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Rapid determination of volatile compounds in grapes by HS-SPME coupled with GC–MS

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Abstract

Volatile compounds of grapes are responsible of varietal aroma. At the moment, methods used for analysis of these compounds are solvent-based, time-consuming and generally require large amounts of sample. In order to obtain an appropriate technique to study grape volatile compounds, HS-SPME method has been developed. The optimal sampling conditions were: 70 °C for 20 min with a 65-μm PDMS/DVB fibre. Sixteen volatile compounds have been quantified in pulp and skins of Muscat grapes. Terpenes, mainly linalool, geraniol and nerol, have been the volatiles present in the highest concentration, since these compounds contribute, to a larger extent, to the aroma of Muscat grapes and wines. So the proposed technique can be used for the characterisation of grape varieties or cultivars and for the determination of the aromatic maturity of grapes.

Keywords: Solid-phase microextraction; Grapes; Skin; Volatile compounds

1. Introduction

Grape volatile compounds are the main contributor to the fresh and fruity note of wines. Compounds responsible for this aroma (terpenes, C_{13}-norisoprenoids, benzene derivatives, and aliphatic alcohols) are present in grapes, mainly in the skin [1]. Concentration of these volatile compounds is different depending on the grape variety, cultural practiques, and climatic or biological factors [2,3].

On the other hand, grape volatile composition can greatly vary during ripening [4]. Traditionally grape maturity corresponds to an optimal sugar/acid ratio, however production of quality white wines requires grapes whose aromatic substances are at a maximal concentration [5]. Monitoring aromatic grape maturation needs a simple and rapid method to analyse volatile compounds in grape using as little amount of sample as possible to enable an adequate sampling.

Volatile compounds of grapes are generally present in trace amounts and require a previous step of isolation and concentration for the subsequent gas chromatography analysis. Most of the proposed methods are based on obtaining must by previously pressing the grapes, and the skin composition is not taken into account. Different methods have been used for isolating volatile compounds from must such as simultaneous distillation-extraction [6] or solid-phase extraction [7,8]. Berry fractions were analysed after homogenisation and centrifugation of 500 g of grapes [9,10].

These methods are based on the use of solvents and present some drawbacks, such as the possibility of sample contamination and the loss of analytes during the concentration step. Additionally they are time-consuming and in general require high temperatures and large amounts of sample.

Dynamic headspace technique has been used to analyse the volatile composition of grapes in order to discriminate different varieties [11].

Solid-phase microextraction (SPME) is a fast, simple and solvent-free technique that, thanks to the appearance of differ-

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ent types of adsorbents with a wide range of polarity, makes it possible to isolate trace compounds of different substrates [12]. SPME has been used in a range of fields including studies of flavours and taints especially for quick screening of the volatile composition of a wide range of products [13].

Traditional fibres (PDMS and PA) have been used to determine terpenes and fermentation compounds in wines [14–18], fruits and juices [19–22], however these fibres present a poor sensitivity for polar compounds.

Mixed coating fibres containing divinylbenzene (DVD), PDMS and carboxen (CAR) or polyethylene glycol (CW), increase the tramping ability of the fibre due to the synergic effect of adsorption and distribution within the stationary phase, producing higher sensitivity than PDMS and PA fibres [23].

In this work, we propose a method for a rapid analysis of volatile compounds of grape (pulp and skin) using HS-SPME-MS. These method can be useful for monitoring grape maturation and to determine grape composition before winemaking. Optimisation of operating conditions and selection of the best fibre were evaluated.

2. Experimental

2.1. Samples

Commercial samples of Muscat grapes were purchased from a local store, and frozen at −20 °C until their analysis. Before isolating the volatile compounds, the grapes were unfrozen at 5 °C under nitrogen.

2.2. HS-SPME conditions

The grapes were manually pealed and the skins were separated from the pulp. Both fractions (skin and pulp) were individually homogenised and analysed.

Three SPME fibre coatings were tested and used: 65 μm PDMS/DVB, 50/30 μm CAR/DVB/PDMS, and 70 μm CW/DVB. Fibres were exposed to the headspace of a 30-mL capped vial, which contained 4 g of skins, or 14 g of pulp, and 1 or 2 μL of internal standard (4-nonanol in EtOH, 1.067 g L^{-1}), respectively, for 20 min at 70 °C. All fibres were supplied by Supelco (Bellefonte, Pennsylvania, USA) and were conditioned by keeping them in the GC injector following instructions from manufacturer.

After this, fibres were desorbed in a split/splitless injector at 250 °C for 5 min (splitless mode 0.8 min).

Operating conditions were optimised realising SPME extractions of real samples (skins) at different adsorption temperatures (40, 50, 60, and 70 °C) and times (10, 20, 30, 40, and 50 min).

