Research Note

Fast Screening Method for Wine Headspace Compounds Using Solid-Phase Microextraction (SPME) and Capillary GC Technique

GY. VAS[1*], K. KÖTELEKY[2], M. FARKAS[3], A. DOBÓ[4], and K. VÉKEY[5]

Solid-phase microextraction (SPME) coupled to capillary gas chromatography-mass spectrometry (GC-MS) was used for determination of volatile wine components. This combination offers a simple, quick, and sensitive approach suitable for characterization of wine aroma compounds without a complicated sample preparation procedure. Wines are characterized by "aromagrams", a set of identified components with corresponding relative abundances. Reproducibility (RSD errors of relative peak abundances) due to the analytical procedure are *ca.* 4%; variations among different samples of the same type of wine from the same region are *ca.* 8%. SPME-GC(-MS) has been shown to yield far larger differences among different wine types (Chardonnay, Muscat Ottonel, and Tramini) and among the same type of wine produced in different regions, showing the utility of the technique in wine analysis.

KEY WORDS: solid-phase microextraction, wine headspace compounds, capillary gas chromatography, GC-MS

Aromas are the most important components of wines; over 1000 aroma compounds have been identified. These compounds originate from the grape, and most are formed during fermentation. Aroma production is influenced by various factors: environment (soil, climate), grape variety, ripeness, fermentation conditions (pH, temperature, yeast flora), the wine production process (enological methods, treatment substances), aging (bottle maturation), *etc.* [10]. Wine aromas contain various classes of compounds such as hydrocarbons, alcohols, terpene alcohols, esters, aldehydes, ketones, acids, ethers, lactones, bases, sulfur-compounds, halogenated compounds, and nitriles [10,14]. Some of these compounds are volatile or highly volatile (hydrocarbons, terpene alcohols), while others have low volatility.

Wines contain aroma compounds in a wide concentration range, some components being present in high concentration (hundreds of mg/L), but most are found at the low mg/L or ng/L level. The low concentration of most volatile components of wine makes extraction and concentration necessary before analysis by high resolution gas-chromatography (HRGC) or by GC-MS. Several extraction-concentration methods have been used, such as liquid-liquid extraction [4,7,9,14], liquid-liquid extraction with ultrasound [2], simultaneous distillation-extraction [8], solid phase extraction [3], and other techniques [5,11,12,15]. These techniques are generally labor-intensive and of relatively low reproducibility. Sample preparation is mainly used to obtain more concentrated samples, but the elimination of interfering substances and simultaneously improving the detection limit for specific compounds is also important. There is, however, no general procedure which is suitable for all purposes.

The specific advantages and disadvantages of these methods are always considered when selecting the most adequate technique for a given problem. Solid Phase Microextraction (SPME) is a new technique for concentration of samples prior to analysis [1,6,16]. Its main advantages are that it is very simple, requires little sample manipulation and is very fast. SPME is a solvent free technique that can be used either for headspace analysis or direct extraction of analytes from liquids. SPME with capillary GC and GC-MS has recently been used for the analysis of wine aromas [13]. Some important fragrance compounds, like ethyl-esters and terpene alcohols, can be enriched selectively during analysis by SPME, depending on the type of extraction fiber. Headspace GC-MS proved to be an excellent technique for aroma characterization: it is selective, sensitive, quick, simple, and relatively inexpensive. Under the experimental conditions employed, detection limits for some components using headspace are in the low ng/L level (ethyl-octanoate, ethyl-decanoate, terpene-alcohols, ß-phenethyl-alcohol), for some other components they are in the low mg/L level (ethyl-acetate, alcohols) [13]. Needless to say, this technique can be used for aroma characterization not only of wines, but of spices, fruits, and other food products. The purpose of the present work is to demonstrate the utility of SPME coupled to GC or to GC-MS analysis for the characterization of wine aromas. Applications from two areas are shown: dependence of the aroma components on the place of origin and on the type of grapes.

Materials and Methods

Samples: Several Muscat Ottonel wine samples, originating from four different regions were analyzed:

[1,2]Research Institute for Viticulture & Enology of Agricultural Ministry, Eger, Hungary; [4,5]Central Research Institute for Chemistry of the Hungarian Academy of Sciences, Budapest, Hungary.

