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Food Chemistry 94 (2006) 143–150
Food Chemistry
www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods

Effect of the matrix volatile composition in the headspace solid-phase microextraction analysis of extra virgin olive oil

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Received 28 April 2004; received in revised form 21 December 2004; accepted 29 December 2004

Abstract

The effectiveness of headspace solid-phase microextraction (HS-SPME) for the quantitative analysis of extra virgin olive oil volatiles was investigated on 44 standard compounds, using an adsorbent polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber. The method's sensitivity was satisfactory, as was its repeatability. However, when the extraction was carried out on mixtures containing all the standard analytes, phenomena of coating saturation and competition between components caused losses in linearity at lower levels of concentration, thus distorting the quantitative evaluation. Coating saturation or displacement between components was also found to be responsible for the bias in the quantitative determinations when extra virgin olive oil samples were analysed. These limitations were overcome by diluting the oil at concentrations not exceeding the total capacity of the fiber coating and until the quantity of displacing compounds was reduced to sufficiently low levels.

Keywords: Extra virgin olive oil; Headspace; SPME; Volatiles

1. Introduction

The fragrant, unique flavour of extra virgin olive oil represents one of the most important qualitative aspects of this vegetable oil, and plays a major role in consumer approval. Although a full description of the organoleptic characteristics of the oil is only obtainable through sensory analysis, the quali–quantitative determination of the volatile compounds can provide very useful information on product quality.

The composition of the headspace of an extra virgin olive oil is highly complex. It is composed of over 100 components, most of which are present in very low concentrations, just a few ppm or even less (Angerosa, Basti, Vito, & Lanza, 1999; Angerosa, Mostallino, Basti, Vito, & Serraiocco, 2000; Morales & Aparicio, 1996). For this reason, an extraction–concentration of volatiles prior to GC analysis is required, usually carried out using dynamic headspace sampling techniques (Angerosa, Di Giacinto, & D'Alessandro, 1997; Morales, Aparicio, & Rios, 1994).

An alternative to dynamic headspace analysis is headspace solid-phase microextraction (HS-SPME). It is a simple, effective adsorption/desorption technique that integrates sampling, extraction, concentration, and sample introduction into a single step without the use of solvents. This method has been successfully employed for analysing flavor compounds in many foods and beverages prior to GC analysis (Harmon, 1997; Steffen & Pawlinszyn, 1996).

In recent years, a number of works have been published on the utilization of HS-SPME for the analysis of vegetable oil volatile compounds. Some were oriented towards the study of off-flavour compounds in refined

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doi:10.1016/j.foodchem.2004.12.039

oil (Doleschall, Recseg, Kemeny, & Kovart, 2003; Jelen, Obuchowska, Zawirska-Wojtasiak, & Wasowicz, 2000; Keszler, Heberger, & Gude, 1998; Palm, 2002), while others more specifically addressed the study of aroma compounds in extra virgin olive oils (Contini, De Santis, Frangipane, Carlini, & Anelli, 2000; Koprivnjak, Conte, & Totis, 2002; Servili, Selvaggini, Jalali, & Montedoro, 1997; Tura, Prenzler, Bedgood, Antolovich, & Robards, 2004; Vichi et al., 2003). In these studies, SPME fibers having either absorbent or adsorbent-coating were used, even if it is now ascertained that the absorbent-type fibers are insufficiently sensitive and thus inadequate for the analysis of olive-oil compounds present at trace levels (Contini et al., 2000; Doleschall et al., 2003; Jelen et al., 2000; Vichi et al., 2003). Consequently, the adsorbent fibers are the only ones utilisable for a global analysis of the headspace of olive oils.

