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Determination of odour-causing volatile organic compounds in cork stoppers by multiple headspace solid-phase microextraction

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Abstract

Multiple headspace solid-phase microextraction (MHS–SPME) coupled with gas chromatography–mass spectrometry has been applied in order to determine 2,4,6-trichloroanisole (2,4,6-TCA), guaiacol, 1-octen-3-ol and 1-octen-3-one in three samples of cork stoppers. These compounds are responsible for cork taint in wine and can modify the organoleptic properties of bottled wine. Variables such as temperature, addition of water, extraction time, and amount of cork were studied. The extractions were performed with a 50/30 μm divinylbenzene–carboxen–polydimethylsiloxane (DVB–CAR–PDMS) fibre for 45 min at 100 °C using 20 mg of cork. For calibration, 50 μL of VOC aqueous solutions were used and the extraction were carried out for 45 min at 75 °C. The limits of detection of the method expressed as ng of VOC per g of cork were 0.3 for 2,4,6-TCA, 7.5 for guaiacol, 1.7 for 1-octen-3-one and 1.9 for 1-octen-3-ol. Relative standard deviation of replicate samples was less than 10%. Significant losses of analytes were observed when the samples were ground at room temperature. Finally, a recovery study was performed and the MHS–SPME results were validated using Soxhlet extraction results.

Keywords: Multiple headspace solid-phase microextraction; Cork stoppers; 2,4,6-Trichloroanisole; Volatile organic compounds; Cork taint

1. Introduction

Cork is the traditional material used to produce stoppers for wine bottling due to its physical properties: flexibility, lightness, impermeability to polar liquids and gases, chemical inertness and resistance to extreme heat. Cork stoppers are obtained from the bark of cork oak (*Quercus suber*), which grows mainly in Mediterranean countries.

Cork is composed [1] of suberin (a macromolecular network insoluble in all solvents), lignin, polysaccharides and extractibles. The presence of chloroanisoles as dichloroanisoles, 2,4,6-trichloroanisole (2,4,6-TCA) or pentachloroanisole (PCA) in cork can be due to the microbial degradation of chlorophenols (used in insecticides and herbicides) or chlorinated solutions (used to bleach cork) [2], and can also be due to contamination during shipping procedures or storage in caves [3]. 2,4,6-TCA has been reported [3,4] as the volatile organic compound that is mainly responsible for the off-odour contamination of wine (also called cork taint). The odour of 2,4,6-TCA is described as musty or mouldy and it can be detected even at low concentrations; its sensory threshold ranges from 10.0 to 40 ng/L [5], although this sensory threshold depends on several factors such as the type of wine. Other compounds such as 1-octen-3-ol, 1-octen-3-one (mushroom aroma), guaiacol (smoky, phenolic aroma), geosmin or 2-methylisoborneol (earthy aroma) have also been reported [2,4] as being responsible for cork taint, and, more recently, 2,4,6-tribromoanisole [6]. The decarboxylation and oxidation of lignin produce vanillic acid [2] and its subsequent degradation causes the presence of guaiacol [7]. 1-octen-3-ol and 1-octen-3-one are mould metabolites arising from the degradation of lipids [2]. All these compounds can migrate to bottled wine, changing its organoleptic properties and undermining its quality.

The analysis of 2,4,6-TCA has been reported in wines and corks by Soxhlet extraction [3,8], microwave extraction [8], shake-flash extraction [9] or thermal desorption [10]. In

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recent years, more modern techniques such as solid phase extraction (SPE) [11], supercritical fluid extraction (SFE) [12], solid-phase microextraction (SPME) [5,13–16] or stir bar sorptive extraction [17] have been also described.

The analysis of solid samples by SPME [18–20] is simple, rapid, easy to automate and recommended for VOCs. Moreover, sample manipulation is reduced and the use of solvents avoided. We recently developed the theory of multiple headspace solid-phase microextraction (MHS–SPME) [21]; this method enables to estimate the total area which correspond to the complete extraction of the analyte by performing several (three or four) HS–SPME consecutive extractions from the same sample. The total area (A_T) is calculated using the following mathematical equation:

$$A_T = \frac{A_1}{1-\beta}$$

where A_1 is the peak area of the first extraction and β is calculated from the linear regression of the logarithms of the individual peak areas:

$$\ln A_i = (i-1)\ln \beta + \ln A_1$$

and A_i is the peak area obtained in the ith extraction. When extraction is exhaustive after a few extractions, the calculus is simplified, and the total area can be directly calculated as the sum of the areas of each individual extraction.

With this procedure the matrix effect can be overcome [22] and calibration can be performed using aqueous solutions (even if solid matrixes are analysed). It has been applied to the analysis of off-odour compounds in packaging materials [21,22], the determination of BTEX in soils samples [23] or the determination of vinyl chloride in polymer [24].