*Corresponding author [E-mail: H12232vas@ella.hu].

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five samples from Eger, Hungary; three samples from Mátra, Hungary; one sample from Siklós, Hungary; and one sample from Trento, Italy. Other wine types, produced in the Eger wine region were also studied (5 Chardonnay and 5 Tramini samples). The wines were fermented under similar fermentation conditions and were from the 1995 vintage.

Sample preparation: Wine samples were studied with the SPME technique according to the following protocol. The sample (125 mL) was placed into a 130 mL sampling bottle. A 100-µm diameter polydimethylsiloxane (PDMS) coated SPME fiber of 10 mm length (Supelco Inc., Bellefonte, PA) was inserted into the head space and held in place for 10 minutes at ambient temperature. During this time the liquid phase (wine) was stirred with a magnetic stirrer. The exact experimental conditions for SPME headspace sampling described above (with the exception of temperature) are not very critical. The large amount of liquid with a small headspace volume was used to minimize changes in the equilibrium in the liquid due to sampling. There are no critical requirements regarding to the sampling bottle, but a teflon valve at the top makes sampling easy. The fiber was then inserted into the GC injector (held at 250°C) for five minutes to desorb the aroma compounds, which were then analyzed by GC (or GC-MS).

Gas-chromatography and mass spectrometry: In the experiments discussed quantitation (peak area measurement) was performed by GC using a FID detector. A Hewlett Packard 5890 series II gas chromatograph equipped with a two-channel Electronic Pressure Control and FID detector was used with a Supelco 30 m x 0.25 mm fused silica capillary column coated with a poly-alkylene-glycol (PAG) stationary phase of 0.25 µm film thickness (Supelco Inc. Bellefonte PA). The PAG phase has a lower polarity, but similar characteristics to the PEG phase, so retention indices are somewhat different. For comparison, retention indices of some aroma components using both PAG and PEG phase are provided in Table 1. This can be advantageous, particularly if some peaks of interest are not resolved on a PEG column. The injector and the FID detector temperatures were 250°C, the splitless purge valve was closed for five minutes, the carrier gas was hydrogen (UCAR, purity 5.5), the gas flow was 1.8 mL/min. The temperature program of the GC was the following: initial temperature, 35°C (5 min hold); first ramp, 5°C/min to 100 °C (0 min hold); second ramp, 3°C/min to 200°C (1 min hold); and third ramp, 10°C/min to 220 °C (0 min hold).

The compounds were identified by mass spectrometric analysis (GC-MS) and by retention indices. In these analyses the same GC with a Hewlett-Packard 5972 MSD mass selective detector in electron impact ionization mode (70 eV) was used. GC run parameters were the same as described above, but the carrier gas was He. Retention indices were calculated from retention times using external calibration, twice a day, utilizing a software written by János Harangi (Hewlett Packard Hungary). The calibration mixture contained 20 aliphatic hydrocarbons (C_6-C_{27}). Day to day reproducibility of retention index determination was ±1 unit.

Results and Discussion

Initial tests [13] have shown the utility of Solid Phase Microextraction (SPME) coupled to capillary GC and GC-MS for the characterization of wine aroma compounds. The prime advantages are the simplicity of sample preparation, and the sensitivity and selectivity of the analysis. In the present study we have used head space analysis (the SPME fiber was inserted into the head space, and not directly into the wine) with an apolar (polydimethylsiloxane coated) SPME fiber. Both head space analysis and extraction by the SPME fiber (and to a smaller degree also detection by FID or MS) are compound-selective. This means that relative peak areas are not equal to the relative concentrations of various wine aroma components. Differences of relative abundances (peak areas) among various wine samples, on the other hand, do represent changes in the composition of wines — so wines can be characterized and compared using peak areas determined by the given experimental setup. The relative peak areas defined this way will be described as "aromagrams" in the following text. Using a different analytical technique (*e.g.*, a different SPME fiber, or immersion of the fiber into the wine) does result in a different aromagram [13]. For this reason aromagrams obtained by the same technique will always (and should) be compared. Using suitable standards SPME-GC(-MS) analysis can be developed in the future to determine absolute concentrations as well, but this has not been attempted here.

