# ANALYSIS OF VOLATILE COMPOUNDS IN THREE GRAPE VARIETIES OF LOCAL SELECTION FROM REPUBLIC OF MOLDOVA

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**ABSTRACT:**
*THE CURRENT STUDY AIMS TO EXPLORE THE AROMATIC COMPOSITION OF THREE GRAPE VARIETIES OF LOCAL SELECTION FROM REPUBLIC OF MOLDOVA. THE GRAPES WERE ANALYZED USING A HEADSPACE (HS) TECHNIQUE COUPLED TO A GAS CHROMATOGRAPHY (GC) SYSTEM COMPOSED FROM A SINGLE QUADRUPOLE MASS-SPECTROMETER (MS) AND A THREE-DIMENSIONAL AUTOMATED SYSTEM FOR THE INJECTION OF SAMPLES. HS COUPLED WITH GC/MS IS FOUND TO BE A SIMPLE, QUICK, AND SENSITIVE APPROACH AND IS SUITABLE FOR CHARACTERIZATION OF GRAPE AROMA COMPOUNDS WITHOUT COMPLICATED SAMPLE PREPARATION PROCEDURES. GRAPE JUICE WAS CHARACTERIZED BY "AROMAGRAMS", A SET OF IDENTIFIED COMPONENTS WITH CORRESPONDING RELATIVE ABUNDANCES. ACCORDING TO COINCIDENCE OF GC RETENTION DATA AND ON THE SIMILARITY OF ODOR WITH STANDARDS WERE IDENTIFIED THE CHEMICAL COMPOUNDS RESPONSIBLE FOR PEAK AREAS.*

**KEY WORDS:** VARIETAL AROMA, TERPENES, HEADSPACE, GAS CHROMATOGRAPHY (GC), MASS-SPECTROMETRY (MS).

## INTRODUCTION

Wine is one of the most complex alcoholic beverages, and its aroma substances are responsible for much of this complexity. More than 1000 volatile compounds could be found in wine[2], but only less than 10 % may contribute to the flavour. Describing the aroma of wines is not a simple task, because aroma compounds have been identified with a wide concentration range varying between hundreds of mg/L to the µg/L or ng/L levels[3].

Depending on the origin, and considering the biotechnological sequence of winemaking, wine flavour can be classified into four different groups: varietal aroma, pre-fermentative aroma, fermentative aroma and post-fermentative aroma[4].

Examining the contribution of volatile compounds to characteristic varietal aromas, Ferreira[5] suggested three patterns. The most obvious is to produce a large amount of distinctive

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[2] Poláskova et al., "Wine flavor: chemistry in a glass". *Chemical Society Reviews*, 37 (2008): 2478-2489.
[3] Ebeler, S.E. "Unlocking the secrets of wine flavor". *Food Reviews International*, 17 (2001): 45–64.
[4] Bayonove et al., „Arômes", 1998, 163–235.
[5] Ferreira et al., "Quantitative determination of the odorants of young red wines from different grape varieties". *Journal of the Science of Food and Agriculture*, 80 (2000): 1659-1667. doi: 10.1002/10970010.

volatiles, which are absent or not detectable in other varieties. The second mechanism is based on non-odorous precursors, such as glycoside or cysteine conjugates which are specific for the particular wine variety. Typically, grape juice has very little flavour and is not varietally distinct. Only a few impact compounds, such as the monoterpenes or the methoxypyrazines, are present in their free form in the grape and in the juice after pressing. In contrast, the majority of varietal aroma compounds are present in a bound form, making them non-volatile and hence they have no odour. A third source for varietal aroma is acid-catalysed rearrangements of odourless or barely volatile compounds yielding highly active odorants.

Some studies have investigated the composition of specific cultivars in an effort to better understand the origins of varietal aroma[6]. Although there have been significant developments in the identification of important odour active compounds, few have been able to identify a defining compound responsible for „varietal character". It is apparent that „varietal character" is dependent not on a particular compound but on the profile of odour active compounds present.

The advent of gas chromatography (GC) and gas chromatography coupled to mass spectrometry (GC/MS) has resulted in an expansion of the identification of aroma compounds in many foodstuffs, including grapes and wine[7].

