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Published version:

DOI:10.1016/j.foodchem.2016.06.047

DIFFERENT COATINGS FOR THE HS-SBSE GRAPE VOLATILE ANALYSIS IN MODEL SOLUTION: PRELIMINARY RESULTS

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ABSTRACT

Head space stir bar sorptive extraction was used to evaluate the efficacy of two stir bar coatings, poly(ethylene glycol) (PEG)-modified silicone (EG-Silicone) and polyacrylate (PA), in comparison with polydimethyl siloxane (PDMS) coating. Two temperatures (0 °C and 25 °C) of the CIS injection system were compared with the aim to propose an analytical procedure faster respect to traditional extractive methods and environmental friendly, avoiding the use of solvents and high-energy consumption for cooling. Fifteen volatiles with different polarities were tested. The PDMS coating showed higher relative areas for terpenes and norisoprenoids. C6 volatiles, benzaldehyde and eugenol, obtained greater area response using EG-Silicone and PA coatings, regardless the temperatures. EG Silicone and PA coatings showed a higher memory effect for a certain compounds, probably due to the lower desorption temperature that EG Silicone and PA coatings can be submitted to, respect to PDMS (220 °C respect to 295 °C).

Keywords: volatiles, PDMS, EG-Silicone, Polyacrylate, grape.

1. Introduction

Aroma is one of the most important characteristics of wine, being related to product quality and consumer acceptance. Among the constituents of wine aroma, the ones from grapes play an important role in determining quality and authenticity. Isolation, identification and quantification of grape volatiles are pivotal to understand key events of vine response to external stimuli. Thus, it is necessary to find rapid and efficient analytical methods that can help technicians and grapegrowers to make the most correct choice at harvest and during winemaking, when time is often the critic point.

Traditional analytical methods require time-consuming extraction and enrichment steps prior to chromatography. Modern techniques in sample preparation have evolved towards simplification by reducing solvents and sample handling. Stir bar sorptive extraction (SBSE; commercially named 'Twister®') (Baltussen, Sandra, David & Cramers, 1999; Baltussen, Cramers & Sandra, 2002) is an extraction technique that is less time consuming respect to traditional extractive techniques and does not employ solvents. Moreover, it has been largely automated. The stir bar is coated with a polymeric extracting phase that traps the volatile analytes in liquid samples (immersion mode) or in headspace. SBSE technique was used to analyze grape and wine volatiles by the immersion mode (Ferrandino, Carlomagno, Baldassarre & Schubert, 2012; Pardo-García, Serrano de la Hoz, Zalacain, Alonso & Salinas, 2014; Zalacain, Marín, Alonso & Salinas, 2007), by the headspace mode (Maggi, Zalacain, Mazzoleni, Alonso & Salinas, 2008) or by both of them (Arbulu, Sampedro, Sánchez-Ortega, Gómez-Caballero, Unceta, Goicolea & Barrio, 2013). In comparison with the immersion mode, the headspace SBSE mode extends the life of the stir bar, especially in complex matrix. The SBSE technique showed analytical simplicity, high reproducibility and very low detection limits. Polydimethyl siloxane (PDMS) was the universal commercially available stationary phase coating material. PDMS is a sorptive material that at temperature above its glass transition temperature acquires a gum-like state with chemical properties such as diffusion and distribution constants similar to those of organic solvents. SBSE-PDMS has a stationary phase volume much more higher respect to SPME-PDMS: the maximum volume of PDMS in a SPME fiber is 0.5 microliter (film thickness of

