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Flavour analysis of Greek white wine by solid-phase microextraction–capillary gas chromatography–mass spectrometry

Jan C.R. Demyttenaere[b,*], Cynthia Dagher[b,c], Pat Sandra[a], Stamatina Kallithraka[c], Roland Verhé[b], Norbert De Kimpe[b]

[a]*Department of Organic Chemistry, Faculty of Sciences, Ghent University, Krijgslaan 281 (S4), B-9000 Ghent, Belgium*
[b]*Department of Organic Chemistry, Faculty of Agricultural and Applied Biological Sciences, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium*
[c]*Mediterranean Agronomic Institute of Chania, Alsyllion Agrokepion, PO Box 85, GR-73 100 Chania, Crete, Greece*

Abstract

Solid-phase microextraction (SPME) was optimised for the qualitative determination of the volatile flavour compounds responsible for the aroma of Greek Boutari wine. Several factors influencing the equilibrium of the aroma compounds between the sample and the SPME fiber were taken into account, including the extraction time, the extraction temperature, the sampling mode (headspace and direct immersion or liquid SPME), and the presence of salt. Four different SPME fibers were used in this study, namely poly(dimethylsiloxane) (PDMS), poly(acrylate), carbowax–divinylbenzene and divinylbenzene–carboxen on poly(dimethylsiloxane). The best results were obtained using the PDMS fiber during headspace extraction at 25 °C for 30 min after saturating the samples with salt. The optimised SPME method was then applied to investigate the qualitative aroma composition of three other Greek wines, namely Zitsa, Limnos and Filoni.

Keywords: Wine; Food analysis; Aroma compounds; Volatile organic compounds

1. Introduction

Aroma substances are important in wine as they contribute to the quality of the final product. The combination of different aroma compounds such as alcohols, esters, organic acids, aldehydes, ketones and terpenes forms the character of wine and differentiates one wine from another [1].

Therefore, several analytical methods have been developed for the extraction and determination of wine flavour compounds. These include purge and trap (i.e. dynamic headspace sampling) [1], liquid–liquid extraction [2], solid-phase extraction using XAD-2 and XAD-7 resins [3], simultaneous extraction–distillation and supercritical fluid extraction [4] amongst others, followed by chromatographic determinations. Each sample preparation procedure is subject to its drawbacks, although offering specific advantages under certain circumstances [5]. Most of these methods are very time consuming, requiring exhaustive concentration steps and gas chromatographs equipped with headspace sampling devices. Therefore, it is evident that there is a need for a more rapid and simple technique that will reduce and eliminate these problems [6]. Solid-phase microex-

*Corresponding author. Tel.: +32(0)-9-264-59-64; fax: +32(0)-9-264-62-43.
E-mail address: jan.demyttenaere@rug.ac.be (J.C.R. Demyttenaere).

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traction (SPME) was developed by Arthur and Pawliszyn to overcome these difficulties [7].

Previously several authors have applied SPME to the analysis of biological samples and volatile compounds in food products [8–10]. The use of SPME in wine analysis first focused on analysis of pesticide residues and other contaminants [11–13] and on studies with standard solutions [14–16].

More recently SPME was reported for varietal characterisation of wines [17] and analysis of the wine bouquet using different fibers [18,19]. Vas et al. [20] reported the use of SPME for fast screening of different wine types (Chardonnay, Muscat Ottonel, and Tramini) and applied SPME for the determination of volatiles from red wines produced by carbonic maceration [21]. SPME has also been applied for the analysis of Portuguese muscatel wines [16] and for the classification of Nebbiolo based wines from Piedmont (Italy) [22]. Begala et al. [23] used headspace SPME for the analysis of the aroma constituents of ''Cannonau of Jerzu'' wine, which is a typical Sardinian product, obtained from only one particular grape variety.

Whiton and Zoecklein [24] studied the optimisation of headspace SPME for the analysis of wine aroma compounds, in which the influence of various parameters, such as sampling time, temperature and sample matrix, on the extraction of ten model flavour compounds was investigated. And more recently the application of SPME to the characterisation of varietal wines, using poly(dimethylsiloxane) as stationary phase, was reported [25].

Headspace SPME has also been applied to the determination of specific trace components, such as diacetyl [26], volatile and low volatile sulphides and disulphides [27–30], oak lactones in barrel aged wines [31], the cork taint compound TCA (2,4,6-trichloroanisole) [32], the fungicides cyprodinil and fludioxonil in Spanish white wines [33], vitispirane in sparkling wine [15] and even 3-alkyl-2-methoxypyrazines in Cabernet Sauvignon and Merlot wines [34].

Compared to traditional techniques, especially solid–liquid extraction, liquid–liquid extraction, static and dynamic headspace analysis and distillation extraction, the SPME method has advantages such as high sensitivity and reproducibility, low cost, solvent-free extraction, no need for previous sample preparation and the possibility of automation [35–37].

A new technique has also been developed recently, namely stir bar sorptive extraction (SBSE) [38]. Unlike SPME, which is ideally suited for flavour profiling and analysis of compounds present at higher concentration, SBSE is more sensitive and can be used for trace and ultratrace analysis, such as the detection of dicarboximide fungicides in white wines and sparkling wines of different origin [39] and the determination of TCA in wines [40].

Although the SPME analysis of German [17–19], Portuguese [16], Spanish [25,34], and Italian [22,41] wines has been described, to date no literature is available on the analysis of Greek wines by SPME. The aim of the current work was therefore the systematic optimisation of SPME for the analysis of Greek white wine. Four Greek white wines were selected (Boutari, Zitsa, Limnos and Filoni) and their different aroma components were identified.

2. Experimental

2.1. Description of the samples

Wine samples were purchased from Ets. Marinopoulos, Chania, Crete, Greece. Four different Greek dry white wines of 1999 vintage were used in the analyses. Moschofilero-Boutari wine (11% alcohol) originates from Peloponnese (Mantinean plateau). It comes from the grape ''Moschofilero'' and is selected from the vineyard ''Mantinia''. Monastiri-Zitsa wine (11.5% alcohol) is produced in strictly limited quantities, only from exceptional years. It comes from the grape ''Debina'', carefully hand-picked and selected from the best vineyards in the Zitsa region. Limnos wine (dry white wine, 12.5% alcohol) is selected from the vineyards of Limnos. It comes from the grape ''Moschato Alexandrias''. It is characterised by its fruity delicious aroma of Muscat of Alexandria. Filoni wine (12% alcohol) originates from Kaminia Limnos. It comes from the grape ''Moschato Alexandrias''. All the

samples were products of appellation of origin and bottled in 750-ml flasks.

2.2. SPME extraction and analysis

The SPME holder, for manual sampling, and fibers used in the analyses were purchased from Supelco (Aldrich, Bornem, Belgium).

