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Headspace solid-phase microextraction analysis of aroma compounds in vinegar
Validation study

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Abstract

Headspace solid-phase microextraction (HS-SPME) was evaluated for analysing aromatic compounds in vinegar. The fibre used (a Carboxen–polydimethylsiloxane fibre), and the analytical conditions had been optimised in a previous work. The HS-SPME procedure developed shows detection and quantitation limits, and linear ranges adequate for analysing this type of compounds. The recoveries obtained were close to 100%, with repeatability values lower than 20%. However, considerable differences have been detected between different fibres. The method was applied to a variety of Sherry wine vinegars.

Keywords: Headspace analysis; Vinegar; Food analysis; Solid-phase microextraction; Validation; Aroma compounds

1. Introduction

Vinegar is used not only as a condiment but also as ingredient in many food products, particularly sauces and dressings. Sherry wine vinegar, produced from Sherry wines following traditional methods of acetification [1], is a wine-derived product of high reputation, much appreciated in gastronomy. Due to the diversity of vinegars on 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.

The market value of this type of product can only be sustained if chemical–physical and/or sensorial parameters are found to express differences in composition on the basis of the origin of the vinegar, manufacturing techniques and commercial type.

The flavour of vinegar depends on the raw materials (white and red wines, cider, malted barley, honey, etc.), the constituents formed during the fermentation and, in some cases, the substances formed during the ageing, so it is logical to suppose that vinegars may be characterised and differentiated by the quantita-

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tive and qualitative analysis of their volatile components.

There are various methods for the capillary GC analysis of volatile components [2–4]. Solid-phase microextraction (SPME) is a recently developed technique [5–8] in which a polymer-coated silica fibre is employed to extract analytes from a variety of matrices, and these are then transferred into the injector of a GC system for analysis. This sample preparation prior to the GC and GC–MS analysis can be carried out by direct immersion of the fibre into the sample (DI-SPME) [9] or by exposure of the fibre to the vapour phase or headspace above a liquid or solid sample (HS-SPME) [10,11].

In a previous paper [12], silica fibre coated with Carboxen–polydimethylsiloxane (CAR–PDMS) was found to be more efficient at extracting the aromatic compounds of vinegar than other fibres such as those coated with polydimethylsiloxane, Carbowax–divinylbenzene, and polydimethylsiloxane–divinylbenzene. Parameters such as extraction time, temperature of the sample during the extraction, ionic strength and sample volume were optimised in order to analyse, using a CAR–PDMS fibre, the aroma compounds in vinegars.

It was also determined that the extraction efficiency is inversely affected by the acetic acid concentration, but since the relative areas (compound area/internal standard area) remain constant, the I.S. may be used for quantitative analysis.

The purpose of the work reported here is to perform the validation of the analytical method for the analysis of this type of compound. The method, after validation, has been applied to various different samples of 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 flavor compounds of varying volatilities and functions in vinegar, using a Carboxen–polydimethylsiloxane fibre.

After validation, the method was applied to a variety of Sherry vinegar samples supplied by different producers and produced by different methods (with and without ageing in wood).

2.2. Chemicals and reagents

All the aroma standards used in this study were supplied by Merck (Darmstadt, Germany) and Sigma (Steinheim, Germany). 4-Methyl-2-pentanol was employed as internal standard. Acetic acid and NaCl were purchased from Scharlau (Barcelona, Spain).

Individual stock standard solutions of each aroma compound were prepared by mass in ethanol.

Taking into account reports by other authors [13,14], that SPME analysis may be influenced by the overall aromatic compounds present in the matrix, a global stock standard solution containing all the analytes was prepared in a synthetic vinegar solution (2 g/l of tartaric acid, 80 g/l of acetic acid, 1 g/l ethyl acetate, and 10 ml/l of ethanol, in Milli-Q water). Working solutions used in further studies were prepared by diluting different amounts of the global standard solution in a synthetic vinegar solution.

All these solutions were stored at 4 °C.

2.3. Sample preparation

For each SPME analysis, a volume of 15 ml of sample (natural or synthetic 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 in Milli-Q water containing 80 g/l of acetic acid). A small magnetic stirring bar was also added. The vial was tightly capped with a PTFE-faced silicone septum and placed in a thermostatted block on a stirrer. After 5 min at 70 °C, the SPME fibre was exposed to the headspace of the sample for 60 min. During this 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.

2.4. Equipment

2.4.1. SPME

The CAR–PDMS fibres (Carboxen–polydimethylsiloxane, 75 μm) used in this study were

purchased from Supelco (Bellefonte, PA, USA). The fibres were conditioned prior to use according to supplier's instructions by inserting them into the GC injector.