The aim of this study is to determine 2,4,6-TCA, guaiacol, 1-octen-3-ol and 1-octen-3-one in corks stoppers by MHS–SPME. The parameters affecting extraction by multiple HS–SPME, such as temperature, addition of water, extraction time and amount of cork, were studied. For calibration, VOCs solutions were prepared in water. Once the features of the method had been established, it was applied in order to analyse three cork stopper samples, and the results obtained using a grinder or a freezer mill to grind the cork samples were compared. Finally, a recovery study was performed and a Soxhlet extraction of the ground samples was carried out to compare the results obtained with the MHS–SPME method.

2. Experimental

2.1. Equipment

Gas chromatographic analyses were performed with a Varian 3900 gas chromatograph with a Varian 2100T MS detector (Walnut Creek, California, USA). Automated SPME injections and liquid injections were carried out with a CombiPAL autosampler (CTC Analytics, Zwingen, Switzerland).

The cork samples were ground using a 6750 Freezer/Mill (SPEX CertiPrep, New Jersey, USA); this mill enables samples to be cooled to cryogenic temperatures using liquid nitrogen and pulverized by magnetically shuttling a steel impactor against two stationary end plugs. Also, part of sample 1 was ground at room temperature using an IKA A10 grinder (IKA Labortechnik, Staufen, Germany).

2.2. Chromatographic conditions

The chromatographic column was a Varian CP8843 WCOT fused silica column (30 m × 0.32 mm i.d. with a 0.25 μm polyethylene glycol phase (CP-WAX 52 CB)) (Walnut Creek, California, USA). For SPME injections, an initial oven temperature of 50 °C for 2 min was used; the temperature was then increased at a rate of 7 °C/min to 230 °C. The GC injector was equipped with a 0.8 mm insert and was maintained at 270 °C with a 1:20 split ratio for an initial time of 0.50 min followed by a 1:50 split ratio. For liquid injections, the oven program started at an initial temperature of 40 °C for 3 min, the temperature was then increased at a rate of 7 °C/min to 230 °C. A 4 mm i.d. liner with glass wood was used and maintained at 270 °C with a 1:10 split ratio. The carrier gas was helium at 1.0 mL/min (99.996%) for both. Ionization was performed by electronic impact (EI) and the electron multiplier was set at 1900 eV. The temperatures used were 200 °C for the trap, 60 °C for the manifold, and 280 °C for the transfer line. The following ions were selected in order to quantify the compounds in SIS (selected ion storage) mode: 55 + 97 for 1-octen-3-one, 57 for 1-octen-3-ol, 195 + 197 for 2,4,6-trichloroanisole, 109 + 124 for guaiacol, 265 for pentachloroanisole (PCA) and 246 for 2,3,4,5-tetrachloroanisole (2,3,4,5-TeCA).

2.3. Chemicals

2,4,6-trichloroanisole (99.9%) from Sigma-Aldrich (St. Louis, Missouri, USA), 1-octen-3-one (97%) from Lancaster (Bischheim-Stasbourg, France), 1-octen-3-ol (≥98%), guaiacol (≥98%) from Fluka (Butch, Switzerland), pentachloroanisole (99.0%) and 2,3,4,5-tetrachloroanisole (99.0%) from Dr. Ehrenstorfer GmbH (Augsburg, Germany) were used to prepare stock solutions in methanol (≥99.8%) from Merck (Darmstadt, Germany). These solutions were stored at −22 °C in sealed vials in order to avoid the loss of volatiles.

Dilutions in ultrapure milli-Q water (Millipore, Bedfords, MA, USA) of 1.9–250 μg/L (depending on the compound) with 0.10% of methanol in all solutions were used for calibration. These solutions were stored at 4 °C in sealed vials without free headspace.

For liquid injections stock solutions of the analytes were prepared in *n*-Pentane (pesticide residues grade) from Scharlau (Barcelona, Spain). Dilutions of 1.2–8800 μg/L (depending on the compound) were used for calibration. 2,6-dichloroanisole (2,6-DCA) (97.5%) from Dr. Ehrenstorfer

GmbH (Augsburg, Germany) was used as internal standard. The standard solution used to calculate volatile losses in the concentration process was prepared with the following concentration of VOCs: 0.59 μg/L for 1-octen-3-ol and 1-octen-3-one, 1.6 μg/L for guaiacol, 22 ng/L for 2,4,6-TCA and 0.3 μg/L for 2,6-DCA.

2.4. Samples

Three types of cork stoppers from different local stores were analysed. The cork samples were ground using a freezer mill, the pre-cooling time prior to grinding was 7 min; grinding run time was 4 min and one cycle was performed at a rate of 10 impacts/s. One part of sample 1 was ground using an IKA A10 grinder for 4 min. Finally, the samples were sieved in order to obtain a 500 μm particle size and stored at −22 °C in order to avoid losses of volatile organic compounds.

