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Differentiation of three grape varieties by using sensory analysis and characterization of the volatile compounds profile of their musts

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Abstract

The primary flavours of musts and wines are generally constituted by the terpenes and terpenoids, norisoprenoids, benzol derivatives, aliphatic and glycosidic substances, carotene substances. The objective of this study was to differentiate among three must samples (Merlot, Cabernet Sauvignon and Feteasca Neagra) based on their volatile profile. The grape samples were obtained from Murfatlar vineyard (Romania). The wines produced from these grapes are labelled "Protected Denomination of Origin" (PDO) and the finding of certain markers for their authenticity should be of great interest for the wine producers. The headspace analysis (GC-MS) was employed along with the α-Prometheus multi-sensor system used for the food quality control (electronic nose), which provides pattern recognition of the flavouring compounds present in the three must samples. The study reveals differences in the volatile chemical compounds profiles of the analysed varieties. This study may be used as a starting point for developing GC-MS and electronic nose valuable methods for the authentication of the grape variety.

Key words: GC-MS, Electronic nose, grapes, volatiles, authenticity.

1. Introduction

Wine production is an important issue for the economy of many regions around the world. In the winery process, the quality control of the raw material (grape must) is important in order to obtain high quality wines (Flamini, R. and Traldi, P. [1]). Generally, wines made from different grape varieties possess a characteristic aroma and flavour, which is attributed, at least in part, to grape-derived flavour-active precursor compounds (Dubourdieu, D.et al. [2]). During fermentation and subsequent wine aging these nonvolatile, odourless compounds undergo transformation into volatile, aromatic compounds (Ugliano, M. and Henschke, P. A. [3]); (Panighel, A. and Flamini, R. [4]). That is why wines show the opportunity for the exploitation of the electronic nose. For recent years, studies have shown increasing interest in finding ways to discriminate wine samples based on various parameters like variety, geographic origin, and sensory properties. Musts are subjected to different treatments (wine making techniques) in order to become wines, therefore they can be ascertained as markers for authenticity and typicality (Antoce, A. O. and Namolosanu, I. [5]). For example, terpenes, carotenoids and pyrazines are grape-derived, while alcohols and C6-aldehydes arise during flavour extraction and prefermentation treatments. Terpenes are derived from isoprene units (2-methyl butadiene). They can break down into compounds with 9, 10, 11, or 13 carbon atoms that are more intense odorants than their precursors. The most important are the C13-norisoprenoid derivatives. The most important aromatic alcohols and aldehydes are those with 6 carbon atoms (saturated and unsaturated). These compounds originate enzymatically during prefermentation treatments via the aerobic oxidation of linoleic and linolenic acid (C18:2 and C18:3) (Moreno, J. and Peinado, R. [6]); (Flamini, R. and Traldi, P. [1]). There are interactions that exist between the grape composition and the must treatment. The precursors of the varietal aroma compounds are present in the grape, but some studies posit that some of them are formed after the action of oxygen, enzymes and yeast (Roland, A. et al. [7]);(Loscos, N.et al. [8]); (Swiegers, J. H. et al. [9]); (Álvarez-Pérez, J. M. et al. [10]). The aim of this study was to assess the possibility to take into account, as quality markers for authenticity, the volatile profiling by using GC-MS and Electronic Nose analysis.

2. Materials and methods

Sample preparation. The must samples were obtained from the Murfatlar vineyard (Romania): Feteasca Neagra, Cabernet Sauvignon and Merlot varieties. The samples were stored in the freezer at -20°C until analysed.

Gas chromatography/mass spectrometry analysis (GC-MS): *Headspace* - the mobile phase used was helium with 1 ml/min flow. The initial oven temperature was maintained 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 (Sorrentino et al. [11]).

Electronic Nose analysis: three must samples were analysed, Feteasca Neagra, Cabernet Sauvignon and Merlot, using the α-Prometheus multi-sensor system for food quality control (electronic nose). Sample preparation was done as follows: 1ml of must was weighed in each vial and three vials were analysed for the same sample. The vials were capped, heated at 35°C in the oven of the apparatus for 300 seconds, under agitation at 500 rpm. The volatile layer that was generated (1000 µl) was injected in the system comprising 18 sensors in order to record the volatile print.

3. Results and discussion

The main chemical constituents of the must samples for the three grape varieties were determined by gas chromatography coupled with mass spectrometry. The components identified by headspace screening of the samples were: butanoic acid, methyl ester; tropilidene; 2-ethyl heptanoic acid; 2,4-dimethyl heptane; 2,4-dimethyl-1-heptene; 4-methyl octane; n-nonane; 2-propyl-1-pentanol; 6-methyl tridecane ; 3,5-dimethyl octane; n-decane; o-cymene; undecane; terpinen-4-ol; linalool; estragole.

According to Table 1, the distribution of the identified compounds is diversified among the different varieties. The results are consistent with the findings of (Câmara, J. S. et al. [12]), (Mamede, M. E. O. and Pastore, G. M. [13]), (Noguerol-Pato, R. et al. [14]), (Bakker, J. and Clarke, R. J. [15]).

