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This is the author's manuscript
Original Citation:
Availability:
This version is available http://hdl.handle.net/2318/1587363 since 2020-04-01T15:49:16Z
Published version:
DOI:10.1016/j.foodchem.2016.06.047
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1	DIFFERENT COATINGS FOR THE HS-SBSE GRAPE VOLATILE ANALYSIS IN
2	MODEL SOLUTION: PRELIMINARY RESULTS
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32 ABSTRACT

33 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 34 35 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 36 37 aim to propose an analytical procedure faster respect to traditional extractive 38 methods and environmental friendly, avoiding the use of solvents and high-energy 39 consumption for cooling. Fifteen volatiles with different polarities were tested. The 40 PDMS coating showed higher relative areas for terpenes and norisoprenoids. C6 41 volatiles, benzaldehyde and eugenol, obtained greater area response using EG-42 Silicone and PA coatings, regardless the temperatures. EG Silicone and PA coatings 43 showed a higher memory effect for a certain compounds, probably due to the 44 lower desorption temperature that EG Silicone and PA coatings can be submitted 45 to, respect to PDMS (220 °C respect to 295 °C).

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

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49 **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 duringwinemaking, when time is often the critic point.

58 Traditional analytical methods require time-consuming extraction and enrichment 59 steps prior to chromatography. Modern techniques in sample preparation have 60 evolved towards simplification by reducing solvents and sample handling. Stir bar 61 sorptive extraction (SBSE; commercially named 'Twister®') (Baltussen, Sandra, 62 David & Cramers, 1999; Baltussen, Cramers & Sandra, 2002) is an extraction technique that is less time consuming respect to traditional extractive techniques 63 64 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 65 liquid samples (immersion mode) or in headspace. SBSE technique was used to 66 67 analyze grape and wine volatiles by the immersion mode (Ferrandino, Carlomagno, Baldassarre & Schubert, 2012; Pardo-García, Serrano de la Hoz, Zalacain, Alonso & 68 69 Salinas, 2014; Zalacain, Marín, Alonso & Salinas, 2007), by the headspace mode 70 (Maggi, Zalacain, Mazzoleni, Alonso & Salinas, 2008) or by both of them (Arbulu, 71 Sampedro, Sánchez-Ortega, Gómez-Caballero, Unceta, Goicolea & Barrio, 2013). In 72 comparison with the immersion mode, the headspace SBSE mode extends the life 73 of the stir bar, especially in complex matrix. The SBSE technique showed analytical 74 simplicity, high reproducibility and very low detection limits. Polydimethyl 75 siloxane (PDMS) was the universal commercially available stationary phase 76 coating material. PDMS is a sorptive material that at temperature above its glass 77 transition temperature acquires a gum-like state with chemical properties such as 78 diffusion and distribution constants similar to those of organic solvents. SBSE-79 PDMS has a stationary phase volume much more higher respect to SPME-PDMS: 80 the maximum volume of PDMS in a SPME fiber is 0.5 microliter (film thickness of

81 100 micrometer) whereas PDMS in SBSE can provide from 24 to 126 microliters of 82 volume. Thus, the sensitivity of PDMS-SBSE is higher, resulting in a higher volatile 83 extraction respect to SPME and in the detection and identification of compounds 84 present at very low concentrations, such as sesquiterpenes (May & Wüst, 2012; 85 Ferrandino, Carlomagno, Baldassarre & Schubert, 2012) or malodors in wastewaters 86 (Loughrin, 2006). PDMS stir bars were also used for the detection of stilbene molecules 87 in wines (Cacho, Campillo, Viñas & Hernández-Córdoba, 2013). However, doubts 88 about its efficaciousness arose due to the PDMS non-polar nature that was thought 89 to limit the applicability of SBSE-PDMS to hydrophobic compounds. Slightly polar 90 or polar coatings have recently been introduced but their use is limited. Cacho, 91 Campillo, Viñas & Hernández-Córdoba (2015) compaired the use of these three 92 different coatings for the determination of volatile terpenes in honey, of chlorophenols 93 and chloroanisoles in wines (Cacho, Campillo, Viñas & Hernández-Córdoba, 2014). 94 EG-Silicone stir-bars were recentely used to meausre ochratoxin A in beers (Nguyen & 95 Dojin, 2014).

96 For the thermo-desorption of the retained compounds on the stir-bar, a specific 97 injector, constituted by a Thermal Desorption Unit (TDU) and of a Cooled Injection 98 System (CIS), is generally used. Different CIS temperatures, such as: -50 °C, (Caven-99 Quantrill & Bouglass, 2011); -30 °C (Martínez-Gil, Garde-Cerdán, Martínez, Alonso & Salinas, 2011; Martínez-Gil, Pardo-García, Zalacain, Alonso & Salinas, 2013); 0 °C 100 101 (Ferrandino, Carlomagno, Baldassarre & Schubert, 2012); 15 °C (Cacho, Campillo, 102 Viñas, & Hernández-Córdoba, 2015); 30 °C (Ochiai, Sasamoto, Ieda, David & Sandra, 103 2014), were used to measure volatiles with the PDMS-SBSE/GC-MS technique, but at 104 present no studies deal with the comparison of specific CIS temperatures, and with 105 different coatings.