2.4.2. Chromatography

The samples were analysed using a GC 8000 chromatograph with a flame ionization detection (FID) system (Fisons Instruments, Milan, Italy).

The injection was made in the splitless mode for 2 min. For the desorption of the analytes inside the GC injection port, the temperature was 280 °C. The GC system 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. 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 to 100 °C. Then it was raised to 210 °C at 3 °C/min and held for 40 min.

The compounds were identified by mass spectrometric analysis. In these analyses, the same GC coupled to a MD 800 mass detector (Fisons Instruments) was used. The mass detector operated in the possible electron impact ionization (EI+) mode at 70 eV in a range of 30 to 450 amu. GC analytical conditions were the same as described above.

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.

3. Results and discussion

3.1. Performance characteristics

3.1.1. Calibration, linearity

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

The [aroma compound/internal standard] peak area ratio for the identified aroma compounds was used for each compound. The range of linearity studied for each compound appears in Table 1. The correlation coefficients were good (r^2>0.99). An excellent linearity was obtained in all cases for the range studied. This was 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).

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

3.1.2. Detection and quantitation limits, recovery and analytical sensitivity

Detection and quantitation limits, and analytical sensitivity (Table 2) were calculated from the calibration curves constructed for each aromatic compound, using the Alamin computer program [15]. Analytical sensitivity is defined by the quotient S_s/b, in which S_s is the residual standard deviation and b is the slope of the calibration curve.

The limits of detection (three times the relative standard deviation of the analytical blank values calculate from the calibration curve) and quantitation (ten times the relative standard deviation of the analytical blank values calculated from the calibration curve) obtained are low enough to determine these compounds in real vinegar samples, taking into account the concentrations found for them from the bibliography [16,17].

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

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

Table 1
Characteristics of the calibration curves

Compound	Linear range (mg/l)	Regression coefficient	Linearity (LOL, %)	Slope±SD	Intercept±SD
n-Butyl acetate	0.009–1.9	0.997	98.8	0.240±0.0029	0.024±0.0023
Ethyl pentanoate	0.006–1.0	0.996	98.6	0.888±0.0125	0.021±0.0053
2-Methyl-1-propanol	1.3–23.0	0.999	99.0	0.017±0.0002	−0.004±0.0019
Isoamyl acetate	0.042–11.0	0.999	99.1	0.493±0.0047	0.013±0.0221
Ethyl hexanoate	0.002–0.098	0.992	97.5	1.540±0.0400	0.017±0.0190
2-Methyl-1-butanol	0.848–31.5	0.994	97.9	0.069±0.0014	0.001±0.0146
Isoamyl alcohol	0.037–50.0	0.999	99.1	0.073±0.0060	0.067±0.0022
3-Hydroxy-2-butanone	22.0–1000.0	0.998	98.8	0.002±0.0000	−0.022±0.0059
2-Furancarboxaldehyde	0.024–3.6	0.999	99.2	0.156±0.0012	0.013±0.0020
Benzaldehyde	0.007–0.605	0.997	99.4	4.400±0.0710	0.123±0.0208
2,3-Butanediol	36.0–380.0	0.997	97.8	0.002±0.0000	−0.064±0.0089
Ethyl decanoate	0.003–0.065	0.997	98.3	43.731±0.7410	0.247±0.0253
Isopentanoic acid	1.0–95.0	0.998	98.6	0.037±0.0010	−0.013±0.0159
Diethyl succinate	0.045–3.0	0.998	98.9	0.192±0.0022	0.024±0.0023
Benzyl acetate	0.011–0.239	0.996	97.3	1.321±0.0410	0.041±0.0045
Ethyl-2-phenyl acetate	0.002–0.089	0.998	98.1	5.361±0.1010	0.057±0.0045
Phenylethyl acetate	0.019–3.3	0.997	98.2	3.722±0.0620	0.387±0.0496
Hexanoic acid	0.054–2.00	0.994	97.9	0.389±0.0082	0.053±0.0076
α-Ionone	0.003–0.065	0.995	97.8	18.391±0.0100	0.096±0.0145
Benzyl alcohol	0.058–1.5	0.998	98.4	0.150±0.0023	0.021±0.0011
2-Phenylethanol	0.853–67.5	0.994	97.4	0.105±0.0020	0.240±0.0505
4-Ethylguaiacol	0.002–0.319	1.000	99.5	2.211±0.0121	0.112±0.0017
Octanoic acid	0.027–1.5	0.999	99.0	1.461±0.0212	0.079±0.0113
4-Ethylphenol	0.009–0.319	0.993	97.4	2.570±0.0720	0.013±0.0027
Decanoic acid	0.002–0.303	0.996	98.8	18.721±0.3311	0.073±0.0454