Recovery experiments were carried out by adding known quantities of the target analytes (between 0.20 and 1.00 ng) to 20 mg of ground cork. 500 μL of two different aqueous solutions of the analytes were added to the cork in the vial. The spiked samples were kept at −22 °C for 1 week until the moment of the analysis.

2.5. Sampling procedure for MHS–SPME

The analytes were extracted using a 50/30 μm divinylbenzene–carboxen–polydimethylsiloxane fibre (Supelco, Bellefonte, PA). For the determination of VOCs in cork stoppers by MHS–SPME the following conditions were used: 20 mg of ground cork, 5 min of pre-incubation at 100 °C at 400 rpm followed by 45 min of extraction at this temperature and three extractions were performed in each sample; except for 1-octen-3-ol, whose conditions were 5 min of pre-incubation at 70 °C at 400 rpm followed by 45 min of extraction at this temperature and suspensions of 20 mg of cork in 0.5 mL of deionisated water were used. For the standard solutions 50 μL of solution were used and three extractions were performed in each sample using 5 min of preheating at 75 °C at 400 rpm followed by 45 min of extraction at this temperature. In all cases, the desorption time was 6 min and 20 mL headspace glass vials and steel caps with 3.0 mm thick teflon/silicone septa were used.

2.6. Soxhlet extraction

The procedure used was similar to the one described by Juanola et al. [9]. Approximately 3 g of ground cork were placed in a cellulose thimble and introduced in a Soxhlet extractor. The extractions were carried out with 200 mL of *n*-pentane for 16 h, and each cycle lasted approximately 9 min. Fifty microlitres of a 1.18 mg/L solution of 2,6-DCA were added to each extract and then concentrated until 400 μL under a N_2 stream and filtered with a syringe 0.45-μm nylon filter (Varian, Walnut Creek, California, USA). The volume of injected extract was 3 μL.

3. Results and discussion

3.1. Selection of MHS–SPME conditions in cork stopper samples.

3.1.1. Type of fibre

One hundred micrometer polydimethylsiloxane (PDMS) fibre and 50/30 μm divinylbenzene–carboxen–polydimethylsiloxane (DVB–CAR–PDMS) fibre have been reported for extracting TCA from wine [5,13,14,16], corks [13] or raisins [25]; while DVB–CAR–PDMS coating shows better sensitivity, PDMS coating displays better repeatability. Since in MHS–SPME it is essential to extract a significant amount of analyte in relation to the total amount in order to observe an exponential decay of peak areas versus the number of extractions, the more sensitive approach was chosen and a 50/30 μm DVB–CAR–PDMS fibre was selected. Fig. 1 shows the chromatogram obtained in full scan for a cork stopper with a 50/30 μm DVB–CAR–PDMS fibre. As can be observed, the analytes gave very small size peaks in relation to other compounds, hence SIS mode was selected in order to increase the signal/noise ratio and improve sensitivity. The chromatographic peaks in SIS mode of 1-octen-3-one, 1-octen-3-ol, 2,4,6-TCA and guaiacol are also shown in Fig. 1.

3.1.2. Temperature and addition of water

An increase in temperature helps analytes to migrate from the solid sample to the headspace of the vial and thus shorter equilibrium times are required. However, temperature also affects the distribution constants of the fibre–gas and sample–gas equilibrium. Hence, if an excessive temperature is used the extraction yield of fibre decreases. The addition of water has been reported to help analytes to migrate to the headspace and achieve homogenous heating [15].

The samples (20 mg of cork) were pre-incubated for 5 min at 400 rpm, the extractions were performed with a 50/30 μm DVB–CAR–PDMS fibre for 45 min, the temperatures ranged from 40 to 140 °C and analyses were performed in triplicate. Suspensions of 20 mg of cork in 0.5 mL of ultrapure milli-Q water were also analysed at 40 and 70 °C (temperatures higher than 70 °C were not studied in order to avoid excessive vapour pressure in the vial). Table 1 shows the relative areas obtained in the HS–SPME determination of VOCs in the cork sample at different temperatures. Relative areas were calculated by assigning a value of 100 to the maximum peak area for each compound and the rest of values were related to this maximum. As can be seen, no common maximum temperature was observed for all compounds. A temperature of 100 °C was selected for further experiments since this temperature provides a maximum for 2,4,6-TCA (the most important VOC for cork taint) and working under these conditions enables the obtainment of good chromatographic signals for 1-octen-3-one and guaiacol. However, at 100 °C, the chromatographic signals of 1-octen-3-ol are close to noise, therefore suspensions of cork in water at