GC/MS is widely used to separate and tentatively to identify and to quantify flavor constituents. Also, there are several techniques that introduce flavor components into GC instruments. These include static headspace (HS) or enrichment techniques such as solid-phase extraction[8], solid phase microextraction[9] or stir bar sorptive extraction[10].

Static HS is a relatively simple technique and can provide sensitivity similar to dynamic purge and trap analysis. The benefits are increased sample throughput, less costly equipment, and simplified operation.

When a GC/ MS method is used, the identification of the individual components is obtained using a mass spectral library. In order to have full confidence of the identity of a compound, standards are necessary to validate the MS findings [11]. The simple way to identify volatile compounds is comparing retention times of the interest peaks with those of pure standard compounds.

Analysis of flavor and odor composition can be challenging. When key components, responsible for specific aroma, are known and quantified, they can be utilized as a tool to optimize viticultural and oenological practices to obtain maximum grape and wine quality.

## EXPERIMENTAL

a) *Samples*

Grapes of three certified clones of *Vitis vinifera* L. *cv*. Startovyi, Viorica and Muscat of Ialoveni were collected in one vineyard of the Practical Scientific Institute of Horticulture and Food Technology from Chişinău, in the 2012 vintage. Fresh grapes were picked at random from whole bunches to give total sample mass of 500 g. Grape samples were frozen

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[6] Schneider et al., "The effect of the site, maturity and lighting of grape bunches on the aromatic composition of Vitis vinifera L. cv. Melon B. berries in Muscadet vineyards", *Bulletin de l'OIV*, 75 (2002): 269-282.
[7] Ohloff, "Importance of minor components in flavors and fragrances". *Perfumer&Flavorist*, 3 (1978): 11–22.
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[10] Baltussen et al., "Stir bar sorptive extraction (SBSE), a novel extraction technique for aqueous samples: Theory and principles", *Journal of Microcolumn Separations*, 11 (1999): 737 - 747.
[11] Hoffmann et al., "Flavor Profiling of Beverages by Stir Bar Sorptive Extraction (SBSE) and Thermal Desorption GC/MS/PFPD", *Gerstel AppNote*, 4 (2000).

immediately after being picked. Prior to analysis, the grapes were thawed, homogenised, and filtered through cheesecloth. The liquid must was then centrifuged at 4ºC (150 rpm) for 15 min.

b) *Experimental conditions:*

The grape juice was analyzed using a Shimadzu GC system composed from a single quadrupole mass-spectrometer GC/MS QP2010SE coupled with a three-dimensional automated system for the injection of samples AOC-5000 (fig. 1). The GC/MS was equipped with a Rtx-5MS (30 m x 0.25 mm; 0.25 µm film thickness) fused silica capillary column. Helium was used as the carrier gas adjusted to 0.8 mL min$^{-1}$; with splitless injection of 1 µL of a hexane solution; injector and interface temperature were 200 ºC; oven temperature programmed was 40-240 ºC at 8 ºC min$^{-1}$. EIMS: detector voltage: 1.3 kV; ion source temperature was 200 ºC. The mass spectrophotometer was operated in the selective ion mode under autotune conditions and the area of each peak was determined by ChemStation software (Agilent Technologies). Analyses were carried out in triplicate.

Statistical analysis was conducted with three replications of the same sample.


Fig. 1. GC/MS QP2010SE coupled with three-dimensional automated system for sample injection AOC-5000

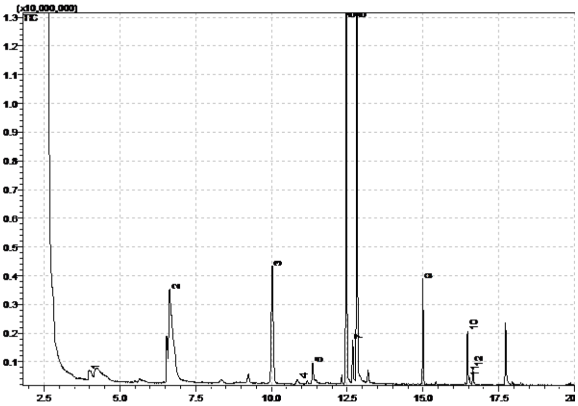
After headspace extraction and GC/MS analysis, mass spectral identifications of compounds was carried out by comparing their GC mass and retention data with those held in the NIST-08 [12] mass spectral library and FFNSC 1.3 [13], a library which was specially developed for flavours and fragrances (available from Shimadzu Europa GmbH). Total ion chromatogram (TIC) and mass fragment of each component was compared with mass spectra from mentioned libraries and confirmed with retention data of compounds. Quantitative data were obtained from the electronic integration of the total ion chromatogram (TIC) peak areas.

