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Cumulative solid phase microextraction sampling for gas chromatography-olfactometry of Shiraz wine

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ABSTRACT

Solid phase microextraction (SPME) coupled to gas chromatography-olfactometry (GC-O) is now commonly used for determination of aroma-active compounds, but the method sensitivity and selectivity is restricted by the small volume and limited type of fibre coating phases. In an attempt to enhance the method performance, a cryogenic trapping (CT) approach was investigated in this study by coupling multiple SPME sampling events for wine headspace using GC-O analysis. By performing multiple SPME sampling employing different chemical polymer coatings, desorbed solute from the integrated sampling is accumulated by the CT at the front section of a Wax separation capillary column prior to chromatographic analysis. Results show that the CT was capable of retaining apolar alkane volatiles of decane and greater, and tested polar alcohols, including methanol. Chromatographic signals eluting later than the ethanol peak were found to progressively increase in response, and correlated well, with the cumulative number of SPME samplings. The approach was developed for GC-O screening of potent odorants in Shiraz wine collected from fibre coatings of polyacrylate (PA) and the triple-phase coated polydimethylsiloxane/divinylbenzene/carboxen (PDC). The aromagram for solute derived from a combined introduction of both PA plus PDC fibres (i.e. sequential fibre introduction into the injector; termed as PADC) compared well to the sum of those sampled by using a single fibre coating alone, which comprised of odorants derived from both fibre coatings. Accumulation in the CT of volatile solutes derived from up to 6 repeat PADC sampling events revealed a similar pattern of their aromagrams, though with stronger olfactory stimulus response. This study demonstrated a simple and effective way for enhancing SPME sensitivity and potentially less discrimination during the analysis of wine volatiles. However, the single dimensional GC separation method requires development of an improved separation strategy to better separate individual compounds.

1. Introduction

The organoleptic properties of wine arise from numerous volatile compounds, and these compounds affect the wine's properties, both positively and negatively. A recent review [1] postulated that verification of odour substances in wine using gas chromatography-olfactometry (GC-O) depends critically on the procedure for isolation of volatiles, as it affects the representativeness of the isolate and the composition of the eluate that is subjected to sensory evaluation. Solid phase microextraction (SPME) has been established as an analyte sampling/enrichment approach for trace compound analysis in various sample matrices. This technique is commonly utilized in wine flavour analysis because of its ease of use, solventless attributes, good reproducibility and small sample volume needed. Due to limited sorbent volume on the fibre coating, extending the extraction time or cooling the fibre coating [2,3] has been applied for improving the analyte mass affinity to the fibre, whilst heating or sonication has been employed during extraction by SPME in order to enhance mobilization of volatiles out of the sample matrix [3].

Significant variation in the range of volatile compounds isolated by SPME has been observed to depend greatly on the properties of fibre coating type [4]. Commercially available SPME fibres commonly comprise polar polyacrylate (PA), carbowax (CW)/polyethylene glycol (PEG) material, or non-polar polydimethylsiloxane (PDMS) material. Blended porous particles carboxen (CAR) and/or divinylbenzene (DVB) with PDMS exhibit composite sorption properties. Searching for the most suitable coating phase is required during SPME method development [5]. A fibre coating comprising a mixture of CAR/DVB/PDMS (PDC) has been used extensively for characterization of wine bouquet. Sun et al. [6] and Robinson et al. [7] reported a non-targeted analysis using such a fibre coating to characterize different wines, by coupling with comprehensive two-dimensional GC time-of-flight

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mass spectrometry. Meanwhile, Ganss et al. [8] employed 2 different fibre coatings, CW/DVB (for polar components such as volatile alcohols, acids, monoterpenes), and CAR/DVB/PDMS (for less polar volatile esters) in order to monitor the changes in the volatile profile during wine fermentation. An unbiased volatile extract is crucial for screening of aroma-impact components in wine by GC-O, so that unambiguous qualitative and quantitative assignment of the wine profile can be achieved. A representative sample of the overall flavour profile may be difficult to achieve by using any one fibre coating alone, and so discrimination of extracted volatiles during the sampling may necessarily arise.

Cryogenic focusing of large volume headspace vapours at the beginning of the separation column has been described by Wylie [9], and Kolb and Liebhardt [10], for enhancing the sensitivity of equilibrium headspace GC. Lipinski [11] demonstrated that an enrichment step could be integrated in SPME/GC analysis, to enhance the limit of detection for the determination of pesticides in water, by using repeated extraction/desorption cycles within an acceptable total extraction time. By performing up to 4 repeat SPME injections consecutively into the GC, the desorbed semi-volatile analytes were trapped and accumulated at the front of the 'cool' GC column (at 40 °C), prior to GC separation. A systematic study of solute accumulation for higher volatility compounds, with application to GC-O, has yet to be investigated.