Analytes are extracted by the absorbent-type fibers through partitioning, and there is virtually no competition between compounds. On the contrary, in adsorbent-type fibers the extraction can be accomplished by capturing the compounds in internal pores which, being irregular, trap the analytes in function of their size (Shirey & Mindrup, 2000). Since the number of pores is limited, the analytes may complete; this can result in a reduction in coating capacity and/or the displacement of some compounds by others (Roberts, Pollien, & Milo, 2000; Shirey et al., 2000). For these reasons, adsorbent-type fibers are characterised by their marked ability to capture small quantities of analytes (particularly indicated for the analysis of trace components) but they may be easily saturated and have a shorter linear range compared to absorbent fibers. Therefore, when adsorbent SPME fibers are utilized, fiber capacity and displacement effects need to be carefully evaluated, because they can prejudice the quantitative determination of compounds.

In the headspace analysis carried out using SPME adsorbent fibers, such as PDMS–DVB and DVB–CAR–PDMS, some researchers have reported competition effects between analytes (Roberts et al., 2000) or anomalous linearity deviations in relation to the sample matrix composition (Mestres, Busto, & Guasch, 2002; Vichi et al., 2003). Nevertheless, no specific studies have as yet been carried out in order to determine, assess and eventually overcome the possible negative effects these phenomena have on quantitative dosage, when the HS-SPME technique is employed for the analysis of extra virgin olive oil flavour compounds.

The aim of the present study was to test the effectiveness of an adsorbent-type SPME fiber for analysing some of the most common volatile flavour compounds of extra virgin olive oil, and to evaluate the possible effects of the matrix volatile composition on the quantitative determination of the compounds tested.

2. Materials and methods

2.1. Oil samples

Five extra virgin olive oil samples, produced in different Italian regions (Puglia, Calabria, Lazio, Umbria and Toscana) were used. They were purchased locally on the market and their origin was certified from the DOP (Protect Denomination of Origin) European quality trademark. The oils were selected for their flavourful characteristics, as resulted by sensory analysis carried out by a panel composed of eight assessors trained to perform the official European sensor analysis of extra virgin olive oils.

Freshly deodorized olive oil, periodically tested for the presence of interfering compounds, was employed as the solvent for diluting the oils and preparing the standard solutions.

2.2. Standard compounds and mixtures

Forty-four standard compounds were tested (Table 1), selected from those typically found in extra virgin olive oil headspace (Servili et al., 1997; Vichi et al., 2003). They were purchased from Sigma Chemical Co. (St. Louis, USA), Aldrich (Steinheim, Germany), Fluka Co. (Buchs, Switzerland), and Merck KGaA (Darmstadt, Germany), selecting the highest purity available on the market which, in all cases, was over 98%. The standards, either individually or in mixtures, were spiked in deodorized olive oil at an initial concentration of around 1000 ppm; the other concentrations were obtained by subsequent dilutions.

2.3. Extraction of volatiles by SPME

Extraction/concentration of volatiles was carried out with a 65 μm PDMS/DVB fiber (Supelco/Co., Bellefonte, PA, USA), according to the conditions previously selected (Contini et al., 2000) though slightly modified. The sample (10 ± 0.01 g) was placed in a 20 mL glass vial equipped with screw cap and silicon septum. SPME fiber was exposed to the headspace of the sample for 90 min at 40 ± 0.5 °C. During this time, the sample was constantly stirred with a magnetic stirrer. After sampling, the fiber was placed into the injection port of the GC system, equipped with a 0.75 mm i.d. liner, where it was desorbed in splitless mode for 7 min at 240 °C. All the analyses were run in duplicate.