Good recoveries have been obtained, only 2,3-butanediol and 3-hydroxy-2-butanone presented low recoveries (60 and 57%, respectively). Rocha et al. [18] found, using a polyacrylate fibre for analysing different chemical classes of the aroma compounds of wine (monoterpenoids, aliphatic and aromatic alcohols, and esters), that the quantitation by SPME was influenced by the overall composition of aromatic compounds. The compounds subjected to most influence were those with a low slope of the GC peak area versus their concentration. 2,3-Butanediol and 3-hydroxy-2-butanone presented the lowest slope values (0.0018, and 0.0015, respectively). These values appear to be related to the characteristics of each compound, such as molecular mass, boiling point, molecular structure, solubility in the liquid matrix, FID response, and tendency to absorption by the fibre coating. For these compounds, the conjunction of all of these characteristics could explain their low recoveries. Further experiments would be necessary to determine the reason for this and to improve their recoveries.

The CAR–PDMS fibre is coated with porous carbon which makes it suitable for analysing more or less volatile compounds at trace levels. It has a higher efficiency than other fibres owing to its coating (its high porosity provides a large surface area), which would explain these good recoveries for practically all the studied compounds under the sampling conditions employed.

3.1.3. Repeatability and reproducibility

In previous studies it had been reported that the repeatability of this type of fibre is low [19,20]. This disadvantage has been also observed for other types of fibre [21]. In earlier work carried out in our laboratory [12], low repeatability was also observed for some of the aromatic compounds considered. Here, the repeatability and reproducibility have been

Table 2
Performance characteristics

Compound	Analytical sensitivity	Detection limit (LOD, mg/l)	Quantitation limit (LOQ, mg/l)	Recovery (%)
n-Butyl acetate	0.035	0.101	0.336	97.4
Ethyl pentanoate	0.022	0.063	0.209	99.7
2-Methyl-1-propanol	0.295	0.853	2.841	99.6
Isoamyl acetate	0.133	0.382	1.270	93.1
Ethyl hexanoate	0.004	0.010	0.034	101.1
2-Methyl-1-butanol	0.060	0.173	0.578	99.4
Isoamyl alcohol	0.078	0.223	0.743	105.1
3-Hydroxy-2-butanone	7.951	22.701	75.802	57.0
2-Furancarboxaldehyde	0.038	0.100	0.355	93.3
Benzaldehyde	0.004	0.011	0.035	90.2
2,3-Butanediol	5.632	16.512	56.102	60.8
Ethyl decanoate	0.002	0.004	0.014	91.9
Isopentanoic acid	1.201	3.470	11.613	94.3
Diethyl succinate	0.037	0.108	0.358	92.1
Benzyl acetate	0.006	0.015	0.051	98.6
Ethyl-2-phenyl acetate	0.002	0.007	0.023	90.6
Phenylethyl acetate	0.027	0.076	0.245	91.9
Hexanoic acid	0.028	0.076	0.274	90.1
α-Ionone	0.002	0.005	0.018	109.0
Benzyl alcohol	0.017	0.049	0.163	90.0
2-Phenylethanol	1.001	2.731	8.001	96.7
4-Ethylguaiacol	0.002	0.006	0.020	93.1
Octanoic acid	0.021	0.059	0.185	90.6
4-Ethylphenol	0.002	0.006	0.020	95.0
Decanoic acid	0.004	0.010	0.038	89.7

evaluated by means of a series of five extractions of a commercial sherry wine vinegar performed using three different fibres. The mean concentration for all the identified aroma compounds, with their relative standard deviation (RSD) were calculated (Table 3). The RSD obtained for each fibre ranges between 2.5 and 20%. The inter-fibre accuracy showed RSD values higher than intra-fibre accuracy (4.6–46%). α-Ionone, 2-methyl-1-butanol, and 3-hydroxy-2-butanone, were the compounds with highest intra-fibre RSD values. For these compounds, the peaks of other compounds interfere with their determination because the retention times are very similar, and this could explain their high intra-fibre RSD values. Fibre-to-fibre variation has been recognised as a problem in quantitative analysis [22]. Considerable differences were observed between the responses of the three CAR–PDMS fibres employed, which corroborates the low reproducibility of this type of fibre. Because of this, the complete set of experiments should be performed with a single fibre or a control sample should be used.