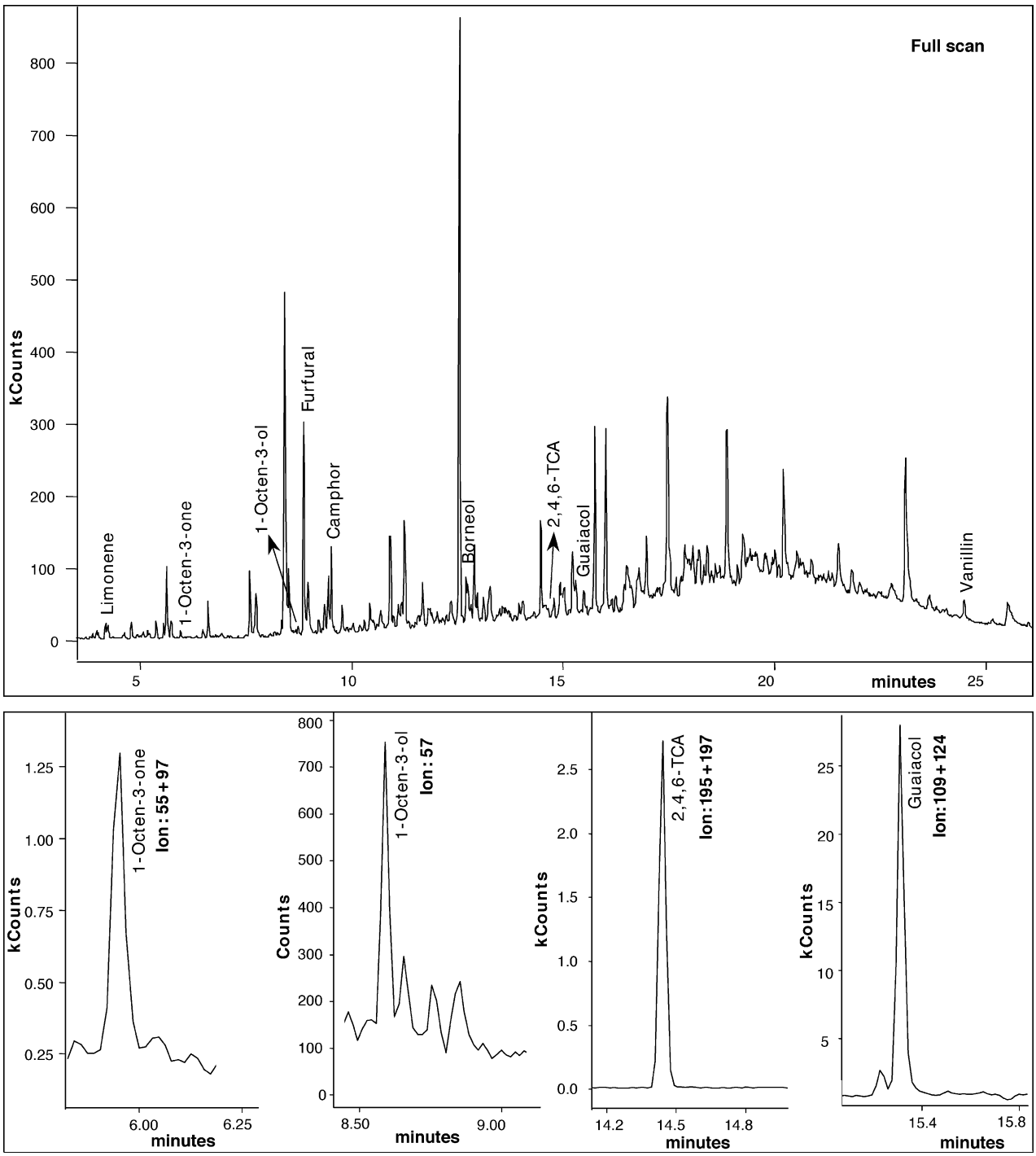

Fig. 1. Chromatogram obtained by HS–SPME–GC–MS for the VOC determination in a cork stopper in full scan and SIS mode.

Table 1
Relative areas[a] obtained in the HS–SPME determination of VOCs in 20 mg of cork using a 50/30 μm DVB–CAR–PDMS fibre at different temperatures

Compound	40 °C	40 °C (Suspension)	70 °C	70 °C (Suspension)	100 °C	125 °C	140 °C
1-Octen-3-one	36 ± 5	34.0 ± 0.7	100 ± 7	58 ± 7	98.6 ± 2.2	26 ± 9	10 ± 5
1-Octen-3-ol	24.0 ± 0.9	44.3 ± 1.7	45.2 ± 0.6	100 ± 3	30.6 ± 0.1	4.1 ± 0.5	1.7 ± 2.4
2,4,6-TCA	9.2 ± 0.8	14.3 ± 0.4	47.7 ± 1.3	65.3 ± 2.3	100.0 ± 1.9	32 ± 7	13.3 ± 0.4
Guaiacol	11.4 ± 1.1	16.3 ± 0.5	47 ± 3	76.4 ± 0.5	86.4 ± 2.4	100 ± 11	85.6 ± 1.4

[a] Mean of three replicates ± standard deviation.

