Investigating the coffee flavour in South African Pinotage wine using novel offline olfactometry and comprehensive gas chromatography with time of flight mass spectrometry

Yvette Naudé* and Egmont R. Rohwer

Department of Chemistry, University of Pretoria, Private Bag X20, Hatfield 0028, Pretoria, South Africa

ABSTRACT

Pinotage wine from several South African wine cellars has been produced with a novel coffee flavour. We have investigated this innovative coffee effect using in house developed solventless sampling and fractionating olfactometric techniques, which are unique in their ability to study synergistic aroma effects as opposed to traditional gas chromatography olfactometry (GC-O) which is designed to, ideally, evaluate single eluting compounds in a chromatographic sequence. Sections of the chromatogram, multiple or single peaks, were recaptured on multichannel open tubular silicone rubber (polydimethylsiloxane (PDMS)) traps at the end of a GC column. The recaptured fractions were released in a controlled manner for offline olfactory evaluation, and for qualitative analysis using comprehensive gas chromatography coupled to time of flight mass spectrometry (GCxGC-TOFMS) for compound separation and identification, thus permitting correlation of odour with specific compounds. A combination of furfural and 2-furanmethanol was responsible for a roast coffee bean-like odour in coffee style Pinotage wines.

This coffee perception is the result of a synergistic effect in which no individual compound was responsible for the characteristic aroma.

*Corresponding author. Tel.: +27 12 420 2517; fax: +27 12 420 4687.

E-mail addresses: yvette.naude@up.ac.za, egmont.rohwer@up.ac.za

1. Introduction

Pinotage is a unique South African red wine cultivar cross-bred from Pinot Noir and Cinsaut (Hermitage). Isoamyl acetate is a key impact odourant responsible for the distinctive character of Pinotage wines [1]. Recently, Pinotage wine from several South African wine cellars has been produced with a novel coffee flavour. This contemporary aroma profile is deliberately derived from a particular combination of Pinotage, alternative toasted wood products and malolactic fermentation.

Traditionally, high-quality red wines are matured in oak barrels [2,3]. Compounds present in the wood are extracted into the wine during the aging process. Volatiles (e.g. oak lactones, guaiacol, 4-methylguaiacol, furfural and 5-methylfurfural) transferred into the wine produce distinctive flavours depending on the type of oak, level of toasting and seasoning [3,4]. Barrels are

176

expensive, have a short lifetime (3-5 years) and maturation is a slow process. Recently, as an alternative to traditional wine aging, the maturation process is accelerated by adding toasted oak chips or staves to wine kept in stainless steel tanks or used barrels [2-4]. By exploiting the larger surface area of these alternative oak products wood derived compounds are transferred rapidly into the wine in relative short periods compared to traditional barrel maturation. A desired flavour profile can be selected depending on the toast level and type of oak product used. For example, descriptions supplied by a manufacturer (XTRAOAK) of these alternative products state that American oak medium toast (AOMT) products may impart a vanilla flavour, American oak medium toast plus (AOMT+) can impart chocolate and coffee notes, French oak medium toast (FOMT) imparts nuances of spice and tobacco, and French oak medium toast plus (FOMT+) imparts subtle smokiness and coffee characteristics.

Gas chromatography olfactometry (GC-O) is traditionally used to investigate individual odour active substances. Aroma perception of the GC effluent is recorded by the human nose, in real time, at the olfactometer outlet. By comparing olfactometric data with chromatographic data, an individual compound can be matched with an odour [5-8]. However, potential synergistic effects cannot be observed when single compounds are evaluated over time. The result of combining single compounds in a complex matrix may be the emergence of a strikingly different sensory perception, completely unrelated to that of the individual compounds alone [9,10]. Addressing the limitations of evaluating rapidly eluting single compounds as opposed to selective combinatorial mixtures, we developed a heart-cut gas chromatographic fraction

collection (GC-FC) technique to study, offline, synergistic perceptions of odourants.

