (*38*), showed higher positive association to factor 1. Other wood-related compounds with high positive association to factor 1 were furfural, 5-methylfurfural, 5-HMF, and vanillin. 2-Phenylethyl alcohol was also highly associated with this factor.

Compounds formed during alcoholic fermentation such as ethyl and acetate esters (*43*) proved to have high positive correlation with factor 2. Even though low, compounds related to usage of oak wood during wine processing, particularly the furfural-derived compounds (*40*), were negatively correlated to factor 2. Because the association of C_6 and C_8 acids demonstrated a positive sign to factor 2, they must have evolved in a similar way to the esters.

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

Higher alcohols such as 1-propanol, isobutanol, *n*-butanol, isoamyl alcohol, and 2-phenylethyl alcohol, which enter the wine medium as secondary products of yeast metabolism (*13*), were positively associated with factors 4 and 5. Another compound positively associated with factor 4 was eugenol. It should be noted that there were other compounds associated with each factor, but their value was not considered because the loading was small.

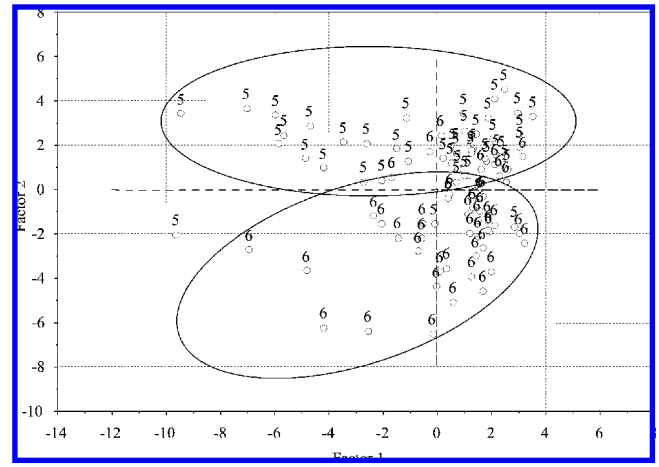

Figure 2. Distribution of Pinotage wines studied in the plane defined by factors 1 and 2 according to vintage. 5 and 6 represent vintages 2005 and 2006, respectively.

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

Advanced PCA Factor Analysis. PCA studies were carried out on the basis of the factors selected above as components for the PCAs. The percentage of the total variability captured was 59.27% (**Table 6**). The interpretation of the volatile pattern and wine characteristics was mainly based on the representation of information contained in factors 1 and 2. Plots of other combinations of factors (components) were also examined (graphs not shown here) even though they did not bring additional information of interest in the wine characterization. It should be noted that, however, these interpretations have to be cautious as the percentage of variance retained with these two factors was quite limited.

The variation in volatile compounds between the two vintages (2005 and 2006) was already highlighted by the plane-defined PCA plot (**Figure 2**). The wines of vintage 2005 were mainly situated at the top zone of the graph, whereas the 2006 vintage wines appeared at the bottom. This observation could be due to seasonal differences during harvesting of the grapes. Obviously, this behavior could not be understood conclusively as some samples appeared in intermediate zones and certain mixing of samples was observed. The study of the distribution of samples according to their geographical origin did not show relevant pattern (**Figure 3**). Unlike the observed grouping between the vintages, the PCA plots did not conform to groupings based on their geographic characteristics of the wines, as they are scattered all over the plane, mostly around the origin of the graph. Hence, the regional classification did not bring additional or complementary conclusions of interest in wine description and characterization as the regions were widely spread with no predominant areas.

