# COMPOUNDS CAPTURED IN $CO_2$ TAMAIOASA ROMANEASCA WINE FERMENTATION

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**Abstract**

The volatile aromas that are lost during the must's fermentation into wine represent a department that is not very much analysed. The capturing and analysing of the volatile compounds that are trapped in the CO2 flow during gas exhaustion of the fermentation stage are the main objectives of the present study. The Tamaioasa romaneasca grapes, harvest of 2011, were processed according to the aromatic wine technology During fermentation, the volatile aromatic compounds were captured using SPE cartridges attached to the airlocks of the fermentation vessels. After the fermentation ended, the extracts were obtained by washing the bed of the SPE cartridges with 2 mL diclormethane. Gas-chromatography coupled with mass-spectrometry was used to identify the captured compounds. The processing technologies influenced the number and quantity of the captured compounds. Esters (isobutyl acetate) and alcohols, as well as aldehydes and terpenes are found in the exhaust air of the fermentation process. The identified compounds are found in trace quantities.

**Key words**: (min. 3 – max. 5): Tamaioasa romaneasca, aroma compounds, exhaust $CO_2$

The aroma compounds are the ones that give wine its “genius”, specificity and individuality. More or less pronounced aromas are the base of each wine’s personality. The aromas evolve during the grape maturation, becoming, from an organoleptic (qualitative) point of view, „personal” for each wine and ecosystem.

The sensoric character of wine is influenced by the grape variety, the maturity degree of the grapes at harvest, the yeast activity, pre-fermentative technologies and aging techniques.

In specific literature, more than 800 aroma compounds are attributed to wine, among which are alcohols, aldehides, ketones, esters, acids and terpenes.

The most important stage of the wine production is the fermentation, the “birth” of the wine. At this time, the second class of aroma compounds is formed, after the varietal aromas and before the aging specific sensorics.

During fermentation, a large volume of $CO_2$ gas is produced, about 260 mL/g glucose. This equates to over 50 times the volume of the juice fermented. Carried off with $CO_2$ are various volatile compounds (Jackson, 2000).

Ethanol loss is estimated to be about 1-1.5% of that produced (Williams and Boulton, 1983), but can vary with sugar use and temperature. Higher alcohols and terpenes are lost to about the same degree. (aprox. 1%).

In contrast, significant losses of both ethyl and acetate esters can occur. Depending on the grape variety and especially the fermentation temperature, up to about 25% of these aromatically important compounds may be lost (Miller et al. 1987). This escape could diminish greatly the fruity characteristics of wine.

This study aims at analysing the volatile aroma compounds that are lost during the fermentation stage, by flowing out together with the exhaust of carbon dioxide (Nasrawi *et al.*, 1990; Muller *et.al.*, 1993), from Tamaioasa romaneasca, one of the most well-known aromatic grape varieties of Romania. These will be harvested by use of SPE (solid phase extraction) cartridges with an ability to retain volatile compounds.

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## MATERIAL AND METHOD

Tamaioasa romaneasca grapes were used, harvested from Cotnari vineyard in 2011. The grapes were processed according to the specific literature (Cotea *et al.*, 1986) for aromatic wine technologies. After desteming and crushing, the marc was macerated for 12 hours; for a better extraction of the aroma compounds commercial pectolytical enzymes (Zymoclaire M®, Sodinal) were added. Pressing of the must was done with a hydraulic press. Selected yeasts (Fermactive Muscat®, Sodinal) were added. The must was transferred into glass vessels for fermentation.

The capturing of the volatile compounds (fig.1) that are lost during fermentation was done as follows: SPE (solid phase extraction) cartridges were conditioned (6 mL diclormethane, 6 mL ethanol and 6 mL ethanol solution (14%) were passed through the C18 bed) and fixed to the fermentation airlocks so that the exhaust CO2 flow, together with the volatile compounds passed through them. The volatile substances were „trapped” in the SPE device for further analysis.

Figure 1 **Capturing of exhaust $CO_2$ and compounds in Tamaioasa romaneasca fermentation process**

After the fermentation process ended, the attached SPEs were disconnected and analysed. The volatile compounds were obtained by washing the SPE bed with 2 mL diclormethane.

