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Isolation of white wine volatiles using different sample preparation methods

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Summary

Three sample preparation methods for gas chromatographic analysis of white wine volatiles were tested. In order to find an adequate replacement for common liquid-liquid extraction using 1,1,1-trichlorofluoromethan, headspace solid-phase microextraction (HS-SPME) and stir bar sorptive extraction (SBSE) were tested. SPME and SBSE sample preparations are characterized by rapid and easy handling, small sample size and the possibility of automation, but the recovery of aroma compounds is restricted because of the discrimination properties of the polymer phase. Unlike SPME, the results obtained by SBSE are more similar to those of liquid-liquid extraction.

K e y w o r d s : white wine, aroma, liquid-liquid extraction, stir bar sorptive extraction, SBSE, solid-phase microextraction, SPME.

Introduction

The presence of aroma compounds in white wine is one of the most important attributes of its final quality. More than 800 volatiles such as alcohols, esters, aldehydes, ketones, volatile acids, terpenes and pyrazines contribute to wine aroma (Etievant 1991, Rapp 1998). The concentration levels of each of these compounds range from several $mg \cdot l^{-1}$ to a few $ng \cdot l^{-1}$. The complexity of the matrix and the low concentration levels require the use of extraction and concentration techniques for analysis. Several sample preparation procedures have been used, such as liquid-liquid extraction, simultaneous distillation-extraction, solid-phase extraction, static head-space sampling, dynamic head-space sampling and other techniques (Hardy 1969, Rapp *et al.* 1976, Nunez *et al.* 1984, Vernin *et al.* 1987, Edwards *et al.* 1990, Ferreira *et al.* 1993, Villen *et al.* 1995, Guth 1997, Schneider *et al.* 1998, Aznar *et al.* 2001, Ortega-Heras *et al.* 2002). Nevertheless the choice of a suitable extraction method for aroma compounds in wine remains a problem that has not yet been satisfactorily resolved.

Liquid-liquid extraction (LE) is still the reference technique for the extraction of volatile compounds from wine because most of the volatiles have a high partition coefficient between the aqueous matrix and the organic phase, *e.g.* 1,1,1-trichlorofluoromethan (Freon 11). Anyway, its main disadvantages are the use of environment-polluting and expensive solvents as well as the high labor cost since there is no possibility of process automation.

Compared to traditional techniques (liquid-liquid extraction, solid-liquid extraction, static and dynamic headspace), the SPME method has some advantages such as easy handling, no need for previous sample preparation, low cost and solvent free extraction (Arthur and Pawliszyn 1990). Recently a new technique, the stir bar sorptive extraction (SBSE) was developed (Baltussen *et al.* 1999). Producer and retailer characterize SBSE as a highly sensitive technique for trace and ultratrace analyses. This technique uses a magnetic stir bar (typically 10 mm length) incorporated in a glass tube and coated with polydimethylsiloxane (PDMS). Upon stirring in a liquid sample matrix, the analytes are partitioned between the matrix and the PDMS phase (quasi liquid) on the stir bar according to their partitioning coefficients. Finally, the stir bar is transferred from the sample to the thermal desorption unit (TDU) coupled with a cold injection system (CIS) as injector to the gas chromatographic column. The desorption process is fully automated. The extraction theory of SBSE and SPME is the same but the volume of the PDMS phase for SBSE is typically 55 µl (ranges from 25-125 µl) and only 0.6 µl for SPME (100 µm PDMS fiber). This affects directly the enrichment of analytes, since their recoveries from liquid samples increase with the volume ratio of the PDMS phase to the sample matrix (Baltussen *et al.* 1999, Bicchi *et al.* 2002).

For several decades liquid-liquid extraction by chlorofluorohydrocarbons (*e.g.* Freon 11) was one of the reference methods for wine and environmental analysis methods, but this compound group is now banned. To characterize wine volatile patterns the above mentioned three sample preparation methods were compared using two different white wines.

Material and Methods

M a t e r i a l : A commercial Croatian Rhine Riesling wine from the Zagorje region produced by Vinko Kihas (Ivanec, vintage 2001) and a new-bred line (internal nomenclature Gf.Ga-52-42) from the Institute of Grapevine Breeding Geilweilerhof (www.bafz.de/siebeldingen) vintage 2000 were studied. The aroma reference substances were supplied by Aldrich (Milwaukee, USA) except for 1-hexanol and 3-decanol, which were obtained from Fluka (Buchs, Switzerland) and ABCR (Karlsruhe, Germany), respectively.