PCA of loadings of the variables based on the first two factors using the concentrations of volatile compounds obtained was also performed (**Figure 4**). This figure shows clearly the association of the compounds with each other as well as with the first two factors. With the exception of a few discrepancies, it revealed some relevant pattern of the volatiles. As can be seen from the graph, the wood-related compounds appear on the top left area of the loading plot. On the other hand, the esters appear on the bottom right of the plot. This indicates that these two groups of compounds are negatively correlated with one another. In a similar way, alcohols are situated on the top

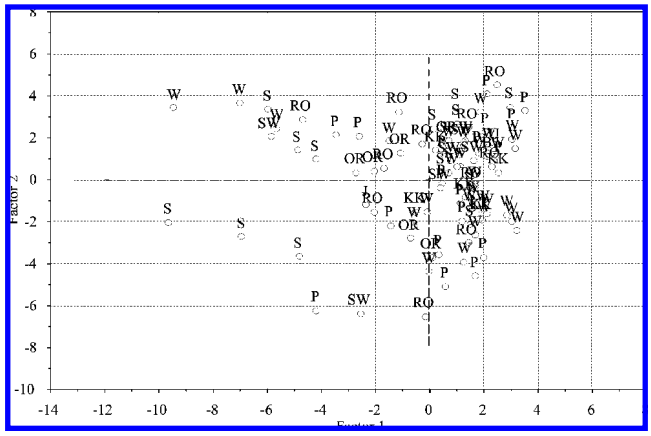

Figure 3. Distribution of Pinotage wines studied in the plane defined by factors 1 and 2 according to their geographic origin. P, S, W, RO, OR, SW, and KK are the codes given to the different regions (for full descriptions of the regions, refer to the text and footnote of **Table 1**).

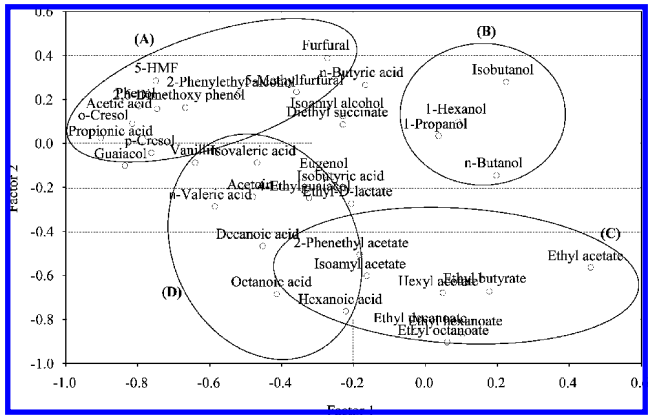

Figure 4. Distribution of volatile components in the plane defined by factors 1 and 2: A, wood-related compounds; B, alcohols; C, esters; D, acids.

right part of the plot, but acids appear on the bottom left of the loading plot. A very similar correlation could be drawn for these two classes of compounds as well.

As an alternative way of comparison among the obtained results, one-way ANOVA was performed. The data analyzed for each vintage and region correspond to the mean concentration obtained for each compound studied. **Table 8** presents the volatile components that showed significant and nonsignificant differences among the various regions and vintages. As a counter check for the ANOVA p value obtained in determining the difference of the mean value of each compound between the two vintages, a Mann−Whitney U (nonparametric) method was applied, whereas for the different regions, the Kruskal−Wallis (nonparametric) method was used. The p values obtained by the nonparametric methods for both vintages and regions were in agreement with the ANOVA p values. However, in specific cases, where it was found that the ANOVA assumptions were violated, the nonparametric p values were reported (**Table 8**).

One-way ANOVA revealed samples with high value among the different wines. For instance, in sample 23 (P23) the concentration of acids was relatively higher in comparison with the rest of the samples. However, for isovaleric, valeric, and decanoic acids the increase in concentration was >2-fold (**Figure 5**). The higher value of the acids in this particular sample could be related to the winemaking practice of that particular supplier, as using different yeasts in the presence of water can promote the production of free fatty acids in wine

Table 8. One-Way ANOVA Carried Out on Quantitative Data To Analyze the Variation of the Mean Concentration of Volatile Components among Regions and Vintages

