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GC-MS HEADSPACE CHARACTERIZATION OF THE VOLATILE PROFILE OF GRAPE SKIN, PULP AND SEED EXTRACTS FOR THREE ROMANIAN VARIETIES

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Abstract

The Wine aroma is one of the most important factors in determining its character and quality. The aromatic compounds are accumulated in the peel and grape, and can pass to wine unmodified or by changing their form.The approach to determine certain markers which are depending mainly on grape variety and cultivation area is very innovative one. In this work, methanolic extracts of grape skin, pulp and seeds of three Vitis vinifera L. *varieties were assessed in order to differentiate among their volatile profiles. The grape samples were obtained from the Murfatlar vineyard (Romania): Cabernet Sauvignon, Merlot and Feteasca Neagra. The wines produced from these grapes are awarded the "Protected Designation of Origin" (PDO) label. The evaluation of the volatile profiles was done by gas chromatography/ mass spectrometric detection. The headspace analysis revealed several monoterpens, alcohols, acids and carbonyl compounds.*
The results are consistent with the methodology for the authentication of the grape variety according to the profile of the volatile compounds, in terms of semi-quantitative interpretation.

Key words*: grapes, GC-MS, headspace, aroma, quality.*

INTRODUCTION

Wine quality and identity are the result of different inter-correlated factors, especially terroir and grape variety, but also viticultural and winemaking techniques. Wine aroma is a key component of the former. It is comprised of certain volatile compounds that belong to different chemical families, arising from the grape metabolism, the yeast fermentation and the aging process (Domínguez and Eduardo, 2010).
The primary varietal flavours are accumulated in the skin and grape through specific processes of the metabolism. They are determined by the genetic nature of the varieties, and by the specific pedological and climatic factors. (Lengyel, 2012). The complex aroma of wine is derived from many sources. The components derived from grapes are responsible for the varietal character. The ability to monitor grape aroma compounds would allow for better understanding of how winemaking techniques influence the final volatile composition (Canuti et al., 2009). Grape aroma is comprised of a large number of volatile compounds including alcohols, esters, acids, terpenes, norisoprenoids, thiols and carbonyl compounds. These aroma compounds are predominantly localized in the skin and many are stored as conjugated in the vacuoles of the skin. The skins contain more than half of the total volatile compounds present in the grape berries. During winemaking, the "free" aroma compounds are released as a result of physical crushing and subsequent chemical and enzymatic hydrolysis enzymes (glycosidases or peptidases).
The volatile composition of grapes is one of the most important factors determining wine character and quality. However, there have been few studies linking volatile composition in grapes to the final volatile composition in the wine. These limitations are due, in part, to the lack of analytical methods that allow for rapid

screening or profiling of multiple volatile compounds that are present at a wide range of concentrations in both grapes and wine (Canuti et al., 2009).

MATERIALS AND METHODS

Sample preparation

The grape samples were collected from the Murfatlar vineyard (Romania): Feteasca Neagra, Cabernet Sauvignon and Merlot varieties. The grapes were sorted in terms of separating the skins, pulp and seeds, and subsequently subjected to solvent extraction, individually, in methanol (70% in distilled water) by maceration for 24h in the dark and at room temperature.

Gas chromatography/mass spectrometry analysis (GC-MS): ***Headspace*** - the mobile phase used was helium with a flow of 1 mL/min. The initial oven temperature was held at 40 °C for 5 min and then increased to 250 °C and held isothermally for 10 min at this temperature. The injection and ion source temperatures were 200 °C and 220 °C respectively, and the injection volume was 1µL in the split mode. Identification of volatile compound was achieved by comparing mass spectra found in the NIST2.0 MS library Database.

RESULTS AND DISCUSSIONS

Although headspace analysis has been widely used for analysis of grape and wine volatiles, static headspace analysis often comes with poor sensitivity for trace volatiles and dynamic headspace analysis suffers from interferences provided by water and ethanol.

Some grapes, like Cabernet Sauvignon, show significant analytical challenges due to the fact that the aroma compounds are present in low concentrations with norisoprenoids, esters, alcohols and aldehydes constituting the majority of the volatiles. There have been not so many applications of HSSPME for profiling aroma volatiles in Cabernet Sauvignon grapes (Canuti et al., 2009).