[12] NIST/EPA/NIH Mass Spectral Library (NIST 08)

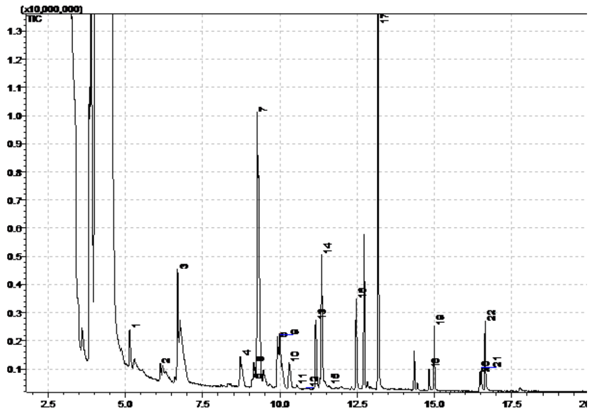
[13] FFNSC ver.1.3 - Flavors and Fragrances of Natural and Synthetic Compounds Library - Mass Spectral Database

## RESULTS AND DISCUSSIONS

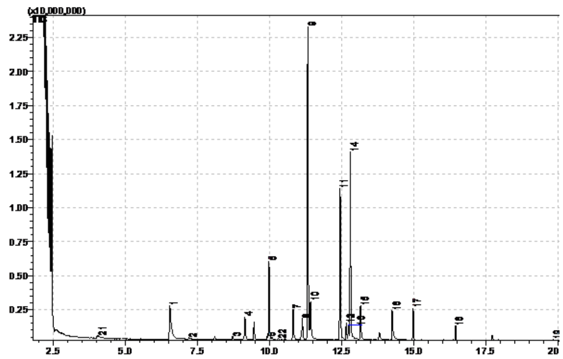
The chromatograms for the identification of volatile compounds (fig. 2) found in three must free fractions were studied. These results of the study offer a very good model for understanding the details of the gas-chromatographic method of analysis. Among the described techniques, HS analysis seems most suitable for a rapid and general characterization of grape and wine aromas.



a) Startovyi



b)Viorica



c) Muscat de Ialoveni

Fig. 2. Total ion chromatogram obtained by the GC/MS with HS extraction of volatile compounds from free fractions of grape must

Almost thirty of the most abundant volatile compounds were identified in the studied three grape juices, including terpenes, alcohols, esters, aldehyde and ketone compounds. The peaks were identified by their mass spectra and retention data. The experimental results are listed in the order of their elution in Table 1. This data indicate that GC/MS analysis using HS technique is a very sensitive technique for the detection of terpene esters and alcohols, which are important odorant compounds. Volatile compounds were quantified as area percentage of total volatiles from GC analyses. Estimation of the quantity of individual components in the samples (in ppm or mg/L) has not been attempted.

Analyzing each grape variety must free fraction in separate, it can be verified that Startovyi and Muscat of Ialoveni fractions have a larger number of detected volatile compounds then Viorica variety, 20 and 12, respectively.

Table 1. Volatile compounds found in three must free fractions: peak number, GC/MS retention data, compound identification, CAS number[14] and significance level

