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Study of chemical and aromatic composition in a Romanian wine *Cabernet Sauvignon*

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Abstract

Gas chromatography and mass spectrometry methods were used to evaluate the main odorants of Cabernet Sauvignon wine from the Dealu Mare vineyard.

Our analyses GC/MS were identified and assayed a total of 24 volatile compounds: 8 esters, 3 alcohols, 3 aromatic alcohols, 1 lactone, 1 terpene, 7 acids and 1 aldehyde. The highest found concentration was of isoamyl alcohol (5460 μg/l). In the ester class were identified in the highest amounts ethyl propanoate (4089 μg/l), ethyl acetate (1805 μg/l) and ethyl butanoate (1735 μg/l). Regarding major alcohol class stands only 3-methyl-1-butanol (5460 μg/l), 1-propanol 10 μg/l. In the aromatic alcohol group were identified benzethanol (890 μg/l) and 2-methoxy-phenol (10 μg/l).

Character of wild vegetation, characteristic of this wine, is due to both methoxy-phenol and limonene, both volatile compounds being found in high concentrations in the species of Muscadinia wine genre.

Keywords: gas chromatography, mass spectrometry, volatile compounds, *Cabernet Sauvignon* wine.

Introduction

Lately there is an increasing demand in the world for advanced quality red wine consumption, because red wines are considered, due to proanthocyanidins content, as having an important therapeutic and anti radical action.

Romania, with favorable climatic conditions, has posed a great potential for producing high quality red wines. *Cabernet Sauvignon*, although originating in France, is well fixed in the range of our country, encountering good conditions for crop growing areas with warmer climates in the South of the country, currently holding an area of about 11 500 ha.

In the terms of Dealu Mare vineyard, *Cabernet Sauvignon* wine is impressive, with personality, is considered the "king" of Romanian red wines. The wines have intense red color, with a beautiful ruby tint, and the flavors are absolutely specific high content of extract recalling the one of wild vegetation, over which is superimposed a noble and complex bouquet, carried out by aging and a harmonious taste.

Our research focused on physical-chemical analysis and then on analytical (GC/MS) ones of *Cabernet Sauvignon* wine from the Dealu Mare vineyard. Many studies have reported results on wine volatile compounds (1,2,3,4,5). However, very few have been devoted to Romanian wine aromas (6,7). Many of these aromas are formed during grape processing (destalking, crushing and pressing) by chemical and enzymatic reactions. Gas chromatography methods have been extensively used in aroma research and allow the determination of odor-active compounds in food.

Material and methods

Cabernet Sauvignon wine was analyzed in terms of physico-chemical characteristics: alcoholic strength (vol% alcohol), sugar content (g/l), total acidity (g/l sulfuric acid), total dry extract (g/l), acidity volatile (g/l acetic acid), total polyphenols (g/l gallic acid), anthocyanins (mg/l), tannins (g/l). All analyses were performed by the standard methods in the field (5).

Chemical tests were followed by organoleptic analysis (tasting laboratory) and GC/MS (gas chromatography coupled with mass spectrometry) analyses for identification and determination of volatile compounds in the wines (8). Determination of volatile aromatic compounds in wine was performed using a Hewlett Packard 5890 gas chromatograph series II coupled to a mass spectrometer Hewlett Packard 5972 series II.

Extraction methods:

Method Principle: based on different solubility, the solution of interest is distributed between two phases, to achieve a balance - equilibrium distribution (partition), when the compositions of both phases remain constant and define two fractions: fine (representing phase which was extracted with a new composition - composition of the equilibrium distribution) and extract (representing the extracted phase extracting enriched, by composition distribution) (9).

