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Analytical Methods

Application of integrated comprehensive/multidimensional gas chromatography with mass spectrometry and olfactometry for aroma analysis in wine and coffee

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ABSTRACT

Component coelution in chromatographic analysis complicates identification and attribution of individual odour-active volatile molecules in complex multi-component samples. An integrated system incorporating comprehensive two-dimensional gas chromatography (GC × GC) and multidimensional gas chromatography (MDGC), with flame ionisation, olfactometry and mass spectrometry detection was developed to circumvent data correlation across different systems. Identification of potent odorants in Shiraz wine and the headspace of ground coffee are demonstrated as selected applications. Multiple solid-phase microextraction (SPME) sampling with GC-O located odour-active regions; GC × GC established the complexity of odour-active regions; MDGC provided high-resolution separation for each region; simultaneous 'O' and MS detection completed the analysis for target resolved peaks. Seven odour regions in Shiraz were analysed with MDGC-O/MS detection, revealing 11 odour volatiles through matching of mass spectrometry and retention indices from both separating dimensions, including acetic acid; octen-3-ol; ethyl octanoate; methyl-2-oxo-nonanoate; butanoic acid, 2-methylbutanoic acid, and 3-methylbutanoic acid; 3-(methylthio)-1-propanol; hexanoic acid; β-damascenone; and ethyl-3-phenylpropanoate. A capsicum odour in ground coffee was identified as 2-methoxy-3-isobutylpyrazine with a 5-fold increase in S/N of the odorant when acquired using a 6-time cumulative SPME sampling approach.

1. Introduction

Understanding the important compounds responsible for food aroma such as in wine and coffee is valuable to improve production processes, such as viticulture practice, vinification, and the roasting process, in order to achieve optimum product quality in these products (Chin, Eyres, & Marriott, 2011; Clarke & Bakker, 2004; Ebeler & Thorngate, 2009; Flament, 2002; Semmelroch & Grosch, 1996; Shimoda & Shibamoto, 1990). Volatile components in such products exhibit complex composition, comprising mainly alcohols, esters, acids, and other minor compounds including norisoprenoid ketones, and sulphur- and nitrogen-containing compounds in the wine, which impact greatly on the global aroma even at sub ppb level (Davis & Qian, 2011; Ebeler & Thorngate, 2009; Pons, Lavigne, Eric, Darriet, & Dubourdieu, 2008; Siebert, Wood, Elsey, & Pollnitz, 2008; Simpson, Capone, & Sefton, 2004). Analysis of the complex composition requires development and exploitation of enhanced separation strategies for disclosing the odour-active volatiles that contribute to unique aroma characteristics.

Often, pre-separation prior to GC–MS is preferred in characterisation of flavour, either by classic acid/base or solvent extraction, or other column chromatography approaches (Qian, Burbank, & Wang, 2007). Fractionation by offline high performance liquid chromatography (HPLC) followed by multidimensional gas chromatography (MDGC)-O/MS was utilised to reveal several impact odorants in wine, such as a blackberry flavour contributed by ethyl 2-hydroxy-4-methylpentanoate ester (Falcao, Lytra, Darriet, & Barbe, 2012), and a strong prune odour by γ-nonalactone, β-damascenone, and 3-methyl-2,4-nonanedione ketones (Pons et al., 2008). Identification of rotundone as the peppery note in wine was performed by coupling solid phase extraction (SPE) with solid phase microextraction (SPME) to selectively concentrate the target component prior to GC–MS detection (Siebert et al., 2008). Schmarr et al. (2010) reported a solid phase extraction (SPE) clean-up procedure followed by online HPLC coupled to MDGC–MS for quantifying 3-alkyl-2-methoxypyrazines in wines and

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musts with complete elimination of the interfering matrix background. These tedious pre-fractionation procedures required longer processing time, with the possibility of artefact formation. Notwithstanding numerous reported techniques, successful aroma analysis demands achieving the greatest possible resolution, with ease of application, whilst also achieving an absence of artefacts and overlapping components generated during the measurement process. Comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC × GC–TOFMS) has been successfully utilised for non-targeted volatile analysis that permits simultaneous analysis of a significantly larger number of compounds found in the wine headspace (Chin et al., 2011; Robinson, Boss, Heymann, Solomon, & Trengove, 2011). The role of MDGC and GC × GC to deliver improved chromatographic resolution has been recently reviewed (Marriott, Chin, Maikhunthod, Schmarr, & Bieri, 2012).

A recent study proposed an integrated GC × GC/MDGC system with simultaneous MS, FID and olfactometry detection, capable of implementing both GC × GC and heart-cut (H/C) MDGC modes within a single unit GC system (Chin, Eyres, & Marriott, 2012b). An analytical strategy using this proposed system for odorants analysis in samples with complex background such as wine was suggested. The present work extends the application of the integrated GC × GC/MDGC system for analysis in several complex co-eluting odour-active regions of aroma extract from Shiraz wine and ground coffee. Cumulative SPME was incorporated for enhanced sensitivity analysis of odour-active compounds. Matching of retention indices (RI) obtained from ^{1}D and ^{2}D separation was used for compound identification together with acquired mass spectrum comparison with established MS database.