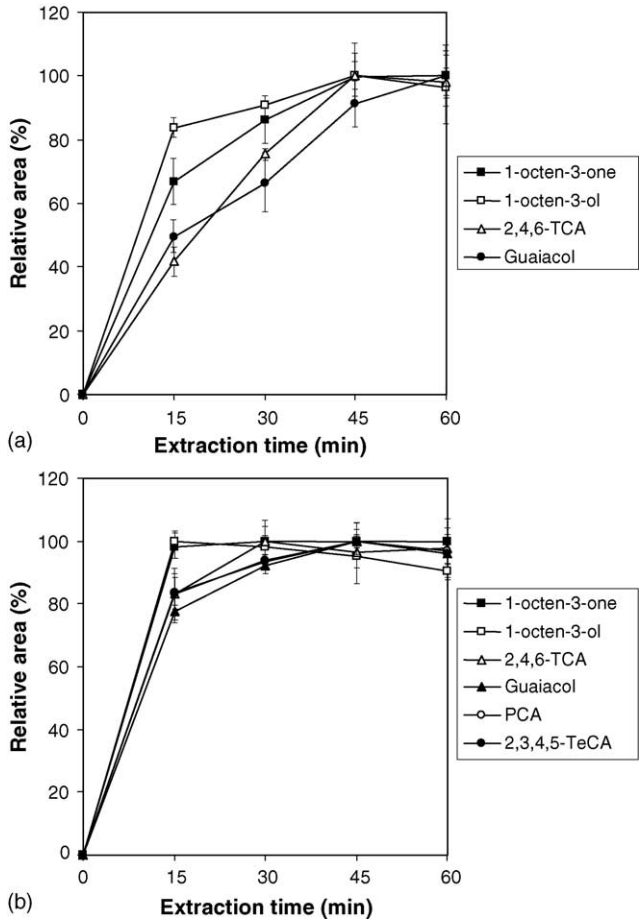

Fig. 2. Influence of the extraction time on the HS–SPME determination of VOCs in (a) cork stoppers at 100 °C and (b) in aqueous solutions at 75 °C using a 50/30 μm DVB–CAR–PDMS fibre.

70 °C were used to determinate this compound in a separate analysis.

3.1.3. Extraction time

Once the temperature had been selected, the influence of extraction time was studied from 15 to 60 min. The results are shown in Fig. 2a. A maximum was observed at 45 min for all compounds with the exception of guaiacol; this time was therefore chosen as the extraction time.

3.1.4. Mass of cork

In order to correctly perform the MHS–SPME, a significant amount of analytes in each extraction had to be removed; in this way an exponential decay of the peak area can be observed with respect to the number of extractions. Table 2 shows the correlation coefficients (R^2) of ln A_i versus $(i-1)$ found for the analytes studied using different masses of cork stopper and using the optimised SPME conditions. The optimum mass of cork is around 20 mg, although good linearity coefficients were found for 2,4,6-TCA from 7 to 50 mg. If the mass is higher, the logarithm of the peak areas does not show a good linearity with the number of extractions and worse coefficients (R^2) are obtained. If it is lower, the chromatographic signals obtained are too small and then worse results are also obtained.

Table 2
Correlation coefficients (R^2) of ln A_i versus $(i-1)$ found for VOCs using different masses of cork stopper

Cork mass (mg)	1-Octen-3-one	1-Octen-3-ol	2,4,6-TCA	Guaiacol
74.2	0.97	0.994	0.98	0.96
48.3	0.98	0.994	0.9990	0.995
23.2	0.991	0.9991	0.9992	0.9991
12.3	0.97	0.97	1	0.997
7.0	0.92	–	0.9998	0.95

(–) Non linear.

3.2. Selection of MHS–SPME conditions in aqueous solutions

3.2.1. Temperature

VOC aqueous standard solutions were prepared for calibration and the effect of extraction temperature was the first parameter studied. Fifty microlitres of aqueous solution were pre-incubated for 5 min at 400 rpm; extractions were performed with a 50/30 μm DVB–CAR–PDMS fibre for 45 min; temperatures ranged from 40 to 75 °C and analyses were performed in triplicate. Temperatures higher than 75 °C were not used in order to avoid excessive vapour pressure in the vial.

Table 3 shows the relative areas obtained in the HS–SPME determination of VOCs in the aqueous solutions at different temperatures. An extraction temperature of 75 °C was selected since it provided a maximum for most of the analytes.