Sample preparation techniques for isolating compounds from wine for analysis are stir bar sorptive extraction (SBSE) [11-14], solid phase microextraction (SPME) [3,14-17], solid phase extraction (SPE) [18,], or liquid-liquid extraction (LLE) [4,18-20]. In the case of SPE or LLE, sensitivity limitations are associated with analysis of microlitre amounts of the diluted final extract. Solvent free sample enrichment provides aroma extracts that are more representative of food aroma when compared to those obtained by solvent extraction. SPME and SBSE are efficient commercial solvent free sorptive extraction alternatives to procedures using solvents. SPME (pioneered by Pawliszyn and co-workers) has a small sorbent volume of 0.6 – 0.9 µl [21], while SBSE (developed by Baltussen and Sandra) provides for sorptive volumes of 25 – 200 µl polydimethylsiloxane (PDMS) [22]. Multichannel open tubular silicone rubber (PDMS) traps, constructed in-house, were used in our study [9,10,23-26] (Fig. 1). The multichannel PDMS trap has a considerably larger volume of 0.6 ml PDMS thereby providing for a greater sample enrichment capacity. The analytes are concentrated by a purge-and-trap method onto a multichannel PDMS trap, followed by thermal desorption of the PDMS trap into a gas chromatograph with flame ionisation detection (GC-FID) or GCxGC-TOFMS.

We report solvent free sampling using PDMS for high capacity analyte enrichment from Pinotage wines, GC fraction collection (heart-cutting) onto

multichannel PDMS traps for offline olfactory evaluation of synergistic effects, and for qualitative analysis by GCxGC-TOFMS for compound separation and identification, thus allowing correlation of odour with specific compounds.

2. Materials and methods

2.1. Wine samples

Three brands of coffee style Pinotage wines (oak staves in tanks, maturation for three to six months) of vintages 2008 (brand 1), 2009 (brand 2), 2010 (brand 3) and 2011 (brand 2), and one traditional style Pinotage wine (second-fill and third-fill French oak barrel maturation for ten months) of vintage 2007 were purchased during 2009-2012 from local supermarkets (Pretoria, South Africa).

2.2. Chemical standards

Furfural (2-furancarboxaldehyde) (purity 99%) and 2-furanmethanol (furfuryl alcohol) (purity 99%) were purchased from Sigma-Aldrich (Pty) Ltd. Kempton Park, South Africa. For linear retention index determination n-alkanes C_6-C_{18} were used (Merck, Pretoria, South Africa).

2.3. Sorptive extraction with multichannel open tubular PDMS traps

Multichannel PDMS traps containing 0.4±0.02 g silicone were prepared based on a technique described by Ortner and Rohwer [23]. The multichannel

PDMS trap was designed to fit a commercial thermal desorber system (TDS 3) available from Gerstel™(Chemetrix, Midrand, South Africa). A bundle of twenty two channels of SIL-TEC™ silicone elastomer medical grade tubing (0.64 mm OD x 0.30 mm ID) (Technical Products, Georgia, United States of America) were arranged, in parallel, inside a 17.8 cm long glass desorption tube (4 mm ID, 6 mm OD) (Fig. 1). The multichannel PDMS trap inside the desorption tube was 55 mm long. The multichannel trap provides a high sample enrichment capacity of 0.6 ml PDMS.

A purge-and-trap sampling method developed in-house [9,10,24] was used to extract the aroma compounds from wines by trapping it on a multichannel PDMS trap. The volatiles were isolated from 50 ml wine inside a 500 ml flat bottomed glass flask. The flask was immersed for 35 min in a water bath (25 °C) after which the sample was purged with 500 ml nitrogen gas at 25 ml/min. The purged volatiles were collected on a multichannel PDMS trap at 35 °C to prevent the condensation of water from the sample onto the trap. The ends of the trap device were capped with glass stoppers during storage. The glass stoppers were secured with tight-fitting polytetrafluoroethylene (PTFE) sleeves. The trapped analytes inside the trap device are not directly exposed to the PTFE sleeves, thereby preventing potential adsorption of analytes onto the Teflon®. The multichannel PDMS traps were desorbed into a GC-FID for heart-cutting, or into a comprehensive GC-TOFMS for compound identification.
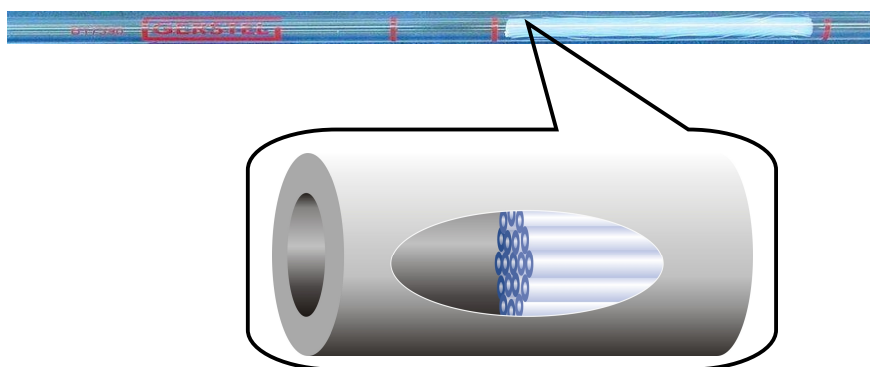
Fig. 1.   Solvent free concentration with silicone rubber (PDMS) tubing. Multichannel trap sampler: a bundle of silicone rubber tubing is inserted, in parallel, into an empty commercial glass desorption tube. The multichannel trap has a sorptive volume of 0.6 ml PDMS.