100 micrometer) whereas PDMS in SBSE can provide from 24 to 126 microliters of volume. Thus, the sensitivity of PDMS-SBSE is higher, resulting in a higher volatile extraction respect to SPME and in the detection and identification of compounds present at very low concentrations, such as sesquiterpenes (May & Wüst, 2012; Ferrandino, Carlomagno, Baldassarre & Schubert, 2012) or malodors in wastewaters (Loughrin, 2006). PDMS stir bars were also used for the detection of stilbene molecules in wines (Cacho, Campillo, Viñas & Hernández-Córdoba, 2013). However, doubts about its efficaciousness arose due to the PDMS non-polar nature that was thought to limit the applicability of SBSE-PDMS to hydrophobic compounds. Slightly polar or polar coatings have recently been introduced but their use is limited. Cacho, Campillo, Viñas & Hernández-Córdoba (2015) compaired the use of these three different coatings for the determination of volatile terpenes in honey, of chlorophenols and chloroanisoles in wines (Cacho, Campillo, Viñas & Hernández-Córdoba, 2014). EG-Silicone stir-bars were recentely used to meausre ochratoxin A in beers (Nguyen & Dojin, 2014).

For the thermo-desorption of the retained compounds on the stir-bar, a specific injector, constituted by a Thermal Desorption Unit (TDU) and of a Cooled Injection System (CIS), is generally used. Different CIS temperatures, such as: -50 °C, (Caven-Quantrill & Bouglass, 2011); -30 °C (Martínez-Gil, Garde-Cerdán, Martínez, Alonso & Salinas, 2011; Martínez-Gil, Pardo-García, Zalacain, Alonso & Salinas, 2013); 0 °C (Ferrandino, Carlomagno, Baldassarre & Schubert, 2012); 15 °C (Cacho, Campillo, Viñas, & Hernández-Córdoba, 2015); 30 °C (Ochiai, Sasamoto, Ieda, David & Sandra, 2014), were used to measure volatiles with the PDMS-SBSE/GC-MS technique, but at present no studies deal with the comparison of specific CIS temperatures, and with different coatings.

The CIS is an injection system with programmed temperature vaporization (PTV) that allows the controlled vaporization of the sample after an enrichment step. The CIS liner can be rapidly heated or cooled. Moderate cooling can be accomplished using a Peltier Cooling Unit but, to achieve more important cooling, liquid nitrogen (N_2) or liquid carbon dioxide (CO_2) are used, increasing the costs and the complexity of the analysis. The analytes on the stir bar can be cooled very quickly, but when the temperature ranges are very wide, more time is required to get the desired temperatures; besides, extreme ranges of temperature imply a more rapid deterioration of the analytical equipment. Thus, analysis are more expensive and a higher energy consumption is required. In most articles, the CIS temperature is a parameter systematically described but not further commented. No studies comparing the effect of different CIS temperatures on the analytical response of the different volatiles have been conducted. We deem necessary to make an effort in the direction of 'energetic' resource reduction also in the laboratory, adapting, when possible, the analytical technique for 'energy saving'.

In the present study, two stir bar coatings: poly(ethylene glycol) (PEG)-modified silicone (EG Silicone) and polyacrylate (PA) were compared to the traditional PDMS stir bar to analyse some grape volatile compounds. The stir bars were used in the HS mode. Two different CIS temperatures (0 ^{0}C and 25 ^{0}C) were assayed to identify running temperatures as high as possible without limiting the analytical results, to propose a more environmental friendly analytical procedure. However, results are still preliminary as validation on musts and wines and on a larger number of molecules is required.

2. Materials and methods

2.1. Chemicals and reagents

Benzaldehyde, β-citronellol, eugenol, geraniol, 1-hexanol, *(E)*-2-hexenal, *(E)*-2-hexen-1-ol, *(Z)*-3-hexen-1-ol, α-ionone, β-ionone, D-limonene, linalool, nerolidol and α-terpineol were purchased from Sigma-Aldrich (Steinheim, Germany). A mixture of commercial standards of high purity grade (>97%) in methanol was prepared, using concentration ranges for each compound commonly found in wines. A model solution was prepared: an aqueous ethanol solution at 12% with 5 g L^{-1} of tartaric acid was and pH adjusted to 3.6 with 1M sodium hydroxide (Zalacain, Marin, Alonso & Salinas, 2007) (Table 1).