Four different fibers were tested in order to find the most suitable for analysis: 100-μm poly(dimethylsiloxane) (PDMS), 65-μm carbowax–divinylbenzene (CW–DVB), 85-μm poly(acrylate) (PA) and 50/30-μm divinylbenzene–carboxen on poly(dimethylsiloxane) (DVB–CAR–PDMS). All the needles were 23 gauge (0.64 mm O.D.) except for the CW–DVB fiber (0.56 mm O.D.). The SPME fibers were conditioned as recommended by the manufacturer at some degrees below each fiber's maximum temperature before they were used for the first time. Before the first daily analysis, the fibers were conditioned for 5 min at 250 °C in the GC injector. For the following analyses, 2 min of desorption after each extraction was used as conditioning time.

The fibers were immersed either in the headspace (HS) or in the liquid phase (direct immersion, DI) of the samples. Depending on extraction conditions, different sample volumes were used. For headspace sampling, an aliquot of 10 ml of wine was transferred into a 22-ml vial, while for the liquid SPME, 17 ml of wine sample was used.

Different parameters were studied including the effect of temperature, of time, of salt addition (3 g/10 ml for saturation), the extraction mode (HS or DI) and the type of fiber. When testing the influence of salt addition on the extraction efficiency, only sodium chloride was selected as salt at saturation level. Indeed, it was very recently established by another group that maximum extraction was obtained from salt saturated samples using NaCl [42]. Constant stirring (800 rpm) was applied in all SPME experiments because it has been suggested that the detection signal was doubled after stirring [28].

The vial containing the wine sample was placed in a thermostated bath adjusted to the different temperatures tested and was sealed with a Black Viton septum (Supelco). After every liquid SPME extraction, the fiber was rinsed with distilled water to remove the excess of polar non-volatile compounds (sugars, phenols, etc.) and dried with a lintfree tissue by carefully dipping before desorption. Thermal desorption of the analytes from the fiber inside the GC injection port was carried out in the split mode (1/10) at a desorption temperature of 250 °C during 2 min, because splitless desorption resulted in overloaded chromatograms with broad and distorted peaks. Once a day a blank test was performed by desorbing the fiber for a second time to check possible carry-over. From these results it was clear that carry-over was negligible and that 2-min desorption time was sufficient to desorb the flavour compounds from the fibers.

2.3. Gas chromatography–mass spectrometry

All samples were analysed with an Agilent 6890 Plus gas chromatograph coupled to a 5973 quadrupole mass spectrometer (Agilent). The gas chromatograph was equipped with an HP-5MS capillary column (30 m×0.25 mm I.D.) coated with a 0.25-μm film of stationary phase (PDMS containing 5% phenyl), and the carrier gas used was helium (1.2 ml min^{-1} constant flow). The GC oven temperature was programmed from 40 °C (held for 1 min) at 5 °C min^{-1} to 180 °C, then at 10 °C min^{-1} to 220 °C (held for 2 min). The injector was a CIS-4 PTV (programmed temperature vaporiser, Gerstel) operating in the split mode (split ratio 1/10). The mass spectrometer was operated in electron impact mode (EI, 70 eV) and the masses were scanned over an m/z range of 40–300 amu (2–20 min) and 40–400 amu (20–35 min). A solvent delay time of 2 min was used, to avoid overloading the mass spectrometer with EtOH.

For identification of the wine flavour compounds, a solution of *n*-alkanes (*n*-octane–*n*-hexadecane) in Et_2O (0.01%, v/v) was co-injected in the GC–MS system after desorption of an SPME extract of wine, and the analysis was performed using a linear temperature program from 40 °C (held for 1 min) to 220 °C at 5 °C min^{-1}, then at 10 °C min^{-1} from 220 °C to 240 °C (2 min). For the representative wine flavour compounds, the Kováts retention indexes

were calculated and compared with the literature [43].

3. Results and discussion

3.1. List of target compounds for SPME extraction of Boutari wine

For optimisation of the SPME parameters, 33 congeners were selected from the Boutari wine aroma. They were divided into groups according to three parameters, namely the relative contribution (main vs. minor compound), functional group (alcohol, ester or miscellaneous, i.e. terpene or organic acid) or volatility (volatile, semivolatile or less volatile), in order to cover different ranges of functionality as well as volatility, in analogy with a comparable study [24]. The Kováts retention indices were also calculated for each peak and compared with the literature [43] in order to ensure the correct identification of the compounds (Table 1).

Table 1
Selection of 33 representative wine flavour compounds for SPME method optimisation

Peak no.	Retention time (min)	Kováts retention index	Compound name	Classification		
				Main/minor	Functional group	Volatility
1	2.95	ND	Isoamyl alcohol	Main	Alcohol	Volatile
2	4.08	806	Ethyl butanoate	Minor	Ester	Volatile
3	5.22	854	Ethyl isopentanoate	Minor	Ester	Volatile
4	5.57	870	1-Hexanol	Minor	Alcohol	Volatile
5	5.77	878	Isoamyl acetate	Minor	Ester	Volatile
6	8.30	972	2,2,6-Trimethyl-6-vinyltetrahydropyran	Minor	Miscell	Volatile
7	8.64	984	Hexanoic acid	Minor	Miscell	Volatile
8	8.91	993	Unknown terpene HC	Minor	Miscell	Volatile
9	9.13	1001	Ethyl hexanoate	Main	Ester	Volatile
10	9.54	1015	Hexyl acetate	Minor	Ester	Volatile
11	9.93	1028	D-Limonene	Minor	Miscell	Volatile
12	10.51	1049	(*E*)-β-Ocimene	Minor	Miscell	Volatile
13	11.69	1088	Terpinolene	Minor	Miscell	Volatile
14	12.04	1099	Linalool	Minor	Miscell	Semivolatile
15	12.39	1113	2-Phenylethyl alcohol	Minor	Alcohol	Semivolatile
16	12.78	1126	Methyl octanoate	Minor	Ester	Semivolatile
17	14.21	1175	Octanoic acid	Minor	Miscell	Semivolatile
18	14.42	1182	Diethyl succinate	Minor	Ester	Semivolatile
19	14.66	1191	α-Terpineol	Minor	Miscell	Semivolatile
20	14.92	1199	Ethyl octanoate	Main	Ester	Semivolatile
21	16.54	1257	2-Phenylethyl acetate	Minor	Ester	Semivolatile
22	16.76	1267	Unknown terpene ester	Minor	Ester	Semivolatile
23	17.13	1281	Vitispirane ($C_{13}H_{20}O$)	Minor	Miscell	Semivolatile
24	17.36	1289	Lavandulyl acetate	Minor	Ester	Semivolatile
25	17.59	1294	Ethyl nonanoate	Minor	Ester	Semivolatile
26	19.08	1354	1,2-Dihydro-1,1,6-trimethylnaphthalene	Main	Miscell	Semivolatile
27	19.50	1366	Decanoic acid	Minor	Miscell	Semivolatile
28	19.99	1389	Ethyl 9-decenoate	Main	Ester	Semivolatile
29	20.21	1397	Ethyl decanoate	Main	Ester	Semivolatile
30	21.46	1448	Isoamyl octanoate	Minor	Ester	Less volatile
31	21.68	1456	Unknown compound	Minor	Miscell	Less volatile
32	24.99	1597	Ethyl dodecanoate	Main	Ester	Less volatile
33	26.13	ND	Isoamyl decanoate	Minor	Ester	Less volatile

HC, hydrocarbon; Miscell, miscellaneous (terpene, ether, organic acid, unknown); ND, not determined.