| Peak no. | Retention time | Compound | CAS no. | Relative peak area, % | | |
|---|---|---|---|---|---|---|
| | | | | Startovyi | Viorica | Muscat of Ialoveni |
| 1 | 4.053 | Isoamyl alcohol | 123-51-3 | - | 1.50 | 1.03 |
| 2 | 5.136 | Ethyl butyrate | 105-54-4 | 2.91 | - | - |
| 3 | 6.131 | Ethyl 2-methyl butyrate | 7452-79-1 | 7.96 | - | 0.86 |
| 4 | 6.550 | Hexanol | 111-27-3 | - | 25.64 | 6.60 |
| 5 | 8.718 | Geranic oxide | 7392-19-0 | 1.99 | - | 2.49 |
| 6 | 9.102 | Myrcene | 123-35-3 | 0.38 | - | - |
| 7 | 9.268 | Ethyl hexanoate | 123-66-0 | 13.81 | - | - |
| 8 | 9.924 | D-Limonene | 5989-27-5 | 2.89 | - | 0.14 |
| 9 | 9.987 | Eucalyptol | 470-82-6 | 5.89 | 12.64 | 7.31 |
| 10 | 10.307 | Ocimene | 502-99-8 | 2.91 | - | 0.17 |
| 11 | 10.544 | γ-Terpinene | 99-85-4 | 0.26 | - | - |
| 12 | 10.852 | Linalool oxide <cis> (furanoid) | 5989-33-3 | - | 0.41 | - |
| 13 | 10.824 | Linalool oxide <trans-> (furanoid) | 11063-78-8 | 0.09 | - | 3.17 |
| 14 | 11.142 | Linalool oxide <cis-> (pyranoid) | 14009-71-3 | - | - | 2.00 |
| 15 | 11.156 | Terpinolene | 586-62-9 | 5.96 | - | - |
| 16 | 11.350 | Linalool | 78-70-6 | 8.71 | 1.37 | 28.32 |
| 17 | 11.428 | Hotrienol | 20053-88-7 | - | - | 3.86 |
| 18 | 11.586 | Rose oxide <trans-> | 4610-11-1 | 0.11 | - | - |
| 19 | 12.455 | p-Menthanone | 10458-14-7 | 5.66 | 23.49 | 14.81 |
| 20 | 12.669 | α-Isomenthone | 491-07-6 | - | 2.37 | 1.49 |
| 21 | 12.744 | Linalool oxide <trans-> (pyranoid) | 14049-11-7 | - | - | 0.99 |
| 22 | 12.809 | DL-Menthol | 89-78-1 | - | 24.14 | 16.63 |
| 23 | 13.163 | α-Terpineol | 98-55-5 | - | - | 3.26 |
| 24 | 13.175 | Ethyl octanoate | 106-32-1 | 30.77 | - | - |
| 25 | 14.261 | Nerol | 106-25-2 | 1.14 | - | 3.09 |
| 26 | 14.990 | Menthyl acetate | 16409-45-3 | 3.14 | 4.73 | 2.65 |
| 27 | 16.461 | β-Damascenone | 23696-85-7 | 0.93 | 2.54 | 0.92 |
| 28 | 16.515 | Ethyl 9-decenoate | 67233-91-4 | 1.07 | 0.32 | - |
| 29 | 16.637 | Ethyl caprate | 110-38-3 | 3.52 | 0.85 | 0.27 |

[14] http://webbook.nist.gov/chemistry/cas-ser.html

As can be seen, the volatile profiles of the varieties are very different among them, which is confirmed by compounds olfactory descriptors [15]. A list of these is shown in Table 2.

Table 2. Volatile compounds found in three must free fractions: peak number, compound identification, odor type and olfactory description[16]