Standard curves were obtained by the SPME extraction under stirring, at optimal sampling conditions, and subsequent injection into the GC system, in duplicate, of synthetic must solutions (100 g of glucose and 100 g of fructose in Milli-Q water, pH adjusted at 3.5), containing defined amounts of the compounds of interest, at different ratios. The compounds used and their concentration range in the solutions are listed in Table 1. Standard mixtures were prepared in a solution of distilled water and 200 g of glucose/fructose (1:1) at pH 3.5.

2.3. GC–MS analysis

GC–MS analysis was performed on an Agilent gas chromatograph model 6890N coupled to a mass selective detector model 5973 *inert*. Compounds were separated on a BP-21 capillary column (50 m × 0.32 mm i.d.; 0.32 μm film thickness). Column temperature was 70 °C (5 min) – 1 °C min^{-1} – 95 °C (10 min) – 2 °C min^{-1} – 190 °C (40 min). Transfer line temperature was 280 °C. Mass detector conditions were: electronic impact (EI) mode at 70 eV; source temperature: 178 °C; scanning rate 1 scan s^{-1}; mass

Table 1
Calibration curves and performance characteristics

Compounds	Linear range (ppm)	Slope	Intercept	r^2	Detection limit (ppm)	Quantitation limit (ppm)
Hexanal	0.10–1.92	0.08	−0.03	0.993	0.071	0.072
(*E*)-2-Hexenal	0.05–1.60	0.06	0.01	0.997	0.022	0.023
1-Hexanol	0.20–24.00	0.05	−0.07	0.995	0.291	0.292
(*Z*)-3-Hexen-1-ol	0.02–1.32	0.02	0.00	0.996	0.024	0.026
(*E*)-3-Hexen-1-ol	0.02–1.32	0.05	0.00	0.992	0.021	0.024
(*E*)-2-Hexen-1-ol	0.10–12.12	0.03	−0.01	0.991	0.087	0.089
trans-Linalool oxide furan	0.01–1.40	0.05	0.00	0.997	0.025	0.034
cis-Linalool oxide furan	0.01–1.40	0.06	0.00	0.998	0.084	0.096
Benzaldehyde	0.02–1.80	0.05	−0.01	0.999	0.042	0.043
Linalool	0.05–6.24	0.59	0.10	0.994	0.035	0.036
α-Terpineol	0.01–1.32	0.82	0.10	0.995	0.026	0.027
Citronellol	0.02–0.46	1.51	0.08	0.995	0.019	0.021
Nerol	0.02–1.32	0.84	−0.03	0.986	0.078	0.079
Geraniol	0.02–1.14	0.76	0.01	0.981	0.017	0.018
Benzyl alcohol	0.05–5.72	0.02	0.00	0.998	0.031	0.034
Phenylethyl alcohol	0.06–7.84	0.04	−0.02	0.994	0.098	0.099

acquisition range: 35–350. Carrier gas was helium at 1 mL min^{-1}.

Identification of the volatile components of grapes was based on comparison of their GC retention times and mass spectra with authentic standards from Sigma-Aldrich and with spectral data from the Wiley G 1035 A library.

3. Results and discussion

3.1. HS-SPME performance

HS-SPME is an equilibrium technique that requires a previous optimisation step of the sampling conditions, in order to obtain high recoveries of volatiles and a good precision of the method. Some of these sampling conditions are extraction temperature and time, and fibre adsorbent phase.

To select the optimum extraction temperature and time, a 50/30 μm CAR/DVB/PDMS fibre was used, since this is one of the last fibres that has been introduced in the market and, due to its nature, it is very suitable for analysing volatile and semivolatile compounds [24–26].

Extraction was carried out at 40, 50, 60, and 70 °C for 10, 20, 30, 40, and 50 min. Fig. 1 shows the result in graphic form, expressed as the sum of areas of all the volatile compounds obtained from skins by use of each set of conditions. The best results were obtained at 70 °C for 20 min.

Extraction temperature and time are critical parameters in the SPME sampling process, such that both have an effect on the equilibrium during extraction. As the time increases (at 60 and 70 °C) the analyte concentration in the fibre also increases, until the equilibrium is reached. Once the equilibrium has been reached, the analyte concentration in the fibre decreases. The same behaviour has been observed in other samples such as oregano [27]. However, when lower temperatures were used, e.g. 40 and 50 °C, the behaviour observed was different, seeing a continuous increase since the equilibrium has not reached in the time of analysis.

As the temperature increases, the recovery of volatile compounds improves, since heating of solid samples help to release analytes into the headspace and facilitate the SPME process [28]. In addition, the equilibrium between the analyte concentration in the fibre and in the sample is reached sooner. For both reasons, a good recovery of volatiles is obtained in a quite short time (20 min) at 70 °C.

Once the adsorption temperature and time were fixed, the trapping ability of the fibre was studied by comparison with other very suitable fibres for analysing volatile compounds: 65 μm PDMS/DVB and 70 μm CW/DVD.