3.2. SPME method optimisation with white Boutari wine

When optimising extraction conditions in any SPME method there are a number of variables that must be considered. The major factors studied in this work include extraction temperature, time, extraction mode (i.e. direct vs. headspace sampling), salt saturation of the samples and fiber coating.

3.2.1. Influence of extraction temperature

The influence of the extraction temperature on the recovery of the volatiles was first investigated using the PDMS fiber, applying headspace extraction of salt saturated samples, since it was assumed that this condition would most clearly demonstrate the effect of temperature. The fiber was inserted in the headspace of the sample vial for 30 min at different temperatures. All the extractions were carried out in triplicate. The results of the comparison of different extraction temperatures are depicted in Table 2.

From these figures it can be concluded that the best extraction temperature was 25 °C. Previous experiments [41] demonstrated that the optimum extraction temperature for SPME analysis in wines was 25 °C. High temperatures are supposed to release more analytes into the headspace, allowing better extraction during the SPME sampling. However, they can adversely affect the absorption of analytes by the coating due to the decrease of partition coefficients and the extraction by the fiber coating decreases as the temperature rises.

Table 3
Influence of temperature on the SPME extraction of wine flavour compounds with a DVB–CAR–PDMS fiber (30 min, headspace sampling with salt saturation)—triplicate analysis

Class of wine flavour compounds	Extraction temperature			
	25 °C		30 °C	
	Peak area	RSD %	Peak area	RSD %
Volatiles	$6.69 \cdot 10^8$	0.95	$5.05 \cdot 10^8$	4.59
Semivolatiles	$1.79 \cdot 10^9$	2.46	$1.66 \cdot 10^9$	16.78
Less volatiles	$4.46 \cdot 10^7$	4.50	$1.80 \cdot 10^7$	31.80
Sum	$2.51 \cdot 10^9$	2.09	$2.19 \cdot 10^9$	13.84

The influence of sampling temperature on extraction efficiency was also determined with the DVB–CAR–PDMS fiber (Table 3). It was found that, as in the previous case (PDMS fiber), more compounds were extracted at 25 °C than at 30 °C, although the results were not significant for the group of semivolatiles, since the standard deviation of the extraction at 30 °C for these compounds was rather high (16.8% RSD). A good reproducibility however was achieved for the extraction of all groups of compounds at 25 °C with this fiber (2.1% RSD).

3.2.2. Influence of extraction time

Since the mechanism of SPME is based on the equilibrium between analyte concentration in the aqueous phase and that in the polymeric phase of the fiber, the optimal time for extraction should be the

Table 2
Influence of temperature on absorption of different wine flavour compounds, classified according to functional group and volatility, during headspace SPME extraction with a PDMS fiber (30-min extraction of salt saturated sample)—triplicate analysis

Class of wine flavour compounds	Extraction temperature					
	25 °C		30 °C		37 °C	
	Peak area	RSD %	Peak area	RSD %	Peak area	RSD %
Alcohols	$2.85 \cdot 10^8$	0.33	$3.08 \cdot 10^8$	12.99	$3.84 \cdot 10^8$	38.81
Esters	$3.19 \cdot 10^9$	9.97	$2.53 \cdot 10^9$	8.99	$4.76 \cdot 10^8$	10.01
Miscellaneous	$1.20 \cdot 10^8$	10.57	$1.92 \cdot 10^8$	66.62	$6.62 \cdot 10^7$	19.48
Volatiles	$6.67 \cdot 10^8$	3.75	$6.62 \cdot 10^8$	25.24	$4.09 \cdot 10^8$	29.85
Semivolatiles	$2.89 \cdot 10^9$	10.28	$2.32 \cdot 10^9$	9.58	$4.85 \cdot 10^8$	14.04
Less volatiles	$3.88 \cdot 10^7$	16.34	$5.11 \cdot 10^7$	28.52	$3.33 \cdot 10^7$	14.55
Sum	$3.60 \cdot 10^9$	9.08	$3.03 \cdot 10^9$	12.84	$9.27 \cdot 10^8$	20.46

Table 4
Influence of extraction time on absorption of wine flavour compounds during headspace SPME extraction, using a PDMS fiber (extraction of salt saturated sample at 25 °C), expressed as peak area—triplicate analysis

Class of compounds	Extraction time (min)		
	15	30	60
Volatiles	$5.77 \cdot 10^8$	$6.67 \cdot 10^8$	$4.87 \cdot 10^8$
Semivolatiles	$1.91 \cdot 10^9$	$2.89 \cdot 10^9$	$2.48 \cdot 10^9$
Less volatiles	$1.95 \cdot 10^7$	$3.88 \cdot 10^7$	$2.23 \cdot 10^7$
Sum	$2.50 \cdot 10^9$	$3.60 \cdot 10^9$	$2.99 \cdot 10^9$
RSD % on sum	2.26	9.08	2.86

time of equilibrium. Different times were examined at optimum temperature (Table 4), using the PDMS fiber in the headspace sampling mode. From these results, it can be concluded that the highest recovery of wine flavour volatiles was obtained after an extraction time of 30 min, although the reproducibility was higher after 60 min (RSD 9.1 and 2.9% after 30 and 60 min, respectively). However, since the total GC–MS analysis time was 35 min, an extraction time of 30 min was selected as the optimum time for further studies. Recently, it was also found by another group that absorption of some wine flavour volatiles decreased after 40 min [42].

3.2.3. Fiber selection

Four different fibers were evaluated using the optimal sampling time (30 min) and temperature (25 °C) to determine which fiber most effectively extracted flavour compounds from wine samples. These fibers were used to extract analytes either in the headspace or liquid sampling mode. In the headspace sampling mode after saturation with salt, the PDMS fiber proved to have a better enrichment capacity than the other fibers used (Table 5). This was also the case when headspace extraction was carried out without salt addition. Under this condition the PA fiber had an extremely low sorption capacity (data not shown).

On the other hand, liquid sampling (direct immersion) resulted in a better performance for the DVB–CAR–PDMS fiber for extracting the different aroma compounds from wine compared to the other fibers (Table 6).

