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Stir bar sorptive extraction of volatile compounds in vinegar: Validation study and comparison with solid phase microextraction

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Abstract

Stir bar sorptive extraction was evaluated for analysing volatiles in vinegar. The procedure developed shows detection and quantitation limits, and linear ranges adequate for analysing this type of compounds. The accuracy obtained was close to 100%, with repeatability values lower than 13%. The extraction efficiency is inversely affected by the acetic acid content. Although the absolute areas decrease, the compound area/internal standard area ratio remains constant, so for quantitative analysis, the acetic acid concentration does not affect the analytical data. The method was compared with a previous SPME method. Similar performance characteristics were obtained for both methodologies, with lower detection and quantitation limits and better repeatability reproducibility values for SBSE. Both analytical methods were used to analyse a variety of vinegars. The results obtained from both methods were in agreement.

Keywords: Stir bar sorptive extraction; Vinegar; Aroma compounds; Validation; Solid phase microextraction

1. Introduction

Vinegar is used not only as condiment but also as ingredient in many food products, particularly sauces and dressings. Several types of vinegars produced from different raw materials and by different production processes can be found in the market. Sherry wine vinegar, produced from sherry wines following traditional method of acetification [1], is a wine-derived product of high reputation, very appreciated in gastronomy. Due to the diversity of vinegars in the market and the increase in demand, it has been considered necessary to investigate reliable analytical methods to establish criteria for determining quality and origin, since objective authentification remains an unresolved issue. Vinegar quality is heavily influenced by flavour compounds. Several hundred of compounds from different families contribute to vinegar flavour. It's chemical and organoleptic properties are determined by the acetification system, the raw material used as substrate and, in some cases, by the wood aging.

The different raw materials and technological procedures that can be used result in a great variety of products of diverse quality and organoleptic properties. Considering that the volatile constituents of any specific vinegar will be determined by these factors, raw material and technological process, it is logical to suppose that they could be characterised and differentiated by the quantitative and qualitative analysis of this type of compounds.

There are various methods for the capillary GC analysis of volatile components [2–4].

Stir bar sorptive extraction (SBSE) is a recently developed technique [5–8] in which a stir bar coated with 50–300 μL of polydimethylsiloxane (PDMS) is employed to extract analytes from a variety of matrices. The extraction mechanism is similar to that of solid phase microextraction (SPME) based on PDMS sorption [9]. A magnetic stirring bar is added to the sample to promote the transfer of analytes to the polymer coating and, after a predetermined extraction period, the analytes are thermally desorbed in the GC injector.

The advantage of SBSE is the much higher mass of PDMS available, which results in high recoveries and a higher sample capacity. The applications developed with SBSE have shown low detection limits and good repeatability [8,10–19], which confirm the great potential of this technique.

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In a previous paper [8], the optimisation of a stir bar sorptive extraction and thermal desorption procedure coupled to capillary gas chromatography–mass spectrometry for the determination of volatile compounds in vinegar was carried out.

For the extraction step, we evaluated the effects of experimental parameters such as sample volume, salting out effect, stirring speed, sampling time and dilution of the sample on the SBSE. For the thermal desorption into the GC, the factors evaluated were desorption temperature, desorption time, helium flow and cryofocusing temperature in the PTV injector. In both cases, the effects of these parameters were evaluated using a two-level factorial design expanded further to a central composite design.

The purpose of the work reported here is to perform the validation of the optimized SBSE analytical method, as well as to compare it with a previous methodology based on SPME technique [9]. The method, after validation, has been applied to various different samples of commercial vinegars.

2. Experimental

2.1. Vinegar samples

A commercial sherry vinegar sample was used to validate the analytical method for determining the various aroma and flavour compounds of varying volatilities and functions in vinegar. This methodology had been optimized in a previous study [8].

After validation, several vinegars from different raw materials were analyzed.

The comparative study was carried out using different commercial vinegar samples.

2.2. Chemicals and reagents

All the aroma standards used in this study were supplied by Merck (Darmstadt, Germany) and Sigma (Steinheim, Germany).

Individual stock standard solutions of each aroma compound were prepared by weight in ethanol. A global stock standard solution containing all the analytes was prepared in a synthetic vinegar solution (2 g L^{-1} of tartaric acid, 80 g L^{-1} of acetic acid, 1 g L^{-1} ethyl acetate, and 10 mL L^{-1} of ethanol, in Milli-Q water). Working solutions used in order to determine the performance characteristics of the SBSE methodology were prepared by diluting different amounts of the global standard solution in a synthetic vinegar solution.

All these solutions were stored at 4 °C.

4-Methyl-2-pentanol was employed as internal standard. NaCl was purchased from Scharlau (Barcelona, Spain).

2.3. SPME

2.3.1. Sample preparation

For each SPME analysis, a volume of 15 mL of vinegar was pipetted and placed into a 50-mL glass vial with 6.14 g of NaCl. Each sample was spiked with 50 μL of a solution of 4-methyl-2-pentanol (2.27 g L^{-1} in Milli-Q water containing 80 g L^{-1} of acetic acid). A small magnetic stirring bar was also added. The vial was tightly capped with a PTFE-faced silicone septum. The vial was placed in a thermostated block on a stirrer. The sample was equilibrated for 5 min at sampling temperature and, after this, the SPME fibre (carboxen-polydimethylsiloxane, CAR-PDMS; 75 μm) was inserted into the headspace. During the sampling time, the sample was stirred at constant speed. After completion of sampling, the fibre was removed from the sample vial and inserted into the injection port of the GC.