2.4.   Heart-cutting with GC-FID: Capturing of single compounds, combinations of compounds, or fractions by GC-FC

The heart-cut procedure followed was previously described [9,10,26]. Briefly, the flame ionisation detector gases are switched off, the FID top assembly and the collector were removed by loosening the knurled brass nut and by taking out the collector prior to heart-cutting; single peaks or fractions were collected from the GC effluent (hydrogen carrier gas) at the end of the GC apolar column by simply placing a multichannel PDMS trap on the inactivated FID flame tip [27].

Experimental linear retention indices (RI_{exp}) were calculated according to the method of Van den Dool and Kratz [28].   Once a wine sample chromatogram was obtained sections of a chromatogram were captured during

consecutive runs. Firstly, RI_{exp} fractions 600-800 (N), 801-899 (M), 901-1016 (KL), 1017-1250 (C), 1300-1800 (DE) were selectively captured on multichannel PDMS traps (Fig. 2) (characters in brackets denote a specific fraction). Thereafter, single peaks and combinations of heart-cuts were collected. After selective capturing of sections, single peaks, or combinations of heart-cuts onto multichannel PDMS traps, the traps were capped with custom-made glass and Teflon® stoppers (section 2.3) and stored for offline olfactometric evaluation or for qualitative analysis by comprehensive GC-TOFMS.

2.5.   Offline olfactometry

The procedure followed for offline olfactometric evaluation of aroma compounds captured on multichannel PDMS traps was previously described by Naudé et al. [9,10]. In short, the whole aroma (not fractionated) was released from multichannel PDMS traps at 20 °C to 140 °C (10 °C/min) and with a flow of nitrogen gas at a flow rate of 20 ml/min in an in-house built portable heating device. Heart-cuts (section 2.4) were released from the multichannel PDMS traps at 140 °C. Two evaluators, participating in the same session, recorded the odour thus released from the multichannel traps.

2.6.   GCxGC-TOFMS

Separation of compounds was performed on a LECO Pegasus 4D GCxGC-TOFMS including an Agilent 7890 GC (LECO Africa (Pty) Ltd., Kempton Park, South Africa). The system included a secondary oven and a

dual stage modulator. Liquid nitrogen was used for the cold jets and synthetic air for the hot jets. Gases were of ultra high purity grade (Afrox, Gauteng, South Africa). Compounds isolated on multichannel PDMS traps were thermally desorbed by heating the traps in a Gerstel™ thermal desorber system (TDS 3) (Chemetrix, Midrand, South Africa) from 25 °C (3 min) at 30 °C/min to 250 °C (30 min) with a desorption flow rate of 80 ml/min at a vent pressure of 13 psi (helium). The TDS transfer line temperature was 350 °C. The desorbed analytes were cryogenically focused at –40 °C using liquid nitrogen and a cooled injection system (CIS 4) with an empty baffled deactivated glass liner. After desorption, a splitless injection (purge on at 3 min, purge flow 50 ml/min, solvent vent mode) was performed by heating the CIS from –40 °C at 10 °C/s to 250 °C and held there for the duration of the GC run.