2.4. GC analysis

The desorbed compounds were separated on a Mega Series 5300 Gas chromatograph (Carlo Erba Instruments, Milan, Italy) equipped with a 60 m × 0.25 mm i.d., film thickness 0.25 μm, AT-WAX column (Alltech

Table 1
Standard compounds tested

No.	Compound
1	Octane
2	Acetone
3	Butanal
4	Ethyl acetate
5	2-Butanone
6	2-Methyl-butanale
7	3-Methyl-butanale
8	Ethanol
9	3-Pentanone
10	1-Penten-3-one
11	1-Propanol
12	2-Butenal
13	Butyl acetate
14	Hexanal
15	2-Methyl-1-propanol
16	Ethyl benzene
17	*E*-2-Pentenal
18	1-Butanol
19	1-Penten-3-ol
20	Heptanal
21	Limonene
22	3-Methyl-1-butanol
23	*E*-2-Hexenal
24	1-Pentanol
25	Hexyl acetate
26	Terpinolene
27	Octanal
28	*E*-2-Penten-1-ol
29	*Z*-3-Hexenyl acetate
30	*Z*-2-Penten-1-ol
31	*E*-2-Heptenal
32	*E*-2-Hexenyl acetate
33	1-Hexanol
34	*E*-3-Hexen-1-ol
35	*Z*-3-Hexen-1-ol
36	Nonanal
37	*E*-2-Hexen-1-ol
38	2,4-Hexadienal
39	*E*-2-Octenal
40	Acetic acid
41	*E*-2-Nonenal
42	1-Octanol
43	Ethyl decanoate
44	1-Nonanol

Ass. Inc., Deerfield IL, USA) and a flame ionization detector at 260 °C. Gas chromatograph conditions were the following: oven temperature was initially kept at 40° for 7 min, then raised by 3 °C min^{-1} to 260 °C. Helium at 0.9 mL min^{-1} (30 cm s^{-1}) was used as the carrier gas.

2.5. Calibration curves and upper limits of the linear range

Calibration curves of the individually-tested standards were drawn by plotting the detector response measured over a series of concentrations ranging from around 0.1 to 20 ppm. Calibration curves of mixtures containing all the analytes were drawn by plotting the detector response measured over a series of concentrations from around 0.5 to 100 ppm total (approximately from 0.12 to 2.3 ppm for each volatile).

The upper limits of the linear range were determined by plotting the peak areas against the concentrations of the individually-tested compound. According to other Authors (Steffen et al., 1996), the range over which the r^2 value was found approximately 0.99, was considered to be the linear range.

2.6. Precision of the method and limit of quantitation (LOQ)

The precision of the method was determined by performing five replicate extractions of single compounds spiked at about 0.1 ppm in deodorized olive oil.

The limits of quantitation were determined on the basis of the calibration straight obtained by exposing the fiber to the headspace of deodorized olive oil, spiked with individual standards at concentrations ranging from about 0.01 to 0.5 ppm.

LOQ was determined as follows:

$$\mathrm{LOQ} = (Y_{\mathrm{LOQ}} - b)/a$$

with

$$Y_{\mathrm{LOQ}} = \text{peak area at LOQ} = b + 10\mathrm{SE}_{x/y},$$

where a is the slope of the calibration curve, b the y-intercept of the calibration curve, $\mathrm{SE}_{x/y}$ is the standard error of linear regression.

3. Results and discussion

From comparative studies effected between SPME fibers having different coating, each tested with standard compounds dissolved in deodorized olive oil, the 2 cm-long DVB–CAR–PDMS and PDMS–DVB fibers showed a greater linearity within a wider interval of concentration (Vichi et al., 2003). These fibers thus result as being the most suitable for quantitative analysis of headspace of olive oil. Notwithstanding a lower extractive efficiency, in this study we opted for testing the PDMS–DVB fiber in as much as it is more versatile. The 2 cm DVB–CAR–PDMS fiber, because of its greater length, in fact, presents a constructive particularity such that it is not recommended for autosampler use; the PDMS–DVB fiber, instead, is utilizable using either manual or automatic injection techniques.

3.1. Calibration curves and upper limits of the linear range

In order to determine the upper limits of the linear range of the individually-tested compounds, the calibration curves of each of the 44 standard volatiles were drawn, employing concentrations ranging from around 0.1 to 20 ppm.