2.3.2. Chromatographic conditions

The samples were analysed using a GC 8000 chromatograph with a FID detector (Fisons Instruments, Milan, Italy).

The injection was made in the splitless mode for 2 min. For analytes desorption inside the GC injection port, the temperature was 280 °C. The GC was equipped with a DB-Wax capillary column (J&W Scientific, Folsom, CA, USA), 60 m × 0.25 mm I.D., with a 0.25 μm coating. The carrier gas was helium at a flow rate of 1.1 mL min^{-1}. The detector temperature was 250 °C. The GC oven was programmed as follows: held at 35 °C for 10 min, then ramped at 5 °C min^{-1} to 100 °C. Then it was raised to 210 °C at 3 °C min^{-1} and held for 40 min.

The compounds were identified by mass spectrometric analysis. In these analyses, the same GC, under the same analytical conditions mentioned before, coupled to a MD 800 mass detector (Fisons Instruments, Milan, Italy) was used. The mass detector operated in EI+ mode at 70 eV in a range of 30 to 450 amu.

The signal was recorded and processed with Masslab software supplied with the Wiley 6.0 MS library. Peak identification was carried out by analogy of mass spectra and confirmed by retention indices of standards when they were available or by retention data from the literature. Quantitative data from the identified compounds were obtained by measuring the relative peak area in relation to that of 4-methyl-2-pentanol, the internal standard.

2.4. SBSE

2.4.1. Sample preparation

The extractions were carried out with 10 mm × 0.5 mm (length × film thickness) PDMS commercial stir bars, supplied by Gerstel (Mülheim a/d Ruhr, Germany). After optimisation, and for each SBSE analysis, a volume of 25 mL of sample (natural and synthetic vinegar) was pipetted and placed into a 100-mL Erlenmeyer flask with 5.85 g of NaCl and 50 μL of a solution of 4-methyl-2-pentanol (2.27 g L^{-1} in Milli-Q water containing 80 g L^{-1} of acetic acid). The Erlenmeyer flask was placed on a magnetic stirrer equipped with 15 positions (Mülheim a/d Ruhr, Germany). The stir bar was stirred at 1250 rpm at 25 °C for 120 min. After removal from the vinegar sample, the stir bar was placed for a few seconds in distilled water in order to remove NaCl and gently dried with a lint-free tissue. Then, it was transferred into a glass thermal desorption tube and then thermal desorption was carried out.

2.4.2. Apparatus

The coated stir bars were thermally desorbed using a commercial TDU thermal desorption unit (Gerstel) connected to a programmed-temperature vaporisation (PTV) injector CIS-4

(Gerstel) by a heated transfer line. The PTV was installed in an Agilent 6890 GC-5973 MS system (Agilent Technologies, Palo Alto, CA, USA). An empty baffled liner was used in the PTV. The thermodesorption unit was equipped with a MPS 2 L autosampler (Gerstel) capable of handling the program for 98 coated stir bars. The desorption temperature was programmed from 40 to 300 °C (held for 10 min) at 60 °C min^{-1} under a helium flow (75 mL min^{-1}) and the desorbed analytes were cryofocused in the PTV system with liquid nitrogen at −140 °C. Finally, the PTV system was programmed from −140 to 300 °C (held for 5 min) at 10 °C s^{-1} for analysis by GC–MS. Capillary GC–MS analyses in the electron impact mode were performed on an Agilent 6890 GC-5973N MS system (Agilent, Little Falls, DE, USA), equipped with a DB-Wax capillary column (J&W Scientific, Folsom, CA, USA), 60 m × 0.25 mm I.D., with a 0.25 μm coating.

2.4.3. Chromatographic conditions

For SBSE, GC analytical conditions were the same as described above. Peak identification was also carried out using the Wiley library. In this case, quantitative data from the identified compounds were obtained by measuring the molecular ion peak area in relation to that of 4-methyl-2-pentanol, the internal standard.

3. Results and discussion

A higher number of volatile compounds were detected in the samples studied by means of SBSE. By SBSE, 47 volatile compounds could be identified whereas by SPME, only 25 were found. Most of these compounds found by SBSE and not by SPME are furanic compounds.

3.1. Performance characteristics

In a previous work [8], the calibration curves for a few relevant compounds were developed in order to estimate the detection limits and repeatability of each one. In the present work, the number of compounds studied have been extended to 47 and a complete validation study has been carried out, studying calibration and linearity, detection and quantitation limits, accuracy, repeatability, reproducibility and the possible matrix effect of the acetic acid content. Fig. 1 shows a typical chromatogram of a sherry wine vinegar obtained by means of SBSE.

3.1.1. Calibration. Linearity

Five levels of concentration were tested in triplicate; these concentrations covered the concentration ranges expected for the various aromatic compounds in vinegars.