	vintages[a]		regions[b]	
compound	F value	p value	F value	p value
ethyl acetate	36.020	0.000[c]	1.463	0.201
ethyl butyrate	11.119	0.001[c]	2.904	0.013[c]
1-propanol	1.996	0.161	1.070	0.387
isobutanol	1.098	0.297	1.456	0.203
isoamyl acetate	6.413	0.013[c]	1.673	0.138
n-butanol	0.574	0.451	1.187	0.322
isoamyl alcohol	3.454	0.066	2.811	0.015[c]
ethyl hexanoate	104.230	0.000[c]	1.385	0.230
hexyl acetate	24.792	0.000[c]	0.386	0.886
acetoin	1.535	0.219	0.492	0.813
ethyl D-lactate	0.377	0.541	1.670	0.139
1-hexanol	0.003	0.958	0.609	0.723
ethyl octanoate	258.540	0.000[c]	0.671	0.673
acetic acid	4.334	0.040[c]	2.213	0.050
furfural	11.905	0.001[c]	1.250	0.290
propionic acid	3.642	0.060	1.074	0.385
isobutyric acid	13.298	0.000[c]	3.136	0.008[c]
5-methylfurfural	3.525	0.064	0.869	0.521
n-butyric acid	5.156	0.026[c]	2.250	0.046[c]
ethyl decanoate	79.238	0.000[c]	0.659	0.683
isovaleric acid	0.276	0.601	1.573	0.165
diethyl succinate	0.000	0.988	0.964	0.455
n-valeric acid	0.501	0.011[d]	0.677	0.669
2-phenethyl acetate	6.971	0.010[c]	1.867	0.096
hexanoic acid	18.856	0.000[c]	0.488	0.816
guaiacol	0.305	0.582	0.763	0.601
2-phenylethyl alcohol	4.673	0.033[c]	4.926	0.000[c]
o-cresol	3.379	0.069	0.403	0.875
phenol	4.042	0.047[c]	0.381	0.889
4-ethylguaiacol	0.758	0.386	1.214	0.307
octanoic acid	14.109	0.000[c]	0.579	0.746
p-cresol	0.503	0.480	0.114	0.995
eugenol	0.934	0.336	1.093	0.374
decanoic acid	3.954	0.050	0.726	0.630
2,6-dimethoxyphenol	2.405	0.124	1.381	0.232
5-(hydroxymethyl)furfural	16.331	0.000[c]	4.453	0.000[c]
vanillin	0.246	0.621	0.535	0.780

[a] Vintages 2005 and 2006. [b] Applied to seven regions P, S, W, RO, OR, KK, and SW (for full descriptions of the regions, refer to the text and footnote of **Table 1**). [c] $p < 0.05$, significant difference. [d] Significant difference confirmed by the nonparametric Mann−Whitney U method.

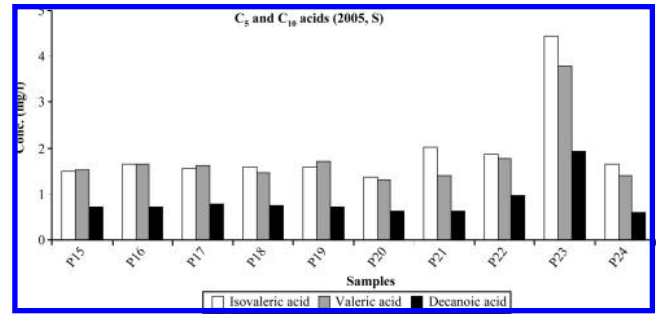

Figure 5. Absolute concentration of isovaleric, valeric, and decanoic acids in 10 samples (P15−P24) from region S of vintage 2005.

(*35*). The observed differences mentioned for the acids were confirmed by running a residual plot and test of homogeneity of variance.

In conclusion, the SBSE method was fast, simple, cost-effective, and reliable for the analysis of the 39 volatile components in Pinotage wines, achieving low LODs and LOQs. The precision obtained for the method was within the acceptable range. Moreover, good calibration curves with a wide linearity range of concentrations for each analyte were obtained. The

method proposed here for the characterization of wines managed to pull out relevant information on the samples analyzed as well as motivating relationships among concentrations of major wine volatiles, and certain wine features such as vintages were deduced.

