# ESTABLISHMENT OF CHEMICAL COMPOUNDS RESPONSIBLE FOR ODORANT AREAS OF THREE WINES FROM LOCAL GRAPE VARIETIES FROM MOLDOVA

**Musteață Grigore, *Furtuna Natalia**

Technical University of Moldova – Chişinău, Moldova

***Furtuna Natalia, furtuna.utm@gmail.com**

**Abstract.** Three wines from local grape varieties from Republic of Moldova were submitted to both sensory and gas chromatography – olfactometry analyses (GC-O). Through descriptive analysis, a set of aroma attributes has been described. In order to identify these odor active compounds, the wines were evaluated using qualitative detection frequency analysis (n=7). The panelists generated in total 697 descriptions distributed in 126 odorant areas (OAs), but only 565 (81 %) distributed in 45 OAs were validated as being representative. According to coincidence of gas chromatographic retention data and on the similarity of odor with standards were identified the chemical compounds responsible for odorant areas.
**Key words:** gas chromatography – olfactometry, detection frequency analysis, odorant area.

## Introduction

Wine, which is produced by fermentation of fresh grapes or must, is one of the most complex alcoholic beverages, and its aroma substances are responsible for much of this complexity. Wine flavor can be classified into three groups: varietal, fermentative and wine ageing aroma. Describing the aroma of wines is not a simple task for researchers, because more than 800 aroma compounds such as alcohols, esters organic acids, aldehydes, ethers, ketones and terpenes, etc., have been identified in them, with a wide concentration range varying between hundreds of mg/L to the μg/L or ng/L levels, and their combinations form the character of wine and differentiates one wine from another [1].

Chromatography is a method used to decompose complex mixtures of chemicals into their constituents. In essence, the method entails the forced transfer of chemical components along an adsorptive or dissolvent material, which usually is packed in a column or which constitutes the inner lining of a column.

When odorous chemicals elude from a capillary column, their presence may be detected by instruments like flame ionization detectors (FID) or by mass spectrometry (MS). Due to large differences in detection thresholds between odorants, the capacity of chemicals to invoke odor sensations at a given concentration level varies strongly. Therefore, relative quantities of the components in the mixture are poor indicators of their relative contributions to the mixture's aroma. A better estimate of each component's contribution to the aroma may be obtained by sensory evaluation of the separated constituents. Thus, by replacing the FID with a sufficiently large panel of subjects that sniff the effluents of the gas chromatograph in an effort to detect and characterize the odor-active chemicals.

The gas chromatography-olfactometry (GC-O) is an analytical method that combines the gas chromatography and sensory evaluation, using the human nose to assess odor components. The human nose has an odor detection limit of about $10^{-19}$ moles, making GC-O an extremely valuable and sensitive tool for odor detection.

After injection, the content of the sample is separated by the chromatographic column. Before leaving the column, the effluent is divided into two parts: the smallest is directed to the instrumental detector, usually a flame ionization detector (FID); the largest part is directed to a smelling device (sniffing port) placed at the evaluator's nose height. Therefore, this method provides simultaneously two signals: the chromatogram of the extract and the recording of odor events perceived by assessors [2].

The odorant areas frequency is correlated to the concentration logarithm of the compound responsible for stimulus. This relationship is based on the hypothesis that, for a certain compound, the perception threshold has a Gaussian distribution. Each assessor must perceive the beginning and the end of the flavor and describe it. The individual aromagrams are summed, yielding the global aromagram where frequency of detection is represented in dependence on time or retention index. The olfactometric indices can be used for ranking odorant areas in function of their olfactory impact [3].

The odorant areas obtained via GC-O are characterized by three parameters: olfactometric index, average linear retention index (LRI) or LRI interval and flavor descriptors. All this information is used later in the identification of compounds.

## Material and method

For analysis were used wines made from Moldavian local grape varieties: Startovyi, Hibernal and Muscat of Ialoveni (harvest 2010) produced at the Practical Scientific Institute of Horticulture and Food Technology from Chişinău.

In order extract aromatic compounds was used the dichloromethane extraction, based on the method proposed by Moio [4].

The olfactometric analysis was performed on 3 extracts by 7 assessors selected in advance and informed that they will analyze three white wines, but no other detail has been specified. The extracts were analyzed by the participants in a different and balanced sequence. Total length of a session was 45 minutes. After injection of the solution into chromatograph column, in order to avoid inhalation of the solvent, the assessor was asked to wait 5 minutes before approaching the nose to the sniffing port.

Gas chromatograph Hewlett-Packard 5890 was equipped with split/splitless injector and DB-1701 capillary column. Simultaneous processing of both signals was performed using EZchrom Elite (Agilent Technologies) and AcquiSniff ® (© INRA).

Linear retention indices (LRI) of chromatographic peaks and odorant events were calculated using a daily injection of a solution of 13 n-alkanes (from $C_7$ to $C_{19}$), analyzed under the same chromatographic conditions as the extracts.