3.2.2. Extraction time

The influence of the extraction time on the HS–SPME of VOCs from aqueous solutions was studied under the same conditions as those described the previous section but temperature was set at 75 °C and the extraction time ranged from 15 to 60 min. The results are shown in Fig. 2b. As can be seen, equilibrium was reached after 30 min except in the case of guaiacol, 2,3,4,5-TeCA and PCA, which presented an equilibrium time of 45 min; this time was therefore chosen as an extraction time for calibration.

Table 3
Relative areas[a] obtained in the HS–SPME extractions of VOCs from aqueous solutions using a 50/30 μm DVB–CAR–PDMS fibre at different temperatures

Compound	40 °C	60 °C	75 °C
1-Octen-3-one	100.0 ± 2.1	91.3 ± 2.2	75 ± 3
1-Octen-3-ol	100.0 ± 2.4	92 ± 3	65 ± 3
2,4,6-TCA	89 ± 12	95 ± 5	100.0 ± 1.4
Guaiacol	53 ± 4	100 ± 6	98.4 ± 1.2
PCA	65 ± 10	90 ± 4	100.0 ± 2.4
2,3,4,5-TeCA	64 ± 9	88 ± 6	100.0 ± 2.0

[a] Mean of three replicates ± standard deviation.

Table 4
Features of the MHS–SPME method

Compound	Studied range (ng)	Linear range (ng)	Slope ± s_m (kCounts × s/ng)	Intercept ± s_b (kCounts × s)	LOD (ng)	R^2	Repeatability[a], (%) (mass level, ng)	Reproducibility[b], (%) (mass level, ng)
1-Octen-3-one	0–3.5	0.07–3.5	4.52 ± 0.08	0.13 ± 0.14	0.03	0.998	6.4 (1.5)	6.6 (1.5)
1-Octen-3-ol	0–3.4	0.06–3.4	2.72 ± 0.06	−0.13 ± 0.10	0.04	0.997	2.7 (1.5)	8.1 (1.5)
2,4,6-TCA	0–7.3	0.013–7.3	13.6 ± 0.4	−1.8 ± 1.2	0.006	0.995	4.9 (2.0)	4.2 (2.0)
Guaiacol	0–12.5	0.25–12.5	17.30 ± 0.16	0.2 ± 0.9	0.15	0.9994	2.5 (1.2)	5.5 (1.2)
PCA	0–6.4	0.24–6.4	4.60 ± 0.20	−0.5 ± 0.7	0.10	0.993	6.2 (2.9)	9.4 (2.9)
2,3,4,5-TeCA	0–6.5	0.04–6.5	6.40 ± 0.24	−0.9 ± 0.8	0.019	0.995	4.2 (2.9)	6.8 (2.9)

s_m = standard deviation of the slope. s_b = standard deviation of the intercept.
[a] Expressed as RSD calculated from ten replicates.
[b] Expressed as RSD of three replicates obtained at different days.

3.3. Features of the method

Once the conditions of the method had been established, the linearity of the total area versus the analyte mass was studied using VOC aqueous solutions. Three extractions were performed in each solution and the total areas were calculated using the linear regression of the logarithms of the individual peak areas.

The range of the mass of analyte studied, the linear range, the limits of detection (LOD), the slope and intercept with their standard deviations, the correlation coefficient (R^2), the repeatability, and the reproducibility obtained for each compound are listed in Table 4. All the compounds showed good linearity in the ranges studied, the correlation coefficients (R^2) found were between 0.993 and 0.9994. Repeatability, expressed as a relative standard deviation, was between 2.5 and 6.4; and reproducibility, calculated as a relative standard deviation from data obtained on different days, was less than 10%.

3.4. Application of the method

Three different cork stopper samples were analysed with the MHS–SPME–GC–MS method developed. Firstly, in order to check whether any volatile compounds had been lost during sample grinding, one of the samples (sample 1) was ground at room temperature using a conventional grinder and at cryogenic temperature using a freezer mill, and the concentration of VOCs found was compared.

The total area of VOCs in corks was calculated by linear regression of the logarithms of the individual peak areas of three consecutive extractions. The concentrations were calculated by interpolation of the total area values obtained for corks in the linear graphs obtained for aqueous solutions. The results are listed in Table 5 and expressed as a nanogram of VOC per gram of cork. PCA and 2,3,4,5-TeCA were not detected in the cork samples under the studied conditions. The levels of 2,4,6-TCA concentration found in cork stoppers were in the same order as the ones reported previously [8,9,11].

