COMPARISON OF LLE, HS-SPME, DI-SPME EXTRACTION METHODS FOR SCREENING THE VOLATILE COMPOUNDS IN FETEASCA NEAGRA WINE

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Abstract. *Liquid-liquid extraction (LLE), headspace solid phase microextraction (HS-SPME) and direct immersion solid phase microextraction (DI-SPME) techniques, followed by capillary gas chromatography-mass spectrometry (GC-MS), were compared for screening the volatile composition in Feteasca Neagra wine. The compounds were identified by comparing the Kovats indexes and the mass spectra included in the NIST Library. The number of compounds extracted using the three procedures decreased in the following order: LLE-GC-MS, DI-SPME-GC-MS, HS-SPME-GC-MS. Despite of the drawbacks, LLE-GC-MS is a useful technique for extraction, separation and identification of volatile compounds in wine samples.*

Keywords: LLE, SPME, Feteasca Neagra, wine aroma

INTRODUCTION

The aroma is one of the most important factors determining the quality of wine and consists of several hundred compounds with concentrations varying between 10^{-4} - 10^{-11} g/l (Rebiere et al., 2010). These compounds belong to very heterogeneous groups such as alcohols, aldehydes, ketones, esters etc. and have different polarities and volatilities (Camara et al., 2006).

Some of the compounds present at low level in wine, need to be extracted and concentrated before analysis. In order to accomplish that, three approaches have been proposed: liquid-liquid extraction (LLE) with methylene chloride, headspace solid phase microextraction (HS-SPME) and direct immersion solid phase microextraction (DI-SPME) prior to GC-MS analysis.

To enrich aroma compounds, liquid-liquid extraction using different solvent/solvent-mixtures (e.g. pentane/ether, pentane/dichloromethane) is suitable (Rebiere et al., 2010). Liquid-liquid extraction is a time consuming procedure, uses large volume of organic solvent, resulting in a diluted extract and give the possibility of solvent cross-contamination (Camara et al., 2006, Mauriello et al., 2009).

The SPME technique use the principle of solventless extraction of partitioning organic compounds, sorption on the stationary phase, then thermal desorption, analytes separation and identification (Chokshi and Christ, 2006).

The goal of this paper was to compare the qualitative aroma compounds profiles of Feteasca Neagra wine using three sampling procedures, followed by capillary gas chromatography-mass spectrometry. The wine samples were collected at Valea Calugareasca vineyard in 2009.

MATERIAL AND METHOD

Liquid-liquid extraction

Wine samples (50 ml) were extracted twice using 10 ml and 5 ml dichloromethane for gas chromatography (Merck, Darmstadt, Germany). The two organic phases obtained were mixed and dried on sodium sulphate (Merck, Darmstadt, Germany), then concentrated with a vacuum rotary evaporator (Laborota 4010, Germany) until they reached a volume of 2-3 ml, then under a stream of pure nitrogen, they where brought to a volume of 1.0 ml. The extract was injected (1 μl) in the GC-MS, according to the method recommended by Perestrelo et al. (2006).

HS-SPME and DI-SPME sampling

The manual SPME holder and the carboxen-polydimethylsiloxane-divinylbenzene (CAR-PDMS-DVB; 50/30 μm x 1 cm) fibre used were purchased from Supelco (Bellefonte, PA, USA).

The fibre coating selected for aroma compounds extraction was CAR-PDMS-DVB according to other studies (Torrens et al., 2004; Riu-Aumatell et al., 2006; Fedrizzi et al., 2007). New fibres were conditioned in the GC inlet, under a helium stream, at 250 °C for 30 min, according to the producer's instructions.

SPME was carried out with 10 ml wine sample in a 20-ml vial closed with a silicone rubber septum with aluminium cap. Salt, NaCl, 4 g was added to adjust the ionic strength. The vial was positioned on a hot plate with magnetic stirrer (IKA Ret, Germany). The operative conditions (temperature, adsorption time, pH) were optimized according to other studies (Fedrizzi et al., 2007). In HS-SPME mode, the fiber was fixed in the headspace above the solution and in DI-SPME mode, the fibre is immersed directly into the sample solution and the analytes are transferred directly from the sample matrix to the extracting phase (Tankeviciute et al., 2004).

The fibre was maintained in the GC injector for 5 min for complete desorption.