It was found that the calibration curves of 27 tested compounds were linear up to the maximum concentration tested whereas the upper limits of the linear range of the other compounds were approximately between 7 and 18 ppm (Table 2).

In order to study the occurrence of saturation effects and displacement between compounds, caused by the simultaneous presence of analytes, standard mixtures containing all 44 tested compounds were analysed. In each mixture, the concentration of individual components was similar, ranging from around 0.012 and 2.3 ppm, for a total concentration of around 0.5 and 100 ppm.

In the ranges considered, when there is no competition nor saturation phenomena, the calibration curve of each analyte should maintain the same slope found for each component. The results of the analysis (data not reported) showed that only seven analytes (ethanol, *Z*-3-hexenyl acetate, nonanal, acetic acid, *E*-2-nonenal and 1-nonanol) were not at all influenced by the presence of other compounds. Linearity of the other 42 compounds was lost at concentrations of mixture ranging between approximately 5 and 50 ppm total. Therefore, the capacity of PDMS/DVB fiber may be inadequate for the quantitative extraction of all the volatiles usually found in extra virgin olive oils, at overall concentrations

Table 2
Upper limits of the linear range, limits of quantitation (LOQs), and CV% of the standard compounds analysed by HS-SPME

No.	Compound	Upper limits of the linear range (ppm)	LOQs (ppb)	CV%
1	Octane	7.1	1.9	4.12
2	Acetone	([a])	29.7	3.92
3	Butanal	([a])	2.0	4.37
4	Ethyl acetate	([a])	4.9	3.29
5	2-Butanone	([a])	18.5	2.39
6	2-Methyl-butanale	([a])	6.5	4.83
7	3-Methyl-butanale	([a])	6.9	1.39
8	Ethanol	([a])	74.9	8.84
9	3-Pentanone	17.5	3.5	8.03
10	1-Penten-3-one	([a])	3.3	8.14
11	1-Propanol	([a])	6.1	6.13
12	2-Butenal	([a])	7.3	3.84
13	Butyl acetate	9.8	3.7	7.90
14	Hexanal	([a])	0.4	3.53
15	2-Methyl-1-propanol	([a])	4.3	5.22
16	Ethyl benzene	12.0	0.9	5.59
17	*E*-2-Pentenal	([a])	3.6	3.33
18	1-Butanol	([a])	2.1	4.06
19	1-Penten-3-ol	([a])	3.6	1.52
20	Heptanal	([a])	9.0	4.97
21	Limonene	17.7	1.6	6.34
22	3-Methyl-1-butanol	17.3	7.4	2.17
23	*E*-2-Hexenal	15.2	4.9	3.94
24	1-Pentanol	17.3	2.8	2.63
25	Hexyl acetate	11.8	7.6	3.99
26	Terpinolene	([a])	7.1	2.47
27	Octanal	14.7	0.9	4.23
28	*E*-2-Penten-1-ol	([a])	5.0	3.66
29	*Z*-3-Hexenyl acetate	([a])	4.9	4.68
30	*Z*-2-Penten-1-ol	([a])	2.8	4.06
31	*E*-2-Heptenal	12.3	5.0	1.61
32	*E*-2-Hexenyl acetate	14.8	3.1	1.88
33	1-Hexanol	12.7	2.5	2.64
34	*E*-3-Hexen-1-ol	15.0	0.7	3.61
35	*Z*-3-Hexen-1-ol	17.1	7.8	4.26
36	Nonanal	([a])	9.9	5.00
37	*E*-2-Hexen-1-ol	15.5	13.4	5.79
38	2,4-Hexadienal	15.4	6.5	2.73
39	*E*-2-Octenal	([a])	4.5	3.97
40	Acetic acid	([a])	12.5	4.62
41	*E*-2-Nonenal	([a])	5.7	5.06
42	1-Octanol	([a])	11.9	4.79
43	Ethyl decanoate	([a])	13.6	7.21
44	1-Nonanol	([a])	6.2	6.30

[a] Linear throughout the tested range (around 0.1–20 ppm).

of about 10–50 ppm (Angerosa et al., 2000; Morales et al., 1996).