3.2. Determination of aromatic compounds in vinegars

This analytical method was used to analyse a variety of Sherry vinegar samples supplied by different producers. Each sample was analysed in triplicate.

The mean results obtained for some of the vinegar samples are shown in Table 4. The major volatile compounds quantified in these samples were 2-methyl-1-propanol, 2- and 3-methyl-1-butanol, 3-hydroxy-2-butanone, 2-phenylethanol, isoamyl acetate, 2,3-butanediol, and isopentanoic acid. 2- and 3-methyl-butanol have been found in other wine vinegars in a range of 10–100 mg/l [17]. The 3-hydroxy-2-butanone content found in this type of samples ranged from 100 to 800 mg/l, with higher

Table 3
Intra- and inter-fibre repeatability study

Compound	Fibre 1 RSD (%)	Fibre 2 RSD (%)	Fibre 3 RSD (%)	Fibres 1–3 RSD (%)	Mean concentration (mg/l)
n-Butyl acetate	11.5	16.1	13.2	13.4	0.683
Ethyl pentanoate	5.4	4.3	6.0	8.0	0.301
2-Methyl-1-propanol	11.9	9.4	10.1	10.4	7.051
Isoamyl acetate	4.5	3.7	2.0	7.7	3.931
Ethyl hexanoate	2.5	7.9	5.0	4.6	0.036
2-Methyl-1-butanol	8.4	17.2	15.2	15.8	5.391
Isoamyl alcohol	10.2	10.2	9.6	9.6	16.920
3-Hydroxy-2-butanone	17.0	6.2	20.0	33.2	192.410
2-Furancarboxaldehyde	5.7	6.5	5.2	19.1	2.410
Benzaldehyde	5.0	5.8	6.1	22.6	0.060
2,3-Butanediol	10.1	7.9	13.1	12.7	236.011
Ethyl decanoate	11.7	12.8	9.6	40.8	0.050
Isopentanoic acid	19.3	12.3	6.3	24.7	55.362
Diethyl succinate	5.0	12.2	8.0	46.5	0.442
Benzyl acetate	10.9	13.2	7.5	21.7	0.080
Ethyl-2-phenyl acetate	2.4	9.0	4.2	16.3	0.049
Phenylethyl acetate	3.6	5.4	7.8	15.2	0.919
Hexanoic acid	16.3	6.4	13.1	24.4	0.497
α-Ionone	25.8	19.0	21.2	37.3	0.032
Benzyl alcohol	15.0	10.2	9.8	26.6	0.263
2-Phenylethanol	13.7	10.4	7.5	17.9	17.221
4-Ethylguaiacol	5.3	6.3	8.2	16.1	0.071
Octanoic acid	6.2	7.1	6.2	7.2	1.391
4-Ethylphenol	5.9	6.9	7.1	18.3	0.063
Decanoic acid	3.2	6.3	4.5	11.1	0.309

concentrations, in general, for vinegars aged in wood (illustrative data only, because of its low recovery). 4-Ethylguaiacol and 4-ethylphenol had already been identified in red wine vinegars [23]. Among the esters identified, the major compounds were diethyl succinate, 2-phenylethyl acetate, isoamyl acetate, and *n*-butyl acetate. These result from the fermentation of alcohols or by the reaction of acids with alcohols during aging. In general, the quantitative data obtained for these compounds in this study agree with the results found in the literature [16,17]. Some compounds identified by other authors in wine vinegar [23] have not been found in the samples considered in this study. This could be due to losses during the SPME process or because these compounds were not present in the studied vinegar samples. Further research is required in order to confirm these facts and to establish the statistically significant differences between wine vinegar ageing in wood and without ageing. This will be the aim of a new study in which, this analytical methodology will also be applied to vinegar samples obtained from different raw materials (white and red wine, cider, malted barley, honey, pure alcohol, etc.) and by different processes of ageing.

4. Conclusions

Under the experimental conditions used in this study, HS-SPME using a Carboxen–polydimethylsiloxane fibre is considered an appropriate technique for the analysis of aroma compounds in vinegars. It is a very simple, solventless and fast technique. The detection and quantitation limits, and the recoveries obtained for various aroma compounds are adequate for their quantitation in wine vinegars. However, the fibre-to-fibre variation should be taken into account.