The internal standard was γ-hexalactone (Sigma-Aldrich, Steinheim, Germany) solution at 1 µL mL^{-1} in absolute ethanol (Merck, Darmstad, Germany).

were employed to prepare model solutions.

2.2. Stir bar coatings

To perform the sorptive extraction process, commercial stir bars coated with polydimethylsiloxane (PDMS Twister®), poly (ethylene glycol) (PEG)-modified silicone (EG Silicone Twister®) and polyacrylate (Acrylate Twister® PA) were used, the last two freely provided by Gerstel (Mülheim and der Ruhr, Germany towards SRA Instruments, Cernusco sul Naviglio, MI, Italy). They consisted of a 10 mm long glass-encapsulated magnetic stir bar with three different external coatings: 24 µL of PDMS, 32 µL of EG Silicone and 25 µL of PA.

2.3. Volatile extraction

The model solution was extracted by headspace (HS-SBSE; Martínez-Gil, Garde-Cerdán, Martínez, Alonso & Salinas, (2011). Twenty-two mL of model solution were placed into a 50 mL headspace vial. For each milliliter of sample, 0.1 g of NaCl and 10 μL of internal standard (γ-hexalactone) were added. HS-SBSE extraction was carried out by exposing the coated stir bar to the vial headspace hermetically closed, stirring the sample was stirred at 500 rpm and heating at 60 ºC for 60 min. After sorption, the stir bar was rinsed with pure water, dried with a lint-free tissue and desorbed.

2.4. Analytical procedure

The sample introduction system was composed of an automated thermal desorption unit (TDU), combined with a MultiPurpose Sampler (MPS) and a Programmed Temperature Vaporization (PTV) Cooled Injection System (CIS-4), from Gerstel . The GC was an Agilent 7890A gas chromatograph coupled to an Agilent 5975C quadrupole mass spectrometer detector (MS, Agilent Technologies, Palo Alto, CA, USA) equipped with a DB-WAX column (J&W 122-7032; 30 m length, 0.25 mm I.D. and 0.25 μm film thickness). The carrier gas was helium with a constant column pressure of 20.75 psi.

Stir bars were thermally desorbed using helium as carrier gas at a flow rate of 75 mL/min; TDU was programmed from 40 °C to 295 °C (5 min) at a rate of 60 °C/min for PDMS and from 40 °C to 220 °C (5 min) at a rate of 60 °C/min for EG Silicone and PA coatings, following the supplier’s recommendations. The analytes were focused on the CIS-4, containing a packed liner (20 mg of Tenax®), at two different temperatures: 25 °C and 0 °C with liquid CO_2 cooling prior to injection.

After desorption and focusing, the CIS-4 temperature was programmed to reach 260 °C (5 min) at 12 °C/s to transfer volatiles onto the analytical column. The stir aliquot of volatiles still sorbed on the the stir bar after desorption and rinsing (‘memory effect’) was evaluated both at 0 and at 25 °C.

The TDU operated in the splitless desorption mode, the CIS-4 operated in PTV solvent vent mode (purge flow to split vent of 80 mL/min, vent 75 mL/min and pressure 20.85 psi). The GC oven temperature was programmed at 40 °C (2 min), raised to 150 °C (10 °C/min, held for 5 min) then to 230 °C (10 °C/min, held 2 min). The MS operated in scan mode (27–300 amu) with ionization energy set at 70 eV. The temperature of the MS transfer line was 230 °C. Each molecule was identified by comparing mass-spectrum with those of the NIST library and chromatographic retention index of each standard. To avoid matrix interferences, the MS quantification was carried out in the single ion monitoring mode using each molecule characteristic m/z value (Table 1).

The HS- SBSE-GC/MS analysis were performed in sevenfold.

2.5. Statistical Analysis

Means and standard errors of the seven analysed replicates were calculated. Data were statistically treated with SPSS (Version 19.0 statistical package for Windows; SPSS, Chicago, IL, USA) and processed using the analysis of variance (ANOVA). Statistical differences among means were evaluated using the Duncan’s test ($P \leq 0.05$). Normalized data underwent a discriminant analysis, using the coating type as differentiating variable.