Table 1. Tentatively identified volatile compounds of the three must samples

% area*			
Compound	**Feteasca Neagra**	**Cabernet Sauvignon**	**Merlot**
butanoic acid, methyl ester	26.14	28.68	12.66
Tropilidene	-	9.19	-
2-ethyl heptanoic acid	10.94	6.7	9.12
2,4-dimethyl heptane	7.64	10.63	7.1
2,4-dimethyl-1-heptene	3.6	-	3.12
4-methyl octane	4.32	9.05	4.83
n-nonane	7.16	6.76	14.02
2-propyl-1-pentanol	4.68		6.11
6-methyl tridecane	4.04	3.33	8.77
3,5-dimethyl octane	4.17	3.25	4.52
n-decane	10.87	10	17.38
o-cymene	3.69	-	2.78
Undecane	2.78	4.09	3.74
Terpinen-4-ol	3.98	3.88	2.47
Linalool	2.81	1.94	3.37
Estragole	3.19	2.52	-

* % 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 must samples

	% Area		
Compounds	FN*	CS*	M*
1. butanoic acid , methyl ester	26.14	28.68	12.66
2. 2-ethyl heptanoic acid	10.94	6.71	9.12
Ratio 1:2	**2.39**	**4.28**	**1.39**
3. Terpinen-4-ol	3.98	3.88	2.47
4. linalool	2.81	1.94	3.37
Ratio 3:4	**1.42**	**2.00**	**0.73**

** FN – Feteasca Neagra; CS – Cabernet Sauvignon; M - Merlot*

In order to get a better understanding of the differences between samples, representative volatile compounds present in grape musts were selected. Their peak area percentages were used to represent ratios grouped as follows: butanoic acid methyl ester and 2-ethyl heptanoic acid, and terpinen-4-ol and linalool, respectively. Table 2 shows the difference between the samples. The ratio between butanoic acid methyl ester and 2-ethyl heptanoic acid is 2.39 for Feteasca Neagra, as compared with the same ratio for Cabernet Sauvignon and Merlot varieties which are 4.28 and 1.39, respectively. The ratio between terpinen-4-ol and linalool is 1.42 for Feteasca Neagra, while for Cabernet Sauvignon and Merlot it is 2.00 and 0.73, respectively. Considering not only the data generated by the NIST library, but also the ratio, a more clear distinction between the samples may be emphasized. The differences among the ratios can be successfully used in the attempt to authenticate wines, based on the profile of the volatile constituents of the musts. The employed GC-MS headspace technique is yet to detect very well all the volatile compounds in grapes. A Headspace Solid Phase Micro Extraction

(SPME) method would cover a wider range of components and show a higher resolution (Flamini, R. and Traldi, P. [1]). Also, it can provide considerable results that can show a fine differentiation among the samples. The electronic nose analysis provides pattern recognition of the flavouring compounds present in the musts (Gardner, D. M. et al. [16]); (Vilanova, M. and Oliveira, J. M. [17]); (Peris, M. and Escuder-Gilabert, L. [18]). For the principal component analysis (Figure 1), there were chosen sensors that are sensitive and react to certain compounds in wine: LY2/AA (alcohols, acetone, ammonium), LY2/Gh (ammonium, amines), LY2/gCTI (hydrogen sulfide), T30/1 (polar compounds), P10/1 (nonpolar compounds: hydrocarbons, ammonium), P10/2 (nonpolar compounds: methane, ethane), PA2 (ethanol, ammonium, amines), P30/1 (hydrocarbons, ammonium, ethanol), P30/2 (hydrogen sulfide, ketones), TA2 (ethanol). The curves generated by the sensors show variations between the samples (data not shown). Applying the PCA analysis for the responses of the sensors, a good differentiation of the samples was obtained, that is 83 (the closer the discrimination is to the value 100, the better the samples are differentiated). It demonstrates that, from the point of view of the volatile composition, the samples are very well separated and they are different (Gardner, D. [19]).

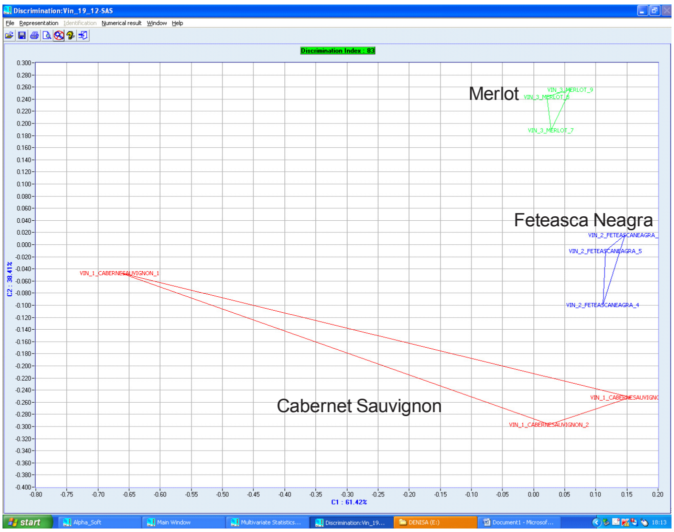

Figure 1. PCA analysis for the three must samples

4. Conclusions

The employed GC-MS Headspace method shows a good differentiation of the must samples. The data expressed as ratios between the peak areas percentages of the same two compounds can serve as marker for the authentication of wines, based on the information supplied by the volatile profile of the musts. The electronic nose analysis shows a fine distinction of the samples, with an 83 discrimination value. The results obtained from the simultaneous use of the GC-MS Headspace and E-nose methods could be successfully ascertained as markers for wine quality and authenticity. This relationship may be also subjected to the area of origin of the grapes and to the applied winemaking technology.

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