106 The CIS is an injection system with programmed temperature vaporization 107 (PTV) that allows the controlled vaporization of the sample after an enrichment 108 step. The CIS liner can be rapidly heated or cooled. Moderate cooling can be 109 accomplished using a Peltier Cooling Unit but, to achieve more important cooling, 110 liquid nitrogen (N₂) or liquid carbon dioxide (CO₂) are used, increasing the costs 111 and the complexity of the analysis. The analytes on the stir bar can be cooled very 112 quickly, but when the temperature ranges are very wide, more time is required to 113 get the desired temperatures; besides, extreme ranges of temperature imply a 114 more rapid deterioration of the analytical equipment. Thus, analysis are more 115 expensive and a higher energy consumption is required. In most articles, the CIS 116 temperature is a parameter systematically described but not further commented. 117 No studies comparing the effect of different CIS temperatures on the analytical 118 response of the different volatiles have been conducted. We deem necessary to 119 make an effort in the direction of 'energetic' resource reduction also in the 120 laboratory, adapting, when possible, the analytical technique for 'energy saving'.

121 In the present study, two stir bar coatings: poly(ethylene glycol) (PEG)-modified 122 silicone (EG Silicone) and polyacrylate (PA) were compared to the traditional 123 PDMS stir bar to analyse some grape volatile compounds. The stir bars were used 124 in the HS mode. Two different CIS temperatures (0 °C and 25 °C) were assayed to 125 identify running temperatures as high as possible without limiting the analytical 126 results, to propose a more environmental friendly analytical procedure. However, 127 results are still preliminary as validation on musts and wines and on a larger 128 number of molecules is required.

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130 **2. Materials and methods**

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132 2.1. Chemicals and reagents

133 Benzaldehyde, β -citronellol, eugenol, geraniol, 1-hexanol, (E)-2-hexenal, (E)-2-134 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 135 mixture of commercial standards of high purity grade (>97%) in methanol was 136 137 prepared, using concentration ranges for each compound commonly found in 138 wines. A model solution was prepared: an aqueous ethanol solution at 12% with 5 139 g L⁻¹ of tartaric acid was and pH adjusted to 3.6 with 1M sodium hydroxide 140 (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).

143 were employed to prepare model solutions.

144

145 2.2. Stir bar coatings

146To perform the sorptive extraction process, commercial stir bars coated with147polydimethylsiloxane (PDMS Twister®), poly (ethylene glycol) (PEG)-modified148silicone (EG Silicone Twister®) and polyacrylate (Acrylate Twister® PA) were used,149the last two freely provided by Gerstel (Mülheim and der Ruhr, Germany towards150SRA Instruments, Cernusco sul Naviglio, MI, Italy). They consisted of a 10 mm long151glass-encapsulated magnetic stir bar with three different external coatings: 24 μL152of PDMS, 32 μL of EG Silicone and 25 μL of PA.

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154 2.3. Volatile extraction

155 The model solution was extracted by headspace (HS-SBSE; Martínez-Gil, Garde-156 Cerdán, Martínez, Alonso & Salinas, (2011). Twenty-two mL of model solution were 157 placed into a 50 mL headspace vial. For each milliliter of sample, 0.1 g of NaCl and 158 10 μ L of internal standard (γ -hexalactone) were added. HS-SBSE extraction was 159 carried out by exposing the coated stir bar to the vial headspace hermetically 160 closed, stirring the sample was stirred at 500 rpm and heating at 60 °C for 60 min. 161 After sorption, the stir bar was rinsed with pure water, dried with a lint-free tissue 162 and desorbed.

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164 2.4. Analytical procedure

165 The sample introduction system was composed of an automated thermal 166 desorption unit (TDU), combined with a MultiPurpose Sampler (MPS) and a 167 Programmed Temperature Vaporization (PTV) Cooled Injection System (CIS-4), 168 from Gerstel. The GC was an Agilent 7890A gas chromatograph coupled to an 169 Agilent 5975C quadrupole mass spectrometer detector (MS, Agilent Technologies, 170 Palo Alto, CA, USA) equipped with a DB-WAX column (J&W 122-7032; 30 m length, 171 0.25 mm I.D. and 0.25 µm film thickness). The carrier gas was helium with a 172 constant column pressure of 20.75 psi.

Stir bars were thermally desorbed using helium as carrier gas at a flow rate of T5 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 CO₂ 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.

183 The TDU operated in the splitless desorption mode, the CIS-4 operated in PTV 184 solvent vent mode (purge flow to split vent of 80 mL/min, vent 75 mL/min and 185 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 2 186 187 min). The MS operated in scan mode (27-300 amu) with ionization energy set at 188 70 eV. The temperature of the MS transfer line was 230 °C. Each molecule was 189 identified by comparing mass-spectrum with those of the NIST library and 190 chromatographic retention index of each standard. To avoid matrix interferences, 191 the MS quantification was carried out in the single ion monitoring mode using each 192 molecule characteristic m/z value (Table 1).