Hence, it can be concluded that the PDMS fiber performed better under the headspace sampling mode

Table 5
Sorption capacity of different fibers for the extraction of wine flavour compounds during headspace SPME extraction, after salt saturation (25 °C, 30 min), expressed as peak area—triplicate analysis

Class of compounds	SPME fiber			
	PDMS	DVB–CAR–PDMS	CW–DVB	PA
Alcohols	$2.85 \cdot 10^8$	$4.55 \cdot 10^8$	$4.77 \cdot 10^8$	$5.11 \cdot 10^8$
Esters	$3.19 \cdot 10^9$	$1.95 \cdot 10^9$	$8.76 \cdot 10^8$	$4.96 \cdot 10^8$
Miscellaneous	$1.2 \cdot 10^8$	$1.08 \cdot 10^8$	59 897 956	46 367 539
Sum	$3.6 \cdot 10^9$	$2.51 \cdot 10^9$	$1.41 \cdot 10^9$	$1.05 \cdot 10^9$
RSD % on sum	9.08	2.09	5.76	3.47

Table 6
Sorption capacity of different fibers for the extraction of wine flavour compounds during liquid SPME extraction (direct immersion, 25 °C, 30 min), expressed as peak area—triplicate analysis

Class of compounds	SPME fiber			
	PDMS	DVB–CAR–PDMS	CW–DVB	PA
Volatiles	$2.38 \cdot 10^8$	$4.12 \cdot 10^8$	$9.20 \cdot 10^7$	$9.12 \cdot 10^7$
Semivolatiles	$1.36 \cdot 10^9$	$1.95 \cdot 10^9$	$3.12 \cdot 10^8$	$6.76 \cdot 10^8$
Less volatiles	$1.84 \cdot 10^7$	$1.94 \cdot 10^7$	$3.93 \cdot 10^6$	$2.63 \cdot 10^6$
Sum	$1.61 \cdot 10^9$	$2.38 \cdot 10^9$	$4.08 \cdot 10^8$	$7.70 \cdot 10^8$
RSD % on sum	12.88	11.41	79.90	14.76

Table 7
Influence of extraction conditions [headspace (HS, no salt/salt) versus liquid (DI)] on the enrichment of different wine flavour compounds classified according to their functional group, during SPME extraction with different fibers (25 °C, 30 min), expressed as peak area—triplicate analysis

Class of compounds	PDMS			DVB–CAR–PDMS			PA			CW–DVB		
	DI	HS-NS	HS-S	DI	HS-NS	HS-S	DI	HS-NS	HS-S	DI	HS-NS	HS-S
Alcohols	$7.12\cdot10^{7}$	$5.94\cdot10^{7}$	$2.85\cdot10^{8}$	$2.78\cdot10^{8}$	$1.54\cdot10^{8}$	$4.55\cdot10^{8}$	$8.13\cdot10^{7}$	$6.22\cdot10^{6}$	$5.11\cdot10^{8}$	$1.26\cdot10^{8}$	$1.15\cdot10^{8}$	$4.77\cdot10^{8}$
Esters	$1.10\cdot10^{9}$	$1.95\cdot10^{9}$	$3.19\cdot10^{9}$	$1.79\cdot10^{9}$	$1.80\cdot10^{9}$	$1.95\cdot10^{9}$	$3.86\cdot10^{8}$	$2.52\cdot10^{7}$	$4.96\cdot10^{8}$	$2.69\cdot10^{8}$	$6.64\cdot10^{8}$	$8.76\cdot10^{8}$
Miscellaneous	$4.41\cdot10^{8}$	$1.67\cdot10^{8}$	$1.20\cdot10^{8}$	$3.15\cdot10^{8}$	$5.64\cdot10^{7}$	$1.08\cdot10^{8}$	$3.03\cdot10^{8}$	$1.33\cdot10^{6}$	$4.64\cdot10^{7}$	$1.37\cdot10^{7}$	$3.54\cdot10^{7}$	$5.99\cdot10^{7}$
Sum	$1.61\cdot10^{9}$	$2.17\cdot10^{9}$	$3.60\cdot10^{9}$	$2.38\cdot10^{9}$	$2.01\cdot10^{9}$	$2.51\cdot10^{9}$	$7.70\cdot10^{8}$	$3.28\cdot10^{7}$	$1.05\cdot10^{9}$	$4.08\cdot10^{8}$	$8.15\cdot10^{8}$	$1.41\cdot10^{9}$
RSD % on sum	12.88	6.28	9.08	11.41	3.89	2.09	14.76	6.00	3.47	79.90	3.21	5.76

with addition of salt whereas the DVB–CAR–PDMS fiber showed a better extraction efficiency during liquid sampling. Moreover, with the latter sampling mode, the 100-μm PDMS fiber poorly extracted polar analytes from the wine (data not shown).

3.2.4. Influence of other extraction conditions

The influence of the extraction mode (headspace vs. direct immersion) was first investigated in triplicate using the PDMS fiber. Therefore, the fiber was either immersed in the liquid sample or exposed to the headspace of the sample (with or without salt addition) at 25 °C for 30 min. All the samples were stirred in order to produce the agitation necessary for efficient transfer of the analytes from the aqueous phase to the fiber. The results are depicted in Table 7.

Adding salt to the sample (3 g/10 ml to obtain saturation) increased the extraction efficiency for all wine components during headspace SPME extraction except for the less volatile compounds since the ionic strength clearly affects the amount of analytes released into the headspace, and hence, the amount of volatile analytes enriched onto the fiber (Table 8). This is in agreement with previous findings [42].

In all the extraction modes applied, the non-polar PDMS fiber proved to be more efficient for the extraction of the semivolatiles and the esters than for the other compounds. Alcohols could only poorly be extracted with headspace SPME without salt addition, whereas the enrichment was much higher after salt saturation (Table 7).

The other three fibers were also examined in triplicate under standard conditions (25 °C for 30 min) using different extraction modes (headspace SPME with and without salt, and direct immersion) (Tables 7 and 8).

A very good reproducibility (RSD 2.1%) in SPME extraction was obtained when the DVB–CAR–PDMS fiber was used in the headspace sampling mode with salt addition (Table 7). This condition also resulted in the highest recovery of alcohols and esters. Miscellaneous compounds (terpenes, fatty acids) however were more efficiently extracted by liquid SPME. It has been reported that with the DVB–CAR–PDMS fiber the minor compounds were

Table 8
Effect of salt addition on the headspace SPME extraction of wine flavour compounds classified according to volatility, using different fibers (25 °C, 30 min), expressed as peak area—triplicate analysis

Class of compounds	PDMS		DVB–CAR–PDMS		PA		CW–DVB	
	No salt	Salt	No salt	Salt	No salt	Salt	No salt	Salt
Volatiles	$1.80\cdot10^{8}$	$6.67\cdot10^{8}$	$3.40\cdot10^{8}$	$6.69\cdot10^{8}$	$4.35\cdot10^{6}$	$4.37\cdot10^{8}$	$1.46\cdot10^{8}$	$4.38\cdot10^{8}$
Semivolatiles	$1.86\cdot10^{9}$	$2.89\cdot10^{9}$	$1.65\cdot10^{9}$	$1.79\cdot10^{9}$	$2.78\cdot10^{7}$	$6.09\cdot10^{8}$	$6.59\cdot10^{8}$	$9.42\cdot10^{8}$
Less volatiles	$1.35\cdot10^{8}$	$3.88\cdot10^{7}$	$2.41\cdot10^{7}$	$4.46\cdot10^{7}$	$6.11\cdot10^{5}$	$7.09\cdot10^{6}$	$9.66\cdot10^{6}$	$3.31\cdot10^{7}$
Sum	$2.17\cdot10^{9}$	$3.60\cdot10^{9}$	$2.01\cdot10^{9}$	$2.51\cdot10^{9}$	$3.28\cdot10^{7}$	$1.05\cdot10^{9}$	$8.15\cdot10^{8}$	$1.41\cdot10^{9}$
RSD % on sum	6.28	9.08	3.89	2.09	6.00	3.47	3.21	5.76

better extracted in the direct immersion mode [44]. These fibers were designated for extraction of highly volatile compounds not usually extracted with PDMS alone.