Figure 2 **Extracting compounds from the exhaust $CO_2$ in Tamaioasa romaneasca fermentation process**

The obtained extract was injected into a Shimadzu GC coupled with a QP2010 Plus mass-spectrometer.

Figure 3 **Identification of compounds from the exhaust $CO_2$ in Tamaioasa romanesca fermentation process**

1000 µL extract are injected into a Supelco SLB 5 ms GC column, 15 m length, column oven temperature 30 °C, injection temperature 250 °C, in splitless mode, initial temperature 30 °C for 1 minute, then if grows at a rate of 8 °C until 240 °C where it stays for 2.75 minutes. The carrier gas was Helium, column flow 0.75 mL/min, ion source temperature 250 °C, interface temperature 250 °C, detector voltage 0.9 kV.

The aroma compounds were determined by means of the NIST 08, Wiley 08 and SZTERP spectrum library. The program lasts for 30 minutes.

## RESULTS AND DISCUSSIONS

Part of obtained chromatogram after analysing the volatile compounds found in the exhaust CO2 flow of fermenting Tămâioasă românească grapes is reproduced here (fig. 4)

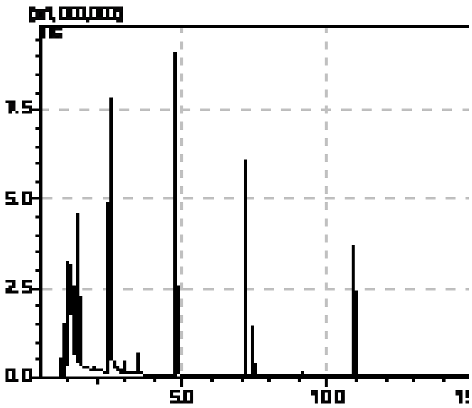


Figure 4 **Chromatogram (part) of exhaust $CO_2$ flow of Tamaioasa romaneasca fermentation**

The compounds indentified in the $CO_2$ exhaust flow are in very small quantities and very volatile, with small masses, being able to get “lost” during the fermentation process.

The identified compounds are from major chemical classes specific to wine: terpenes, alcohols, esters, as well as aldehydes and ketones (Table 1). The compounds are characterised by their area.

Table 1

**Compounds identified in exhaust $CO_2$ flow**

| No. | Retention time | Area | Identified compound |
|---|---|---|---|
| ALCOHOLS | | | |
| 1 | 0.853 | 2644912 | Ethanol |
| 2 | 1.409 | 5960398 | Isobutyl alcohol |
| 3 | 2.115 | 83905 | 2-Pentanol |
| 4 | 2.490 | 28722055 | 1-Butanol, 3-methyl |
| TERPENES | | | |
| 5 | 2.777 | 91743 | Ledene |
| 6 | 9.122 | 68539 | Linalool |
| ESTERS | | | |
| 7 | 1.304 | 6977678 | Ethyl Acetate |
| 8 | 2.998 | 382659 | Isobutyl acetate |
| 9 | 3.407 | 821344 | Butanoic acid, ethyl ester |
| 10 | 4.694 | 11708 | Vinyl butyrate |
| 11 | 4.763 | 14828018 | Isoamyl acetate |
| 12 | 7.160 | 7998043 | Hexanoic acid, ethyl ester |
| 13 | 7.430 | 1850434 | 1-Hexyl acetate |
| 14 | 10.918 | 4790696 | Octanoic acid, ethyl |
| 15 | 11.177 | 29498 | Octyl acetate |
| 16 | 11.869 | 8932 | Isopentyl hexanoate |
| 17 | 13.133 | 34742 | Methyl tridecanoate |
| ALDEHYDES | | | |
| 18 | 1.637 | 410101 | Butanal, 3-methyl- |
| 19 | 2.353 | 97231 | 1,1-Diethoxyethane |
| KETONES | | | |
| 20 | 1.921 | 60830 | Methyl propyl ketone |

Among the terpenes, linalool and lebdene were identified in the exhaust $CO_2$ of the fermenting Tămâioasă românească must.

Linalool is a component of many essential oils, including orange, lavender, rose, rosewood, and coriander. It is a naturally occurring terpene alcohol chemical found in many flowers and spice plants with many commercial applications, the majority of which are based on its pleasant scent (floral, with a touch of spiciness).