2. Materials and methods

2.1. Materials

Shiraz wine (Lindeman's, Victoria, Australia; 2010 Vintage) was purchased from a local wine retailer. Coffee powder (Harris coffee, NSW, Australia) was purchased from a local store. SPME fibres consisting of polar polyacrylate (PA), the blended porous particle phase carboxen and divinylbenzene with polydimethylsiloxane (PDC), and holders for manual sampling were gifts from Supelco (Sigma–Aldrich St. Louis, MO, USA). Hexanoic acid, octen-3-ol and β-damascenone (>98% purity) were gifts from Australian Botanical Product (Hallam, Australia). Standard compounds with purity >97% included butanoic acid, 2-methylbutanoic acid, 3-methylthiopropanol, ethyl 3-phenylpropanoate, and a saturated alkane series from C9 to C22 were obtained from either Fluka or Aldrich (Sigma–Aldrich). GC grade hexane, analytical grade sodium chloride (NaCl), and acetic acid were obtained from Merck Chemical Co. (Merck KGaA, Darmstadt, Germany).

2.2. Cumulative SPME sampling

Cumulative SPME sampling for wine was performed manually as previously described (Chin, Eyres, & Marriott, 2012a). Repeated sampling of 2 × PA + 2 × PDC with 30 min extraction time for each fibre (denoted as 2 × PADC) were sequentially desorbed in the injector for 3 min each, and volatiles were accumulated in a cryotrap (CT) installed just after the injector for cryofocussing. The sampling protocol required separate fibres to be placed in the sample headspace at 3 min intervals, each for the 30 min sampling period, then individually desorbed, in order to minimise total analysis time. Up to six separate PDC fibres were used for evaluation of coffee aroma, allowing up to 6 × PDC accumulations (40 min extraction time each) with desorption as above, for trace analysis. Thus the total extraction/desorption time was 6 × 3 min + 40 min. The GC programme commenced 3 min after the final SPME desorption step into the GC injector, immediately followed by switching off the CT CO_2 supply.

2.3. GC-O, GC × GC, Heart-cut-MDGC-O/MS analysis

An integrated GC × GC/MDGC system as described previously (Chin et al., 2012b) was used for this study. The system (Supplementary information Fig. S1) comprises an Agilent 7980/5975C series GC–MS (Agilent Technologies, Nunawading, Australia) retrofitted with a liquid CO_2 CT device (SGE Scientific, Ringwood, Australia), a SGE olfactory port (ODO II model, SGE Scientific, Ringwood, Australia), an Agilent G2855A Deans switch device (DS), an Agilent G3180B 2-way effluent splitter (ES), and an Everest model Longitudinally Modulated Cryogenic System (LMCS; Chromatography Concepts, Doncaster, Australia). For the Shiraz wine sample, a polar DB-FFAP column (30 m length × 0.25 mm ID × 0.25 μm film thickness (d_f); Agilent Technologies), a non-polar BPX5 (0.9 m × 0.10 mm ID × 0.10 μm d_f; SGE Analytical Science) and non-polar DB-5 ms column (30 m × 0.25 mm ID × 0.25 μm d_f; Agilent Technologies) were used for the ^{1}D, short ^{2}D$_S$ and long ^{2}D$_L$ separation columns respectively. For the coffee sample, an ionic liquid phase SLB-IL59 column (30 m × 0.25 mm ID × 0.20 μm d_f; Supelco), a mid-polar VF-200 ms (30.0 m × 0.25 mm ID × 0.25 μm d_f) and a microbore VF-200 ms (1.0 m × 0.10 mm ID × 0.10 μm d_f; both from Agilent Technologies) was applied as ^{1}D, long ^{2}D$_L$ and short ^{2}D$_S$ columns respectively. The SGE CT served for solute trapping of SPME desorbed analytes at the inlet end of the ^{1}D column, as well as trapping of H/C fractions at the front end of the long ^{2}D$_L$ column. The CT of the LMCS was positioned near the beginning of the short ^{2}D$_S$ column for GC × GC operation.

The inlet of the ^{1}D column was connected to a split/splitless injector whilst the ^{1}D outlet and both ^{2}D column inlets were connected to the DS. Effluent from the short ^{2}D$_s$ column outlet was split equally via a Y-union and 2 deactivated fused silica (DFS) capillaries (55 cm × 0.10 mm ID) to a flame ionisation detector (FID) and an olfactory port respectively. Meanwhile, the effluent from the long ^{2}D$_L$ column outlet was equally split by the ES device operated at 30.0 psi and directed to both the MS detector via a DFS (80 cm × 0.10 mm ID) transfer line heated at 240 °C and another DFS (75 cm × 0.10 mm ID) to the olfactory port respectively. The same olfactory port was used for each channel, and the two separate channel DFS transfer lines both terminated at the nose cone of the olfactory port.

The inlet pressure was initially applied at 48.5 psi during SPME desorption, and then ramped to 51.0 psi immediately after commencing the GC programme giving a flow rate of 2.0 mL/min at 150 °C in ^{1}D, with the DS held constant at 47.5 psi. Following ^{1}D separation of the SPME extract, the DS diverted the solute to either O/FID via ^{2}D$_S$ without operating the LMCS (for GC-O/FID), or to O/MS dual detection via ^{2}D$_L$ (for GC-O/MS). Two experienced panelists were used to evaluate the odour active regions during olfactometry analysis. The GC inlet was set at 250 °C, with helium carrier gas and split vent open after 2 min. The oven programme was set at 60 °C for 2 min, increased to 120 °C (20 °C/min), then to 250 °C (3 °C/min) with 5 min hold. GC × GC–FID analysis was conducted by operating the LMCS CT at −20 °C with a modulation period (P_M) of 4 s. The FID was operated at 250 °C, with acquisition rate of 20 Hz and 100 Hz for 1D GC and GC × GC analysis respectively.

To perform H/C MDGC operation, a 2-step sequence was programmed for the H/C event. The required switching time (0.4 min duration for each H/C zone) was entered into the events software in accordance with GC-O/FID analysis for the target