The column set consisted of a 30 m x 0.25 mm ID x 0.25 µm df Rxi 5SilMS (5% phenyl, 95% dimethylpolysiloxane) apolar column as the primary column (^{1}D) joined to a 1.8 m x 0.15 mm ID x 0.15 µm df Rtx-200 (trifluoropropyl methyl polysiloxane) intermediate polarity secondary column (^{2}D) (Restek, Bellefonte, PA, USA). The primary column was connected to the secondary column with a presstight column connector (Restek, Bellefonte, PA, USA). The primary oven temperature programme was 40 °C (3 min) at 10 °C/min to 300 °C (1 min). The GC run time was 30 min. The secondary oven was programmed identical to the primary oven, but offset by + 5 °C. The modulator temperature offset was 20 °C. The modulation period was 5 s with a hot pulse time of 1.20 s. The carrier gas (helium) velocity was 30 cm/s (1 ml/min) in the constant flow mode. The MS transfer line temperature was set at 280 °C. The ion source

temperature was 200 °C, the electron energy was 70 eV in the electron impact ionization mode (EI+), the data acquisition rate was 100 spectra/s, the mass acquisition range was 40–350 atomic mass units (amu), and the detector voltage was set at 1700 V. Identification of compounds was based on comparison to authentic standards.

3. Results and discussion

3.1. Multichannel PDMS traps

A simple, cheap, non-hazardous solvent free sampling technique using PDMS for analyte enrichment from wine was developed (Fig. 1). The total amount of sorbed analytes was introduced into a GC providing for increased sensitivity. Compared to commercial sorption devices the multichannel PDMS trap provides a larger sample enrichment capacity of 0.6 ml PDMS. Multichannel PDMS traps may be assembled inside any of the commercial thermal desorption tubes available. Additional advantages of the multichannel PDMS traps over commercial sorption devices are their open tubular structure, multichannel flow and associated low pressure drop [23].

3.2. Heart-cutting by GC-FC and offline olfactometry

Figure 2 depicts selective heart-cutting by GC-FC from the complex sample chromatogram. Complicated instrumental set-ups, sophisticated equipment or valves were not required. The FID collector was removed prior to

heart-cutting. The FID flame gases (hydrogen and air) were switched off. PDMS containing sorbed analytes from wine was desorbed and during this apolar separation of the complex mixture, fractions were selectively collected from the GC effluent onto a secondary PDMS multichannel trap. The secondary multichannel trap was placed, by hand, on the inactivated flame tip prior to peak/fraction elution and removed once the peak/fraction had eluted. The multichannel PDMS trap containing a heart-cutted section of a chromatogram was then desorbed in an offline olfactometer for the controlled release of compounds. The open tubular structure of the multichannel PDMS trap and low pressure drop associated with multichannel flow are features that were not only particularly amenable to the recapturing of chromatographic fractions from the GC effluent during a GC run, but also for the controlled release of trapped compounds in an offline olfactometer (Fig. 2). Most of the effluent of the FID passes through the trap without special sealing arrangement that would otherwise complicate the GC-FC procedure. The advantage of a low pressure drop is not offered by conventional packed traps.