Abundant peaks observed in the chromatograms have been labelled from 1 to 14, their retention indices are shown in Table 1. Chromatograms have been obtained from various wine samples; an example is shown in Fig. 1A (a Chardonnay wine from the Eger region). Figure 1B shows that over 100 peaks can easily be quantified - those over *ca.* 0.01% of the most abundant peak in the aromagram.

The reproducibility of peak area measurements, *i.e.*, the error introduced by the analytical method, has been determined using a given batch of Muscat Ottonel wine from the Eger region. This has been sampled and

Table 1. List of selected and identified compounds.

No.	Compound name	Ret. index on PAG col.	Ret. index on PEG col.
1	Isobutanol	1044	1110
2	Isoamyl acetate	1079	1128
3	3-Methyl-1-butanol	1158	1223
4	Ethyl hexanoate	1190	1240
5	Hexanol	1299	1366
6	3-Hexen-1-ol	1302	1302
7	Ethyl octanoate	1390	1440
8	Linalool	1474	1561
9	Linalyl acetate	1486	1563
10	Ethyl decanoate	1588	1649
12	Citronellol	1695	1786
13	Geraniol	1763	1870
14	Phenethyl alcohol	1802	1932

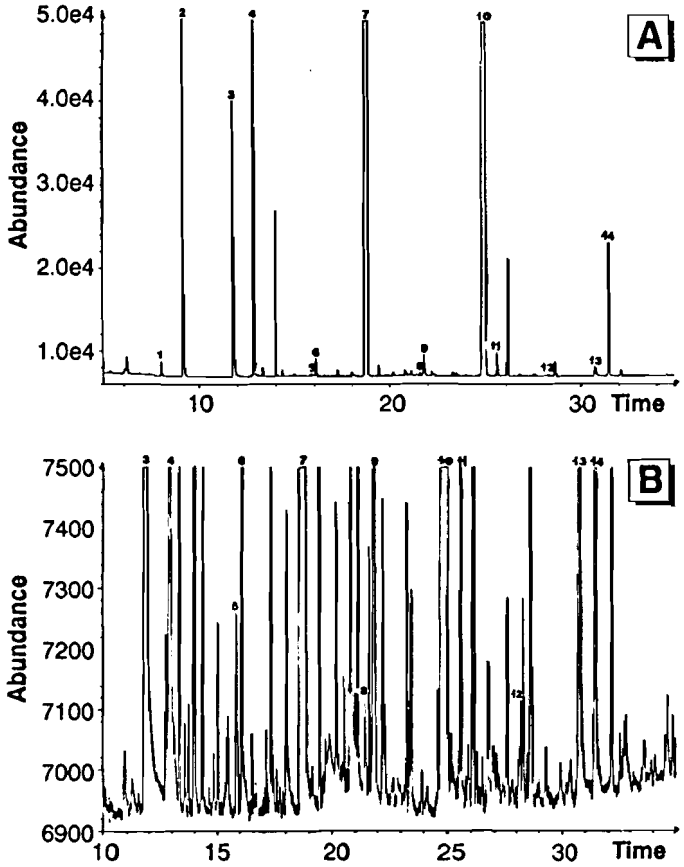

Fig. 1. (A)Head-space chromatogram of Chardonnay wine from Eger region (Hungary). (B) Part of the chromatogram 1/a multiplied by a factor of 75.

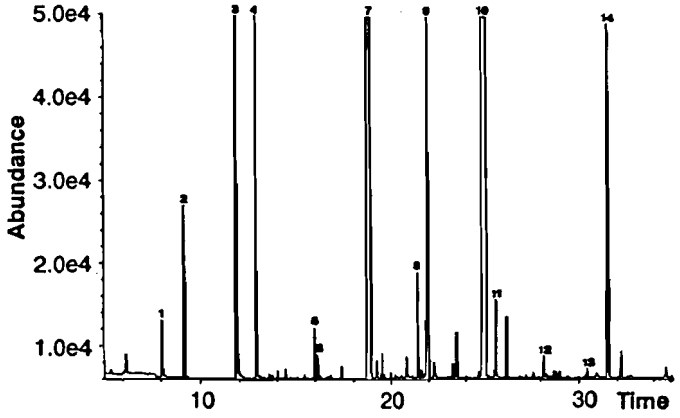

Fig. 2. Head-space chromatogram of Muscat Ottonel wine from Eger region (Hungary).

measured five different times, peaks smaller than 0.1% were not considered in this paper. The reproducibility of the measurements (relative standard deviation, RSD) is in the range of 1% to 10% depending on the components selected. Detailed results on selected peaks are shown in Table 2, the "average" RSD for them is 3.6%.