The (volatile compound/internal standard) molecular ion peak area ratio for the identified volatile compounds was used for each compound. The range of linearity studied for each compound appears in Table 1. For those compounds with LOQ values higher than the lowest points of these ranges, the linearity evaluation was carried out in agreement with their LOQ values. The correlation coefficients were good ($r^2 > 0.98$). An excellent linearity was obtained in all cases for the range studied. This was

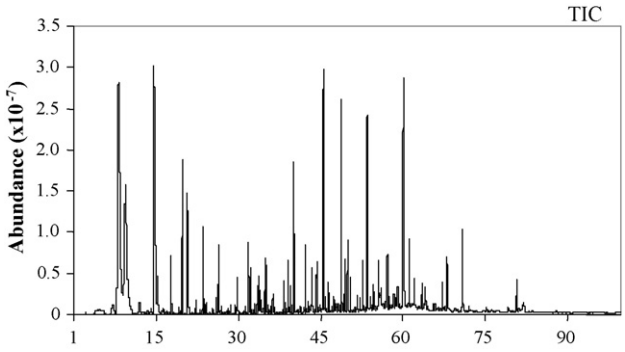

Fig. 1. Total ion chromatogram obtained for a vinegar sample by means of SBSE. Retention times (min): Ethyl isobutyrate (13.62); propyl acetate (13.99); isobutyl acetate (15.76); ethyl butyrate (16.84); *n*-butyl acetate (18.38); ethyl isopentanoate (18.46); hexanal (18.70); isobutanol (19.71); isopentyl acetate (20.57); ethyl pentanoate (20.77); 1-butanol (21.84); *trans*-2-hexenal (24.01); isoamyl alcohol (23.84); 2-methyl-1-butanol (24.12); ethyl hexanoate (24.65); hexyl acetate (25.80); 3-hydroxy-2-butanone (26.62); *cis*-3-hexenyl acetate (27.59); ethyl lactate (28.51); hexan-1-ol (28.87); *cis*-3-hexen-1-ol (30.04); *trans*-2-hexen-1-ol (30.82); ethyl octanoate (31.87); 2-furaldehyde (32.87); benzaldehyde (35.15); isobutyric acid (36.84); 5-methyl-2-furaldehyde (36.95); 2-acetyl-5-methylfuran (38.54); butyric acid (38.89); isovaleric acid (40.28); diethyl succinate (40.58); α-terpineol (41.51); benzyl acetate (42.64); ethyl-2-phenyl acetate (44.59); phenylethyl acetate (45.95); hexanoic acid (46.57); benzyl alcohol (47.03); 2-phenylethanol (49.21), 2-ethyl hexanoic acid (50.17); 4-ethylguaiacol (52.87); octanoic acid (53.75); eugenol (57.21); 4-ethylphenol (57.36); 5-acetoxymethyl-2-furaldehyde (58.00); decanoic acid (60.39); diethyl ftalate (63.87); 5-hydroxymethyl-2-furaldehyde (68.90).

also corroborated by the "on-line linearity (LOL)", with values higher than 97% (Table 1). This parameter is determined by the following equation in which RSD(b) is the relative standard deviation of the slope (expressed as a percentage).

$$\mathrm{LOL}(\%) = 100 - \mathrm{RSD}(b)$$

Similar results, with LOL values higher than 97%, were also obtained for SPME [9].

3.1.2. Detection and quantitation limits and accuracy

Detection and quantitation limits were calculated from the calibration curves constructed for each volatile compound, using the Alamin Computer Program [20].

The limits of detection (three times the relative standard deviation of the analytical blank values calculated from the calibration curve) and quantitation (ten times the relative standard deviation of the analytical blank values calculated from the calibration curve) obtained (Table 2) are low enough to determine these compounds in real vinegar samples, taking into account their concentrations found in the studied samples. For SPME [9], the detection and quantitation limits obtained were higher than those obtained by SBSE. Several authors have established that SBSE exhibits a great potential with very low detection limits [8,10].

In order to check the accuracy of this analytical method, the technique of standard additions was used. A sample of representative vinegar was taken as the matrix and known quantities of the global standard solution were added at five levels and in triplicate. The slopes of the lines thus obtained for each of the aromatic compounds were compared with the corresponding slopes obtained in the calibration with standards (t criterion).