The results of each individual data processing were presented in Excel tables where the LRI peak, the assessor codes, the extract codes and their respective descriptors were indicated. Therefore, 21 tables with olfactometric data were obtained (3 wines x 7 assessors), that subsequently were submitted to mathematical processing. Mathematical processing of olfactometric data was performed using Matlab ® (The Mathwork Inc.), which implements an iterative mathematical function to get a table that contains the number of detections for each tandem wine/odorant area.

## Results and discussions

Initially the wines were submitted to sensory analysis sessions (Table 1). Though considerable dispersion of responses, it was achieved conclusive data. The intensity of wine aroma was appreciated with values within a range from 62.5 to 75 pts out of 100.

***Table 1.* Descriptors set out by tasters during the sensory evaluation**

| The wine | Types of aromas | | | |
|---|---|---|---|---|
| | Floral | Fruity | Vegetal | Spicy |
| Startovyi | Honey | Pear, apple, lemon | Freshly cut hay | Pepper, coconut |
| Hibernal | Basil, thyme | Pomelo, grapefruit | Herbaceous | Laurel leaves, paprika |
| Muscat of Ialoveni | Muscat intense, acacia flower | Citrus, pineapple | Celery | Nutmeg |

The olfactometric study, using frequency detection method, generated 21 individual aromagrams. The number of odorant events related to each wine is situated between 228 (Muscat of Ialoveni) and 238 (Hibernal), meaning that for three wines, seven assessors had spotted 697 events. The assessors, with some exceptions, have described each event with only one term, the report terms / events being nearly 1.1.

In order to process data obtained by using Matlab® software, it was previously set an eliminatory threshold. This corresponds to the value of first quartile of distribution, i.e., to consider an odorant area as representative it must contain at least 5 odor events. Of the totality of 697 odor events, 565 (81%) were distributed within 45 odorant areas that contain at least 5 events per area. Consequently, the areas with the number of events lower than the eliminatory threshold have been removed. Consequently, the areas with the number of events lower than the eliminatory threshold have been removed (Fig. 1).

The results obtained by GC-O analysis were summarized in Table 2.