The Pinotage wines were first evaluated for a coffee aroma by releasing the whole aroma (not fractionated), after purge-and-trap sample preparation, from multichannel PDMS traps in an offline olfactometer (section 2.5). Odour descriptors and the temperature at which an odour was perceived were recorded. The traditional Pinotage wine displayed notes of chocolate box, banana, sweet, fruity, smoky, tobacco and toasty. The coffee style wines had notes of chocolate box, banana peel, smoky, burnt rubber, roast coffee bean,
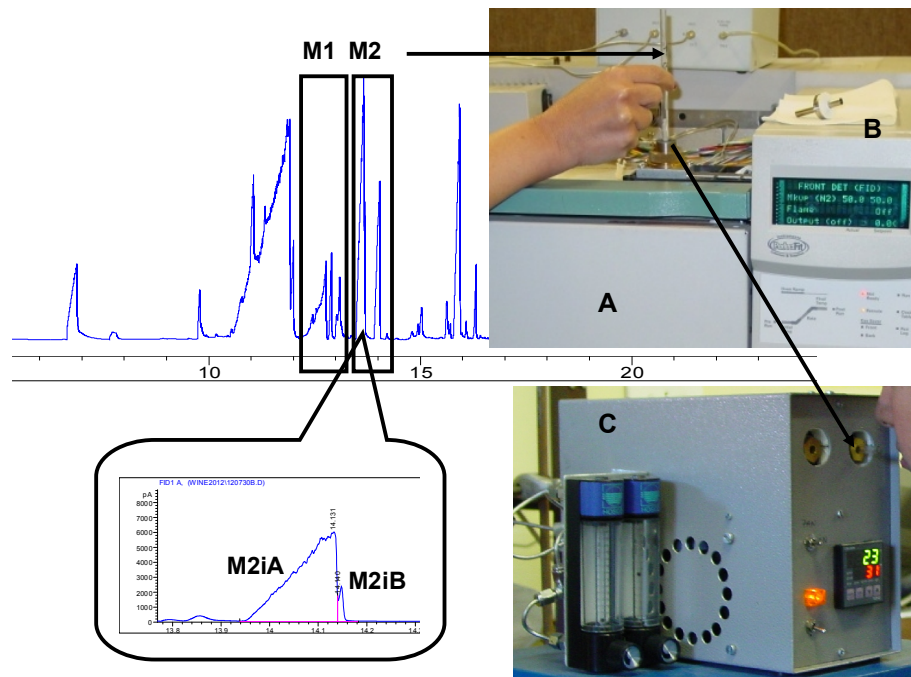
Fig. 2. Heart-cutting and offline olfactometry: identifying the fraction of a chromatogram responsible for a coffee odour. Sections of the chromatogram are captured from the GC effluent on a multichannel silicone rubber trap (A). The trap is rested on the flame tip of an inactive FID during collection. The collector is removed prior to heart-cutting (B). The odour is released from the trap at 140 °C and with a flow of nitrogen gas (20 ml/min) in an offline olfactometer.

savoury, charred veld and leather. Various heart-cuts of the coffee style wine sample chromatogram were then collected from the GC effluent onto secondary multichannel PDMS traps. GC elution temperatures, corresponding to offline olfactometric temperatures at which an odour was perceived, served as a guideline to determine the heart-cut windows. The fractions thus collected represented experimental retention indices (RI_{exp}) 600-800 (N), RI_{exp} 801-899

(M), RI_{exp} 901-1016 (KL), RI_{exp} 1017-1250 (C) and RI_{exp} 1300-1800 (DE) (characters in brackets denote a specific fraction).

For each fraction the heart-cut aroma was released from the secondary multichannel PDMS trap in an offline olfactometer at 140 °C with a flow of nitrogen gas (20 ml/min). Fruity, sweet, caramel, chocolate notes were described for fraction N (RI 600-800); sweet-fruity, banana peel, sweet-smoky, savoury, roast coffee bean, burnt rubber and charred wood were described for fraction M (RI 801-899); sour cream, cotton candy, smoky, sweet, fruity were described for fraction KL (RI 980-1016); savoury, smoke, cocoa powder-like, leather and tobacco were described for fraction C (RI 1017-1250), while fraction DE (1300-1800) was described as burnt rubber, dusty, citrus, exotic sweet floral, black fruit.

Fraction M (RI_{exp} 801-899), in which the coffee bean-like note was detected, was fractionated further to establish the exact GC range in which the compounds responsible for the coffee note eluted. Fraction M was heart-cut into fractions M1 and M2 (Fig. 2). Fraction M1 (RI_{exp} 801-860) was described as sweet-fruity, and fraction M2 (RI_{exp} 870-899) exhibited a roast coffee bean-like note. Fraction M2, the roast coffee bean-like fraction, was again fractionated further into heart-cut M2$_{iA}$ (RI_{exp} 874) and heart-cut M2$_{iB}$ (RI_{exp} 875) (Fig. 2). The aroma from heart-cut M2$_{iA}$, released in the offline olfactometer, was described as sweet-smoky. The aroma from heart-cut M2$_{iB}$, released in the offline olfactometer, was described as char, burnt. The roast coffee bean perception

was not noted for any of the two heart-cuts. A roast coffee bean-like odour was described only for a combination of heart-cuts M2$_{iA}$ and M2$_{iB}$.