Table 1
Characteristics of the calibration curves

Compound	Studied range ($\mu g\,L^{-1}$)	Regression coefficient	Linearity (LOL, %)	Slope ± SD	Intercept ± SD
Ethyl isobutyrate	13.13–1093.75	0.9992	99.20	0.0030 ± 0.00001	0.0248 ± 0.01100
Propyl acetate	3.11–777.75	0.9957	98.25	0.0003 ± 0.00002	0.0064 ± 0.00181
Isobutyl acetate	29.69–2226.75	0.9900	98.47	0.0035 ± 0.00011	0.2970 ± 0.08621
Ethyl butyrate	2.35–141.06	0.9956	97.91	0.0035 ± 0.00010	0.0322 ± 0.00482
Ethyl isopentanoate	2.42–727.65	0.9990	99.02	0.0061 ± 0.00012	0.4369 ± 0.01910
n-Butyl acetate	8.62–862.00	0.9979	98.55	0.0008 ± 0.00003	0.0642 ± 0.00431
Hexanal	37.82–945.50	0.9953	97.56	0.0009 ± 0.00003	0.0620 ± 0.01070
Isobutanol[a]	0.47–35.09	0.9958	97.94	$5 \times 10^{-6} \pm 1 \times 10^{-7}$	−0.0025 ± 0.00163
Isopentyl acetate	106.61–3345	0.9980	97.27	0.0009 ± 0.00002	0.5393 ± 0.04171
Ethyl pentanoate	0.109–10.90	0.9956	98.07	0.0030 ± 0.00010	0.0013 ± 0.00032
1-Butanol	22.50–305.91	0.9994	98.76	0.0010 ± 0.00004	−0.0161 ± 0.00274
trans-2-Hexenal	47.19–1179.75	0.9975	98.83	0.0003 ± 0.000011	0.031 ± 0.0032
2-Methyl-1-butanol[a]	1.42–142.20	0.9876	97.51	$1 \times 10^{-5} \pm 1 \times 10^{-6}$	0.1203 ± 0.03314
Isoamyl alcohol[a]	0.48–100.00	0.9919	97.81	$2 \times 10^{-5} \pm 2 \times 10^{-6}$	0.0133 ± 0.00421
3-Hydroxy-2-butanone[a]	3.38–2706.24	0.9973	98.71	$7 \times 10^{-7} \pm 2 \times 10^{-8}$	0.0436 ± 0.00232
Ethyl hexanoate	0.15–153.50	0.9983	99.02	0.0083 ± 0.00012	0.0154 ± 0.00452
Hexyl acetate	0.12–35.31	0.9976	98.67	0.0486 ± 0.00064	0.0366 ± 0.00861
cis-3-Hexenyl acetate	0.36–108.15	0.9989	99.16	0.0290 ± 0.00030	0.1362 ± 0.01060
Ethyl lactate[a]	0.13–33.71	0.9941	97.94	$2 \times 10^{-}5 \pm 3 \times 10^{-6}$	0.0166 ± 0.00491
Hexan-1-ol	22.65–566.25	0.9986	99.47	0.0041 ± 0.00001	0.0007 ± 0.00172
cis-3-Hexen-1-ol	16.29–1221.75	0.9987	98.86	0.0003 ± 0.00001	0.0009 ± 0.00200
trans-2-Hexen-1-ol	46.50–2823.75	0.9961	98.23	0.0005 ± 0.00003	0.5294 ± 0.01002
Ethyl Octanoate	0.11–380.75	0.9994	99.22	0.0607 ± 0.00342	0.0862 ± 0.00923
2-Furaldehyde[a]	1.03–15.25	0.9990	98.99	$3 \times 10^{-5} \pm 2 \times 10^{-7}$	0.0101 ± 0.00052
Benzaldehyde	1.96–196.00	0.9988	98.91	0.0005 ± 0.00006	0.0061 ± 0.00040
Isobutyric acid[a]	2.43–121.26	0.9953	97.82	$1 \times 10^{-5} \pm 2 \times 10^{-6}$	0.0107 ± 0.01791
5-Methyl-2-Furaldehyde	9.02–2310.00	0.9944	97.99	0.0002 ± 0.00003	0.0170 ± 0.00420
2-Acetyl-5-Methylfuran	1.46–1216.50	0.9978	98.75	0.0011 ± 0.00004	0.0087 ± 0.00670
Butyric acid[a]	1.92–47.88	0.9963	97.86	$2 \times 10^{-5} \pm 1 \times 10^{-6}$	−0.0238 ± 0.01100
Isovaleric acid[a]	2.84–283.74	0.9944	97.62	$8 \times 10^{-6} \pm 1 \times 10^{-7}$	0.2888 ± 0.02252
Diethyl succinate[a]	0.05–1.11	0.9953	97.56	0.0009 ± 0.00004	0.0169 ± 0.01181
α-Terpineol	0.67–66.84	0.9983	98.79	0.0109 ± 0.00011	−0.0077 ± 0.00362
Benzyl acetate	0.75–751.00	0.9932	97.38	0.0020 ± 0.00012	0.1021 ± 0.01642
Ethyl-2-phenyl acetate	4.78–47.80	0.9953	97.20	0.0207 ± 0.00063	0.0473 ± 0.01590
Phenylethyl acetate[a]	0.14–4.70	0.9952	97.53	0.0024 ± 0.00010	1.7399 ± 0.13011
Hexanoic acid[a]	0.12–2.72	0.9967	97.98	0.0001 ± 0.00004	0.0045 ± 0.00253
Benzyl alcohol	25.18–5812.00	0.9980	98.79	$2 \times 10^{-5} \pm 1 \times 10^{-6}$	0.0014 ± 0.00064
2-Phenylethanol[a]	2.13–21.28	0.9967	97.97	0.0001 ± 0.00002	0.1483 ± 0.02385
2-Ethyl Hexanoic acid	11.63–387.50	0.9960	97.79	0.0096 ± 0.00020	0.1198 ± 0.03210
4-Ethylguaiacol	7.15–206.50	0.9961	97.80	0.0202 ± 0.00041	−0.0033 ± 0.00790
Octanoic acid[a]	0.06–6.41	0.9990	98.98	0.0004 ± 0.00002	0.0788 ± 0.01062
Eugenol	1.41–236.60	0.9980	98.81	0.0145 ± 0.00023	−0.0217 ± 0.01710
4-Ethylphenol	14.60–321.20	0.9956	97.65	0.0014 ± 0.00004	0.0041 ± 0.00521
5-Acetoxymethyl-2-furaldehyde	28.17–704.25	0.9968	97.99	0.0001 ± 0.00001	0.0003 ± 0.00012
Decanoic acid	6.73–1682.50	0.9966	97.94	0.0021 ± 0.00001	0.1282 ± 0.03471
Diethyl ftalate	1.12–37.21	0.9983	98.53	0.0934 ± 0.00131	0.0536 ± 0.01364
5-Hydroxymethyl-2-furaldehyde[a]	2.30–325.8	0.9972	98.46	$5 \times 10^{-7} \pm 2 \times 10^{-8}$	0.0023 ± 0.00011

LOL (%, on-line linearity): 100 − RSD(*b*), RSD(*b*) is the relative standard deviation of the slope (expressed as a percentage).
[a] $mg\,L^{-1}$.

In general, no significant differences were found between them at a significance level of 5%.

Table 2 gives the data for the accuracy of each compound, determined by the slope of the line plotting the concentration found against the concentration expected.

Good accuracy values have been obtained, only isobutanol and *trans*-2-hexen-1-ol presented lower values, 85.01% and 80%, respectively.