The limits of detection in cork samples expressed as ng of VOC per gram of cork were calculated by dividing the LODs shown in Table 4 by 20 mg (mass of cork sample) and were 1.7 for 1-octen-3-one, 1.9 for 1-octen-3-ol, 0.3 for 2,4,6-TCA and 7.5 for guaiacol. These LODs can be even lower if 50 mg of cork are used instead of 20 mg (see Table 2). The relative standard deviations found in the cork stopper samples were below 10%, except in sample 3. This sample did not show exponential decay of the peak area with the number of extractions using 20 mg of sample; this was probably due to the presence of other volatile organic compounds in the matrix that compete with the analytes to be retained by the fibre. An exponential decay was observed when 5–7 mg of sample 3 were used.

The results obtained for sample 1 revealed that volatile organic compounds were lost when grinding was carried out at room temperature. The use of a freezer mill is therefore strongly recommended for grinding cork samples. For example, the concentration of 2,4,6-TCA obtained was five times higher when the sample was cryogenically ground.

3.5. Recovery study

In order to validate the method, two natural cork samples spiked with the target analytes were analysed. Two additions were performed to the samples and the concentrations and recoveries found for the spiked samples can be seen in Table 6. The recoveries found were around 100% in most of the cases in spite of the different behaviour of native analytes and spiked analytes. Taylor [12] reported that spiking the surface of cork may not reflect true recoveries from corks where the target analytes have further penetrated into the cork material. Only the recovery of 1-octen-3-ol was slightly higher than 100%.

Table 5
VOC concentrations[a] (ng VOC/g cork) found in three cork stopper samples by MHS–SPME–GC–MS

Compound	Sample 1		Sample 2	Sample 3
	Freezer mill	IKA A10 grinder		
1-Octen-3-one	34 ± 4	16.8 ± 1.6	14.9 ± 1.2	57 ± 6
1-Octen-3-ol	41.5 ± 1.8	39.9 ± 1.0	30.6 ± 1.0	101 ± 6
2,4,6-TCA	11.80 ± 0.16	2.19 ± 0.10	2.03 ± 0.12	2.5 ± 0.7
Guaiacol	411 ± 33	182 ± 17	380 ± 15	–

[a] Mean value ± standard deviation (four replicates).

Table 6
Recovery of target analytes in spiked ground cork

	Compound	Mass (ng)			Recovery[a] (%)
		Cork	Added	Found	
Sample 1 (addition 1)	1-Octen-3-one	0.68	0.23	1.12 ± 0.02	123 ± 2
	1-Octen-3-ol	0.83	0.23	1.03 ± 0.06	97 ± 5
	2,4,6-TCA	0.24	0.20	0.46 ± 0.01	104.4 ± 2.0
	Guaiacol	8.22	0.50	9.42 ± 0.85	108 ± 10
Sample 1 (addition 2)	1-Octen-3-one	0.68	0.46	1.25 ± 0.04	109 ± 4
	1-Octen-3-ol	0.83	0.45	1.32 ± 0.05	103 ± 4
	2,4,6-TCA	0.24	0.39	0.66 ± 0.01	104.7 ± 2.4
	Guaiacol	8.22	1.00	10.14 ± 0.48	110 ± 5
Sample 2 (addition 1)	1-Octen-3-one	0.30	0.23	0.52 ± 0.05	98 ± 10
	1-Octen-3-ol	0.61	0.23	0.81 ± 0.03	97 ± 3
	2,4,6-TCA	0.04	0.20	0.25 ± 0.02	106 ± 7
	Guaiacol	7.60	0.50	8.59 ± 0.81	106 ± 10
Sample 2 (addition 2)	1-octen-3-one	0.30	0.46	0.74 ± 0.05	98 ± 7
	1-Octen-3-ol	0.61	0.45	0.95 ± 0.06	90 ± 6
	2,4,6-TCA	0.04	0.39	0.45 ± 0.03	105 ± 7
	Guaiacol	7.60	1.00	9.03 ± 0.6	105 ± 7

[a] Mean value ± standard deviation (three replicates).

3.6. Determinations of VOCs in n-pentane solutions

The features of the method were established using different standard solutions of the analytes in *n*-pentane but with the same concentration of 2,6-DCA (148 μg/L). The range of the concentration studied, the linear range, the limit of detection (LOD), the slope and intercept with their standard deviations, the correlation coefficient (R^2) and the reproducibility obtained for each compound are shown in Table 7.

The extracts obtained by Soxhlet were too diluted to detect the analytes, and were therefore concentrated under a N_2 stream until 400 μL. This step can be critical because analytes are volatile and analyte losses can occur in the extract concentration process, these losses will differ according to the volatility and concentration of each compound. To prevent this from happening, correction factors were calculated for each compound using a standard solution containing a concentration of analytes similar to the expected one according to the MHS–SPME results. Two hundred millilitres of standard solution were concentrated up to 400 μL and then analysed to determine the real concentration in the concentrated extract. 1-octen-3-ol and 1-octen-3-one were not detected in the extract. Thus, it can be concluded that these analytes were lost in the concentration process and would not have been detected even if they had been extracted by Soxhlet. The rest of the analytes appeared in the extract but at lower-than-expected concentrations; the correction factors for 2,4,6-TCA and guaiacol were 1.14 and 1.17, respectively.