Ledene is the main constituent of labdanum oil. Rockrose *Cistus ladaniferus* has an odoriferous resin called labdanum. It has a warm, sweet-resinous, woody-ambery odour. It is one of perfumery's classic ingredients. The volatile ledene was also correlated to the increase in the spoilage bacteria population in foods. It has not has not been reported by others in wine until now.

Molecular weight is 204.356 g/mol, boiling temperature is 263.9°C at 760 mmHg.

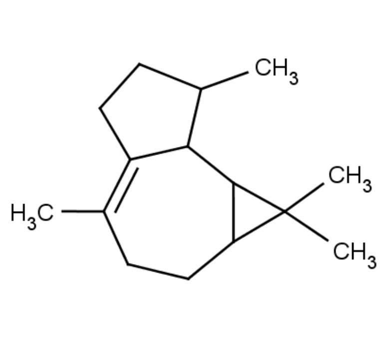


Figure 5 **Molecular strucutre of ledene**

From the volatile alcohols class, 2-nitroethanol, ethanol, isobutyl alcohol, 2-pentanol and 1-butanol, 3-methyl- were identified. All of the mentioned alcohols have a strong solvent smell, except 3-methyl-1-butanol that is a main ingredient in the production of banana oil, an ester found in nature and also produced as a flavouring substance in industry.

The identified esters are ethyl acetate, isobutyl acetate, butanoic acid, ethyl ester, vinyl butyrate, isoamyl acetate, hexanoic acid ethyl ester, 1-hexyl acetate, octanoic acid, ethyl ester, octyl acetate, isopentyl hexanoate and methyl tridecanoate.

The esters of fatty acids (ethyl hexanoate, ethyl octanoate) have specific aromas, those of old cheese and barnyard.

Isoamyl acetate smells nice, of bananas and melon, is characteristic of cool-fermented whites.

Ethyl acetate is ubiquitous in fresh, young white wines made from grapes that are not too ripe, though not necessarily unripe and has the aroma of green apples.

Butanal, 3-methyl- or isovaleraldehyde is a ketone with a fruity, fatty, animal, almond odour, that could also make an important contribution to the herbaceous notes.

Acetal (1,1-diethoxyethane) is a volatile compound found in wine and produced during fermentation. It is responsible for a biscuity or cookie like aromas.

Methyl propyl ketone has a specific odour, being one of the recognized compounds that form the aroma bouquet (Ján Farkaš, 1988).

## CONCLUSIONS

During fermentation of grape must, an important number of aroma compounds, from almost all chemical classes specific to wine aroma, are lost in the exhaust $CO_2$ flow.

A SPE trap has succeeded in capturing aroma compounds from the exhaust $CO_2$ flow of fermenting must.

Further research will analyse the possibility of separating the identified compounds and their reuse as aroma additives in certain food and cosmetic industries.

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## REFERENCES

**Cotea, V.D., Sauciuc, J.,1988,** *Tratat de oenologie, vol. 2*, Ed. Ceres, Bucureşti.

**Ján Farkaš, 1988**, *Technology and Biochemistry of Wine*, Volume 2, CRC Press, ISBN: 2881240704, Czech Republic

**Ron S. Jackson, 2000***, Wine Science: Principles, Practice, Perception,* second edition, 2000, Academic Press, California, USA.

**Miller, G.C., Amon, J.M., Simpson, R.F., 1987**, *Loss of aroma compounds in carbon dioxide effluent during white wine fermentation*, Food Technology Australia 39, 246-249, 253.

**Muller C.J., V.L. Wahlstrom and K.C. Fugelsang. 1993**, *Capture and use of volatile flavor constituents emitted during wine fermentatation*. In Beer and Wine Production. American Chemical Society. Washington, D.C.

**Nasrawi, C.W., Wahlstrom, V.L., Fugelsang, K.C., Muller C.J., 1990**, *Capture and utilization of emission control volatiles*, Presented at Grape Day. California State Univ. Fresno, Nov. 3rd. CATI Publication #901102.

**Williams, A.A. and Boulton, R.B., 1983**, *Modeling and prediction of evaporative ethanol loss during fermentation*, Am.J.Enol.Vitic 34, 234 – 242.

***www.wine-pages.com/guests/tom/taste2.htm

***www.wikipedia.org