3.3. Identification of the compounds in the roast coffee bean-like fraction M2 by GCxGC-TOFMS

Comprehensive GC-TOFMS revealed the presence of furfural and of 2-furanmethanol in heart-cut M2 (Fig. 3). The headspace of authentic standards of furfural and 2-furanmethanol was then captured, either separately or in combination, onto multichannel PDMS traps (volumes were sucked through traps with a 60 ml gastight syringe). The odour was released from the traps in an offline olfactometer (section 2.5). Furfural was described as sweet and almond-like, while 2-furanmethanol was described as savoury, burnt and ether-like. Thereafter, the headspace of furfural and of 2-furanmethanol was combined in varying ratios. The odour of combinations of furfural:2-furanmethanol released from multichannel PDMS traps was described as almond, coffee, smoky and savoury for a ratio of 1:1; almond and dark roasted coffee bean for a ratio of 1:2; and roasty-dusty and coffee beans for a ratio of 1:4.
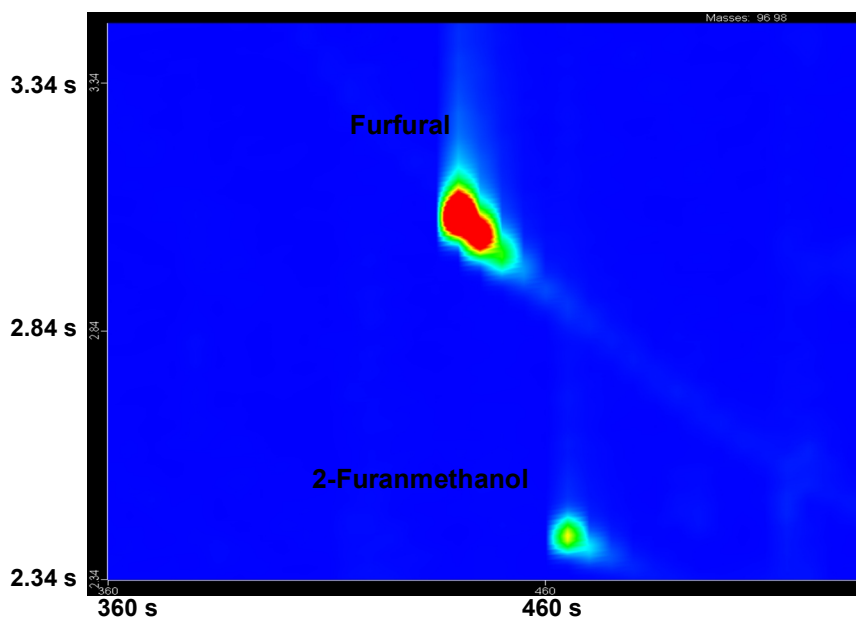
Fig. 3. Contour plot of a reconstructed ion chromatogram (96 + 98 amu) shows the presence of furfural (440 s, 3.070 s) and 2-furanmethanol (465 s, 2.430 s) in heart-cut M2. See 2.6 for instrumental conditions.

Furfural and 2-furanmethanol are amongst the key odourants thought to be particularly responsible for a coffee aroma in brewed coffee [29]. In brewed coffee furfural is described as cooked pea, smoky; and 2-furanmethanol is described as burnt, unpleasant [29]. In general, furfural is described as pungent, but sweet and almond-like [30], while in oak extracts it is described as caramel-like [8]. 2-Furanmethanol is well associated with the burnt and bitter note of dark-roasted coffees [30]. Thus, furfural was responsible for the sweet-smoky note in heart-cut $M2i_A$, and 2-furanmethanol was responsible for the burnt note in heart-cut $M2i_B$.
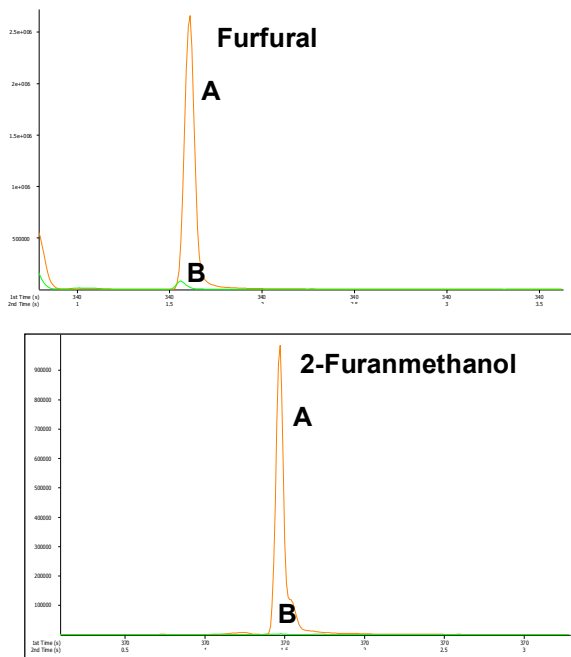
Fig. 4. Reconstructed ion chromatograms of furfural (96 amu) and 2-furanmethanol (98 amu) in a coffee style Pinotage wine (oak staves in tanks, maturation for three months) (orange chromatogram A) compared to a traditional style Pinotage wine (used French oak barrel maturation for ten months) (green chromatogram B). See 2.6 for instrumental conditions.