Compared to the PDMS and DVB–CAR–PDMS fibers, the absorption capacity (expressed as peak area) of the PA and CW–DVB fibers was rather poor (Table 7), although the alcohols were preferentially enriched by the PA fiber during headspace extraction after salt saturation. In the latter case, the reproducibility was also high (RSD 5.8 and 2.4% for the enrichment of the alcohols and esters, respectively).

For the CW–DVB fiber the extraction during liquid sampling (DI) was poor compared to that in the headspace sampling mode (Table 7). Again, the extraction recovery was higher from the salt saturated sample than from the non-salted sample during headspace extraction. Compared to the other fibers, CW–DVB showed the poorest reproducibility, especially in direct immersion mode (RSD 79.9%). With this latter sampling technique, the PA fiber showed a better overall performance in absorbing wine aroma compounds than the CW–DVB fiber.

The effect of salt addition on the extraction efficiency was further investigated, comparing the recovery of the volatile, semivolatile and less volatile compounds using the other fibers, DVB–CAR–PDMS, PA and CW–DVB (Table 8). From these data, it can be concluded that the necessity to saturate the samples with salt prior to headspace SPME extraction, was most pronounced when the PA fiber was used. This effect can also clearly be demonstrated when it is related to the class of compounds (alcohols, esters, miscellaneous) (Table 7). A dramatic increase (80-fold increase) in the recovery of the alcohols during headspace SPME could be observed after saturation with salt. These results are comparable with those obtained by other groups [31,42].

It can be concluded that saturation with salt resulted in a much higher extraction efficiency during headspace SPME sampling. This effect was more important for more polar compounds (alcohols) than for esters and terpenes, and was more pronounced for the ‘‘polar’’ fibers (CW–DVB and PA) than for the ‘‘non-polar’’ fiber (PDMS) and the combined phase, based on adsorption (DVB–CAR–PDMS).

It has to be remarked as well that headspace SPME extraction using the PDMS fiber showed some additional advantages over liquid extraction with DVB–CAR–PDMS. The latter fiber was very sensitive to glycerin and organic acids (octanoic and decanoic acid) which are characterised by wide overlapping peaks (Fig. 1), since for this analysis a non-polar capillary column was used. On the other

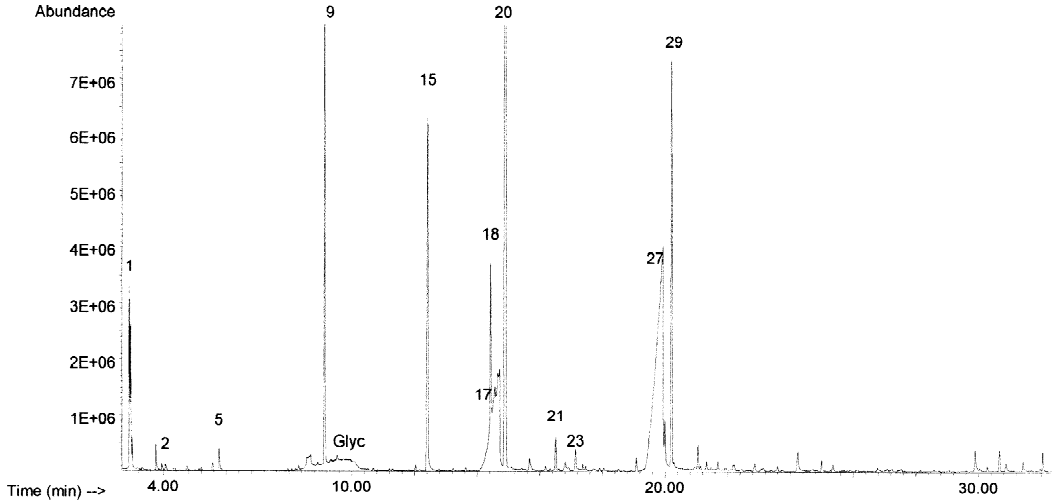

Fig. 1. Chromatogram of Boutari wine sample extracted using DVB–CAR–PDMS fiber in the liquid sampling mode without salt addition to the sample and at 25 °C for 30 min. Peak numbers refer to the compounds listed in Table 1. Glyc, glycerin; peaks 17 and 27 are fatty acids.

hand, this fiber could be used in combination with a polar stationary phase capillary column to analyse the acid profile of the wine. A better chromatogram was obtained using the PDMS fiber in headspace sampling mode (Fig. 2).

3.3. Other wines

Three other types of Greek wine (see Experimental section) were qualitatively and semi-quantitatively analysed using the best sampling condition, i.e. headspace sampling (salt saturation) with the PDMS fiber at 25 °C for 30 min. In the Zitsa wine 42 compounds were positively identified (based on mass spectrum and retention index), while in the Limnos and Filoni wines 60 compounds were identified (Tables 9–10).

The dominating monoterpene alcohols, particularly for Limnos and Filoni varieties, were linalool, citronellol and α-terpineol. These terpene alcohols in wine contribute to the flowery and pleasant, sweet and citrus odours, respectively, of wine [45,46]. The higher alcohols, fatty acids and esters are the most important groups of the yeast-synthesised aroma substances of the fermentation bouquet, whereby the alcohols quantitatively predominated in the three types of wine (isoamyl alcohol and 2-methylbutyl alcohol). The isoamyl alcohol contributes to the alcohol odour, whereas 1-hexanol resembles the green, grassy odour [45]. Also the presence of (*Z*)-3-hexenol contributes to the odour of freshly cut grass [45].

Ethyl esters of fatty acids and acetates of higher alcohols were dominating esters in the three wine varieties. The amount of fatty acid ethyl esters is known to increase significantly during ageing [47]. Although the wines were still young (vintage 1999) when they were analysed (February–April, 2001), ethyl decanoate, octanoate and hexanoate predominated in the three wines. Other esters of importance were isoamyl acetate and ethyl sorbate in Limnos and Zitsa wines.

The presence of 2-phenylethyl alcohol in the three wine varieties can give the wine a rose-like flavour [47]. The compound (*E*)-β-damascenone was identified in Limnos wine (relative contribution of the headspace, 0.05%). This compound belongs to the rose ketones class. Damascenone is believed to originate from the breakdown of the carotenoid neoxanthin by a complex pathway [48]. It has an odour threshold of 0.002 ppb in water and has been described as flower like.