For SPME [9], good accuracy values were obtained with the exception of 2,3-butanediol and 3-hydroxy-2-butanone, with 60% and 57%, respectively.

3.1.3. Repeatability and reproducibility

The repeatability and reproducibility have been evaluated by means of a series of five extractions of a commercial sherry wine vinegar performed using two different twisters. The mean concentration for all the identified volatile compounds, with their relative standard deviation (RSD) were calculated (Table 2). The RSD obtained for each twister ranges between 2.88% and 12.90%. The inter-twister accuracy showed RSD values similar to intra-twister accuracy (2.85–11.95%). Only, ethyl octanoate showed higher intra- and inter-twister RSD values.

Table 2
Performance characteristics

Compound	Detection limit (LOD, $\mu g\,L^{-1}$)	Quantitation limit (LOQ, $\mu g\,L^{-1}$)	Accuracy (%)	Repeatability (RSD, %)	Reproducibility (RSD, %)
Ethyl isobutyrate	3.31	10.98	99.61	5.39	5.43
Propyl acetate	0.43	1.41	99.16	4.13	4.43
Isobutyl acetate	10.01	32.32	99.13	4.85	3.73
Ethyl butyrate	0.71	2.35	99.52	5.27	3.92
Ethyl isopentanoate	0.78	2.54	99.12	10.85	9.97
n-Butyl acetate	2.25	7.34	99.14	9.43	6.97
Hexanal	20.00	65.44	98.82	–	–
Isobutanol	150.22	500.12	85.01	2.97	3.42
Isopentyl acetate	33.00	110.23	98.44	4.68	9.17
Ethyl pentanoate	0.09	0.29	99.03	–	–
1-Butanol	4.13	13.09	99.23	–	–
trans-2-Hexenal	30.12	100.10	98.83	–	–
2-Methyl-1-butanol	1000.22	3328.07	99.43	12.90	10.62
Isoamyl alcohol	220.11	733.7	92.16	2.88	3.27
3-Hydroxy-2-butanone	2200.33	7332.66	99.26	9.49	7.46
Ethyl hexanoate	0.05	0.15	99.79	4.88	11.66
Hexyl acetate	0.05	0.15	99.11	11.47	11.78
cis-3-Hexenyl acetate	0.10	0.31	99.08	–	–
Ethyl lactate	50.01	165.23	99.82	–	–
Hexan-1-ol	8.60	27.67	98.91	–	–
cis-3-Hexen-1-ol	7.83	25.70	99.03	–	–
trans-2-Hexen-1-ol	20.01	66.60	80.00	–	–
Ethyl Octanoate	0.04	0.13	99.15	17.00	19.00
2-Furaldehyde	0.5	1.65	99.04	5.53	4.06
Benzaldehyde	0.52	1.52	99.36	4.18	3.79
Isobutyric acid	888.00	2878.32	98.64	5.04	7.06
5-Methyl-2-Furaldehyde	4.51	14.99	98.65	6.41	4.25
2-Acetyl-5-methylfuran	0.34	1.12	99.71	–	–
Butyric acid	783.11	2609.77	99.2	9.70	11.95
Isovaleric acid	800.04	2660.87	98.97	4.98	6.32
Diethyl succinate	34.12	113.54	99.76	4.51	3.42
α-Terpineol	0.35	1.15	99.35	8.76	6.86
Benzyl acetate	0.22	0.73	99.57	5.28	5.08
Ethyl-2-phenyl acetate	1.13	3.66	99.08	9.12	6.39
Phenylethyl acetate	43.99	145.45	99.30	3.13	4.01
Hexanoic acid	50.00	165.12	99.40	4.55	10.84
Benzyl alcohol	10.01	31.21	99.16	3.24	2.91
2-Phenylethanol	641.07	2136.50	99.07	4.95	3.97
2-Ethyl Hexanoic acid	5.00	15.99	99.30	8.69	8.03
4-Ethylguaiacol	2.22	7.30	99.43	3.43	2.85
Octanoic acid	32.11	106.99	99.00	9.80	10.73
Eugenol	0.35	1.15	98.79	4.57	3.61
4-Ethylphenol	5.12	16.12	98.16	7.21	6.13
5-Acetoxymethyl-2-Furaldehyde	10.01	33.12	99.23	–	–
Decanoic acid	2.12	7.01	98.82	4.90	4.37
Diethyl Ftalate	0.47	1.54	98.58	9.62	6.87
5-Hydroxymethyl-2-Furaldehyde	100.00	332.32	99.59	9.95	10.78

Repeatability (same twister, $n = 5$) and reproducibility (different twisters, $n = 5$).

For SPME [9], higher inter-fibre (2.5–20%) and intra-fibre (4.6–46%) accuracy values were obtained with considerable differences observed among the chromatographic responses obtained for each fibre and volatile compound.

These results corroborate the higher reproducibility of SBSE.

3.1.4. Matrix effect

Since acetic acid is one of the major constituents of vinegars and it exhibits a high volatility, it may compete with the volatile compounds in the extraction.