3. Results and Discussion

Each compound relative area was calculated as the ratio between its response area and the response area of the internal standard after HS-SBSE-GC/MS analysis, setting the CIS at 0 °C and 25 °C (Tables 2 and 3). Standard errors were very low, regardless the type of Twister® coating used, demonstrating high precision and reproducibility of the methodology.

Compounds whose Log K_{ow} was near 3 are the most apolar (Table 1), so it would be expected that they were more retained by the PDMS due to its nonpolar phase; the other two coatings (more polar) were expected to exhibit higher affinity for compounds with Log K_{ow} around 1.

CIS temperature at 0 °C.

When the CIS temperature was set at 0 °C (Table 2), EG Silicone coating showed a behaviour similar to PDMS for *(E)*-2-hexenal and *(E)*-2-hexen-1-ol. On the contrary, more apolar compounds showed lower relative areas, whereas more polar compounds such as benzaldehyde, 1-hexanol and *(Z)*-3-hexen-1-ol showed higher values with a higher percentage of recovery in EG Silicone respect to PDMS coatings.

PA coating showed lower relative areas for most compounds respect to PDMS; only for 1-hexanol similar relative areas in PA and PDMS coatings were obtained. Benzaldehyde, *(E)*-2-hexen-1-ol and *(Z)*-3-hexen-1-ol were the only compounds showing an opposite trend, as they displayed higher relative areas in PA respect to PDMS; so, the highest percentages of recovery were detected. Similarly to EG Silicone coating, also in PA coating a relation between the percentages of recovery and the compound polarities was detected, as higher relative areas respect to PDMS were detected for compounds with lower LogK_{ow}.

CIS temperature at 25 °C.

Comparing EG Silicone and PDMS coatings with the CIS temperature set at 25 °C (Table 3) resulted in significant differences for most compounds except for *(E)*-2-hexenal, D-limonene and linalool. Higher relative areas and percentage recoveries were detected for benzaldehyde, 1-hexanol, *(E)*-2-hexen-1-ol and *(Z)*-3-hexen-1-ol; lower values were detected for all remaining compounds. Again, it emerged that compounds with lower LogK_{ow} were more efficaciously sorbed onto more polar coatings.

Comparison of the CIS temperatures.

With PDMS coating, individual compound relative areas were always significantly higher when the CIS was set at 25 °C respect to 0 °C, except for D-limonene (Table 3). For EG Silicone stir bars, setting the CIS at 25 °C increased the relative area values for all compounds except for α-terpineol. For PA, setting the CIS at 25 °C increased the relative area values of 1-hexanol, *(E)*-2-hexenal, *(E)*-2-hexen-1-ol, *(Z)*-3-hexen-1-ol and of α-ionone. However, areas were lower respect to those obtained with PDMS.

Generally, for all three types of coatings, standard errors were always lower when the CIS temperature was set at 25 °C.

Compounds with Log K_{ow} between 1.58 and 1.82 were more efficaciously sorbed by EG Silicone and PA coatings even though the standard errors were always higher, regardless the CIS temperature. For the other studied compounds, PDMS showed higher relative areas.

Area detection and quantification limits.

We calculated the limits of the relative detection area and of the relative quantification area for each compound (ADL and AQL, respectively) detected by using the three coatings operating with the two CIS temperatures (Table 4); to do that we used a procedure similar to the one used to calculate detection and quantification limits when working with concentrations. ADL and AQL were calculated based on a signal to noise ratio of 3 and 10. Using areas is less appropriate respect to using concentrations; however, we deem preliminary information reported in this study useful and informative. In fact, a number of recent studies reported analytical areas from SBSE-GC/MS analysis for specific compounds (Ochiai, Sasamoto, Ieda, David & Sandra, 2013; Gilart, Miralles, Mercé, Borrull & Fontanals, 2013; Gilart, Mercé, Borrull & Fontanals, 2014). The necessity to report information expressed in areas rather than in concentrations is tied to the lack of standards of many molecules and/or to their high costs.