M e t h o d s : L i q u i d - l i q u i d e x t r a c t i o n : Volatiles from wine (250 ml) were extracted with approximately 40 ml 1,1,1-trichlorofluoromethane (Freon 11) using a liquid-

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liquid extractor for 20 h at room temperature as described by Rapp *et al.* (1976). 3-Decanol (0.1 ppm v/v) was added as internal standard before extraction. Immediately before analysis the extract was concentrated to 100 µl by distilling off the solvent on a Vigreux column (20 cm length, 1 cm ID). Because of the high vapour pressure of freon extracts, an aliquot of 1 µl was injected manually into the GC using a cooled vial and syringe.

Head-space SPME: The wine volatiles were sampled by HS-SPME with a 100 µm PDMS fiber (Supelco, Bellefonte USA) using an MPS2 autosampler from Gerstel (Mühlheim an der Ruhr, Germany). The internal standard was added to the sample of wine (200 ml), resulting in a concentration of 0.1 ppm (v/v). An aliquot of 10 ml was placed into a 20 ml headspace vial containing solid NaCl p.a. (3 g) and capped with a crimp cap and teflon-lined septum. Equilibration time before absorption was 10 min at 35 °C and shaking (300 rpm). The fiber was exposed to the wine headspace for 15 min at 35 ºC with further shaking. Thermal desorption followed for 2 min in the injector (splitless mode) at 250 ºC and, afterwards, additional thermal cleaning (3 min at 250 ºC, split ratio 1:10).

Stir bar sorptive extraction: A stir bar with 0.5 mm film thickness and 10 mm length coated with polydimethysiloxan (PDMS) was used (Gerstel, Mülheim an der Ruhr, Germany). The wine sample with internal standard prepared as mentioned above (10 ml) and the stir bar were placed in a 20 ml headspace vial. The vial was sealed with a stopper. The stir bar was used at 350 rpm at room temperature for 45 min. After removal from the wine sample, the stir bar was gently dried with a lint-free tissue and then transferred into a glass tube for thermal desorption and subsequent GC analysis. The parameters for the thermal desorption unit (TDU) and the cold injection system (CIS) were the following: thermal desorption at 280 °C, cryo trapping at -80 °C.

Gas chromatographic analyses: The analyses were performed with an Agilent Technologies 6890 gas chromatograph equipped with a flame ionization detector (FID). Compounds were separated on a polar column HP INNOWax, 0.25 mm ID x 30 m length x 0.5 µm film thickness. The FID temperature was 250 ºC. Helium was used as a carrier gas with a column flow rate of 1.1 ml· min^{-1}. Temperature program: 40 ºC (3 min), from 40 to 200 ºC at 3 ºC· min^{-1} and 15 min at 200 ºC.

The wine compounds were identified by parallel running of mass spectrometric analyses (GC/MS) and by retention indices. For identification the same GC with an Agilent 5973 MSD in the electron impact ionization mode (70 eV) was used. GC run parameters were the same as described above. For identification of compounds the Wiley 138, NIST 02 and HPCH 1607 (Allured Corp., USA) libraries were used.

Results and Discussion

Liquid-liquid extractions with various Freons were frequently used for wine aroma analyses (Hardy 1969, Rapp *et al.* 1976, Ferreira *et al.* 1993, Zhou *et al.* 1996). The technical effort is low but the process is characterized by high labor cost and relatively large sample sizes; moreover automation is difficult due to the special properties of the solvent. SPME and SBSE are innovative methods which require a more complex technical equipment. The adsorption process from the liquid sample to the SPME fiber contains two phase transfers: between liquid and gas phase and, additionally, between gas phase and fiber (quasi-liquid phase). The adjustment of the equilibria between the three phases is very sensitive to several parameters, *e.g.* temperature, polarity and concentration of analytes, solvent content of the matrix, volume ratio of gas phase and liquid, shaking or stirring. The complex interaction of all these parameters requires a strict standardization of the sample preparation process. Therefore an autosampler is required for quantification (or semi-quantification as described in this paper). SBSE is more robust to the mentioned parameters than SPME because of the immersion technique and the higher volume (about 100-fold) of the polymer adsorption phase. A disadvantage of this technique is the very complex and expensive technical system for thermal desorption including a cold injection system.