In a previous study [9], using SPME, it was found that the extraction efficiency was influenced by acetic acid content. So, in order to check this possible source of interference, the same amount of volatile compounds (alcohols, aldehydes, esters and acids) were added to five synthetic vinegar samples with different acetic acid content (0–90 $g\,L^{-1}$). Three extractions were analysed for each of these synthetic samples. The data obtained show that the higher the acetic acid concentration, the lower the extraction efficiency (Fig. 2). Although the absolute areas decrease, the

Table 3
Volatile compounds ($\mu g\,L^{-1}$) found in vinegars

Compound	Balsamic		Apple		Sherry			Tarragon	Pedro Ximenez	White wine
	1	2	3	4	5	6	7	8	9	10
Ethyl isobutyrate	17.29	24.36	0.208[b]	4.43[b]	51.38	104.06	15.24	12.86	531.80	40.10
Propyl acetate	497.57	330.58	nd	246.58	305.77	178.92	136.30	nd	466.88	111.34
Isobutyl acetate	1427.2	977.8	847.5	1591.0	941.07	489.92	440.40	210.60	920.63	1620.4
Ethyl butyrate	27.20	14.50	48.25	762.72[a]	20.99	34.01	13.37	1.03[b]	285.60	28.19
Ethyl isopentanoate	14.15	14.13	19.40	20.59	196.00	344.66	92.97	55.55	766.94[a]	170.93
n-Butyl acetate	1.23[b]	1.27[b]	4.02[b]	836.71	37.7	76.65	10.70	3.32[b]	229.05	19.34
Hexanal	nd	nd	661.63	392.37	nd	nd	nd	nd	nd	nd
Isobutanol[c]	10.42	8.07	4.78	6.12	4.13	2.13	3.32	nd	4.86	7.11
Isopentyl acetate	3257.1	1882.5	386.99	3230.4	2776.0	1549.2	1171.7	609.77	2682.7	5204.4[a]
Ethyl pentanoate	0.095[b]	0.292	0.669	1.51	0.256[b]	0.495	0.227[b]	0.101[b]	2.833	0.481
1-Butanol	40.96	51.06	59.64	103.22	96.27	98.18	77.75	54.3	68.96	62.82
trans-2-Hexenal	14.15[b]	205.23	332.69	109.82	149.40	115.04	1.83[b]	117.93	377.41	29.88[b]
2-Methyl-1-butanol[c]	24.85	21.96	8.63	11.10	12.20	8.08	3.55	0.424[b]	21.83	27.66
Isoamyl alcohol[c]	29.64	23.13	9.61	23.50	12.57	10.12	8.11	5.36	16.77	26.28
3-Hydroxy-2-butanone[c]	1326.9	898.69	284.41	309.61	871.62	265.29	706.08	441.97	309.37	415.87
Ethyl hexanoate	0.999	0.399	10.30	7.84	10.20	8.70	1.42	4.71	25.60	2.57
Hexyl acetate	0.140[b]	0.172	89.23	401.40[a]	0.314	1.95	0.039[b]	0.021[b]	6.80	0.694
cis-3-Hexenyl acetate	nd	nd	2.27	26.34	0.016[b]	0.867	1.41	96.76	2.34	0.478
Ethyl lactate[c]	9.23	5.49	0.052[b]	0.221	0.999	2.22	0.274	0.521	21.64	0.301
Hexan-1-ol	30.87	34.83	1869.6[a]	6984.1[a]	12.73[b]	24.11[b]	9.52[b]	5.36[b]	138.60	nd
cis-3-Hexen-1-ol	18.75[b]	20.08[b]	40.18	851.54	27.79	51.97	34.05	2158.8[a]	34.58	77.06
trans-2-Hexen-1-ol	nd	nd	150.12	1941.6	nd	nd	nd	nd	50.00[b]	nd
Ethyl Octanoate	0.079[b]	0.100[b]	0.352	4.32	0.423	1.25	0.742	nd	1.88	1.39
2-Furaldehyde[c]	9.66	13.46	5.08	2.82	1.97	15.69[a]	1.93	11.59	9.11	3.08
Benzaldehyde	77.53	22.27	116.28	51.62	343.91[a]	197.14	137.97	159.39	142.01	388.83[a]
Isobutyric acid[c]	22.50	34.58	46.60	29.21	67.86	39.61	38.65	14.48	73.43	38.43
5-Methyl-2-Furaldehyde	2778.4[a]	2802.5[a]	13.12[b]	nd	118.61	244.92	122.58	2.09[b]	1371.7	328.52
2-Acetyl-5-methylfuran	35.38	63.74	0.501[b]	nd	9.87	4.40	3.92	nd	10.34	5.47
Butyric acid[c]	7.73	12.25	5.23	8.29	16.86	7.28	16.00	4.22	14.01	8.18
Isovaleric acid[c]	18.39	51.25	77.73	3.03	169.04	93.43	68.48	17.01	163.27	94.29
Diethyl succinate[c]	1.05	1.36	0.041[b]	0.135	0.122	0.109[b]	0.178	0.012[b]	1.85[a]	0.251
α-Terpineol	14.63	32.64	0.710[b]	2.77	1.94	0.9131	2.14	5.92	11.75	2.91
Benzyl acetate	1.71	15.11	0.501[b]	0.032[b]	100.19	112.34	10.57	0.321[b]	299.50	22.26
Ethyl-2-phenyl acetate	8.05	9.42	1.59	1.22[b]	34.92	56.85[a]	12.22	14.00	143.58[a]	18.65
Phenylethyl acetate[c]	1.12	1.20	nd	0.185	1.53	1.02	0.597	0.804	1.24	2.33
Hexanoic acid[c]	1.52	2.18	2.71	4.96[a]	3.61[a]	1.49	1.52	0.956	2.47	1.64
Benzyl alcohol	699.42	1207.1	67.28	58.99	1630.5	1975.5	747.87	333.42	5676.6	732.74
2-Phenylethanol[c]	14.91	16.36	4.55	5.24	15.66	11.29	8.63	8.41	12.74	20.50
2-Ethyl Hexanoic acid	0.112[b]	nd	nd	6.04[b]	66.83	0.121[b]	23.92	nd	nd	22.01
4-Ethylguaiacol	40.58	37.53	nd	nd	56.99	11.69	28.33	20.11	3.97[b]	24.22
Octanoic acid[c]	1.41	1.24	0.6771	8.06[a]	1.71	0.960	0.810	0.6763	1.15	1.02
Eugenol	4.15	4.91	5.66	122.06	7.66	5.76	5.16	47.64	4.17	4.92
4-Ethylphenol	115.23	151.03	0.876[b]	nd	209.54	126.34	100.33	32.53	50.61	61.15
5-Acetoxymethyl-2-furald.	4192.0[a]	4284.5[a]	50.76	nd	284.06	256.72	55.08	nd	4067.6[a]	471.00
Decanoic acid	305.71	112.92	9.88	1505.7	109.75	82.49	84.61	64.75	52.93	54.74
Diethyl Ftalate	0.326[b]	0.966[b]	1.14[b]	1.54	5.84	4.49	3.82	0.805[b]	2.30	6.22
5-Hydroxymethyl-2-furald.[c]	268.45	379.29[a]	6.67	nd	12.28	16.53	3.30	6.73	295.74	30.64