GC-MS analysis

Chromatographic analyses were performed with an 6890 Agilent gas chromatograph (Agilent Technologies), equipped with a 30 m x 0.25 mm i.d., 0.25 film thickness, HP-5 MS, (5%-Phenyl)-methylpolysiloxane (Agilent Technologies) fused silica capillary column, connected to an 5975 B Agilent mass spectrometer (Agilent Technologies). Splitless injection was used. The initial oven temperature was set to 40 °C and the temperature was increased to 220 °C, at 5° min^{-1}. The injector temperature was 250 °C and the transfer line was held at 220 °C. Mass

spectra were recorded after electronic impact (EI) ionisation. The mass-to-charge ratio range (*m/z*) used was 45-400. The ion source temperature was set to 180 °C.

RESULTS AND DISCUSSION

The total ion current (TIC) chromatograms obtained for Feteasca Neagra wine sample by three analytical procedures: LLE-GC-MS, HS-SPME-GC-MS and DI-SPME-GC-MS, respectively, are shown in Figures 1-3.

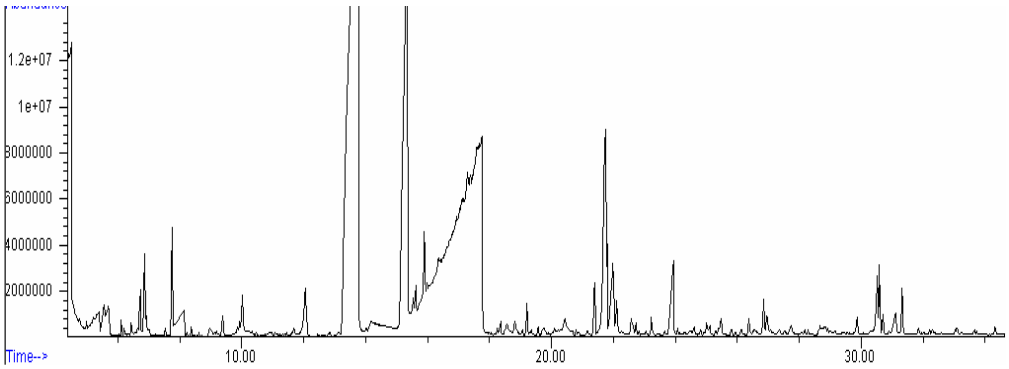

Fig. 1. TIC chromatogram for the LLE-GC-MS analysis of Feteasca Neagra wine

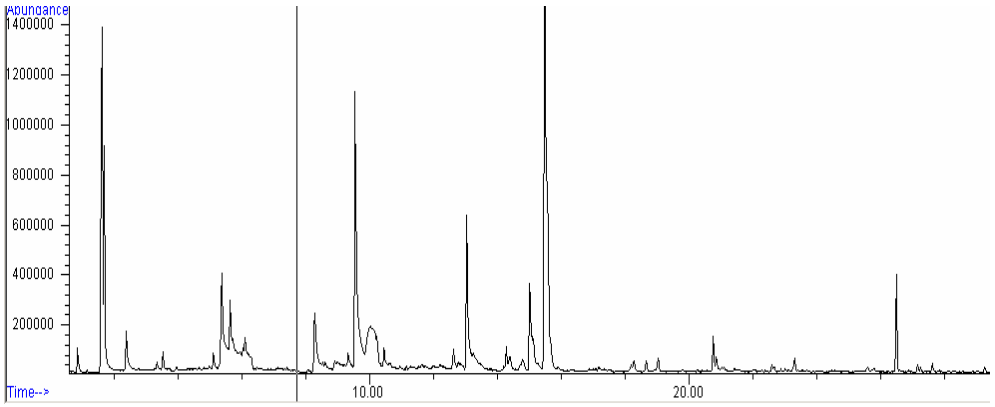

Fig. 2. TIC chromatogram for the HS-SPME-GC-MS analysis of Feteasca Neagra wine

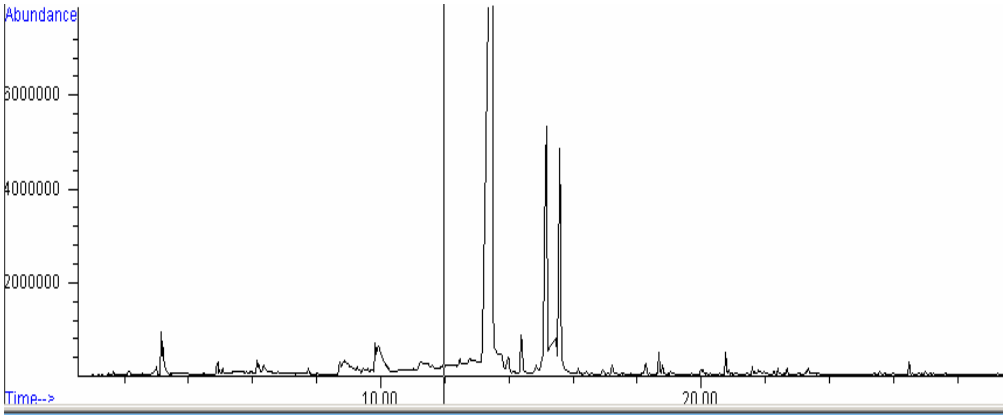

Fig.3. TIC chromatogram for the DI-SPME-GC-MS analysis of Feteasca Neagra wine

Identification of volatile compounds was performed using NIST Library and mass spectral databases and correlation with Kovats indexes reported in literature.