It is known that recovery rates of different extraction techniques may differ widely (Krumbein and Ulrich 1996). Fig. 1 shows three typical gas chromatograms obtained by application of the different extraction methods. Peak numbers were assigned in order of retention time, and identification was based on comparison with GC/MS and library search. Obviously, the peak number and the peak intensities

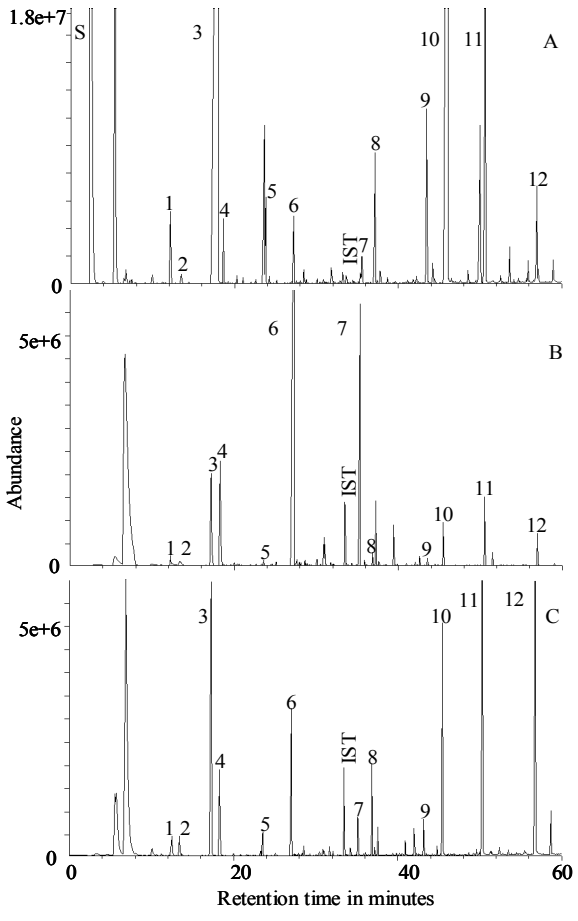

Fig. 1: Gas chromatograms of Rhine Riesling wine obtained by three sample preparation methods. **A**: LE; **B**: HS-SPME; **C**: SBSE.

are maximum with LE. The figure shows that major compounds are detected by all three methods. The results obtained by the SBSE method are more similar to the LE results, except for the abundance of several compounds, *e.g.* 3-methyl-1-butanol and 2-phenylethanol. The concentration of these compounds is higher than that of all other compounds in wine. The SBSE method is also more efficient than the solid phase microextraction. Although the adsorption phases are identical (PDMS) there are differences between results obtained by HS-SPME and SBSE. SPME was used as headspace technique, whereas the stirring bar was immersed in the liquid phase. With SBSE nearly the same compounds were extracted as by HS-SPME including numerous additional compounds, mostly semivolatiles.

Summed up 69, 40 and 60 peaks were identified by LE, SPME and SBSE, respectively (Tab. 1). Generally, HS-SPME is more sensitive to ethyl esters of hexanoic, octanoic and decanoic acids and, especially, terpenes such as terpinolene, nerol, β-ionon, γ-terpineol and a few esters that were not determined by SBSE. Investigations recently showed that the differences between SBSE and SPME were dependent on compounds: For very apolar substances SBSE is more effective than SPME because of the much higher amount of PDMS phase. With increasing polarity this difference be-

Table 1

Qualitative analysis of volatiles by GC-MS and library search using three different sample preparation methods