Procedure:

- Use a separating funnel of 500 ml volume, with a catch clips on a stand; in the funnel place 200 ml of wine to be analyzed and then add 40 ml of methylene chloride, which is solvent extraction;
- Funnel stopper, remove the ring and keep it with both hands, so to ensure a continuous and simultaneous fixation of the valve in the closed position and the stopper;
- Funnel, shake vigorously 5 min. (experience has shown that the distribution equilibrium is reached after that time);
- Decompression is performed by turning the funnel with the stopper down and slowly opening the valve, the pressure inside the funnel is balanced by atmospheric pressure, mixing operation is resumed;
- Collect the lower layer after layer demarcation;
- Repeated three times off the job, picking the lower layer in the same cup (extract concentration);
- Na_2SO_4 is added over the aqueous extract to absorb excess water collected by rinsing the solution.
- Extract is made by bubbling nitrogen concentration and maintaining the sample on ice bath. The extract is concentrated to give an amount of 1 ml.

1µl from each extract was injected into an HP 5-MS capillary column with dimensions: 30 m (column length) x 0.25 mm (diameter) x 0.25 mm (film thickness). Column temperature: 30° C for 10 min., followed by temperature gradient 10 ° min^{-1} up to 80 ° C, then gradient of 25° C/min. up to 250° C where stationed 10 minutes. Detector and injector temperatures are: 280° C and 250° C resp. Carrier gas is He, flow-0.5 ml min^{-1}.

MSD conditions are: temperature 180 °C ion source, ionization energy 70 eV, mass limit of 20-400 amu, electronic multiplier voltage 1700V, scan rate 1.60 s^{-1}.

Injection mode: split, opening after 60sec, and the split flow: 20 ml min^{-1}.

Quantitative determination and identification of volatile compounds based on the comparison of retention indices (RI), mass spectra and the estate of odors. Identification is based on the standard MS library Wiley.

Research results

I. Chemical and organoleptic analyses of wine

Cabernet Sauvignon wine was analyzed in terms of key physical, chemical and organoleptic parameters, and results are presented in Tables 1 and 2.

Table 1. Analysis of chemical composition of *Cabernet Sauvignon* wine

Physico-chemical properties	Concentration
Total acidity (g/l sulfuric acid)	4.3
Sugars (g/l)	3.8
Alcohol (vol% alcohol)	13.1
Glycerol (g/l)	9.0
Total Extract (g/l)	31.2
Volatile acidity (g/l acetic acid)	0.7

Table 2. Composition of *Cabernet Sauvignon* wine polyphenols

Physico-chemical properties	Concentration
Total polyphenols (g/l gallic acid)	3.3
Tannins (g/l)	3.9
Anthocyanins (mg/l)	665

In terms of wine sensory analysis is a quality red wine, intense red color with ruby tint and a specific flavor of wild vegetation associated with a complex bouquet, fruit and herbs.

II. Analysis of GC / MS of wines. Identification of volatile compounds in the wines

Volatile compounds were extracted with methylene chloride and 1ml extract was concentrated by bubbling nitrogen ice bath. The amount of wine used for extraction was 200 ml. After applying the method of extraction, the extract obtained was injected into a gas chromatograph coupled with a mass spectrometer to identify volatile compounds. The results are presented below.

Table 3. Volatile compounds determined in *Cabernet Sauvignon* wine

Nr. crt.	Compound	Peak	Retention time (min)	Aria
1	Ethyl acetate	1	3.43	1997942035
2	Acetic Acid	3	5.35	11275431
3	3-hydroxy-2-butanone	4	5.86	24048640
4	3-methyl 1-butanol	6	8.04	6949959120
5	Ethyl propanoate	7	12.13	451936764
6	1-hexanol	9	14.30	19346040

7	Pentanoic acid	10	15.24	8498728
8	γ-butyrolactone	11 +12	15.46 +15.82	18444174 +454731047
9	3-hydroxy-ethyl butanoate	13 +14	15.95+15.98	2003440+6404180
10	Ethyl acetate	15	16.59	2181196
11	1-propanol	16	16.75	10262895
12	Ethyl Hexanoate	17	16.84	10901198
13	1-limonene	18	17.20	2091834
14	Hexanoic acid	19	17.43	14145466
15	Benzmethanol	20	17.57	8273135
16	2-methoxy-phenol	22	17.98	4673969
17	Heptanoic acid	23	18.11	4602993
18	Benzetanol	24	18.37	1795892425
19	Ethyl Butanoate	26	18.63	194102945
20	Octanoic acid	27	18.80	39160067
21	Ethyl Butanoate	28	19.11	1143025910
22	Methyl Hexanoate	43	22.39	4959142
23	Hexanoic acid	44	22.61	9354873
24	Octanoic acid	49	23.69	6462029