The analysis of chromatograms revealed different number of separated compounds, according to analytical procedure used: 23 for HS-SPME-GC-MS, 35 for DI-SPME-GC-MS and 49 compounds for LLE-GC-MS. The compounds were selected according to Qualifier value (Qual) higher than 85 and are presented in Table 1, for all the three investigated extraction techniques.

The main compounds obtained using HS-SPME-GC-MS procedure belonged to ethyl esters (octanoic acid, ethyl ester; hexanoic acid, ethyl ester; butanoic acid, ethyl ester; decanoic acid, ethyl ester; butanedioic acid, diethyl ester), higher alcohols (1-butanol, 3-methyl; 1-butanol, 2-methyl; phenylethyl alcohol), acetates (1-butanol, 3-methyl-, acetate) and carbonyl compounds (benzaldehyde).

Using DI-SPME-GC-MS technique the obtained main compounds belonged to more numerous classes: ethyl esters (hexanoic acid, ethyl ester; butanedioic acid, diethyl ester; octanoic acid, ethyl ester; decanoic acid, ethyl ester; butanedioic acid, diethyl ester; ethyl hydrogen succinate), acetates (ethyl acetate; 1-butanol, 3-methyl-acetate), higher alcohols (1-butanol, 3-methyl-; 1-butanol, 2-methyl-, (+/-); phenylethyl alcohol; benzeneethanol, 4-hydroxy-; 6-octen-1-ol, 3,7-dimethyl-, (R)-; 1-phenoxypropan-2-ol), lactone (butyrolactone), fatty acids (n-decanoic acid), sulphur compounds (2-hydroxyethyl butyl sulfide; 2-pentanethiol).

The most numerous compounds were obtained using LLE-GC-MS procedure. The predominant compounds were ethyl hydrogen succinate (area=25.9306), phenylethyl alcohol (area=24.4545) and butanedioic acid, diethyl ester (area=9.3156).

Table 1

Summary of the Feteasca Neagra wine volatiles analysed by HS-SPME-GC-MS, DI-SPME-GC-MS, LLE-GC-MS