No.	Compound	LE	HS-SPME	SBSE
1	ethyl butanoate	+	+	+
2	ethyl 2-methylbutanoate	+	n.d.	+
3	ethyl 3-methylbutanoate	+	n.d.	+
4	2-methyl-1-propanol	+	+	+
5	3-methyl 1-butanol acetate	+	+	+
6	1-butanol	+	n.d.	n.d.
7	ethyl 2-butenoate	+	n.d.	n.d.
8	methyl hexanoate	+	n.d.	+
9	3-methyl 1-butanol	+	+	+
10	ethyl hexanoate	+	+	+
11	γ-terpinene	+	n.d.	n.d.
12	1-pentanol	+	n.d.	n.d.
13	2-methylbutyl butanoate	+	n.d.	n.d.
14	hexyl acetate	+	+	+
15	terpinolene	n.d.	+	n.d.
16	3-hydroxy-2-butanone	+	n.d.	n.d.
17	(*Z*)-3-hexen-1-ol acetate	n.d.	n.d.	+
18	4-methyl 1-pentanol	+	n.d.	+
19	3-methyl 1-pentanol	+	+	+
20	ethyl heptanoate	+	+	+
21	ethyl 2-hydroxypropanoate	+	+	+
22	1-hexanol	+	+	+
23	(*E*)-3-hexen-1-ol	+	n.d.	+
24	(*Z*)-3-hexen-1-ol	+	n.d.	+
25	2-nonanone	n.d.	n.d.	+
26	methyl octanoate	+	+	n.d.
27	ethyl 2-hydroxybutanoate	+	n.d.	n.d.
28	(*Z*)-2-hexen-1-ol	+	n.d.	n.d.
29	ethyl 2-hydroxy-3-methylbutanoate	+	n.d.	n.d.
30	ethyl octanoate	+	+	+
31	(*Z*)-linalool oxide	+	n.d.	+
32	γ-terpineol	n.d.	+	n.d.
33	acetic acid	+	+	+
34	isoamyl hexanoate	n.d.	+	n.d.
35	3-decanone	n.d.	+	+
36	nerol oxide	+	+	+
37	2-ethyl-1-hexanol	+	+	+
38	ethyl 3-hydroxybutanoate	+	n.d.	n.d.
39	1-(4-methoxyphenol)-1,3-butanedione	n.d.	n.d.	+
40	nerol	n.d.	+	n.d.

Tab. 1 continued

No.	Compound	LE	HS-SPME	SBSE
41	α-ionone	+	+	n.d.
54	ethyl decanoate	+	+	+
55	diethyl fumarate	n.d.	n.d.	+
42	2-methyltetrahydrothiophen-3-one	+	n.d.	n.d.
43	propanoic acid	+	n.d.	n.d.
44	linalool	+	+	+
45	1-octanol	+	+	+
46	2-methylpropanoic acid	+	n.d.	n.d.
47	5-methylfurfural	n.d.	n.d.	+
48	diethyl malonate	+	n.d.	+
49	methyl decanoate	n.d.	+	n.d.
50	2-undecanone	n.d.	+	n.d.
51	terpinen-4-ol		n.d.	+
52	3,7-dimethyl-1,5,7-octatrien-3-ol	+	n.d.	+
53	butanoic acid	+	n.d.	n.d.
56	1-nonanol	n.d.	n.d.	+
57	3-methylbutyl octanoate	n.d.	+	n.d.
58	3-methylbutanoic acid	+	n.d.	n.d.
59	diethyl succinate	+	+	+
60	ethyl 9-decenoate	+	+	+
61	α-terpineol	+	+	+
62	2,6-dimethyl-3,7-octadiene-2,6-diol	+	n.d.	n.d.
63	methionol	+	n.d.	n.d.
64	1-dodecanol	n.d.	n.d.	+
65	diethyl glutarate	+	n.d.	+
66	ethyl phenylacetate	+	+	+
67	2-phenylethyl acetate	+	+	+
68	β-damascenone	n.d.	+	+
69	ethyl dodecanoate	+	+	+
70	hexanoic acid	+	+	+
71	2-phenyethanol	+	+	+
72	(*E*)-2-hexenoic acid	+	n.d.	n.d.
73	2,6-dimethyl-7-octene-2,6-diol	+	n.d.	n.d.
74	4-ethylguaiacol+	n.d.	+	
75	diethyl malate	+	n.d.	+
76	ethyl 3-hydroxypentadecanoate	n.d.	+	n.d.
77	octanoic acid	+	+	+
78	ethyl 3-hydroxytridecanoate	n.d.	n.d.	+
79	ethyl 3-phenyl-2-propenoate	+	+	+
80	γ-undecalactone	n.d.	n.d.	+
81	nonanoic acid	n.d.	n.d.	+
82	4-ethylphenol	+	n.d.	n.d.
83	4-vinylguaiacol	+	n.d.	+
84	tetradecanoic acid	n.d.	n.d.	+
85	decanoic acid	+	+	+
86	9-decenoic acid	+	n.d.	+
87	hexadecanoic acid	n.d.	n.d.	+
88	2,3-dihydrobenzofuran	n.d.	n.d.	+
89	δ-dodecalactone	n.d.	n.d.	+
90	2-furancarboxylic acid	+	n.d.	n.d.
91	ethyl citrate	+	n.d.	n.d.
92	dodecanoic acid	+	n.d.	+
93	benzeneacetic acid	+	n.d.	n.d.
94	methyl vanillate	+	n.d.	n.d.