1 and 2, balsamic vinegars; 3 and 4, apple vinegars; 5–7, sherry wine vinegars; 8, tarragon vinegar; 9, Pedro Ximenez vinegar; and 10, white wine vinegar.
furald.: furaldehyde
[a] Values out of the studied range.
[b] Values lower than LOQ; nd, not detected.
[c] $mg\,L^{-1}$.

compound area/I.S. area ratio remains constant and the relative standard deviations are less than 15%. In general, for quantitative analysis, the I.S. may be used, so the acetic acid concentration does not affect the analytical data.

3.2. Determination of volatile compounds in vinegars

This analytical method was used to analyse several vinegar samples supplied by different producers. Each sample was analysed in triplicate.

Table 4
Comparison of SPME and SBSE for the determination of volatile compounds in vinegars

Compound	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	y = SBSE; x = SPME
n-Butyl acetate[a]	78.15 74.92	25.52 23.99	8.65 8.34	nd nd	nd nd	$y = 1.0743x - 0.0027$ ($r^2 = 0.9999$)
Isoamyl alcohol[a]	63.16 65.18	279.27 283.56	235.56 232.14	74.19 73.14	130.30 128.45	$y = 0.9756x - 0.1980$ ($r^2 = 0.9812$)
Isobutanol[a]	4.03 4.00	8.20 7.75	5.06 5.01	8.03 8.04	4.07 4.02	$y = 1.0348x - 0.0480$ ($r^2 = 0.9908$)
Isopentyl acetate[a]	3.20 3.03	2.37 2.46	3.56 3.87	3.59 3.50	0.90 0.92	$y = 0.9506x + 0.0489$ ($r^2 = 0.9787$)
Ethyl pentanoate	19.01 19.01	nd nd	nd nd	3.10 3.03	25.00 24.11	$y = 1.0287 - 0.001$ ($r^2 = 0.9988$)
2-Methyl-1-butanol[a]	10.21 9.76	6.16 6.17	8.01 7.87	19.76 20.46	6.17 6.24	$y = 0.9513x + 0.4387$ ($r^2 = 0.996$)
Isoamyl alcohol[a]	9.70 8.91	5.61 5.17	6.41 6.11	16.28 17.89	2.19 2.13	$y = 0.9163x + 0.8125$ ($r^2 = 0.9938$)
3-Hydroxy-2-butanone[a]	508.74 509.51	424.76 451.79	322.02 302.10	120.55 130.86	443.13 438.14	$y = 0.9924x - 0.7747$ ($r^2 = 0.9868$)
cis-3-Hexenyl acetate	0.41 nd	nd nd	nd nd	nd nd	0.51 nd	–
2-Furaldehyde[a]	5.31 4.72	7.72 7.71	5.89 5.81	3.98 4.40	2.72 2.48	$y = 0.9454x + 0.3226$ ($r^2 = 0.9953$)
Benzaldehyde[a]	0.06 0.07	0.12 0.13	0.68 0.62	0.04 0.05	0.40 0.40	$y = 1.0937x - 0.0113$ ($r^2 = 0.9962$)
Isovaleric acid[a]	16.26 14.62	25.19 25.65	146.61 134.93	74.47 72.35	180.84 178.06	$y = 1.0360x + 0.5134$ ($r^2 = 0.9980$)
Diethyl succinate[a]	0.21 0.22	2.79 2.89	0.40 0.46	1.49 1.91	0.31 0.28	$y = 0.9141x - 0.0132$ ($r^2 = 0.9817$)
Benzyl acetate	7.05 7.45	1.91 2.12	21.44 28.99	1.91 2.45	5.34 5.99	$y = 0.8911x - 0.0287$ ($r^2 = 0.9908$)
Ethyl-2-phenyl-acetate	11.82 12.56	1147.77 145.49	24.96 24.22	9.18 9.58	nd	$y = 1.0184x - 0.0004$ ($r^2 = 0.9990$)
Phenylethyl acetate[a]	0.21 0.21	0.21 0.22	0.18 0.17	1.97 2.22	4.16 4.05	$y = 0.9874x - 0.0485$ ($r^2 = 0.9848$)
Hexanoic acid[a]	1.23 1.13	1.33 1.35	2.46 2.55	0.30 0.27	0.95 0.90	$y = 0.9400x + 0.0929$ ($r^2 = 0.9939$)
Benzyl alcohol[a]	1.93 2.13	0.47 0.48	1.86 1.95	2.73 2.80	0.47 0.44	$y = 0.9380x + 0.0325$ ($r^2 = 0.9961$)
2-Phenylethanol[a]	31.05 33.50	11.02 12.12	58.23 62.09	22.49 22.18	19.55 20.24	$y = 0.9169x + 0.7095$ ($r^2 = 0.9972$)
4-Ethylguaiacol	9.30 9.56	154.51 161.63	125.56 126.82	nd nd	nd nd	$y = 0.9608x + 0.0056$ ($r^2 = 0.9942$)
Octanoic acid[a]	0.15 0.14	1.94 1.78	0.60 0.54	1.81 1.95	2.16 2.26	$y = 0.9444x + 0.0691$ ($r^2 = 0.9866$)
4-Ethylphenol	237.54 267.81	62.84 56.16	749.38 798.90	130.05 129.80	12.04 14.22	$y = 0.9309x + 0.0025$ ($r^2 = 0.9990$)
Decanoic acid	55.21 56.41	31.14 32.61	131.12 129.53	126.21 124.68	92.04 91.50	$y = 0.9404x + 0.0043$ ($r^2 = 0.9905$)