Simple chemometric techniques such as FA, PCA, and one-way ANOVA were used for processing the data. The role of volatile profiles in the characterization of wine origin was limited. Contents of certain volatiles were somewhat characteristic of a given vintage. The relationship between volatile components and the vintages was certainly substantial. Comparatively, esters were higher in vintage 2006. On the other hand, their corresponding acids were higher in vintage 2005. Volatile phenols showed very comparable results between the two vintages. The aromatic aldehydes, furfural and 5-methylfurfural, which are primarily formed in wood during the toasting process, were slightly lower in 2005 vintage compared to 2006. However, whiskey lactone, especially the cis-isomer, was lower in vintage 2006. Even though there is no clear conclusion, the above observations could be due to variation in either geographical origin or the winemaking practice. Because we do not have the detailed history of the wines, we were unable to make a correlation among the different volatiles and their method of production. The statistical approach taken for characterizing the wine samples in terms of their volatiles provides insight for further study.

ACKNOWLEDGMENT

We thank the South African Young Wine Competition for supplying the wine samples. We also thank the Institute of Wine Biotechnology, University of Stellenbosch, for storing the wine samples. In adition, we thank Dr. Martin Kidd from the Centre for Statistical Consultation, University of Stellenbosch, for his valuable contribution on the statistical analysis, Andreas Tredoux for his input on instrumental assistance, and Tesfamariam Hagos for his assistance on the data analysis.

LITERATURE CITED

(1) Marais, J. Effect of different wine-making techniques on the composition and quality of Pinotage wine. I. Low-temperature skin contact prior to fermentation. *S. Afr. J. Enol. Vitic.* **2003**, *24*, 70–75.

(2) Van Wyk, C. J.; Augustyn, O. P. H.; De Wet, P.; Joubert, W. A. Isoamyl acetate—a key fermentation volatile of wines of *Vitis vinifera* cv Pinotage. *Am. J. Enol. Vitic.* **1979**, *30*, 167–173.

(3) Selli, S.; Cabaroglu, T.; Canbas, A.; Erten, H.; Nurgel, C.; Lepoutre, J. P.; Gunata, Z. Volatile composition of red wine from cv. Kalecik Karasi grown in central Anatolia. *Food Chem.* **2004**, *85*, 207–213.

(4) Diez, J.; Dominguez, C.; Guillen, D. A.; Veas, R.; Barroso, C. G. Optimisation of stir bar sorptive extraction for the analysis of volatile phenols in wines. *J. Chromatogr., A* **2004**, *1025*, 263–267.

(5) Escudero, A.; Gogorza, B.; Meluäs, M. A.; Ortiän, N.; Cacho, J.; Ferreira, V. Characterization of the aroma of a wine from Maccabeo. Key role played by compounds with low odor activity values. *J. Agric. Food Chem.* **2004**, *52*, 3516–3524.

(6) Alves, R. F.; Nascimento, A. M. D.; Nogueira, J. M. F. Characterization of the aroma profile of Madeira wine by sorptive extraction techniques. *Anal. Chim. Acta* **2005**, *546*, 11–21.

(7) Castro, R.; Natera, R.; Benitez, P.; Barroso, C. G. Comparative analysis of volatile compounds of 'fino' sherry wine by rotatory and continuous liquid−liquid extraction and solid-phase microextraction in conjunction with gas chromatography−mass spectrometry. *Anal. Chim. Acta* **2004**, *513*, 141–150.

(8) Kotseridis, Y.; Baumes, R. Identification of impact odorants in Bordeaux red grape juice, in the commercial yeast used for its fermentation, and in the produced wine. *J. Agric. Food Chem.* **2000**, *48*, 400–406.

(9) Del Alamo Sanza, M.; Dominguez, I. N.; Carcel, L. M. C.; Gracia, L. N. Analysis for low molecular weight phenolic compounds in a red wine aged in oak chips. *Anal. Chim. Acta* **2004**, *513*, 229–237.

(10) Santos, J. P.; Arroyo, T.; Aleixandre, M.; Lozano, J.; Sayago, I.; Garcia, M.; Fernandez, M. J.; Ares, L.; Gutierrez, J.; Cabellos, J. M. A comparative study of sensor array and GC-MS: application to Madrid wines characterization. *Sens. Actuators B* **2004**, *102*, 299–307.

(11) Tat, L.; Comuzzo, P.; Stolfo, I.; Battistutta, F. Optimization of wine headspace analysis by solid-phase microextraction capillary gas chromatography with mass spectrometric and flame ionization detection. *Food Chem.* **2005**, *93*, 361–369.