n.d. = not detectable (the detection limit is about 0.5 ppb v/v calculated with 3-decanol as the internal standard).

comes more accentuated (Baltussen *et al.* 1999). The advantage of HS-SPME and SBSE methods is that the volatiles are extracted from wine without organic solvents. Therefore, in their chromatograms the solvent peak does not appear (peak S in Fig. 1). This may be important for detection of some interesting peaks that elute with the solvent peak, for example ethyl acetate (Ortega-Heras *et al.* 2002). HS-SPME, which is suitable for rapid analyses of wine, needs low sample size and the process can be completely automated. To compare methods Tab. 2 summarises the compounds determined in two wines.

In Rhine Riesling wine the following compounds had higher peaks: ethyl 2-hydroxybutanoate, linalool, 4-ethylguaiacol, ethyl 9-decenoate, ethyl 3-phenyl-2-propenoate, 9-decenoic acid and dodecenoic acid, while the components ethyl-3-hexenoic acid, (*E*)-2-hexen-1-ol, 3-methylbutyl octanoate and benzoic acid were not detected (the detection limit is approximately 0.5 ppb v/v calculated with 3-decanol as the internal standard).

Tabs 3 and 4 show results of semi-quantitative analyses of these wine volatiles as relative concentrations related to the internal standard (0.1 ppm v/v). In Fig. 2 the results of semi-quantitation are visualized for esters, alcohols and acids. The standard deviations and standard errors depend on the individual substances and the concentration patterns of the matrix. The median standard error over all 12 substances increased in the order LE (4.7 %), SBSE (5.7 %) and SPME (14.8 %). The low reproducibility of SPME is due to the special concentration pattern of the wine matrix. Major compounds like 3-methyl-1-butanol and 2-phenylethanol may block the limited adsorption sites of the fiber polymer. As a consequence, components with high volatility and low concentration (3-methyl-1-propanol and isoamyl acetate) are discriminated and extracted with low reproducibility. In Rhine

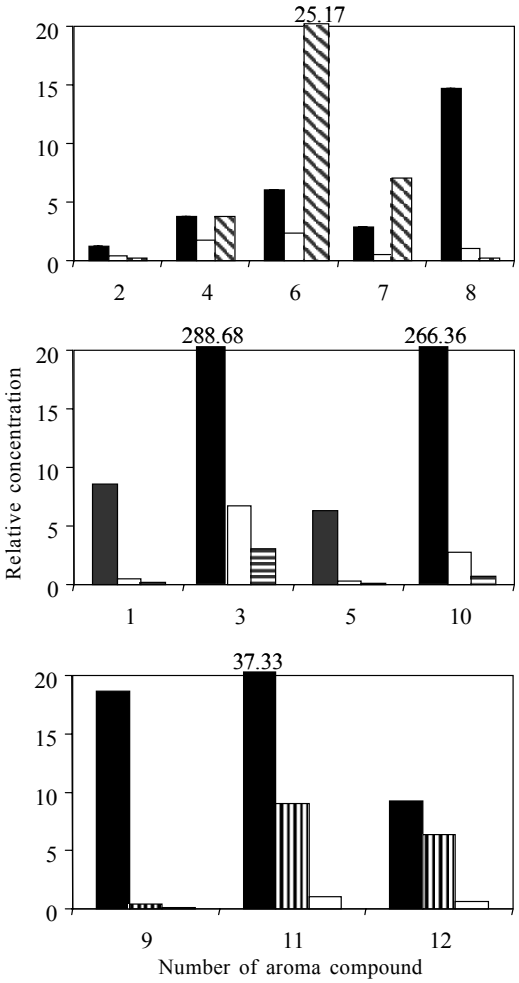

Fig. 2: Relative concentrations of 12 volatiles in Rhine Riesling wine. Black bars: LE; white bars: SBSE; striped bars: SPME. The x-axis represents the compound numbers (see Tab. 2).