193The HS- SBSE-GC/MS analysis were performed in sevenfold.

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195 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 ($P \le 0.05$). Normalized data underwent a discriminant analysis, using the coating type as differentiating variable.

202

203 **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.

209 Compounds whose Log K_{ow} was near 3 are the most apolar (Table 1), so it would 210 be expected that they were more retained by the PDMS due to its nonpolar phase; 211 the other two coatings (more polar) were expected to exhibit higher affinity for 212 compounds with Log K_{ow} around 1.

213 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.

220 PA coating showed lower relative areas for most compounds respect to PDMS; 221 only for 1-hexanol similar relative areas in PA and PDMS coatings were obtained. 222 Benzaldehyde, (E)-2-hexen-1-ol and (Z)-3-hexen-1-ol were the only compounds 223 showing an opposite trend, as they displayed higher relative areas in PA respect to 224 PDMS; so, the highest percentages of recovery were detected. Similarly to EG 225 Silicone coating, also in PA coating a relation between the percentages of recovery 226 and the compound polarities was detected, as higher relative areas respect to PDMS were detected for compounds with lower LogKow. 227

228 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)*-2hexenal, 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.

236 *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 Dlimonene (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)*-2hexen-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.

250 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

254 that we used a procedure similar to the one used to calculate detection and 255 quantification limits when working with concentrations. ADL and AQL were 256 calculated based on a signal to noise ratio of 3 and 10. Using areas is less 257 appropriate respect to using concentrations; however, we deem preliminary 258 information reported in this study useful and informative. In fact, a number of 259 recent studies reported analytical areas from SBSE-GC/MS analysis for specific 260 compounds (Ochiai, Sasamoto, Ieda, David & Sandra, 2013; Gilart, Miralles, Mercé, 261 Borrull & Fontanals, 2013; Gilart, Mercé, Borrull & Fontanals, 2014). The necessity 262 to report information expressed in areas rather than in concentrations is tied to 263 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 0.53×10^{-6} (nerolidol, EG Silicone) and 12.83×10^{-6} (D-limonene, PDMS); AQL values for the same temperature between 2.05×10^{-6} (nerolidol, PA) and 35.27×10^{-6} (α -terpineol, PDMS).

When the CIS temperature was set at 25 °C ADL values ranged between 268 269 0.97x10⁻⁶ (nerolidol, PA) and 8.29x10⁻⁶ (1-hexanol, PA), whereas AQL values from 270 3.77x10⁻⁶ (nerolidol, EG Silicone) to 27.62x10⁻⁶ (1-hexanol, PA). The area limit 271 values for the identification and quantification of these compounds show an 272 adequate sensitivity for the two CIS temperatures and the three coatings. However, 273 we consider that the two tested coatings (Table 2 and 3) do not offer important 274 improvements of analytical results respect to PDMS, except for benzaldehyde, 1-275 hexanol, (E)-2-hexenal, (E)-2-hexen-1-ol, (Z)-3-hexen-1-ol, particularly when the 276 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 277 centroid distances for CIS 25 °C and CIS 0 °C are the shortest, meaning that results 278

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.

284 Stir bar memory effect

285 Another important aspect in HS-SBSE-GC/MS analysis is to show that 286 compounds are as much as possible desorbed from the stir bar after analysis to 287 avoid the memory effect. EG Silicone and PA coatings showed a higher memory 288 effect for 1-hexanol, (Z)-3-hexen-1-ol, (E)-2-hexen-1-ol, linalool, α -terpineol and 289 nerolidol respect to PDMS coatings at both CIS temperatures (Figure 2). This 290 higher memory effect could be due to the lower desorption temperature that EG 291 Silicone and PA coatings require respect to PDMS (220 °C the former two, 295 °C 292 the latter).

293

4. Conclusions

295 SBSE used in the HS mode allowed a highly reliable and reduced time-consuming 296 detection of some wine volatiles of grape origin. EG-Silicone and Polyacrylate, 297 being more polar respect to PDMS, resulted to be more efficient in the 298 measurement of polar volatiles, with Log K_{ow} minor than 1.82, particularly when 299 the CIS temperature was set at 25 °C. As CIS temperature set at 25 °C always 300 allowed the detection of larger volatile relative areas, regardless the coating type, 301 we deem not necessary to use a forced cryo-focalization at very low temperatures 302 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 CO₂ is expensive for the laboratory.

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306 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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311 References

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- 373

- 374 **Figure 1.** Discriminant analysis between the three type of coatings (PDMS, EG Silicone
- and PA) and the two CIS temperatures (0 °C and 25 °C).
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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
- 380 memory effects in EG Silicone and PA Twister® respect to PDMS. 1, 1-hexanol; 2, (Z)-
- 381 3-hexen-1-ol; 3, (*E*)-2-hexen-1-ol; 4, linalool; 5, α -terpineol; 6, nerolidol.