Some artefacts, e.g. phthalates and butylated hydroxytoluene, were also observed in the wines. It is

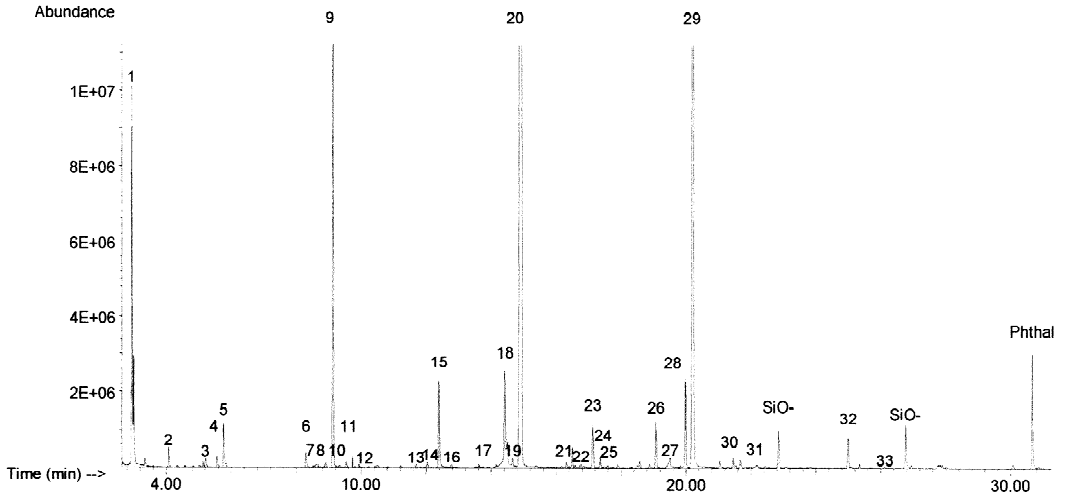

Fig. 2. Chromatogram of Boutari wine sample extracted using PDMS fiber in the headspace sampling mode with salt addition to the sample and at 25 °C for 30 min. Peak numbers refer to the compounds listed in Table 1. SiO represents PDMS-fiber material (siloxanes); Phthal, phthalates.

Table 9
Average peak area and relative peak areas (RPA) of Boutari and Zitsa wines (average of triplicate analysis)

No.	RT (min)	Compound	Boutari		Zitsa	
			Average peak area	RPA (%)	Average peak area	RPA (%)
1	2.67	Ethyl propanoate	$1.53 \cdot 10^6$	0.04	$6.87 \cdot 10^5$	0.04
2	2.95	Isoamyl alcohol	$1.74 \cdot 10^8$	4.56	$9.53 \cdot 10^7$	5.04
3	3.00	2-Methyl-1-butanol	$4.66 \cdot 10^7$	1.22	$2.37 \cdot 10^7$	1.25
4	3.35	Ethyl isobutanoate	$3.92 \cdot 10^6$	0.10	$2.57 \cdot 10^6$	0.14
5	4.08	Ethyl butanoate	$9.57 \cdot 10^6$	0.25	$8.19 \cdot 10^6$	0.43
6	5.14	Ethyl 2-methylbutanoate	$2.52 \cdot 10^6$	0.07	$9.73 \cdot 10^5$	0.05
7	5.21	Ethyl isovalerate	$4.85 \cdot 10^6$	0.13	$2.51 \cdot 10^6$	0.13
8	5.26	(*Z*)-3-hexen-1-ol	$1.97 \cdot 10^5$	0.01	$2.11 \cdot 10^5$	0.01
9	5.56	1-Hexanol	$6.14 \cdot 10^6$	0.16	$3.16 \cdot 10^6$	0.17
10	5.77	Isoamyl acetate	$2.49 \cdot 10^7$	0.65	$3.01 \cdot 10^7$	1.59
11	5.84	2-Methylbutyl acetate	$1.95 \cdot 10^6$	0.05	$1.14 \cdot 10^6$	0.06
12	8.30	2,2,6-Trimethyl-6-vinyltetrahydropyran	$8.16 \cdot 10^6$	0.21	$2.84 \cdot 10^5$	0.02
13	8.66	Hexanoic acid	$4.76 \cdot 10^6$	0.12	$1.73 \cdot 10^6$	0.09
14	9.13	Ethyl hexanoate	$3.73 \cdot 10^8$	9.76	$1.54 \cdot 10^8$	8.13
15	9.54	Hexyl acetate	$3.69 \cdot 10^6$	0.10	$3.10 \cdot 10^6$	0.16
16	9.93	Limonene	$1.34 \cdot 10^6$	0.04	ND	–
17	10.51	(*E*)-β-Ocimene	$1.80 \cdot 10^6$	0.05	ND	–
18	11.24	Sorbic acid	ND	–	$8.86 \cdot 10^6$	0.47
19	11.69	Terpinolene	$1.98 \cdot 10^6$	0.05	ND	–
20	12.01	Ethyl sorbitate	ND	–	$1.14 \cdot 10^8$	6.02
21	12.04	Linalool	$4.54 \cdot 10^6$	0.12	ND	–
22	12.39	2-Phenylethyl alcohol	$5.15 \cdot 10^7$	1.35	$8.66 \cdot 10^6$	0.46
23	12.78	Methyl octanoate	$1.47 \cdot 10^6$	0.04	$1.15 \cdot 10^6$	0.06
24	13.63	Nerol oxide	$1.86 \cdot 10^6$	0.05	$3.31 \cdot 10^5$	0.02
25	14.42	Diethyl succinate	$7.80 \cdot 10^7$	2.04	$1.49 \cdot 10^7$	0.79
26	14.51	Octanoic acid	$3.19 \cdot 10^7$	0.83	$1.30 \cdot 10^7$	0.69
27	14.66	α-Terpineol	$6.59 \cdot 10^6$	0.17	$5.92 \cdot 10^5$	0.03
28	14.93	Ethyl octanoate	$1.66 \cdot 10^9$	43.54	$7.17 \cdot 10^8$	37.90
29	15.10	Decanal	ND	–	$9.63 \cdot 10^5$	0.05
30	16.32	Isopentyl hexanoate	$3.80 \cdot 10^6$	0.10	$1.12 \cdot 10^6$	0.06
31	16.50	2-Phenylethyl acetate	$1.26 \cdot 10^7$	0.33	$1.73 \cdot 10^6$	0.09
32	16.85	(*E*)-cinnamaldehyde	$3.27 \cdot 10^5$	0.01	$7.12 \cdot 10^5$	0.04
33	17.13	Vitispirane	$2.90 \cdot 10^7$	0.76	$6.37 \cdot 10^6$	0.34
34	17.36	Lavandulyl acetate	$8.05 \cdot 10^6$	0.21	$2.86 \cdot 10^5$	0.02
35	17.59	Ethyl nonanoate	$1.51 \cdot 10^6$	0.04	$4.33 \cdot 10^5$	0.02
36	18.33	Methyl decanoate	$7.93 \cdot 10^5$	0.02	$7.27 \cdot 10^5$	0.04
37	19.08	1,2-Dihydro-1,1,6-trimethylnaphthalene	$3.27 \cdot 10^7$	0.86	$4.71 \cdot 10^6$	0.25
38	19.51	*n*-Decanoic acid	$1.75 \cdot 10^7$	0.46	$6.16 \cdot 10^6$	0.33
39	19.99	Ethyl 9-decenoate	$6.66 \cdot 10^7$	1.74	$3.66 \cdot 10^6$	0.19
40	20.23	Ethyl decanoate	$1.04 \cdot 10^9$	27.33	$6.00 \cdot 10^8$	31.71
41	21.45	Isoamyl octanoate	$8.66 \cdot 10^6$	0.23	$5.28 \cdot 10^6$	0.28
42	21.53	2-Methylbutyl octanoate	$1.86 \cdot 10^6$	0.05	$9.18 \cdot 10^5$	0.05
43	23.11	BHT	$9.40 \cdot 10^5$	0.02	$8.75 \cdot 10^6$	0.46
44	24.99	Ethyl dodecanoate	$2.02 \cdot 10^7$	0.53	$2.41 \cdot 10^7$	1.27
45	26.13	Isoamyl decanoate	$7.54 \cdot 10^5$	0.02	$1.14 \cdot 10^6$	0.06
46	30.67	Phthalate	$3.36 \cdot 10^7$	0.88	$9.92 \cdot 10^6$	0.52
	–	Unidentified compounds	$2.69 \cdot 10^7$	0.70	$9.11 \cdot 10^6$	0.48
		Sum total±RSD %	$3.82 \cdot 10^9 \pm 3.94\%$		$1.89 \cdot 10^9 \pm 2.40\%$	