The main chemical constituents for the separate grape samples (skin, pulp, seeds) for the three grape varieties were determined by gas chromatography coupled with mass spectrometry.

The components identified by headspace screening of the extracts were: 1) butanoic acid, methyl ester; 2) tropilidene; 3) 2-ethyl heptanoic acid; 4) 3-ethylhexane; 5) 3-methyl-2-heptanol; 6) 2,4-dimethyl-1-heptene; 7) n-octane; 8) n-nonane; 9) 2-propyl-1-pentanol; 10) tridecane; 11) 3,5-dimathyloctane; 12) n-decane; 13) o-cymene; 14) undecane; 15) c-terpinen; 16) 3-methyldecane; 17) hexadecane; 18) estragole; 19) phenol,2,4-di-tert-butyl.

As can be seen from Table 1, the distribution of the identified compounds is diversified among the different parts of the grape.

The results are consistent with the findings of (Doneva-Sapceska et al., 2006), (Nechita, 2010), (Gomez et al., 1994), (Ashok Kumar and Vijayalakshmi, 2011), (Lamorte et al., 2007), (Nirmala and Narendhirakannan, 2011), (Welke et al., 2013), (Tamborra and Esti, 2010).

After the GC-MS screening of the volatile profile of the extracts, the same compounds for each of the three grape varieties were selected. The proportion of the peak area of each of these compounds is presented after the integration. The same 4 volatiles were taken into consideration in order to represent the ratio between them. From the tables there can be observed the difference between the certain ratios (Example: skin – the ratio between butanoic acid, methyl ester and estragole is 2.011 for Feteasca Neagra, as compared with the same ratio for Cabernet Sauvignon and Merlot varieties which are 11.373 and 7.572, respectively).

As long as the headspace volatile profiling is a qualitative one and, at the same time, a semi-quantitative one, it cannot be based on the simple comparison of the identified individual compounds, because the difference between the areas of the same component detected in separate samples is not conclusive and visible.

Thus, the ratio between the same two compounds supply a more clear distinction between the grape varieties.

The differences among the ratios can be successfully used in the attempt to authenticate wines, based on the profile of the volatile constituents.

The employed headspace technique is yet to be able to detect very well all the volatile compounds in grapes. An SPME-headspace (solid phase micro-extraction) method would cover a wider range of components and show a higher resolution.

Still, it can provide considerable results that can show a fine differentiation among grape samples.

Table 1. Tentatively identified volatile compounds of the skin, pulp and seeds extracts from Feteasca Neagra, Cabernet Sauvignon and Merlot grapes, respectively

	Feteasca Neagra			Cabernet Sauvignon			Merlot		
	% area								
Compound	skin	pulp	seeds	skin	pulp	seeds	skin	pulp	seeds
1	15.35	24.79	30.38	33.21	34.8	30.81	22.34	32.83	45.45
2		3.52	5.6				2.67		
3	6.76			7.18			2.24	6.24	
4		6.1	7.45	6.71	6.23	16.66	4.99	4.16	6.64
5		4.14	8.08	6.43	6.02		2.15		
6		2.27							
7		3.66	4.53	5.64	8.51	5.86	4.91	3.1	5.87
8	6.44	5.99	6.32	7.37	9.11	10.64	12.11	9.42	11.37
9	3.35		3.88						2.1
10	4.96	4.37		3.41	3.6		6.27	4.41	1.79
11	8.68	5.2	4.29		3.97	5.06	5.8	6.08	3.96
12	20.2	15.7	8.14	11.86	14.97	10.18	20.1	17.32	10.5
13	9.12	5.82	5.89	3.68	3.93	5.42	2.21	3.02	3.38
14	5.17	6.51		5.44		6.57			4.86
15	8.2	5.42	4.81	6.14	4.63	3.87	3.41	4.56	4.1
16	4.13	2.62					3.17	2.59	
17			1.97				1.99		
18	7.63	3.82	4.14	2.92	4.23		2.95	2.87	
19			4.51			4.93	2.69	3.41	

% area – the percentage of the peak area as proportion of the total area of the integrated peaks (100%)

Table 2. Ratios of the area percentages for the three grape varieties taken into study - ***skin***