While the loss of linearity detected at high total concentrations is plausibly due to the saturation of fiber sites, the reduction in the linear range observed at low total concentrations is probably due to displacement phenomena between analytes. In such complex mixtures, it is difficult to identify the analytes responsible for displacement and the components they displace. It should be noted that the concentrations of the compounds in the tested mixtures do not reflect the natural composition of extra virgin olive oils, where, except for *E*-2-hexenal, all the other volatiles are at very low concentrations; moreover, many other compounds besides those we tested, may be present in the oil's headspace.

3.2. Precision of the method and limits of quantitation

The CV% values and the quantitation limits of each of the 44 volatiles are reported in Table 2.

The repeatability of the method depended on the compound; on average, the CV value was 4.4%, ranging between 1.4% and 9.7%, similar to the findings of other Authors (Vas, Koteleky, Farkas, Dobo, & Vekey, 1998).

The method enabled the quantification of volatiles at concentrations below 10 ppb, for the most part; only ethanol and acetone had very high limit of quantitation, 74.9 and 29.7 ppb, respectively.

3.3. Sensitivity of the method

The slope of the calibration curve in the linear range gives an indication of the sensitivity of the analytical method (Fig. 1). At the same concentration, compounds with higher slopes gave a higher GC response and the method enabled small variations in concentration to be better appreciated. HS-SPME analysis was found to be particularly sensitive with respect to octane and butyl acetate, whereas it was less sensitive with regard to acetic acid, acetone, ethanol and ethyl decanoate.

3.4. Quantitative analysis of extra virgin olive oils

In order to evaluate matrix volatile composition effects when oil samples are analysed, and determine whether the possible effects of competition between analytes can be overcome by diluting the sample matrix, SPME analysis of five flavourful extra virgin olive oils, undiluted and diluted with deodorized olive oil (1:2; 1:5; 1:7; 1:10), was carried out. It was indeed observed that competition phenomena occur once the concentration of the single compound exceeds the upper limit of the linear range (Roberts et al., 2000; Shirey et al., 2000), and that the displacing could be controlled by reducing the possibility of fiber overloading. Alternative methods to reducing the amount of analyte adsorbed on the coating are: reduction of the exposure time of the fiber; reduction of the volume of oil sample or dilution of the sample. SPME extraction performed on fixed sample volumes in more or less diluted form, depending on requirements, is the only means of enabling the optimisation of every single analysis, using the same calibration curves for all the analyses effected. This was the reason why we preferred to test the effectiveness of dilution rather than resort to other ways of reducing the amount of analytes captured by the fiber.