As is to be expected, furfural was relatively higher in the wines exposed to toasted wood staves, compared to the wine aged in a used barrel (Fig. 4). Relative high levels of 2-furanmethanol were present in Pinotage wines exposed to alternative wood products (chips or staves), while it was not detected in the used barrel aged Pinotage wine (Fig. 4). Furfural in wine is oak-derived, whilst 2-furanmethanol does not directly originate from oak [4,31]. In wine furfural may, chemically or microbiologically, convert to 2-furanmethanol [4]. Although 2-furanmethanol was reported to occur in Spanish red wine exposed to toasted oak chips [31], in traditional aged Shiraz [13], in Shiraz

190

exposed to oak chips and micro-oxygenation [32] and in Merlot [17], there are no reports of 2-furanmethanol occurring in Pinotage wines (possibly because published studies [11-13,15,16,18] did not include Pinotage wines exposed to alternative wood products). A roast coffee aroma of certain red Bordeaux wines was attributed to the presence of 2-furanmethanethiol [33]. However, this chemical compound was not detected in the Pinotage wines analysed here.

4. Conclusions

Multichannel PDMS traps were used for solvent free extraction of compounds from wines and for collection of heart-cuts at the end of a GC column. Heart-cuts thus collected were released from the multichannel PDMS traps in an offline olfactometer which permitted the study of synergistic aroma effects. Heart-cuts were desorbed into a comprehensive GC-TOFMS for compound identification, thereby allowing correlation of aroma with specific compounds. A simple combination of furfural and 2-furanmethanol was, for the first time, shown to contribute to a roast coffee bean-like odour in coffee style Pinotage wines. This coffee bean-like perception is the result of a synergistic effect in which no individual compound was responsible for this particular aroma.

References

[1]   C.J. van Wyk, O.P.H. Augustyn, P. de Wet, W.A. Joubert, Am. J. Enol. Vitic. 30(3) (1979) 167.

[2]   D. de Beer, E. Joubert, J. Marais, W. du Toit, B. Fourie, M. Manley, S. Afr. J. Enol. Vitic. 29(1) (2008) 39.

[3]   E. Koussissi, V.G. Dourtoglou, Ageloussis, Y. Paraskevopoulos, T. Dourtoglou, A. Paterson, A. Chatzilazarou, Food Chem. 114 (2009) 1503.

[4]   A.B. Boutista-Ortín, A.G. Lencina, M. Cano-López, F. Pardo-Mínguez, J.M. López-Roca, E. Gómez-Plaza, Aust. J. Grape Wine Res. 14 (2008) 63.

[5]   T.E. Acree, J. Barnard, D.G. Cunningham, Food Chem. 14 (1984) 273.

[6]   W. Grosch, Flav. Fragr. J. 9 (1994) 147.

[7]   L. Moio, E. Chambellant, I. Lesschaeve, S. Issanchou, P. Schlich, P. Etiévant, Ital. J. Food Sci. 3 (1995) 265.

[8]   M. Consuelo Díaz-Maroto, E. Guchu, L. Castro-Vázquez, C. de Torres, M. Soledad Pérez-Coello, Flavour Fragr. J. 23 (2008) 93.