Various wine samples obtained from the same region (Eger) and same wine type (Muscat Ottonel) were also studied. The wines were fermented under similar fermentation conditions, and were from the 1995 vintage. The differences among the aromagrams of the five different wine samples studied were small, on average only two times higher than the reproducibility of the analytical technique. It seemed reasonable therefore to characterize the variation in peak intensities by relative standard deviations, as used above and the results are shown in Table 2. These values characterize the errors connected to the analytical method, to sampling, and to small, unintentional variations in cultivation, fermentation, place of origin within a wine region. In the following text these errors will be referred to as "sampling" errors. Very similar RSD values were obtained using three different Muscat Ottonel wine samples from the Mátra region (Hungary) the results are also shown in Table 2.

The aromagrams of various wine types show large and characteristic differences; Chardonnay, Muscat Ottonel, and Tramini type wines originating from the Eger region were compared (in each case five different samples). SPME-GC chromatograms of these wines (one of each type) are shown in Figures 1, 2, and 3; areas of the major peaks are listed in Table 3. Relative standard deviations due to sampling errors (as

Table 2. Reproducibility of peak area (peak areas normalized to the peak of ethyl octanoate) measurements using the SPME-GC technique.

No.	Compound name	Rel. peak area	RSD% Repr. of technique using single MO wine	RSD% between different MO wines from Eger region	RSD% between different MO wines from Mátra region
1	Isobutanol	0.75	2.40	3.02	2.65
2	Isoamyl acetate	2.30	1.50	3.23	2.14
3	3-Methyl-1-butanol	9.50	5.00	5.64	4.22
4	Ethyl hexanoate	6.42	4.40	5.42	6.22
5	Hexanol	0.65	1.10	6.45	6.15
6	3-Hexan-1-ol (Z)	0.27	4.70	3.46	4.45
7	Ethyl octanoate	100.00			
8	Linalool	1.13	2.70	5.30	4.32
9	Linalyl acetate	7.30	8.30	14.30	0.52
10	Ethyl decanoate	102.00	0.80	11.20	8.75
11	Terpineol	1.03	2.20	13.20	9.45
12	Citronellol	0.30	1.80	15.42	2.46
13	Geraniol	0.15	4.70	11.40	0.20
14	Phenylethyl alcohol	7.60	7.30	7.80	8.20
	Average RSD%		3.60	8.14	6.90

MO = Muscat Ottonel.

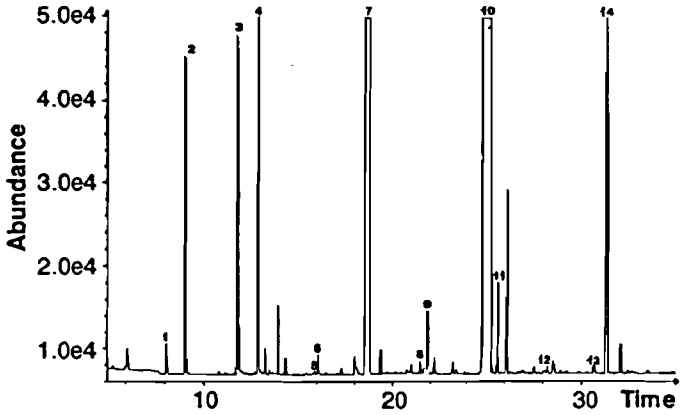

Fig. 3. Head-space chromatogram of Tramini wine from Eger region (Hungary).

defined above, also shown in Table 3) are similar to those discussed above (between 5% and 10% on average, less than 20% even in the worst case). The pattern of main aroma components is significantly different for the three wine types. Among the main aroma components listed in Table 3 in five cases (isoamyl-acetate, hexanol, linalool, linalyl-acetate, and citronellol) there are over 10 fold differences in relative concentrations — 100 times larger, than that due to sampling errors. While it is not surprising that the taste (aromagrams) of these wines is different, it is significant and encouraging, that a simple analytical procedure, like SPME-GC, is capable of quantifying these differences.