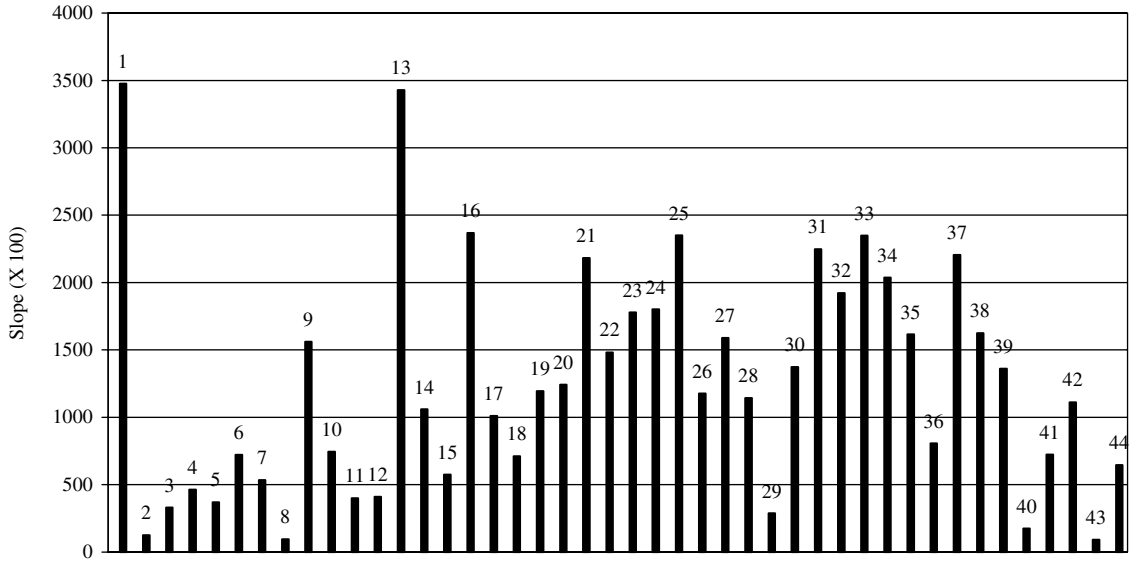

Fig. 1. Slope values of the calibration curves obtained by plotting the peak areas of compounds against the concentrations. The numbers are identified in Table 1.

The chromatograms obtained from the GC analysis of an undiluted and a 1:7 diluted extra virgin olive oil are given in Fig. 2. Peak identification was based on both comparing the retention times of the standard compounds and fortifying the oils with the tested analytes. An additional control was carried out, by comparing the order of elution of the compounds with those obtained from the bibliography.

In Table 3, the results of the quantitative dosage carried out on the extra virgin olive oil sample with the highest total concentration of the tested analytes (about 44 ppm total) are reported. The findings showed that the