(12) Calleja, A.; Falque, E. Volatile composition of Mencia wines. *Food Chem.* **2005**, *90*, 357–363.

(13) Ortega-Heras, M.; Gonzalez-Huerta, C.; Herrera, P.; Gonzalez-Sanjose, M. L. Changes in wine volatile compounds of varietal wines during ageing in wood barrels. *Anal. Chim. Acta* **2004**, *513*, 341–350.

(14) Arthur, C. L.; Pawliszyn, J. J. Solid phase microextraction with thermal desorption using fused silica optical fibers. *Anal. Chem.* **1990**, *62*, 2145–2148.

(15) Majors, R. E.; David, F.; Tienpont, B.; Sandra, P. Stir-bar sorptive extraction of trace organic compounds from aqueous matrices. *LCGC North Am.* **2003**, *21*, 1–8.

(16) Baltussen, E.; Sandra, P.; David, F.; Cramers, C. A. Stir bar sorptive extraction (SBSE), a novel extraction technique for aqueous samples: theory and principles. *J. Microcol. Sep.* **1999**, *11*, 737–747.

(17) Tienpont, B.; David, F.; Bicchi, C.; Sandra, P. High capacity headspace sorptive extraction. *J. Microcol. Sep.* **2000**, *12*, 577–584.

(18) Coelho, E.; Perestrelo, R.; Neng, N. R.; Câmara, J. S.; Coimbra, M. A.; Nogueira, J. M. F.; Rocha, S. M. Optimisation of stir bar sorptive extraction and liquid desorption combined with large volume injection−gas chromatography−quadrupole mass spectrometry for the determination of volatile compounds in wines. *Anal. Chim. Acta* **2008**, *624*, 79–89.

(19) Zhou, Y.; Riesen, R.; Gilpin, C. S. Comparison of Amberlite XAD-2/Freon 11 extraction with liquid/liquid extraction for the determination of wine flavor components. *J. Agric. Food Chem.* **1996**, *44*, 818–822.

(20) Falque, E.; Fernandez, E.; Dubourdieu, D. Volatile components of Loureira, Dona Branca, and Treixadura wines. *J. Agric. Food Chem.* **2002**, *50*, 538–543.

(21) Spranger, M. I.; Climaco, M. C.; Sun, B.; Eiriz, N.; Fortunato, C.; Nunes, A.; Leandro, M. C.; Avelar, M. L.; Belchior, A. P. Differentiation of red winemaking technologies by phenolic and volatile composition. *Anal. Chim. Acta* **2004**, *513*, 151–161.

(22) Marti, M. P.; Busto, O.; Guasch, J. Application of a headspace mass spectrometry system to the differentiation and classification of wines according to their origin, variety and ageing. *J. Chromatogr., A* **2004**, *1057*, 211–217.

(23) Pozo-Bayon, M. A.; Pueyo, E.; Martin-Alvarez, P. J.; Polo, M. C. Polydimethylsiloxane solid-phase microextraction−gas chromatography method for the analysis of volatile compounds in wines: Its application to the characterization of varietal wines. *J. Chromatogr., A* **2001**, *922*, 267–275.

(24) Weldegergis, B. T.; Tredoux, A. G. J.; Crouch, A. M. Application of a headspace sorptive extraction method for the analysis of volatile components in South African wines. *J. Agric. Food Chem.* **2007**, *55*, 8696–8702.

(25) Bianchi, F.; Careri, M.; Mangia, A.; Musci, M. Retention indices in the analysis of food aroma volatile compounds in temperature-programmed gas chromatography: database creation and evaluation of precision and robustness. *J. Sep. Sci.* **2007**, *30*, 563–572.

(26) Mahajan, S. S.; Goddik, L.; Qian, M. C. Aroma compounds in sweet whey powder. *J. Dairy Sci.* **2004**, *87*, 4057–4063.