Mean values ($\mu g\,L^{-1}$, $n = 2$). First value for each compound was obtained by SBSE.
nd, not detected.
[a] $mg\,L^{-1}$.

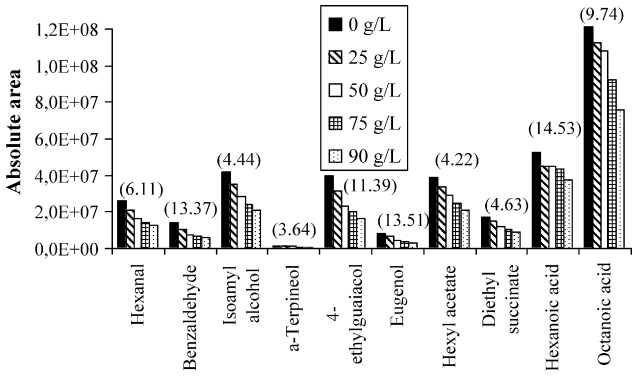

Fig. 2. Absolute molecular ion peak areas obtained for synthetic vinegar samples with different acetic acid content. Relative standard deviations in brackets (%) of the mean values of the relative areas (molecular ion peak area/internal standard area).

The mean results obtained for some of the vinegar samples are shown in Table 3. The major volatile compounds quantified in these samples were isobutanol, 2-methyl-1-butanol, 3-methyl-1-butanol, 2-furaldehyde, 3-hydroxy-2-butanone, isovaleric acid, 2-phenylethanol, and 5-hydroxymethyl-2-furaldehyde.

Among the esters identified, which result from the fermentation of alcohols or by the reaction of acids with alcohols during aging, the major compounds were diethyl succinate, phenylethyl acetate, isopentyl acetate, and isobutyl acetate.

2- and 3-methyl-butanol have been found in other wine vinegars in a range of 10–100 mg L^{-1} [21]. The 3-hydroxy-2-butanone content found ranged from 265.29 mg L^{-1} for sherry vinegars to 1326.9 mg L^{-1} for balsamic vinegars. A high content of 3-hydroxy-2-butanone in apple vinegars have been observed by other authors [22]. It was justified as a consequence of a low aeration during the acetification process. Palacios et al. [23] found that the 3-hydroxy-2-butanone content increased in sherry vinegars during their aging in wood as a consequence of the transformation of butyleneglycol into this compound during the process of the acetic fermentation and, of the general water loss produced during this period by evaporation. In our case, vinegars 1 and 2, balsamic vinegars with a long period of aging in wood, exhibit the highest contents in this compound.

Hexanal and *trans*-2-hexen-1-ol were only found in apple vinegars. Balsamic and Pedro Ximenez vinegars showed, as can be expected on the basis of their production process, very high contents in 2-furaldehyde, 5-methyl-2-furaldehyde, 5-acetoxymethyl-2-furaldehyde, and 5-hydroxymethyl-2-furaldehyde.

A further research is required in order to establish the statistically significant differences among vinegars obtained from different raw materials (white and red wine, cider, malted barley, honey, pure alcohol, etc.) and by different processes of aging.

3.3. Comparison of SBSE and SPME for the analysis of volatile compounds in vinegar

The SBSE analytical method validated here has been compared with a SPME methodology previously optimized by authors for the analysis of volatile compounds in vinegars [9]. Both analytical methods were used to analyse five vinegars supplied by different producers. The results obtained for these samples are shown in Table 4. Only volatile compounds analyzed by both methodologies appear in this table. Not all the compounds measured by SBSE are detected using the SPME technique, but those which are quantified by both methods show similar concentration values. In the column, a slope of one means perfect correlation between SBSE and SPME. As can be seen, the regression coefficients (r^2) for analysis by SBSE and SPME for the compounds quantified always exceeded 0.9800 (Table 4), indicating that results obtained from both methods are in agreement. Nevertheless, SBSE method is capable to study a higher amount of compounds.

4. Conclusions

Under the experimental conditions used in this study, SBSE can be considered an appropriate technique for the analysis of volatile compounds in vinegars. It is a very simple and solventless technique. The detection and quantitation limits, and the accuracies obtained for various volatile compounds are adequate for their quantitation in vinegars. Acetic acid competes in the extraction, but for quantitative analysis, the internal standard may be used. This technique is in good agreement with SPME technique and, in general, exhibits better sensitivity and reproducibility values.

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