Probably more important for practical purposes, is that the aromagrams show large and characteristic differences between wines grown in different regions. Aromagrams of Muscat Ottonel wines originating in four different regions (Eger, Mátra, and Siklós in Hungary; and Trento, Italy) were compared. The relative peak abundances selected peaks are listed in Table 4. Data shown in Table 4 clearly indicate, that the concentration of aroma components vary among regions to a far larger extent, than warranted by sampling errors. In eight cases (out of the 14 listed in Table 4) the difference among the abundances is over a factor of two, while sampling errors never exceed 20% (RSD). This result strongly suggests that SPME-GC(-MS) can provide valuable analytical clues relating to the place of origin of a wine sample.

Table 3. Comparison of Chardonnay, Muscat Ottonel, and Tramini wines from the Eger region based on relative peak abundances obtained by the SPME-GC technique.

No.	Compound name	Chardonnay	RSD%	Tramini	RSD%	Muscat Ottonel	RSD%
1	Isobutanol	0.65	2.75	0.56	2.14	0.79	3.02
2	Isoamyl acetate	38.16	4.25	1.03	3.12	2.32	3.23
3	3-Methyl-1-butanol	23.9	4.82	4.85	4.15	9.54	5.64
4	Ethyl hexanoate	19.11	5.17	6.21	4.7	6.29	5.42
5	Hexanol	0.56	6.75	0.04	5.12	0.67	6.45
6	3-Hexan-1-ol (Z)	0.33	4.12	0.12	3.79	0.24	3.46
7	Ethyl octanoate	100.00		100.00		100.00	
8	Linalool	0.93	5.6	0.11	4.7	1.17	5.3
9	Linalyl acetate	0.32	17.2	0.23	11.2	7.32	14.3
10	Ethyl decanoate	84.05	14.2	73.3	12.4	104.00	11.2
11	Terpineol	0.12	8.73	0.9	9.75	1.009	13.4
12	Citronellol	0.02	10.29	0.14	11.2	0.29	15.42
13	Geraniol	0.04	7.65	0.1	9.5	0.13	11.4
14	Phenylethyl alcohol	4.77	8.2	6.34	8.4	7.53	7.8

Table 4. Comparison of Muscat Ottonel wines produced in different regions based on relative peak abundances obtained by the SPME-GC technique.

No.	Compound name	Eger (n = 5)	Mátra (n = 3)	Siklós	Trento	Sampling error (SD%)
1	Isobutanol	0.79	1.34	0.3	0.28	2.84
2	Isoamyl acetate	2.32	4.16	6.95	4.5	2.69
3	3-Methyl-1-butanol	9.54	18.9	5.57	7.32	4.93
4	Ethyl hexanoate	6.29	11.87	7.75	6.55	5.82
5	Hexanol	0.67	0.82	10.5	1.08	6.3
6	1-Hexan-1-ol (Z)	0.24	0.69	0.17	0.25	3.96
7	Ethyl octanoate	100.00	100.00	100.00	100.00	
8	Linalool	1.17	0.59	1.08	0.69	4.81
9	Linalyl acetate	7.32	0.26	0.13	1.9	12.41
10	Ethyl decanoate	104.00	60.82	71.0	130.00	9.98
11	Terpineol	1.09	1.42	0.99	1.71	11.33
12	Citronellol	0.29	0.1	0.57	0.05	13.94
13	Geraniol	0.13	0.23	0.15	0.13	10.8
14	Phenylethyl alcohol	7.53	7.52	4.65	1.64	8.0

Conclusions

Solid phase microextraction is a fast inexpensive and user friendly extraction method which can be combined with GC or GC-MS analysis. The technique is suitable for the characterization of wine headspace components without any further sample preparation. Results presented show excellent reproducibility of the analytical technique (*ca.* 4% RSD of peak abundances), and small variations among different batches wines produced in a region (5% to 10% RSD). SPME-GC-MS has shown to be capable of distinguishing different wine types and wines produced in different regions.

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