Table 2

Volatile compounds used for comparison of sample preparation methods

No.	RT (min)	RI	Compound	Ident.	Literature
1	12.14	1105	2-methyl-1-propanol[1]	MS	Guth 1997, Lee *et al.* 2003
2	13.46	1135	3-methyl-1-butanol acetate	MS	Guth 1997, Lee *et al.* 2003, Demyttenaere *et al.* 2003
3	17.89	1235	3-methyl-1-butanol	MS, RT	Guth 1997, Demyttenaere *et al.* 2003
4	18.58	1251	ethyl hexanoate	MS, RT	Guth 1997, Lee *et al.* 2003, Demyttenaere *et al.* 2003
5	23.79	1368	1-hexanol	MS, RT	Guth 1997, Lee *et al.* 2003, Demyttenaere *et al.* 2003, Perez-Coello *et al.* 2003
6	27.23	1449	ethyl octanoate	MS, RT	Guth 1997, Lee *et al.* 2003, Demyttenaere *et al.* 2003
7	35.56	1656	ethyl decanoate	MS, RT	Lee *et al.* 2003, Demyttenaere *et al.* 2003, 20, 21
8	37.14	1698	diethyl succinate	MS	Demyttenaere *et al.* 2003, Perez-Coello *et al.* 2003
9	43.46	1877	hexanoic acid	MS, RT	Guth 1997, Lee *et al.* 2003, Demyttenaere *et al.* 2003, Perez-Coello *et al.* 2003
10	46.03	1952	2-phenylethanol	MS, RT	Guth 1997, Lee *et al.* 2003, Demyttenaere *et al.* 2003
11	50.62	1975	octanoic acid	MS	Lee *et al.* 2003, Perez-Coello *et al.* 2003
12	56.95	1922	decanoic acid	MS	Guth 1997, Lee *et al.* 2003, Demyttenaere *et al.* 2003, Perez-Coello *et al.* 2003

[1] On the wax column 2-methyl-1-propanol is eluted together with a small amount of an unknown compound. MS - identification by MS and library search, RT -identification by coelution of authentic references.

Table 3

Results of GC-FID analyses of Rhine Riesling wine obtained by three different sample preparation methods

Compound	Liquid-liquid extraction	HS-SPME	SBSE
3-methyl-1-propanol	8.6 ± 0.71	0.2 ± 0.08	0.5 ± 0.03
3-methyl-1-butanol acetate	1.2 ± 0.10	0.2 ± 0.13	0.4 ± 0.03
3-methyl-1-butanol	288.7 ± 23.30	3.1 ± 0.18	6.7 ± 0.39
ethyl hexanoate	3.8 ± 0.27	3.8 ± 0.33	1.8 ± 0.05
1-hexanol	6.4 ± 0.40	0.1 ± 0.07	0.3 ± 0.02
ethyl octanoate	6.0 ± 0.14	25.2 ± 1.13	2.4 ± 0.06
ethyl decanoate	2.9 ± 0.09	7.0 ± 0.87	0.5 ± 0.01
diethyl succinate	14.7 ± 0.11	0.2 ± 0.01	1.1 ± 0.05
hexanoic acid	18.7 ± 0.11	0.1 ± 0.01	0.4 ± 0.04
2-phenylethanol	266.4 ± 3.96	0.7 ± 0.06	2.8 ± 0.13
octanoic acid	37.3 ± 1.43	1.0 ± 0.06	9.1 ± 0.37
decanoic acid	9.2 ± 0.55	0.6 ± 0.08	6.3 ± 0.63

Results represent means of a 7-fold replication calculated as relative concentrations related to an internal standard (0.1 ppm v/v). Data are means ± standard deviation (SD).

Table 4

Results of GC-FID analyses of Gf.Ga-52-42 wine obtained by three different sample preparation methods

Compound	Liquid-liquid extraction	HS-SPME	SBSE
3-methyl-1-propanol	6.2 ± 0.23	0.2 ± 0.02	0.5 ± 0.12
isoamyl acetate	0.7 ± 0.02	0.2 ± 0.04	0.4 ± 0.07
isoamyl alcohol	286.2 ± 8.92	4.0 ± 0.35	10.6 ± 2.21
ethyl hexanoate	2.5 ± 0.08	2.4 ± 0.15	1.9 ± 0.10
1-hexanol	8.2 ± 0.25	0.2 ± 0.01	0.6 ± 0.10
ethyl octanoate	3.5 ± 0.05	14.0 ± 0.70	2.5 ± 0.05
ethyl decanoate	1.2 ± 0.07	2.6 ± 0.21	0.2 ± 0.01
diethyl succinate	17.0 ± 0.16	0.2 ± 0.03	1.5 ± 0.24
hexanoic acid	14.4 ± 0.11	0.1 ± 0.02	0.4 ± 0.08
2-phenylethanol	123.3 ± 1.50	0.4 ± 0.09	1.9 ± 0.32
octanoic acid	25.5 ± 0.62	0.6 ± 0.12	6.1 ± 0.75
decanoic acid	4.2 ± 0.21	0.2 ± 0.04	3.1 ± 0.29

For details see Tab. 3.