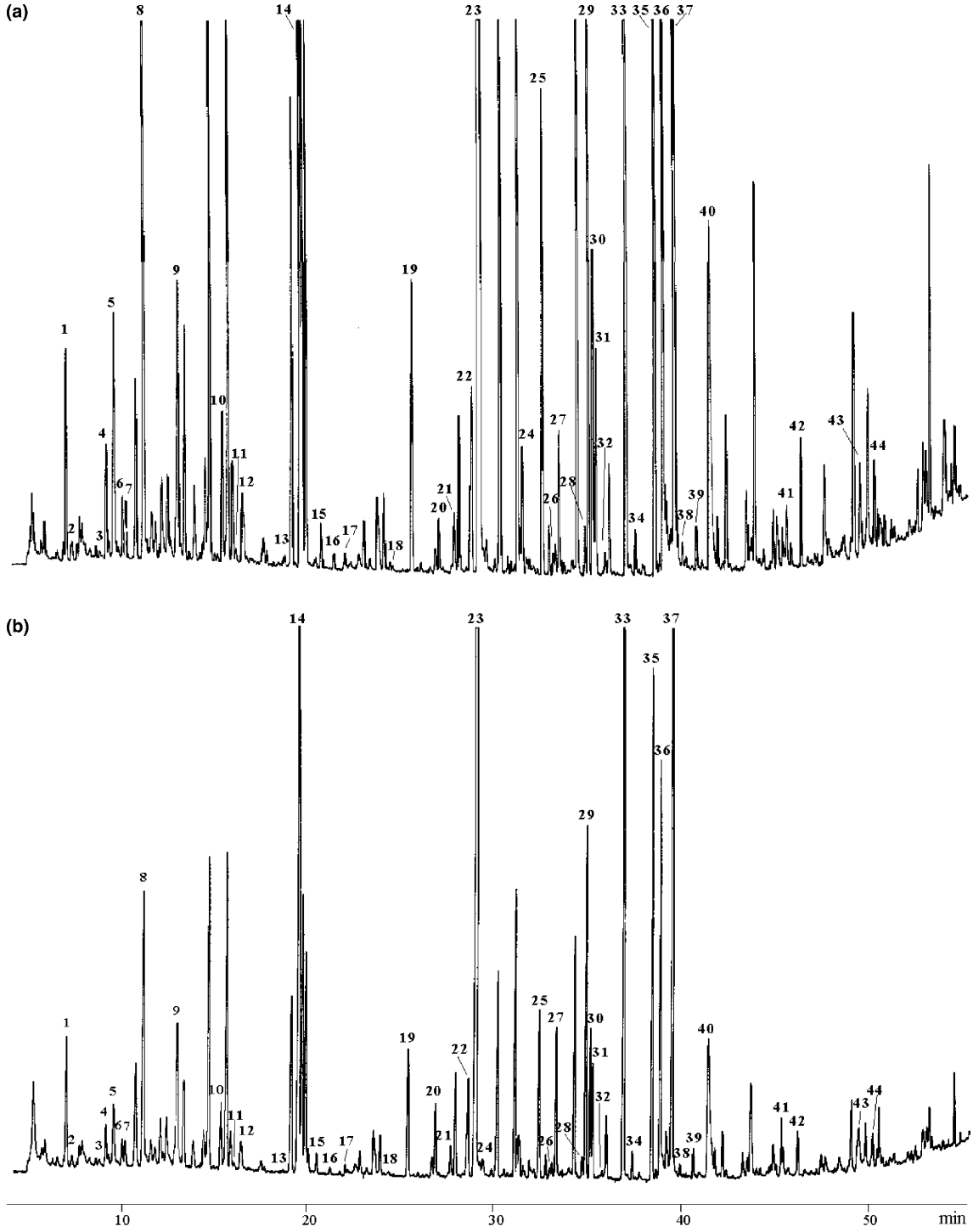

Fig. 2. Chromatograms resulting from the HS-SPME analysis of an undiluted (a) and 1:7 diluted (b) extra virgin olive oil. For peak identification, see Table 1.

volatiles quantified on both undiluted and insufficiently diluted samples were underestimated and that the underestimation varied to a lesser or greater extent in relation to the analyte. Similar results were obtained for the other extra virgin olive oils tested. A stable estimation of the target volatiles was obtained using a minimum dilution of 1:7, whereas the further dilution (1:10) did not produce quantitative increases. The same dilution, 1:7, was also effective in the case of the other extra virgin olive oils analysed, which were constituted of flavourful oils (as resulted by sensory analysis), with quite a high total concentration of investigated volatile compounds (comprised between 35 and 43 ppm).

The results indicate that, by diluting the oil to concentrations not exceeding the total capacity of the fiber coating and until the quantity of displacing compounds is reduced to sufficiently low levels, interferences can be removed and a quantitative dosage of the tested compounds is made possible. However, oil dilution may excessively reduce the quantity of some particularly low-concentrated analytes. In the example reported, butanal was quantifiable only in undiluted oil (although

Table 3
Volatile compounds content (ppm) of an undiluted and diluted extra virgin olive oil analysed by HS-SPME