The analyses identified 24 aromatic compounds: 8 belong to the class of esters, 3 alcohols, 3 aromatic alcohols belong to the group, 1 lactone, 1 terpene, 7 acids and 1 aldehyde.

The calculation of the concentration of identified volatile compounds

The total quantity of standard: 0.0083 g α-terpineol + 0.0058 g butyl alcohol + 0.0908 g ethyl acetate + 0.0555 g γ-butyrolactone + 0.1025 g benzyl alcohol + 0.0038 g 1,4 - butanediol = 0.2667 g.

0.2667 g standard	0.0083 g α-terpineol
100 g standard	x = 3.112%
0.2667 g standard	0.0058 g Butyl alcohol
100 g standard	y = 2.174%
0.2667 g standard	0.0908g and ethyl acetate
100 g standard	z = 34.045%
0.2667 g standard	0.0555g γ-butyrolactone
100 g standard	w = 20.809%
0.2667 g standard	0.1025 g benzyl alcohol
100 g standard	j = 38.432%
0.2667 g standard	0.0038 g 1,4-butanediol
100 g standard	k = 1.424%
2 x 1240080175	34.05% ethyl acetate

1600350889	q = 21.971% ethyl acetate
100 g extract	21 971 g ethyl acetate d
1µl x 1.336 g / ml	p = 0.293 • 10^{-3} g = 0.293 mg of ethyl acetate
200 ml wine	0.293 mg ethyl acetate
1000 ml wine	t = 1.465 mg / l ethyl acetate

The area of a standard appropriate ethyl acetate: 1240080175 where: 1.336 g/cm ^{3} = density of the methylene chloride extract. For calculating the esters concentration was used range of ethyl acetate, to calculate the lactones was used the area of γ-butyrolactone, to calculate aliphatic alcohols was used the area of isoamyl alcohol, for aromatic alcohols was used area of benzyl alcohol and for terpene alcohols was used the area of alcohol-α terpineol.

Table 4. Esters concentration in *Cabernet Sauvignon* wine

Nr.crt.	**Compound**	**Concentration** (µg/l)
1	ethyl acetate	1805
2	ethyl propanoate	4089
3	3-hydroxy-ethyl butanoate	10
4	ethyl acetate	2
5	ethyl hexanoate	15
6	ethyl butanoate	295
7	ethyl butanoate	1735
8	ethyl hexanoate	7
9	Total	7958

In the esters class was identified in the highest amount the ethyl propanoate (4089 µg/l), ethyl acetate (1805 µg/l) and ethyl butanoate (1735 µg/l). In this wine, in the esters class was also identified a compound 3-hydroxy-ethyl butanoate 10 µg/l muscadin flavor.

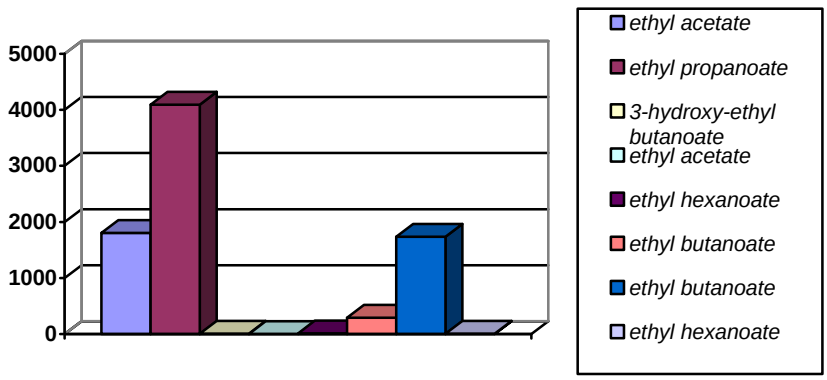

Fig. 1. The main esters of a *Cabernet Sauvignon* wine

Table 5. Concentration of aliphatic alcohols in *Cabernet Sauvignon* wine

Nr.crt.	Compound	Concentration (μg/l)
1	3-methyl-1-butanol	5460
2	1-hexanol	15
3	1-propanol	10
4	Total	5485