[9]   Y. Naudé, M. van Aardt, E.R. Rohwer, J. Chromatogr. A 1216 (2009) 2798.

[10]   Y. Naudé, E.R. Rohwer, in: R. Marsili (Ed.), Flavor, Fragrance, and Odor Analysis, Second Edition, Taylor & Francis Group LLC, Boca Raton, Florida, 2012, pp. 93.

[11]   B.T. Weldegergis, A.G.J. Tredoux, A.M. Crouch J. Agric. Food Chem. 55 (2007) 8696.

[12]   B.T. Weldegergis, A.M. Crouch, J. Agric. Food Chem. 56 (2008) 10225.

[13]   A. Tredoux, A. de Villiers, P. Majek, F. Lynen, A. Crouch, P. Sandra, J. Agric. Food Chem. 56 (2008) 4286.

[14]   A. de Villiers, P. Alberts, A.G.J. Tredoux, H.H. Nieuwoudt, Anal. Chim. Acta 730 (2012) 2.

[15]   J. Vestner, S. Malherbe, M. du Toit, H.H. Nieuwoudt, A. Mostafa, T. Górecki, A.G.J. Tredoux, A. de Villiers, J. Agric. Food Chem. 59 (2011) 12732.

[16]   B.T. Weldegergis, A. de Villiers, C. McNeish, S. Seethapathy, A. Mostafa, T. Górecki, A.M. Crouch, Food Chem. 129 (2011) 188.

[17] J.E. Welke, V. Manfroi, M. Zanus, M. Lazarotto, C.A. Zini, J. Chromatogr. A 1226 (2012) 124.

[18] B.T. Weldegergis, A.M. Crouch, T. Górecki, A. de Villiers, Anal. Chim. Acta 701 (2011) 98.

[19] P. Schreier, F. Drawert, A. Junker, J. Agric. Food Chem. 24(2) (1976) 331.

[20] A. Rapp, Nahrung 42 (1998) 351.

[21] Z. Zhang, J. Pawliszyn, Anal. Chem. 65 (1993) 1843.

[22] E. Baltussen, P. Sandra, F. David, C. Cramers, J. Microcolumn Sep. 11 (1999) 737.

[23] E.K. Ortner, E.R. Rohwer, J. High Resolut. Chromatogr. 19 (1996) 339.

[24] M.J. Lim Ah Tock, M.Tech. dissertation (2009) Aroma analysis of alcoholic beverages using multi-channel silicone rubber traps, Tshwane University of Technology, Pretoria, South Africa [online]. Available:http://libserv5.tut.ac.za:7780/pls/eres/wpg_docload.download_file?p_filename=F485852604/Lim,%20M.J.%20Ah%20Tock.pdf (Accessed 30 July 2012).

[25] Y. Naudé, E.R. Rohwer, Anal. Chim. Acta 730 (2012) 112.

[26] Y. Naudé, E.R. Rohwer, Anal. Chim. Acta 730 (2012) 120.

[27] E.R. Rohwer, M.J. Lim Ah Tock, Y. Naudé, SA provisional patent application ZA 2006/07538 (2006).

[28] H. van den Dool, P.D. Kratz, J. Chromatogr. 11 (1963) 463.

[29] S-T. Chin, G.T. Eyres, P.J. Marriott, J. Chromatogr. A 1218 (2011) 7487.

[30] I. Flament, Coffee Flavour Chemistry, John Wiley & Sons, West Sussex, England, 1st ed., 2002, pp. 81.

[31] B. Fernández de Simón, E. Cadahía, M. del Álamo, I. Nevares, Anal. Chim. Acta 660 (2010) 211.

[32] L.M. Schmidtke, thesis (2011) Effects of Micro-oxygenation on Shiraz Wine, Agricultural and Wine Sciences, Charles Sturt University, Australia [online]. Available: http://researchoutput.csu.edu.au/R/-?func=dbin-jump-full&object_id=29361&local_base=GEN01-CSU01 (Accessed 25 October 2012).

[33] T.Tominaga, L.Blanchard, P. Darriet, D. Dubourdieu, J. Agric. Food Chem. 48 (2000) 1799.