BHT, butylated hydroxytoluene; ND, not detected.

Table 10
Average peak area and relative peak areas (RPA) of Limnos (average of triplicate analysis) and Filoni (duplicate) wines

No.	RT (min)	Compound	Limnos		Filoni	
			Average peak area	RPA (%)	Average peak area	RPA (%)
1	2.67	Ethyl propanoate	$1.47 \cdot 10^6$	0.06	$1.05 \cdot 10^6$	0.06
2	2.96	Isoamyl alcohol	$1.86 \cdot 10^8$	7.08	$2.13 \cdot 10^8$	12.53
3	3.01	2-Methyl-1-butanol	$5.38 \cdot 10^7$	2.05	$6.77 \cdot 10^7$	3.99
4	3.35	Ethyl isobutanoate	$3.56 \cdot 10^6$	0.14	$6.98 \cdot 10^6$	0.41
5	3.60	Isobutyl acetate	$4.37 \cdot 10^5$	0.02	$6.36 \cdot 10^5$	0.04
6	3.67	2,3-Butanediol	$2.05 \cdot 10^5$	0.01	$1.39 \cdot 10^6$	0.08
7	3.87	1,3-Butanediol	$7.97 \cdot 10^4$	0.01	$4.29 \cdot 10^5$	0.03
8	4.08	Ethyl butanoate	$8.44 \cdot 10^6$	0.32	$7.09 \cdot 10^6$	0.42
9	4.34	Ethyl lactate	$4.01 \cdot 10^6$	0.15	$2.31 \cdot 10^6$	0.14
10	5.14	Ethyl 2-methylbutanoate	$1.43 \cdot 10^6$	0.05	$2.64 \cdot 10^6$	0.16
11	5.22	Ethyl isovalerate	$3.52 \cdot 10^6$	0.13	$5.62 \cdot 10^6$	0.33
12	5.25	(*Z*)-3-Hexen-1-ol	$6.15 \cdot 10^5$	0.02	$4.97 \cdot 10^5$	0.03
13	5.57	1-Hexanol	$4.50 \cdot 10^6$	0.17	$4.88 \cdot 10^6$	0.29
14	5.78	Isoamyl acetate	$4.35 \cdot 10^7$	1.66	$2.20 \cdot 10^7$	1.30
15	5.85	2-Methylbutyl acetate	$3.05 \cdot 10^6$	0.12	$2.61 \cdot 10^6$	0.15
16	8.31	2,2,6-Trimethyl-6-vinyltetrahydropyran	$8.36 \cdot 10^6$	0.32	$8.66 \cdot 10^6$	0.51
17	8.57	Hexanoic acid	$3.15 \cdot 10^6$	0.12	$1.85 \cdot 10^6$	0.11
18	8.91	Herboxide	$3.64 \cdot 10^6$	0.14	$3.23 \cdot 10^6$	0.19
19	9.14	Ethyl hexanoate	$2.00 \cdot 10^8$	7.64	$1.07 \cdot 10^8$	6.32
20	9.54	Hexyl acetate	$4.02 \cdot 10^6$	0.15	$1.76 \cdot 10^6$	0.10
21	9.81	*p*-Cymene	$4.16 \cdot 10^5$	0.02	$2.80 \cdot 10^5$	0.02
22	9.97	Limonene+	$4.16 \cdot 10^6$	0.16	$1.74 \cdot 10^6$	0.10
23		2-ethyl-1-hexanol (not resolved)				
24	10.24	(*Z*)-β-Ocimene	$1.26 \cdot 10^6$	0.05	$2.94 \cdot 10^5$	0.02
25	10.50	(*E*)-β-Ocimene	$2.50 \cdot 10^6$	0.10	$1.29 \cdot 10^6$	0.08
26	11.24	*trans*-Linalool oxide	ND	–	$1.56 \cdot 10^6$	0.09
27	11.28	Sorbic acid	$1.29 \cdot 10^7$	0.49	0.00·00	0.00
28	11.70	Terpinolene	$7.48 \cdot 10^6$	0.29	$2.38 \cdot 10^6$	0.14
29	12.00	Ethyl sorbate	$3.22 \cdot 10^7$	1.23	ND	–
30	12.04	Linalool	$3.86 \cdot 10^7$	1.47	$1.54 \cdot 10^7$	0.91
31	12.18	3,7-Dimethyl-1,5,7-octatrien-3-ol	$3.98 \cdot 10^6$	0.15	$1.82 \cdot 10^6$	0.11
32	12.40	2-Phenylethyl alcohol	$7.36 \cdot 10^7$	2.81	$7.47 \cdot 10^7$	4.40
33	12.78	Methyl octanoate	$2.24 \cdot 10^6$	0.09	$8.36 \cdot 10^5$	0.05
34	13.63	Nerol oxide	$4.93 \cdot 10^6$	0.19	$7.96 \cdot 10^6$	0.47
35	14.28	Octanoic acid	$2.22 \cdot 10^7$	0.85	$1.47 \cdot 10^7$	0.87
36	14.42	Diethyl succinate	$4.05 \cdot 10^7$	1.55	$5.16 \cdot 10^7$	3.04
37	14.65	α-Terpineol	$1.74 \cdot 10^7$	0.66	$1.55 \cdot 10^7$	0.91
38	14.90	Ethyl octanoate	$9.96 \cdot 10^8$	37.98	$5.64 \cdot 10^8$	33.21
39	15.09	Decanal	$9.35 \cdot 10^5$	0.04	$1.02 \cdot 10^6$	0.06
40	15.71	Citronellol	$1.79 \cdot 10^6$	0.07	$1.35 \cdot 10^6$	0.08
41	16.33	Isopentyl hexanoate	$1.70 \cdot 10^6$	0.06	$9.71 \cdot 10^5$	0.06
42	16.50	2-Phenylethyl acetate	$3.74 \cdot 10^7$	1.43	$2.22 \cdot 10^7$	1.31
43	16.85	(*E*)-Cinnamaldehyde	$1.14 \cdot 10^6$	0.04	$5.13 \cdot 10^5$	0.03
44	17.14	Vitispirane	$7.45 \cdot 10^6$	0.28	$1.09 \cdot 10^7$	0.64
45	17.37	Lavandulyl acetate	$2.55 \cdot 10^7$	0.97	$1.43 \cdot 10^7$	0.84
46	17.60	Ethyl nonanoate	$6.72 \cdot 10^5$	0.03	$8.64 \cdot 10^5$	0.05
47	18.33	Methyl decanoate	$1.66 \cdot 10^6$	0.06	$2.86 \cdot 10^5$	0.02
48	18.94	(Iso)butyl octanoate	$6.14 \cdot 10^5$	0.02	$2.93 \cdot 10^5$	0.02
49	19.08	1,2-Dihydro-1,1,6-trimethylnaphthalene	$3.20 \cdot 10^6$	0.12	$5.41 \cdot 10^6$	0.32