Riesling wine these compounds had the highest standard errors with 31.2 % and 69.1 %, respectively.

Fig. 3 shows a correlation analysis using the 12 volatile concentrations as parameters and the three methods as variables. For Gf.Ga-52-42 wine the following coefficients (on the 5 % level) were found; LE versus SPME: 0.07; LE versus SBSE: 0.80 and SBSE versus SPME: 0.20. Also the results of correlation analysis point out the higher similarity of LE and SBSE results compared to those of SPME.

In conclusion, these investigations demonstrate the advantages of the liquid-liquid extraction technique for volatile isolation regarding recovery range of analyte polarity, reproducibility and robustness. Since chlorinated solvents are banned now worldwide for reasons of environmental protection, liquid-liquid extraction will be performed only with non-chlorinated solvents. Unfortunately these solvents have a lower extraction capability than Freons. In spite of the disadvantages of SPME and SBSE, both techniques are profitable for special purposes. The adsorption methods can be applied nearly fully automated. Additionally, the absence of organic solvents and the possibility of using very small sample sizes enable the application of these methods for rapid screenings of huge numbers of samples as, for instance, in metabolomics (Ulrich *et al.* 2003). Moreover, using SPME, the chromatographic separation of some terpenes is improved.

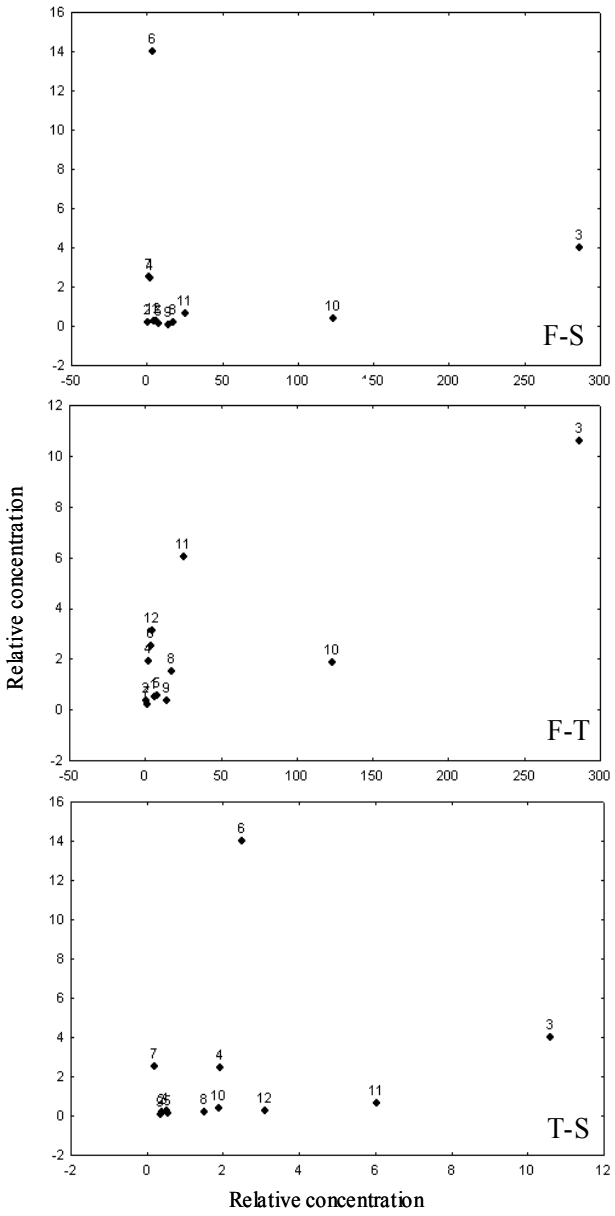

Fig. 3: Results of a correlation analysis with Gf.Ga-52-42 wine. The axes represent the relative concentrations. **F-S**: LE versus SPME; **F-T**: LE versus SBSE; **T-S**: SBSE versus SPME.

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