Crt. No.	HS-SPME-GC-MS		DI-SPME-GC-MS		LLE-GC-MS	
	Peak area	Compound	Peak area	Compound	Peak area	Compound
1	0.5723	Benzene	0.0821	Ethyl Acetate	0.0397	1-Pentanol
2	0.6824	Heptane	0.1582	Benzene	0.2516	Butanoic acid, ethyl ester
3	10.9586	1-Butanol, 3-methyl-	0.8505	1-Butanol, 3-methyl-	0.4198	4-Butoxy-2-butanone
4	6.1504	1-Butanol, 2-methyl-	0.7368	1-Butanol, 2-methyl-, (+/-)-	0.4202	Butanoic acid
5	1.2932	Toluene	0.3174	Benzene, 1,3-dimethyl-	0.0816	1-Propanol, 3-ethoxy-
6	0.2728	Butanoic acid, ethyl ester	0.1925	p-Xylene	0.0530	1-Pentanol, 3-methyl-
7	0.4695	Tetrachloroethylene	0.5293	1-Butanol, 3-methyl-, acetate	0.1059	3-Hexen-1-ol, (Z)-
8	0.4999	Ethylbenzene	0.0178	Benzene, 1,3-dimethyl-	0.0800	Butanoic acid, 3-methyl-
9	5.5001	p-Xylene	0.1334	4-Ethylbenzoic acid, 2-methylpropyl ester	0.5991	1-Butanol, 3-methyl-, acetate
10	4.6865	1-Butanol, 3-methyl-, acetate	0.1654	Butyrolactone	0.1562	1-Butanol, 2-methyl-, acetate
11	2.7157	Benzaldehyde	2.6834	Benzaldehyde	0.1387	Butanoic acid, 4-hydroxy-
12	0.4236	Benzene, 1,3,5-trimethyl-	0.1385	alpha-D-Galactopyranoside, methyl	0.0569	Butanoic acid, 3-hydroxy-, ethyl ester
13	11.8938	Hexanoic acid, ethyl ester	2.7836	Hexanoic acid, ethyl ester	0.4412	Hexanoic acid, ethyl ester
14	0.4883	2-Decen-1-ol, (E)-	0.0193	Diglycerol	0.0633	Benzyl Alcohol
15	0.7495	Hexadecane	0.0465	2-Hydroxyethyl butyl sulfide	0.1454	Ethyl dl-2-hydroxycaproate
16	5.5072	Phenylethyl Alcohol	1.3551	2-Pentanethiol	0.7651	1-Hexene, 4-ethyl-
17	3.072	Butanedioic acid, diethyl ester	51.2627	Phenylethyl Alcohol	0.0376	Butanedioic acid, ethyl methyl ester
18	21.6714	Octanoic acid, ethyl ester	0.709	N-(3-Methylbutyl)acetamide	24.4545	Phenylethyl Alcohol
19	0.3088	Naphthalene, 2-methyl-	9.6862	Butanedioic acid, diethyl ester	0.1433	N-(3-Methylbutyl)acetamide
20	0.3812	Tridecane	3.8347	Ethyl hydrogen succinate	9.3156	Butanedioic acid, diethyl ester
21	0.3527	7H-Dibenzo[b,g]carbazole, 7-methyl-	8.5947	Octanoic acid, ethyl ester	25.9306	Ethyl hydrogen succinate

Crt. No.	HS-SPME-GC-MS		DI-SPME-GC-MS		LLE-GC-MS	
	Peak area	Compound	Peak area	Compound	Peak area	Compound
22	0.9071	Decanoic acid, ethyl ester	0.0657	6-Octen-1-ol, 3,7-dimethyl-, (R)-	0.0329	Propanoic acid, 2-methyl-, hexyl ester
23	0.3884	Tetradecane	0.1902	1-Phenoxypropan-2-ol	0.2112	3-Acetyl-2-oxo-1,3-oxazolidine
24			0.284	Acetic acid, 2-phenylethyl ester	0.0453	Butanedioic acid, dibutyl ester
25			0.1684	n-Decanoic acid	0.4738	Sulfurous acid, isobutyl 2-pentyl ester
26			0.4781	Decanoic acid, ethyl ester	0.0384	Decanoic acid, ethyl ester
27			0.1091	Tetradecane	0.3898	Propanoic acid, 2-methyl-, butyl ester
28			0.0455	Naphthalene, 2,6-dimethyl-	1.2838	Benzeneethanol, 4-hydroxy-
29			0.1790	Butanedioic acid, diethyl ester	0.2493	Oxalic acid, 2-phenylethyl propyl ester
30			0.2278	Benzeneethanol, 4-hydroxy-	0.0896	Butanedioic acid, diethyl ester
31			0.0587	2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-	0.0311	4-(2,6,6-Trimethylcyclohexa-1,3-dienyl)but-3-en-2-one
32			0.0542	1,1'-Biphenyl, 4-methyl-	0.1396	Ethanone, 1-(4-hydroxy-3-methoxyphenyl)-
33			0.059	Benzoic acid, 4-hydroxy-3-methoxy-, ethyl ester	0.0316	Phenol, 2,4-bis(1,1-dimethylethyl)-
34			0.1038	Isoquinolinium, 2-[(aminocarbonyl)amino]-, hydroxide, inner salt	1.1498	Acetamide, N-(2-phenylethyl)-
35			0.0395	3,5-di-tert-Butyl-4-hydroxybenzaldehyde	0.0441	Ethylparaben
36					0.0778	3-Hydroxy-4-methoxybenzoic acid
37					0.0324	Megastigma trienone