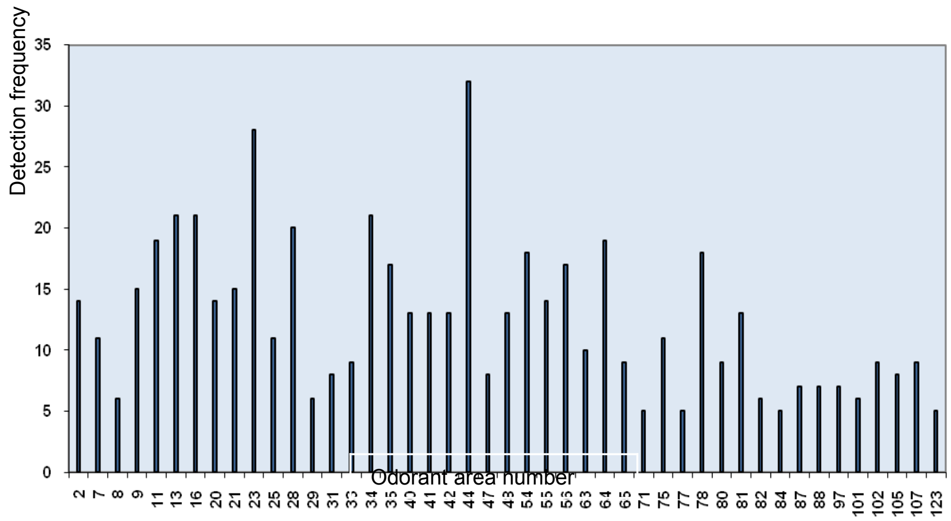


***Fig. 1.*** Global aromagram of studied wines

***Table 2.*** Characteristic of representative odorant areas for studied wines

| Area number[1] | LRI[2] | Detection frequency | Odorant area description | Chemical compounds responsible for odorant areas [3] |
|---|---|---|---|---|
| 2 | 695 | 14 | Yoghurt, cream, butter | 1,1-dietoxiethane |
| 7 | 766 | 11 | Fruity, solvent | ethyl acetate |
| 8 | 770 | 6 | Vinegar, pungent | acetic acid |
| 9 | 778 | 15 | Fruity, brandy | ethyl propanoate |
| 11 | 816 | 19 | Strawberries, pineapple | ethyl 2-methylpropanoate |
| 13 | 845 | 21 | Cocoa, chocolate, yeasty | 3-methylbutan-1-ol |
| 16 | 862 | 21 | Tutti frutti, strawberries, | ethyl butyrate |
| 20 | 906 | 14 | Fruity, kiwi, pineapple | ethyl 2-metilbutanoate |
| 21 | 912 | 15 | Fruit candy, linden, verbena | ethyl 3-metilbutanoate |
| 23 | 938* | 28 | Peanuts, roasted, banana, pear | 2-metilfuran-3-thiol / isobutyl acetate / isoamyl acetate |
| 25 | 957 | 11 | Cheese | butanoic acid |
| 28 | 1009 | 20 | Cheese, rancid | 3-methylbutanoic acid |
| 29 | 1014 | 6 | Apple, cheese | 2-methylbutanoic acid |
| 31 | 1027 | 8 | Woody, fresh herbs, lime | *alpha*-pinene |
| 33 | 1053 | 9 | Cooked potatoes, gnocchi | 3-methylthiopropanal |
| 34 | 1060 | 21 | Fruit candy, apple, citrus | ethyl hexanoate |
| 35 | 1074 | 17 | Black currant buds | 4-mercapto-4-methylpentan-2-one |
| 40 | 1149 | 13 | Flowers | methyl octanoate |
| 41 | 1154 | 13 | Sulfurous, plastic | hexanoic acid |
| 42 | 1174 | 13 | Fruity, balsamic | ethylfuran-2-carboxylate |
| 44 | 1194* | 32 | Lily of the valley, lavender, citrus, marshmallows | 2-phenylacetaldehyde / linalool |
| 47 | 1235 | 8 | Caramel, chocolate | guaiacol |
| 48 | 1240 | 13 | Cotton candy, caramel | furaneol |
| 54 | 1284 | 18 | Honey, rose, lilac | 2-phenylethanol |
| 55 | 1292 | 14 | Flowers | *alpha*-terpineol |
| 56 | 1305 | 17 | Caramel, cotton candy | homofuraneol |
| 63 | 1350 | 10 | Cheese, smoky, dusty | octanoic acid |
| 64 | 1357 | 19 | Spicy, curry, fennel | sotolon |
| 65 | 1371 | 9 | Bergamot, citrus | 3-sulfanylhexyl acetate |
| 71 | 1432 | 5 | Licorice | dehydro-ar-ionene (TDN) |
| 75 | 1473 | 11 | Floral, herbaceous | ethyl 3-phenylpropanoate |
| 77 | 1489 | 5 | Chemical, pharmaceutical | 4-vinylphenol |
| 78 | 1494 | 18 | Balsamic, clove, curry | 4-vinylguaiacol |
| 80 | 1508 | 9 | Polyfloral honey | *beta*-damascenone |
| 81 | 1512 | 13 | Prune, floral, smoky | phenylacetic acid |
| 82 | 1518 | 6 | Clove | eugenol |
| 84 | 1529 | 5 | Spicy | methyleugenol |
| 87 | 1545 | 7 | Mineral | 2,6-dimethoxyphenol |
| 88 | 1550 | 7 | Floral, herbaceous | ethyl dihydrocinnamate |
| 97 | 1619 | 7 | Fruity, punch | ethyl cinnamate |

*Continuie Table 2*

| Area | LRI[2] | Detection | Odorant area description | Chemical compounds |
|---|---|---|---|---|

| number[1] | | frequency | | responsible for odorant areas [3] |
|---|---|---|---|---|
| 101 | 1644 | 6 | Sulfurous, fermented | decanoic acid |
| 102 | 1662 | 9 | Vanilla | vanillin |
| 105 | 1728 | 8 | Mulled wine, balsamic | methyl vanillate |
| 107 | 1748 | 9 | Coconut | *delta*-decalactone |
| 123 | 1909 | 5 | Fruity, candy | tyrosol |

1 – Odorant areas that contain at least 5 events per area;
2 – Average LRI in DB-1701 capillary column (30 m x 0.32 mm x 1 μm).
3 – Identification based on coincidence of gas chromatographic retention data and on the similarity of odor with standards [5], [6].
* – co-eluted chemical compounds

This table contains the number attributed to the detected odorant areas (OA), the linear retention indices (LRI), the identity of the compounds and the main odor descriptors of the wines. According to the presented data, OA no. 44 (linalool and/or 2-phenylacetaldehyde) and OA no. 23 (2-metilfuran-3-thiol and/or isobutyl acetate / isoamyl acetate) have the highest average of detection frequency, probably due to co-elution of several chemical compounds. The OA no.13 (3-methylbutan-1-ol), OA no.16 (ethyl butyrate) and OA no.34 (ethyl hexanoate) were also highly detected probably due to their low perception threshold or their high concentration.

## Conclusions

Olfactometry analysis (GC-O) allows the selection of odorant compounds using human analyzer, sequentially combining gas chromatography (instrumental analysis) and sensory perception (subjective analysis), thus being a very precious technique for detection of compounds with higher detection threshold than their concentration in wine, and thereby solving some problems in the aroma analysis.

The study presented here has shown that the wines from local grapes from Republic of Moldova (Startovyi, Hibernal and Muscat of Ialoveni) posses a large amount of odorants detectable by olfactometric studies.

The central method of this research was the olfactometry analysis by using the detection frequency method to generate 21 individual aromagrams, which were later summed into a global aromagram for all three wines.

According to mathematical processing of experimental data using Matlab® software, it was established that out of 697 odor events spread in 123 odorant areas, 565 (81%) were distributed within 45 odorant areas that contain at least 5 events per area.

In spite of some limitations, The GC-O approach used in the study arises as a valid tool for determining the existence of intense odorants of wine.

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