Table 2 shows the area percentage of the volatile compounds identified in a skin sample of Muscat grape extracted with the three fibres tested. PDMS/DVB and CAR/DVB/PDMS fibres extracted a similar proportion of volatile compounds, mainly of those responsible of the typical aroma of Muscat grapes, e.g. linalool, geraniol and nerol, however PDMS/DVB fibre achieved to obtain smaller R.S.D.s, improving the reproducibility of the technique. CW/DVB fibre presented a good ability to trap C6-aldehydes, but it was not suitable to extract terpenes and other volatile compounds present in a lower concentration and higher R.S.D. values were obtained. The more polar compounds are best extracted by polar fibres like CW/DVB [29–31], whereas PDMS/DVB fibre shows the highest sensitivity for aroma compounds of medium and low volatility [32], and it has been used for analysis of aroma compounds in fruits [33,34] and wines [35].

3.2. Calibration curves and performance characteristic

Six levels of concentration were tested in duplicate and regression lines were calculated for each compound, calibration data are shown in Table 1. Linear range covered the volatile compound concentrations expected in the samples and a good regression coefficient (r^2) was obtained in all the cases.

Detection and quantification limits calculated from the baseline of a standard solution chromatogram (as three and ten times the noise of the base line, respectively) were good

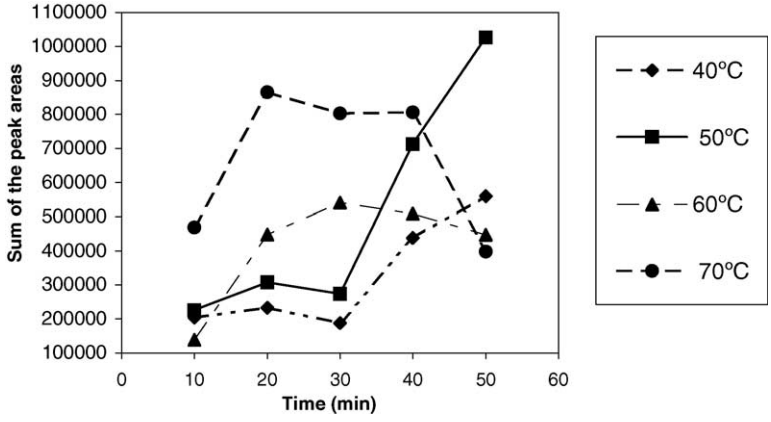

Fig. 1. Effect of sampling conditions (adsorption temperature and time) on the recovery of skin aroma compounds.

Table 2
Volatile composition (%) and R.S.D.s of skin of Muscat grapes obtained by HS-SPME using three different fibres

Compound	PDMS/DVB		CAR/DVB/PDMS		CW/DVB	
	Mean	R.S.D. (%)	Mean	R.S.D. (%)	Mean	R.S.D. (%)
Hexanal	5.6	3.1	6.2	5.3	19.6	27.5
(*E*)-2-Hexenal	3.6	7.3	4.3	21.0	9.0	10.3
1-Hexanol	0.6	4.8	0.6	4.4		
(*E*)-2-Hexen-1-ol	1.3	5.7	0.9	8.6	0.5	
trans-Linalool oxide furan	0.6	3.1	0.4	11.0	0.1	
cis-Linalool oxide furan	0.5	0.7	1.0	13.1	0.2	
Benzaldehyde	0.5	0.3	0.6	15.2		
Linalool	54.5	2.4	50.4	6.1	46.4	3.8
α-Terpineol	0.2	5.9	1.3	3.0	0.4	
trans-Linalool oxide pyran	1.4	5.2	2.6	1.5	1.5	1.2
cis-Linalool oxide pyran	0.9	4.5	1.2	5.3		
Citronellol	1.7	0.7	1.8	6.8	1.0	38.4
Nerol	9.8	2.2	9.6	7.8	5.0	11.8
Geraniol	17.5	5.4	17.3	7.9	15.1	14.7
Benzyl alcohol	0.8	5.3	1.0	14.6		
2-Phenylethanol	0.7	5.9	0.8	6.8	1.0	41.1

enough to analyse the volatile compounds considered in grapes.

3.3. Analysis of volatile compounds in pulp and skins of Muscat grapes

Fig. 2 shows the total ion chromatogram obtained for a pulp sample of Muscat grapes with the PDMS/DVB fibre at the optimal sampling conditions. Sixteen compounds were identified in pulp and skins of Muscat grapes (Table 3), including C6-alcohols and aldehydes, terpenes and benzenic compounds. The major compounds were linalool, geraniol, and nerol that are responsible for the typical floral aroma of Muscat grapes and contribute to the aroma of their wines. The importance of terpenes as grape and wine aroma substances is well known, and their distribution can be used for the characterisation of grape varieties [1].