Table 4
Concentrations of aromatic compounds (mg/l) and standard deviations found in Sherry wine vinegars with (samples 1–4) and without (samples 5–8) ageing in wood

Concentration±SD (mg/l) Compound	1	2	3	4	5	6	7	8
n-Butyl acetate	1.2±0.05	1.8±0.01	1.4±0.21	1.0±0.13	1.3±0.12	2.8±0.21	0.9±0.12	0.7±0.09
Ethyl pentanoate[c]	633±93.1	678±30.5	356±63.4	323±34.3	110±4.3	68±2.1	84±5.2	91±3.4
2-Methyl-1-propanol	8.8±2.10	16.1±2.81	8.69±1.22	7.6±2.22	8.5±1.93	13.0±1.65	8.0±1.01	12.8±2.86
Isoamyl acetate	7.7±0.60	7.0±0.71	5.9±0.90	4.9±0.04	5.2±0.51	13.3±1.00	3.0±0.87	2.7±0.87
2-Methyl-1-butanol	52.1[a]±2.00	8.9±0.59	3.0±0.21	3.5±0.86	4.1±0.57	6.1±1.09	3.0±0.48	5.9±0.78
3-Methyl-1-butanol	52.8[a]±1.88	21.8±2.01	17.3±0.68	26.1±1.12	9.0±1.27	39.6±2.20	13.0±1.59	15.2±0.50
Ethyl hexanoate[c]	36±2.1	nd	36±12.2	nd	55±8.7	nd	nd	75±12.2
3-Hydroxy-2-butanone	830±50.1	344±51.0	567±35.1	81±17.8	287±25.3	113±13.5	179±25.0	292±18.9
2-Furancarboxaldehyde	0.4±0.02	1.5±0.85	8.0[a]±1.02	0.1[b]±0.00	0.6±0.09	2.2±0.02	0.4±0.02	1.2±0.04
Benzaldehyde[c]	137±27.0	106±36.2	1070[a]±70	54±5.0	489±46.1	276±18.6	44±0.1	166±38.3
α-Ionone[c]	38±12.2	19±7.8	23±11.5	nd	nd	18±9.6	20±9.9	nd
2,3-Butanediol	284±23.1	260±12.6	108±15.1	95±1.0	353±10.3	137±14.1	375±7.5	177±9.1
Ethyl decanoate[c]	8[b]±1.2	16±2.5	31±9.6	51±7.8	54±10.2	nd	20±7.0	21±3.0
Isopentanoic acid	36.1±1.50	67.8±2.31	51.0±0.88	20.1±2.10	71.1±3.09	51.6±5.11	72.3±2.40	79.8±3.11
Diethyl succinate	2.0±0.56	1.0±0.10	2.6±0.78	0.6±0.09	1.1±0.04	0.6±0.15	1.5±0.21	1.7±0.31
Benzyl acetate[c]	75±12.3	163±23.5	223±75.4	52±5.4	224±69.1	54±0.1	138±33.4	109±12.4
Ethyl-2-phenyl acetate[c]	132±32.2	40±5.4	43±7.8	55±1.0	55±0.6	25±7.4	48±7.4	49±4.1
Phenylethyl acetate	1.2±0.21	1.4±0.05	4.8[a]±0.98	0.5±0.13	3.1±0.54	2.2±0.36	1.5±0.05	2.1±0.13
Hexanoic acid[c]	39[b]±8.7	374±87.0	730±52.3	63[b]±10.2	603±35.1	438±87.3	521±74.2	3150±60
Benzyl alcohol[c]	421±12.8	808±45.3	656±4.1	81[b]±7.0	613±24.3	73[b]±10.3	294±48.1	1980±50.1
2-Phenylethanol	29.7±1.21	18.1±0.50	52.9±1.01	12.2±0.92	32.7±1.17	27.9±0.32	21.1±0.59	69.4[a]±1.41
4-Ethylguaiacol[c]	62±5.0	25±1.4	18±2.0	3[b]±1.2	37±1.5	24±4.8	49±3.1	90±6.5
Octanoic acid	1.0±0.10	1.0±0.08	1.6±0.32	0.7±0.21	1.6±0.08	1.5±0.32	1.2±0.12	1.1±0.23
4-Ethylphenol[c]	111±36.0	218±45.7	244±63.2	82±4.1	193±54.4	186±9.3	353±12.3	565[a]±9.2
Decanoic acid[c]	184±21.3	91±1.2	308±2.3	57±4.1	124±5.3	48±1.2	137±21.3	566[a]±9.1

[a] Values out of the studied range.
[b] Values lower than LOQ.
[c] Values in μg/l; nd: not detected.

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