Crt. No.	HS-SPME-GC-MS		DI-SPME-GC-MS		LLE-GC-MS	
	Peak area	Compound	Peak area	Compound	Peak area	Compound
38					0.0214	Phenol, 3,4,5-trimethoxy-
39					0.0527	Succinoic acid, 2-hydroxy-3-methyl-, diethyl ester
40					0.0312	p-Hydroxycinnamic acid, ethyl ester
41					0.0538	Ethyl-.beta.-(4-hydroxy-3-methoxy-phenyl)-propionate
42					0.0387	Benzoic acid, 4-hydroxy-3-methoxy-, ethyl ester
43					0.6396	p-Hydroxycinnamic acid, ethyl ester
44					0.4803	Heptanoic acid, 2-phenylethyl ester
45					0.3384	Phthalic acid, isobutyl octyl ester
46					0.0649	Ethyl (2E)-3-(4-hydroxy-3-methoxyphenyl)-2-propenoate
47					0.0461	n-Hexadecanoic acid
48					0.0360	Hexadecanoic acid, ethyl ester
49					0.0479	Octadecanoic acid, ethyl ester

CONCLUSIONS

Many aroma compounds from wine can be screened with the three investigated analytical methods: LLE-GC-MS, HS-SPME-GC-MS and DI-SPME-GC-MS. The most compounds were extracted using LLE-GC-MS procedure. Despite of the disadvantages, LLE-GC-MS is a useful technique for screening volatile compounds in wine samples.

The SPME technique is an advanced methodology for rapid determination of aroma compounds and is "environmentally friendly" due to the absence of any organic solvents involved in the analysis. HS-SPME and DI-SPME are simple and fast techniques, easy to use and permit simultaneous sample extraction and analytes' enrichment. SPME is an attractive alternative to classical LLE for identification of volatile compounds in wine.

The SPME technique represents an important developmental area and can be further used for residue analysis in different sample matrix: water or waste water, soil, food.

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REFERENCES

1. Camara, J. S., M. A. Alves, and J. C. Marques (2006). Multivariate analysis for the classification and differentiation of Madeira wines according to main grape varieties. Talanta, 68:1512-1521.

2. Chokshi, K., and I. Christ (2006). Comparative SPDE and SPME studies for analysis of off-flavors in wines, Chromsys LLC, Alexandria VA. Available at: http://www.chromsys.com/

3. Fedrizzi, B., G. Versini, I. Lavagnini, G. Nicolini, and F. Magno (2007). Gas chromatography–mass spectrometry determination of 3-mercaptohexan-1-ol and 3-mercaptohexyl acetate in wine: A comparison of headspace solid phase microextraction and solid phase extraction methods. Analytica Chimica Acta, 596:291-297.

4. Mauriello, G., A. Capece, M. D'Auria, T. Garde-Cerdan, and P. Romano (2009). SPME–GC method as a tool to differentiate VOC profiles in Saccharomyces cerevisiae wine yeasts. Food Microbiology, 26:246–252.

5. Perestrelo, R., A. Fernandes, F. F. Albuquerque, J. C. Marques, and J.S Camara (2006). Analytical characterization of the aroma of Tinta Negra Mole red wine: Identification of the main odorants compounds. Analytica Chimica Acta, 563:154-164.

6. Rebière, L., A. C. Clark, L. M. Schmidtke, P. D. Prenzler, G. R. Scollary (2010). A robust method for quantification of volatile compounds within and between vintages using headspace-solid-phase micro-extraction coupled with GC–MS. Application on Semillon wines. Analytica Chimica Acta, 660:149–157.

7. Riu-Aumatell, M., J. Bosch-Fuste, E. Lopez-Tamames, and S. Buxaderas (2006). Development of volatile compounds of cava (Spanish sparkling wine) during long ageing time in contact with lees. Food Chemistry, 95:237–242.

8. Tankeviciute, A., E. Adomaviciute, R. Kazlauskas, and V. Vickackaite (2004). Solid phase microextraction of esters: comparison of headspace and direct extraction. Chemija, 15(2):21-26.

9. Torrens, J., M. Riu-Aumatell, E. Lopez-Tamames, and S. Buxaderas (2004). Volatile compounds of red and white wines by headspace-solid-phase microextraction using different fibers. Journal of Chromatographic Science, 42(6):310-316.