Compounds	% Area		
	F.N.	C.S.	M.
1 butanoic acid, methyl ester	15.35	33.21	22.34
2. estragole	7.63	2.92	2.95
Ratio 1:2	**2.011**	**11.373**	**7.572**
3. o-cymene	9.12	3.68	2.21
4. c-terpinen	8.2	6.14	3.41
Ratio 3:4	**1.112**	**0.599**	**0.648**

Table 3. Ratios of the area percentages for the three grape varieties taken into study - ***pulp***

Compounds	% Area		
	F.N.	C.S.	M.
1 butanoic acid, methyl ester	24.79	34.8	32.83
2. estragole	3.82	4.23	2.87
Ratio 1:2	**6.489**	**8.226**	**11.439**
3. o-cymene	5.82	3.93	3.02
4. c-terpinen	5.42	4.63	4.56
Ratio 3:4	**1.073**	**0.848**	**0.662**

Table 4. Ratios of the area percentages for the three grape varieties taken into study - ***seeds***

Compounds	F.N.	C.S.	M.
1 butanoic acid , methyl ester	30.38	30.81	45.45
2. decane	8.14	10.18	10.5
Ratio 1:2	**3.732**	**3.026**	**4.328**
3. o-cymene	5.89	5.42	3.38
4. c-terpinen	4.81	3.87	4.1
Ratio 3:4	**1.224**	**1.400**	**0.824**

CONCLUSIONS

Headspace analysis (both static and dynamic) has been widely used for analysis of grape and wine volatiles.

The volatile composition of grapes is one of the most important factors determining wine character and quality.

The ratio between two or more same compounds provide a clear differentiation between grape varieties.

There have been few studies linking volatile composition in grapes to the final volatile composition in the wine.

Wine quality is the result of different inter-correlated factors, among which the grape variety plays an important role.

REFERENCES

Ashok Kumar, K., and Vijayalakshmi, K. (2011). GC-MS analysis of phytochemical constituents in ethanolic extract of Punica granatum peel and Vitis vinifera seeds. *International Journal of Pharma and Bio Sciences*, *2*, 461–468.

Canuti, V., Conversano, M., Calzi, M. L., Heymann, H., Matthews, M. a., and Ebeler, S. E. (2009). Headspace solid-phase microextraction-gas chromatography-mass spectrometry for profiling free volatile compounds in Cabernet Sauvignon grapes and wines. *Journal of Chromatography A*, *1216*, 3012–3022.

Domínguez, a. M., and Eduardo, A. (2010). Gas chromatography coupled with mass spectrometry detection for the volatile profiling of Vitis Vinifera CV. Carménère wines. *Journal of the Chilean Chemical Society*, *55*, 385–391.

Doneva-Sapceska, D., Dimitrovski, A., Milanov, G., and Vojnovski, B. (2006). Free and Potentially Volatile Monoterpenes in Grape Varieties. *Bulletin of the Chemists and Technologists of Macedonia*, *25*(1), 51–56.

Gomez, E., Martinez, a, and Laencina, J. (1994). Localization of free and bound aromatic compounds among skin, juice and pulp fractions of some grape varieties. *Vitis*, *4*, 1–4.

Lamorte, S. A., Gambuti, A., Genovese, A., and Moio, L. (2007). Volatile components of Vitis vinifera L. cvs. Uva di Troia, Aglianico and Fiano at different stages of ripening. *30th World Congress of Vine and Wine*, 1–6.

Lengyel, E. (2012). Primary aromatic character of wines. *Acta Universitatis Cibiniensis Series E: Food Technology*, *16*(1), 3–18.

Nechita, C.-B. (2010). *Contributions to the study of VOLATILES COMPOUND OF GRAPES AND wines obtained in Cotnari vineyard*.

Nirmala, J. G., and Narendhirakannan, R. T. (2011). in Vitro Antioxidant and Antimicrobial Activities of Grapes (Vitis Vinifera . L), *3*(4), 242–249.

Tamborra, P., and Esti, M. (2010). Authenticity markers in Aglianico, Uva di Troia, Negroamaro and Primitivo grapes. *Analytica Chimica Acta*, *660*, 221–226.

Welke, J. E., Manfroi, V., Zanus, M., Lazzarotto, M., and Alcaraz Zini, C. (2013). Differentiation of wines according to grape variety using multivariate analysis of comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometric detection data. *Food Chemistry*, *141*(4), 3897–3905.