Regarding major class stands only alcohols 3-methyl-1-butanol (5460 μg/l), but it is mentioned 1-propanol (10 μg/l), alcohol was not identified other wines analyzed. Isopropyl alcohol in wine is between 10-100 μg/l.

The group is highlighted benzethanol aromatic alcohols (890 μg/l) and 2-methoxy-phenol (10 μg/l).

Table 6. Concentration of aromatic alcohols in *Cabernet Sauvignon* wine

Nr.crt.	Compound	Concentration (μg/l)
1	Benzmetanol	2
2	2-methoxy-phenol	1
3	Benzetanol	390
4	Total	393

Table 7. Concentration of terpenes in *Cabernet Sauvignon* wine

Nr. crt.	Compound	Concentration (μg/l)
1	1-limonene	0.3
2	Total	0.3

On terpenes class the *Cabernet Sauvignon* wine is only where was identified 1-limonene (0.3 μg/l). In the lactones class was identified γ-butyrolactone in the amount of 120 μg/l.

Table 8. Lactone concentration in *Cabernet Sauvignon* wine

Nr.crt.	Compound	Concentration (μg/l)
1	γ-butyrolactone	120
2	Total	120

Conclusions

1. Studies have focused on *Cabernet Sauvignon* wine obtained at vineyard Dealu Mare, a large region with a vocation for obtaining high quality red wines and especially for this wine;

2. Gas chromatography and mass spectrometry methods have shown aromatic profile of *Cabernet Sauvignon* wine specific to area Dealu Mare;

3. The *Cabernet Sauvignon* wine produced in the vineyard Dealu Mare is a wine with high alcohol concentration, dry, thus leading to depletion by fermentation of sugars and an acid taste good balance;

4. The wine boasts a total extract high value, that quality leads to a full-bodied *Cabernet* wine, mining, with personality, rich in phenolic compounds, both the tannins and anthocyanins;

5. Although rich in phenolic compounds this wine is distinguished by fine tannins and softness, qualities influenced probably by the quantity of glycerol contained, high concentration of anthocyanins leads to an intense red wine with blue shades noticed the young wine;

6. The wine tasting presented a characteristic flavor, strong, wild vegetation and berries;

7. Our GC/MS analyses have identified 24 volatile compounds: 8 esters, 3 alcohols, 3 aromatic alcohols, 1 lactone, 1 terpene, 7 acids and 1 aldehydes. The highest concentration was found in isoamyl alcohol (5460 μg/l);

8. Class esters was identified in the highest amount of ethyl propanoate (4089 μg/l), ethyl acetate (1805 μg/l) and ethyl butanoate (1735 μg/l). In this wine, the class was also identified esters and compound 3-hydroxy-ethyl butanoate (10 μg/l) muscadin flavor;

9. Regarding senior alcohols class only 3-methyl-1-butanol (5460 μg/l) stands, but it is mentioned 1-propanol (10 μg/l), alcohol which was not identified in the other analyzed wines. Isopropyl alcohol in wine is between 10-100 μg/l. In the aromatic alcohols group are highlighted benzethanol (890 μg/l) and 2-methoxy-phenol (10 μg/l);

10. In the wine was identified limonene, terpene found in high concentrations in *Muscadinia species,* the caracter of wildlife vegetation could be due to this compound;

11. These studies should be repeated in order to determine what remains common from one year to another and in various centers of Dealu Mare vineyard.

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