(27) Fang, Y.; Qian, M. Aroma compounds in Oregon Pinot Noir wine determined by aroma extract dilution analysis (AEDA). *Flavour Fragrance J.* **2005**, *20*, 22–29.
(28) Acree, T.; Arn, H. Kovats retention indices Flavornet, 2004; http://www.flavornet.org/f_kovats.html.
(29) Qian, M. C.; Wang, Y. Seasonal variation of volatile composition and odor activity value of 'Marion' (*Rubus* spp. hyb) and 'Thornless Evergreen' (*R. laciniatus* L.) blackberries. *J. Food Sci.* **2005**, *70*, C13−C20.
(30) Bell, W. A. Examination of aroma volatiles formed from thermal processing of Florida reconstituted grapefruit juiceM.Sc. thesis, University of Florida, 2004; p 43.
(31) Jarauta, I.; Cacho, J.; Ferreira, V. Concurrent phenomena contributing to the formation of the aroma of wine during aging in oak wood: an analytical study. *J. Agric. Food Chem.* **2005**, *53*, 4166–4177.
(32) Diaz-Maroto, M. C.; Sanchez-Palomo, E.; Perez-Coello, M. S. Fast screening method for volatile compounds of oak wood used for aging wines by headspace SPME-GC-MS (SIM). *J. Agric. Food Chem.* **2004**, *52*, 6857–6861.
(33) Webster, D. R.; Edwards, C. G.; Spayd, S. E.; Peterson, J. C.; Seymour, B. Influence of vineyard nitrogen fertilization on the concentrations of monoterpenes, higher alcohols, and esters in aged Riesling wines. *Am. J. Enol. Vitic.* **1993**, *44*, 275–284.
(34) Rankine, B. C. Formation of higher alcohols by wine yeasts, and relationship to taste thresholds. *J. Sci. Food Agric.* **2006**, *18*, 583–589.
(35) Nykanen, L. Formation and occurrence of flavor compounds in wine and distilled alcoholic beverages. *Am. J. Enol. Vitic.* **1986**, *37*, 84–96.
(36) Reynolds, A. G.; Edwards, C. G.; Cliff, M. A.; Thorngate, J. H., III.; Marr, J. C. Evaluation of yeast strains during fermentation of Riesling and Chenin blanc musts. *Am. J. Enol. Vitic.* **2001**, *52*, 336–344.
(37) Lilly, M.; Bauer, F. F.; Lambrechts, M. G.; Swiegers, J. H.; Cozzolino, D.; Pretorius, I. S. The effect of increased yeast alcohol acetyltransferase and esterase activity on the flavour profiles of wine and distillates. *Yeast* **2006**, *23*, 641–659.
(38) Moreno, N. J.; Azpilicueta, C. A. Binding of oak volatile compounds by wine lees during simulation of wine ageing. *LWT* **2007**, *40*, 619–624.
(39) Pollnitz, A. P.; Pardon, K. H.; Sefton, M. A. Quantitative analysis of 4-ethylphenol and 4-ethylguaiacol in red wine. *J. Chromatogr., A* **2000**, *874*, 101–109.
(40) Morales, M. L.; Benitez, B.; Troncoso, A. M. Accelerated aging of wine vinegars with oak chips: evaluation of wood flavour compounds. *Food Chem.* **2004**, *88*, 305–315.
(41) Romano, P.; Palla, G.; Caligiani, A.; Brandolini, V.; Maietti, A.; Salzano, G. Evaluation of stereoisomers of 2,3-butanediol and acetoin to differentiate *Saccharomyces cerevisiae* and *Kloeckera apiculata* wine strains. *Biotechnol. Lett.* **2000**, *22*, 1947–1951.
(42) Romano, P.; Granchi, L.; Caruso, M.; Borra, G.; Palla, G.; Fiore, C.; Ganucci, D.; Caligiani, A.; Brandolini, V. The species-specific ratios of 2,3-butanediol and acetoin isomers as a tool to evaluate wine yeast performance. *Int. J. Food Microbiol.* **2003**, *86*, 163–168.
(43) Herraiz, T.; Ough, C. S. Formation of ethyl esters of amino acids by yeasts during the alcoholic fermentation of grape juice. *Am. J. Enol. Vitic.* **1993**, *44*, 41–48.

Received for review May 20, 2008. Revised manuscript received September 11, 2008. Accepted September 15, 2008. This work was funded by the WineTech, South Africa and University of Stellenbosch.

JF8015706