3.7. Soxhlet extraction

Once the method was established, three natural contaminated samples ground with a freezer mill were extracted by Soxhlet to compare these results with the MS–SPME ones. The solvent used was n-pentane [8,9] and the internal standard was 2,6-DCA [9] (this compound was not found in the previous analysis of the samples).

After the extractions, 2,6-DCA was added to the extracts obtained and they were concentrated up to 400 μL under a N_2 stream. Before injection, the extracts were filtered to remove a suspension that appeared in the concentration process.

The concentration obtained for 2,4,6-TCA and guaiacol in the three cork samples is shown in Table 8; neither 2,3,4,5-TeCA nor PCA were detected. Statistical test

Table 7
Features of the method VOCs in *n*-pentane

Compound	Studied range (μg/L)	Linear range (μg/L)	Slope ± s_m (L/μg)	Intercept ± s_b	LOD (μg/L)	R^2	Reproducibility[a], (%) (concentration level, μg/L)
1-Octen-3-one	0–1100	26–1100	$(8.13 \pm 0.18)10^{-3}$	0.08 ± 0.08	10	0.997	10 (200)
1-Octen-3-ol	0–1100	9–1100	$(6.65 \pm 0.20)10^{-3}$	0.07 ± 0.09	6	0.995	10 (200)
2,4,6-TCA	0–60	2.0–60	$(16.0 \pm 0.4)10^{-3}$	0.009 ± 0.007	0.9	0.997	7 (15)
Guaiacol	0–8800	27–8800	$(21.7 \pm 0.6)10^{-3}$	3.6 ± 2.4	14	0.996	4 (2000)
PCA	0–850	4–850	$(5.5 \pm 0.4)10^{-3}$	0.04 ± 0.05	3	0.995	3 (200)
2,3,4,5-TeCA	0–550	3–550	$(8.47 \pm 0.22)10^{-3}$	0.07 ± 0.05	1.3	0.997	5 (200)

s_m = standard deviation of the slope and s_b = standard deviation of the intercept.
[a] Expressed as RSD of four replicates obtained at different days.

Table 8
VOC concentrations[a] (ng VOC/g cork) found in three cork stopper samples by Soxhlet extraction

Compound	Sample 1	Sample 2	Sample 3
2,4,6-TCA	10.5 ± 2.1	2.3 ± 0.7	2.75 ± 0.15
Guaiacol	450 ± 35	452 ± 80	240 ± 60

[a] Mean value ± standard deviation (three replicates).

(F-test and t-test) was used to verify that MHS–SPME and Soxhlet gave the same mean values for the determination of 2,4,6-TCA and guaiacol: for 2,4,6-TCA the values of t were 1.08 (critical value 4.30), 0.64 (critical value 4.28) and 0.56 (critical value 2.57) for sample 1, 2 and 3 respectively; and for guaiacol the values of t were 1.52 (critical value 2.57) and 1.53 (critical value 4.27) for sample 1 and 2 respectively, the parameter α_C was 0.05. Therefore, both methods provided the same concentration values for 2,4,6-TCA and guaiacol.

Both methods gave similar reproducibility levels. MHS–SPME displayed higher sensitivity, Soxhlet required a concentration step so that the concentration of analytes was over the detection limits.

Soxhlet extraction has many drawbacks, the main one being that is non-selective. Consequently, extracts contain other compounds that soil the liner, the chromatographic column and the ion trap. As a result, purges and blanks are needed after each injection of the extracts to make sure that neither impurities nor analyte remains appear in the chromatograms. Extraction takes much longer than SPME extraction, consumes large volumes of solvent, and a concentration process that lasts several hours is also needed. Moreover, 1-octen-3-ol and 1-octen-3-one cannot be measured by Soxhlet.

4. Conclusions

The method developed enables the quantification of four compounds responsible for cork taint in cork stoppers. Moreover, calibration can be carried out in aqueous solutions since the matrix effect is removed. This alternative method is cheaper than other extraction techniques such as supercritical fluid extraction, avoids the use of organic solvents of Soxhlet extraction and substantially reduces sample manipulation. It also enables the determination of 1-octen-3-ol and 1-octen-3-one, which cannot be measured by Soxhlet because they are lost in the concentration step.

Analytes were lost when grinding was carried out at room temperature, therefore the use of a freezer mill or freezing the samples with liquid nitrogen prior to grinding is recommended.

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