ADL values calculated when the CIS was set at 0 °C ranged between $0.53\text{x}10^{-6}$ (nerolidol, EG Silicone) and $12.83\text{x}10^{-6}$ (D-limonene, PDMS); AQL values for the same temperature between $2.05\text{x}10^{-6}$ (nerolidol, PA) and $35.27\text{x}10^{-6}$ (α-terpineol, PDMS).

When the CIS temperature was set at 25 °C ADL values ranged between $0.97\text{x}10^{-6}$ (nerolidol, PA) and $8.29\text{x}10^{-6}$ (1-hexanol, PA), whereas AQL values from $3.77\text{x}10^{-6}$ (nerolidol, EG Silicone) to $27.62\text{x}10^{-6}$ (1-hexanol, PA). The area limit values for the identification and quantification of these compounds show an adequate sensitivity for the two CIS temperatures and the three coatings. However, we consider that the two tested coatings (Table 2 and 3) do not offer important improvements of analytical results respect to PDMS, except for benzaldehyde, 1-hexanol, (E)-2-hexenal, (E)-2-hexen-1-ol, (Z)-3-hexen-1-ol, particularly when the CIS was set at 25 °C. The discriminant analysis (93.9 % of explained variance on function 1 and 6.1 on function 2, Figure 1) supported these conclusions: the centroid distances for CIS 25 °C and CIS 0 °C are the shortest, meaning that results obtained with the PDMS are very similar, regardless the CIS temperature. On the contrary, PA centroids are the most distant and EG Silicone presented an intermediate situation. We can conclude that using the CIS at 0 °C or at 25 °C brought to quite different analytical results when EG Silicone and PA coatings were used.

Stir bar memory effect

Another important aspect in HS-SBSE-GC/MS analysis is to show that compounds are as much as possible desorbed from the stir bar after analysis to avoid the memory effect. EG Silicone and PA coatings showed a higher memory effect for 1-hexanol, *(Z)*-3-hexen-1-ol, *(E)*-2-hexen-1-ol, linalool, α-terpineol and nerolidol respect to PDMS coatings at both CIS temperatures (Figure 2). This higher memory effect could be due to the lower desorption temperature that EG Silicone and PA coatings require respect to PDMS (220 °C the former two, 295 °C the latter).

4. Conclusions

SBSE used in the HS mode allowed a highly reliable and reduced time-consuming detection of some wine volatiles of grape origin. EG-Silicone and Polyacrylate, being more polar respect to PDMS, resulted to be more efficient in the measurement of polar volatiles, with Log K_{ow} minor than 1.82, particularly when the CIS temperature was set at 25 °C. As CIS temperature set at 25 °C always allowed the detection of larger volatile relative areas, regardless the coating type, we deem not necessary to use a forced cryo-focalization at very low temperatures for the assessment of the volatiles tested in this work. This is an important point to focus on for SBSE-GC/MS analysis as cryo-focusing, particularly when performed by liquid CO_2 is expensive for the laboratory.

Ackwoledgement

Authors thank SRA Instruments Italia for freely providing the EG-Silicone and PA stir bars. The work was conducted in the frame of the agreement between UNITO and Provincia di Cuneo.

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Figure 1. Discriminant analysis between the three type of coatings (PDMS, EG Silicone and PA) and the two CIS temperatures (0 ºC and 25 ºC).

Figure 2. Overlay of HS-SBSE-GC-MS chromatograms obtained after a second injection of each Twister® in the Total Ion Chromatogram (TIC) mode. A = CIS temperature at 0 °C; B = CIS temperature at 25 °C. Some molecules showed higher memory effects in EG Silicone and PA Twister® respect to PDMS. 1, 1-hexanol; 2, *(Z)*-3-hexen-1-ol; 3, *(E)*-2-hexen-1-ol; 4, linalool; 5, α-terpineol; 6, nerolidol.