A higher concentration of some aroma compounds in skins than in pulp has been described [4]. In general, volatile composition of skins has been studied as compounds ceded to the must or synthetic solutions, but no rapid method to evaluate the actual skin or pulp composition has been done.

Table 3 shows the concentration of volatile compounds in the pulp and skins. The higher concentration of aroma compounds in skins justify the use of must skin contact before fermentation as a suitable technique to increase aromatic substances in musts and wines, specially in neutral grape varieties with low terpenic components [36].

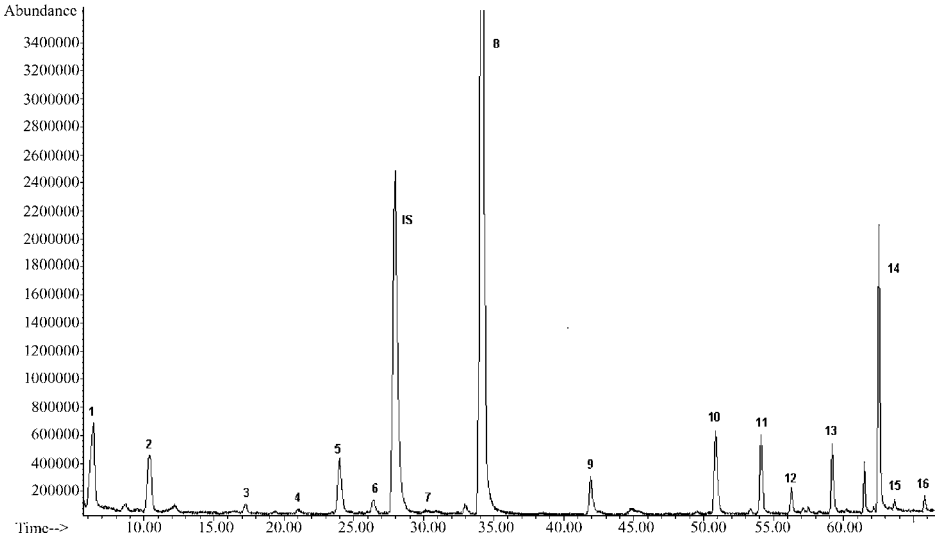

Fig. 2. Total ion chromatogram of pulp from Muscat grapes obtained by HS-SPME using a 65-μm PDMS/DVB fibre. Time scale in minutes. Peak identification: 1, hexanal; 2, (*E*)-2-hexenal; 3, 1-hexanol; 4, (*E*)-2-hexen-1-ol; 5, *trans*-linalool oxide furan; 6, *cis*-linalool oxide furan; 7, benzaldehyde; 8, linalool; 9, α-terpineol; 10, *trans*-linalool oxide pyran; 11, *cis*-linalool oxide pyran; 12, citronellol; 13, nerol; 14, geraniol; 15, benzyl alcohol; 16, 2-phenylethanol.

Table 3
Concentration of volatile compounds identified in pulp and skins of Muscat grapes

Compound	Pulp		Skins	
	Mean ($\mu g\,kg^{-1}$)	R.S.D. (%)	Mean ($\mu g\,kg^{-1}$)	R.S.D. (%)
Hexanal	394.7	4.4	2291.4	10.5
(*E*)-2-Hexenal	313.8	4.1	1477.6	4.6
1-Hexanol	283.3	0.3	689.4	4.2
(*Z*)-3-Hexen-1-ol	n.d.	–	n.d.	–
(*E*)-3-Hexen-1-ol	90.3	10.9	n.d.	–
(*E*)-2-Hexen-1-ol	128.7	7.3	1082.2	5.8
trans-Linalool oxide furan	347.2	10.1	920.9	10.1
cis-Linalool oxide furan	111.4	13.0	n.d.	–
Benzaldehyde	n.d.	–	557.9	4.9
Linalool	462.6	1.8	2353.8	1.4
α-Terpineol	40.8	12.6	n.d.	–
trans-Linalool oxide pyran[a]	2.6	2.8	115.5	5.0
cis-Linalool oxide pyran[a]	0.8	2.7	21.4	8.9
Citronellol	n.d.	–	29.5	7.7
Nerol	29.0	1.5	378.3	11.7
Geraniol	119.3	3.4	1462.0	1.7
Benzyl alcohol	102.1	4.6	1653.2	7.6
Phenylethyl alcohol	142.7	0.1	3316.9	7.7

n.d.: no detected.
[a] Assuming response factor equal to 1.

4. Conclusions

HS-SPME using a PDMS/DVB fibre is a fast and useful method for quantification of volatile compounds in skins and pulp, allowing a rapid screening of aroma compounds in grapes of different varieties or cultivars. This method can also be used to determine the aromatic maturity in grapes in order to decide the optimal harvest date.

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