Efficient profiling of wine aroma composition should ideally detect as many analytes as possible in a single analytical run, requiring effective sample extraction prior to instrumental analysis. Development of a volatile sampling method with greater sensitivity and more representative extraction efficiency whilst exploiting the simplicity of SPME, is a desirable goal. This work aims to study the cumulative effect of multiple SPME sampling hyphenated with GC-O for aroma analysis of Shiraz wine. Combination of multiple volatile extract sampling steps from similar or different fibres, including PDC and PA, were tested for wine analysis by cryotrapping at the column head prior to separation. GC-O, with detection frequency (DF) sensory analysis, was used for screening of potent aroma compounds. Compounds were tentatively identified using GC–MS and retention index matching.

2. Materials and methods

2.1. Materials

A sample of Shiraz wine produced in year 2010 (Lindeman's, Victoria, Australia) was purchased from a local wine retailer. SPME fibres and holders for manual sampling were obtained from Supelco (Sigma–Aldrich, St. Louis, MO, USA), saturated alkane (C6–C23 except C11) and primary alcohol series (C1, C2, C4, C6, C8 and C10) were obtained from Sigma–Aldrich. Sodium chloride (NaCl) for salting-out was obtained from Merck Chemical Co. (Merck KGaA, Darmstadt, Germany).

2.2. GC-O analysis

An Agilent 6890N GC (Agilent Technologies, Nunawading, Australia) retrofitted with a SGE olfactory port (ODO II model, SGE Scientific, Ringwood, Australia) was used as illustrated in Fig. S1. Separation of volatiles was performed using an Agilent HP-INNOWax capillary column (15 m × 0.32 mm I.D. × 0.5 μm d_f). The inlet section of the column was passed though an SGE liquid CO_2 cryogenic cold trap (CT) located 10 cm after the injector, while the effluent from the column outlet was split using a Y-union to 2 deactivated fused silica capillary tubings, one (50 cm length × 0.10 mm I.D.) directed to the flame ionization detector (FID) and the other (1 m × 0.18 mm I.D.) directed to the olfactory port respectively. The split ratio of effluent to FID and olfactory port was measured to be 1:5 respectively. The GC inlet was set at 260 °C, with splitless sampling for 2 min. A hydrogen carrier flow rate of 1.0 mL/min was initially applied during SPME desorption, and then ramped to 3.0 mL/min immediately after commencing the GC program. The lower initial carrier flow rate was used to improve solute holding capacity by the CT until the desired number of injections was made. The GC oven was programmed at 40 °C (3 min hold), increased to 180 °C (5 °C/min), then to 240 °C (12 °C/min; hold 8 min). Six panellists were screened and selected to conduct the GC-O sniffing. GC-O sniffing commenced at 4 min (after ethanol elution) and concluded at 35 min. The described odour perception of the separated effluent by each panellist was recorded and computed as detection frequency (DF), according to previous study [12]. During GC-O data analysis, aroma peaks detected by three or more assessors (i.e. DF ≥ 3; ≥50% of assessors) were selected as significant potent odourants as previously described [13].

2.3. GC–MS analysis

Compound identification was conducted using an Agilent 5975 C GC–MSD system. Helium carrier (99.999% purity) with flow rate of 1.0 mL/min initially, was increased immediately to 3.0 mL/min once the program started. The same column set and configuration was used for separation of volatiles, with the CT located 10 cm from the inlet end of the column, and the outlet was connected to the MSD through a transfer line heated at 280 °C. Electron ionisation mode (70 eV; 230 °C) and a mass scan range from 40 to 350 m/z were used. MS data processing utilized the automated mass spectral deconvolution and identification system (AMDIS) program version 2.66, and NIST MS matching library version 2.0f.

2.4. SPME sampling of wine volatiles

Single SPME sampling experiments were carried out using a 20 mm length PDMS/DVB/CAR (PDC) fibre, or a 10 mm length PA fibre. A 15 mL volume of wine was transferred into a 30 mL glass vial together with 4.5 g NaCl and a magnetic stirring bar (8 mm). Extraction was performed by piercing the vial septum and exposing the fibre coating to the headspace of the vial for 45 min under vigorous stirring (500 rpm) at room temperature (~25 °C).

For cumulative SPME sampling, each sample vial was pre-equilibrated at least 5 min whilst extraction of multiple samples by manual SPME was performed close to simultaneously. Each fibre was exposed for 30 min offset by 3 min intervals such that the fibre exposure time was equal when introduced into the GC inlet for desorption. The GC program run commenced 3 min after the final SPME desorption step into the injector. Various accumulation experiments for SPME sampling were as follows: Most experiments employed an extraction with PDC and PA fibres, with both fibres sequentially desorbed into the injector and volatiles collected in the CT. Cumulative sampling of 1× PA + 1× PDC (equivalent to a SPME sorption experiment separately conducted with both fibres) is denoted 1× PADC. Cumulative sampling with multiple injections of 6× PA + 6× PDC is denoted 6× PADC. The PADC acronym refers to the combination of 4 different coating materials, i.e. PDMS–polyacrylate–DVB–Carboxen. These two experimental sampling conditions were contrasted here, although other combinations were also tested. Liquid CO_2 was supplied to the CT device 5 min prior to the first fibre desorption and switched off once all the SPME desorptions/collections were completed and the GC separation program commenced.