No.	Compound	Oil dilution				
		–	1:2[a]	1:5[a]	1:7[a]	1:10[a]
1	Octane	0.027	0.057	0.112	0.114	0.110
2	Acetone	0.121	0.187	0.299	0.304	0.301
3	Butanal	0.004	([b])	([b])	–	–
4	Ethyl acetate	0.129	0.149	0.235	0.231	0.238
5	2-Butanone	0.369	0.492	0.544	0.549	0.540
6	2-Methyl-butanale	0.040	0.045	0.054	0.055	([b])
7	3-Methyl-butanale	0.046	0.072	0.089	0.117	0.120
8	Ethanol	3.944	4.881	5.449	6.355	6.350
9	3-Pentanone	0.114	0.248	0.324	0.398	0.402
10	1-Penten-3-one	0.119	0.187	0.286	0.283	0.283
11	1-Propanol	0.043	0.054	0.064	0.065	0.062
12	2-Butenal	0.045	0.323	0.391	0.387	0.393
13	Butyl acetate	0.005	0.008	([b])	([b])	–
14	Hexanal	0.483	0.792	1.919	2.273	2.269
15	2-Methyl-1-propanol	0.037	0.058	0.067	0.070	0.068
16	Ethyl benzene	0.006	0.008	0.010	0.009	([b])
17	*E*-2-Pentenal	([b])	0.022	0.042	0.042	0.040
18	1-Butanol	([b])	–	–	–	–
19	1-Penten-3-ol	0.160	0.251	0.335	0.380	0.377
20	Heptanal	0.017	0.036	0.103	0.140	0.138
21	Limonene	0.021	0.032	0.049	0.048	0.050
22	3-Methyl-1-butanol	0.070	0.112	0.163	0.177	0.179
23	*E*-2-Hexenal	([c])	9.852	13.429	16.079	16.110
24	1-Pentanol	0.018	0.026	0.030	0.045	0.044
25	Hexyl acetate	0.133	0.195	0.223	0.220	0.225
26	Terpinolene	0.028	0.040	0.049	0.050	([b])
27	Octanal	0.041	0.050	0.058	0.060	0.058
28	*E*-2-Penten-1-ol	0.026	0.039	0.048	0.058	0.058
29	*Z*-3-Hexenyl acetate	2.084	2.949	3.394	3.388	3.391
30	*Z*-2-Penten-1-ol	0.157	0.269	0.386	0.383	0.381
31	*E*-2-Heptenal	0.061	0.086	0.137	0.139	0.140
32	*E*-2-Hexenyl acetate	0.009	0.014	0.024	0.023	([b])
33	1-Hexanol	0.582	1.050	1.268	1.666	1.659
34	*E*-3-Hexen-1-ol	0.010	0.021	0.040	0.039	([b])
35	*Z*-3-Hexen-1-ol	0.342	0.599	0.685	0.884	0.880
36	Nonanal	0.359	0.386	0.587	0.591	0.588
37	*E*-2-Hexen-1-ol	0.921	1.532	2.031	2.278	2.281
38	2,4-Hexadienal	0.020	0.036	0.066	0.125	0.123
39	*E*-2-Octenal	0.017	0.027	0.052	0.063	0.060
40	Acetic acid	2.336	3.367	5.064	5.345	5.350
41	*E*-2-Nonenal	0.074	0.187	0.269	0.272	0.270
42	1-Octanol	0.068	0.080	0.095	0.096	([b])
43	Ethyl decanoate	0.126	0.149	0.184	0.186	0.183
44	1-Nonanol	0.157	0.167	0.251	0.249	0.247

[a] The values were calculated by multiplying the concentration (estimated on the basis of the calibration curve) by the dilution factor.
[b] Concentration below the LOQ.
[c] Concentration over the upper limit of the linear range.

probably underestimated) and the concentration of butyl acetate was lower than the quantitation limit, when 1:5 or higher dilutions were used.

Furthermore, the results of this study suggest some of further considerations in a broader sense. With regards to the quantitative HS-SPME analysis, effected on particularly complex matrixes, as food products generally are, it is indispensable that the method is accurately tested on the basis of the specifically adopted operative conditions. One must verify the sensitivity of a given adsorbent coating, but also its global capacity and the eventuality that displacement between analytes can occur. The common habit of selecting analytical conditions so as to maximize the quantity of extracted compounds, without adequate controls, may in fact lead to gross quantitative errors.

4. Conclusion

SPME is a simple and useful technique for the analyse of extra virgin olive oil flavour compounds. PDMS/DVB coating is effective for extracting many compounds at trace level, but its capacity is limited and it may give rise to displacement phenomena, controllable by resorting to a suitable dilution of the oil sample.

Acknowledgement

This study was supported by a grant from the Provincial Administration of the Lazio region, within the framework of a finalised ''PRAL'' research project.

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