Table 10. Continued

No.	RT (min)	Compound	Limnos		Filoni	
			Average peak area	RPA (%)	Average peak area	RPA (%)
50	19.43	Decanoic acid	$8.45 \cdot 10^6$	0.32	$4.83 \cdot 10^6$	0.28
51	19.92	(*E*)-β-Damascenone	$1.36 \cdot 10^6$	0.05	$2.08 \cdot 10^5$	0.01
52	19.99	Ethyl 9-decenoate	$9.98 \cdot 10^6$	0.38	$2.50 \cdot 10^7$	1.47
53	20.20	Ethyl decanoate	$6.09 \cdot 10^8$	23.22	$2.75 \cdot 10^8$	16.22
54	21.46	Isoamyl octanoate	$4.37 \cdot 10^6$	0.17	$2.34 \cdot 10^6$	0.14
55	21.54	2-Methylbutyl octanoate	$6.44 \cdot 10^5$	0.02	$4.29 \cdot 10^5$	0.03
56	21.98	2,6-di-tBu-*p*-benzoquinone	$2.59 \cdot 10^6$	0.10	$3.46 \cdot 10^6$	0.20
57	22.09	BHT	$3.15 \cdot 10^6$	0.12	$5.95 \cdot 10^6$	0.35
58	23.11	BHT	$1.65 \cdot 10^6$	0.06	$3.38 \cdot 10^6$	0.20
59	24.99	Ethyl dodecanoate	$2.11 \cdot 10^7$	0.80	$1.26 \cdot 10^7$	0.74
60	26.13	Isoamyl decanoate	$8.16 \cdot 10^5$	0.03	$7.74 \cdot 10^5$	0.05
61	30.66	Phthalates	$1.48 \cdot 10^7$	0.56	$2.01 \cdot 10^7$	1.19
	–	Unidentified compounds	$6.83 \cdot 10^7$	2.61	$6.99 \cdot 10^7$	4.12
		Sum total±RSD %	$2.62 \cdot 10^9 \pm 5.14\%$		$1.70 \cdot 10^9 \pm 2.88\%$	

BHT, butylated hydroxytoluene; ND, not detected.

believed that these contaminants originate from plastic containers or barrels. The origin of some other compounds like 2,2,6-trimethyl-6-vinyltetrahydropyran and 1,2-dihydro-1,1,6-trimethylnaphthalene was also not clear. 2,2,6-Trimethyl-6-vinyltetrahydropyran is a known decomposition product from linalool, formed at low pH [49,50].

From these results it can be concluded that SPME is a very appropriate sampling technique to distinguish the different Greek white wines selected in this study based on their headspace profile. In Table 11 the relative composition of the wines, i.e. contribution of esters, alcohols, terpenes and miscellaneous compounds, is summarised for the four analysed Greek wines.

4. Conclusion

Solid-phase microextraction is a suitable sampling technique for the analysis of aroma compounds in wine providing a simple, fast, sensitive and reproducible alternative to the traditional methods, such as liquid extraction or dynamic headspace (purge and trap).

Poly(dimethylsiloxane) (PDMS) was the most suitable fiber for the SPME analysis of wine, when headspace sampling was applied, whereas DVB–CAR–PDMS was a good fiber when liquid extraction (DI) was performed. The extraction by CW–DVB and PA fibers resulted in low recoveries of wine flavours and suffered from low reproducibility.

Table 11
Relative composition of the wines: % esters, alcohols, terpenes and miscellaneous compounds

Class of compounds	Boutari			Zitsa			Limnos			Filoni		
	Peak area	RSD %	RPA (%)	Peak area	RSD %	RPA (%)	Peak area	RSD %	RPA (%)	Peak area	RSD %	RPA (%)
Esters	$3.32 \cdot 10^9$	2.86	86.83	$1.69 \cdot 10^9$	3.02	89.10	$2.01 \cdot 10^9$	7.14	76.55	$1.11 \cdot 10^9$	4.89	65.63
Alcohols	$2.78 \cdot 10^8$	2.60	7.29	$1.31 \cdot 10^8$	3.09	6.91	$3.19 \cdot 10^8$	5.34	12.14	$3.62 \cdot 10^8$	5.36	21.34
Terpenes	$4.63 \cdot 10^7$	15.71	1.21	ND	–	0.00	$1.87 \cdot 10^8$	5.02	7.11	$1.13 \cdot 10^8$	7.49	6.68
Miscell	$1.79 \cdot 10^8$	27.58	4.67	75 481 018	14.48	3.98	$1.1 \cdot 10^8$	25.31	4.19	$1.08 \cdot 10^8$	6.48	6.35
Sum	$3.82 \cdot 10^9$	3.93	100.00	$1.89 \cdot 10^9$	2.40	100.00	$2.62 \cdot 10^9$	5.14	100.00	$1.70 \cdot 10^9$	2.88	100.00

Miscell, miscellaneous compounds (organic acids, ethers, unknown compounds, etc.); ND, not detected; RSD %, relative standard deviation (%) on peak area; RPA, relative peak area (% share).

From the data discussed in this study, the best extraction procedure, using the PDMS fiber, was the following: headspace sampling (solution saturated with 3 g/10 ml NaCl), 30-min extraction time at a temperature of 25 °C. During extraction, 10-ml aliquots of wine in 22-ml vials were vigorously stirred (800 rpm). In the case of liquid sampling with DVB–CAR–PDMS however, 17-ml aliquots of wine were extracted in 22-ml vials with direct immersion of the fiber in the wine, which was not saturated with salt, and stirred at 800 rpm.

Moreover, SPME was also successfully applied to qualitatively discriminate different Greek white wines.

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