| Peak no. | Compound | Odor type | Olfactory descriptors |
|---|---|---|---|
| 1 | Isoamyl alcohol | fermented | Fusel, alcoholic, cognac, fruity, banana |
| 2 | Ethyl butyrate | fruity | Sweet, fruity, tutti frutti, lifting and diffusive |
| 3 | Ethyl 2-methyl butyrate | fruity | Fruity, estry and berry with fresh tropical nuances |
| 4 | Hexanol | herbal | Pungent, etherial, fusel oil, fruity and alcoholic, sweet with a green top note |
| 5 | Geranic oxide | herbal | Sweet, floral, citrus with woody, cooling, minty and camphoreous nuances |
| 6 | Myrcene | spicy | Terpy, herbaceous, woody with a rosy celery and carrot nuance |
| 7 | Ethyl hexanoate | fruity | Sweet, fruity, pineapple, green banana nuance |
| 8 | D-Limonene | citrus | Sweet, citrus and peely orange, fresh |
| 9 | Eucalyptol | herbal | Eucalyptus, herbal, camphor |
| 10 | Ocimene | floral | Warm, floral herb, flower sweet |
| 11 | γ-Terpinene | terpenic | Terpy, sweet, citrus, with tropical and lime nuances |
| 12 | Linalool oxide <cis-> (furanoid) | earthy | Earthy, floral, sweet, woody |
| 13 | Linalool oxide <trans-> (furanoid) | fruity | Sweet, floral, creamy |
| 14 | Linalool oxide <cis-> (pyranoid) | citrus | Citrus green |
| 15 | Terpinolene | herbal | Sweet, fresh, piney citrus with lemon peel nuance |
| 16 | Linalool | floral | Citrus, orange, floral, terpy, waxy and rose |
| 17 | Hotrienol | tropical | Sweet, tropical, fennel, ginger |
| 18 | Rose oxide <trans> | floral | Rose |
| 19 | p-Menthanone | mentholic | Minty |
| 20 | α-Isomenthone | mentholic | Mentholic, cooling, minty and camphoreous |
| 21 | Linalool oxide <trans-> (pyranoid) | floral | Floral honey |
| 22 | DL-Menthol | mentholic | Peppermint, cool woody |
| 23 | α-Terpineol | floral | Lilac, citrus, woody floral, lily of the valley |
| 24 | Ethyl octanoate | fruity | Waxy, sweet, pineapple and fruity with creamy |
| 25 | Nerol | floral | Fresh, citrus, floral, green, sweet, lemon/lime and waxy with a spicy depth citrus magnolia |
| 26 | Menthyl acetate | mentholic | Tea cooling, minty, fruity, berry |
| 27 | β-Damascenone | floral | Woody, sweet, fruity, green floral nuances |

[15] http://www.thegoodscentscompany.com/
[16] http://www.thegoodscentscompany.com/

| | | | |
|---|---|---|---|
| 28 | Ethyl 9-decenoate | fruity | Fruity fatty |
| 29 | Ethyl caprate | waxy | Sweet, waxy, fruity, apple |

Numerous studies have reported that the terpenoid compounds could be used analytically for varietal characterization. It is known that terpene compounds are secondary plant constituents[17]. In the juice of Startovyi, Viorica and Muscat of Ialoveni grape varieties, the presence of a large range of monoterpenes ($C_{10}H_{16}$) was identified: geranic oxide (bois de rose oxide), myrcene, D-limonene, eucalyptol, ocimene, γ-terpinene, linalool oxide, terpinolene, linalool, (E)-rose oxide, p-menthanone, α-isomenthone, DL-menthol, α-terpineol, nerol, menthyl acetate. All these monoterpenes earlier were identified in other grapes of flavoured varieties[18]. The terpene monohidroxilic alcohols (terpenols) are the most important compounds with flavoring potential, because these represent volatile free flavors from aromatic type grapes. From the quantitative point of view, they represent about 40-50% of volatile odorant compounds with a very low perception threshold (0.1-0.5 mg/L)[19].

In the present study, the grape juice Startovyi is caracterized by a large amount of esters (ethyl 2-methyl butyrate, ethyl hexanoate, ethyl octanoate) that explains the tropical-fruity aroma specific for this variety. Muscat of Ialoveni grapes are distinguished by a huge amount of linalool (28.32 % from all the volatiles), specific for Muscat varieties and also by a great concentration of p-menthanone and DL-menthol, that gives it minty and camphoreous notes.

## CONCLUSION

This study demonstrates that the HS technique coupled with GC/MS analysis is well suited for a qualitative and semi-quantitative analysis of aroma compounds in grapes.

The presence of terpenes, in their different forms, in grape juices represents an enormous potential in a way to increase the varietal characteristics of the wines, contributing the final product with higher floral and fruit-like characteristics. Actually, there is sufficient information and tools to study the presence of terpenes and their evolution in grape juices and wines, but it is not yet possible to translate all of the acquired knowledge to the wineries because an efficient methodology to improve the terpene content of the wines has not been found because of their instability over time.

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[18] Sanchez-Palomo et al., "Rapid determination of volatile compounds in grapes by HS-SPME coupled with GC–MS", *Talanta*, 66 (2005): 1152-1157.

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