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IDENTIFICATION OF MAIN VOLATILE COMPOUNDS FROM THE MUST OF HYBRID GRAPES GROWN IN ROMANIA

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Abstract

Grape must originated from American hybrids Vitis labrusca (first generation) were analyzed by Gas Chromatography/Mass Spectrometry (GC/MS) and Gas Chromatography/Olfactometry (GC/O) to identify the main compounds that characterize aroma of these vine varieties.
Were detected a number of 29 compounds, from which 23 were identify as: volatile aldehydes and acids, ketones, esters, alcohols, terpenes. The responsible compounds with the specific character of grape labrusca are o-Aminoacetophenone, compound which give that foxy aroma of these hybrids and 2,5-dimethyl tetra hydro (2H)-3-furanone, known for having that sweet candy and candy-floss aroma. Other compounds identified in higher concentrations were the hydroxyl esters, they contributed at that "fruity" character of labrusca grapes.

Key words: *Chromatography/Mass Spectrometry, Olfactometry, grape hybrids, Vitis labrusca.*

INTRODUCTION

Romania is well known and appreciated for her wines, occupying 13th in world rankings of winemakers, with approximate 5 million hl of annual wine product. Cultivated area of noble vine has 243.000 ha, of which about 200.000 ha is an area of vines dedicated for wine production.
However a huge area of vines, cultivated exclusively on amateur winegrowers lands (in particular from village areas) is represented by older vines belonging to the American species (interspecific hybrids).
The interspecific hybrids divided by generation, in American old hybrids (first generation), represented by varieties brought from the American continent, created before the invasion of phylloxera in Europe: *Noah*, *Isabelle*, *Lidia*, *Delaware*, *Othello* etc; Euro x American hybrids (second generation, result of crossing between hybrids from first generation with noble vines): *Seibel 1, Seibel 1000, Terras 20, Rayon d'or* etc, to which adds varieties with biological resistance, resulted from multiple crossings between noble vines with American hybrids (Grecu V., 2010).
The biggest areas belong to amateur winegrowers from villages, are cultivated with hybrids from first and second generation. There are many studies about grape flavor of *Vitis vinifera*, as those which aim the interspecific hybrids cultivated on the American continent, but there is no research on flavor of older interspecific hybrids cultivated in Romania (Allen M. et all., 1991; Visan L. et al., 2007; 2015).
Gas Chromatography/Olfactometry methods (GC/O) have been used in aroma research and enable the determination of odor-active compounds in food (Williams P. J. and Allen M. S., 1996; Tominaga T. et all. 1998; 2000). Olfactometric techniques, aroma extract dilution analysis (AEDA) and Charm analysis are commonly applied in characterization of aromatic compounds in foods, inclusively in juices, grape must and wine (Serot Th., 2001).
In AEDA dilutions in series of aromatic extract are analyzed through Gas Chromatography/ Olfactometry methods (GC/O) to determine the dilution factor of flavor.
Our studies have focused on identifying and characterizing of aromatic volatile compounds from first generation of hybrids must, resulting from crossing American species *Vitis labrusca* with *Vitis vinifera* (*V.labrusca* x *V. vinifera*), using the continuous extraction liquid-liquid and identification of the aromatic compounds specific of these hybrids through AEDA method.

MATERIALS AND METHODS

Juice Preparation

Grapes from *Vitis labrusca* hybrids, cultivated in southern Romania (Dambovita) were harvested at full maturity on 18 September 2016; maturity was estimated by content in sugars (degrees Brix), titrable acidity and berry size.

After harvesting, grapes were crushed and pressed with a laboratory winepress. The must was homogenized, filtered and stored at -18°C prior to extraction of volatile compounds.

Extraction of Volatile Compounds

At extraction of the volatile compounds was used a continuous extractor liquid-liquid. Two hundred milliliters of juice (containing internal standard IS: 1-heptanol) placed in a conical flask, was extracted with 5 mL of distilled dichloromethane (Merck, Darmstadt, Germany) by stirring for 30 min at 0°C and then centrifuged for 15 min at 10000 g.

The extract was dried with 4 g sodium sulfate and stored at -18°C until analysis (Chandary S. et all., 1984; Baek H.H., 1997).

Gas Chromatography/Mass Spectrometry (GC/MS)

The GC/MS system includes a Hewlett Packard 5890 Series II gas chromatograph and a Hewlett Packard 5971 mass spectrometer. Each extract was injected 1 µl in the spitless mode (200°C injector temperature, 60 sec valve delay) into a capillary column (DB-Wax, 30 m length x 0.32 mm internal diameter x 0.5 µm film thickness).

The flow rate of helium (carrier gas) was 1 mL/min. Oven temperature was programmed from 50 to 200°C at a rate of 3°C/min with initial and final hold times of 5 and 50 min (Guth H., 1997).

Mass spectrometer, conditions were: ion source temperature: 280°C; ionization energy: 70 eV; mass range: 30-350 a.m.u.; electron multiplier voltage: 2100 V; scan rate: 2.2 sec^{-1}.

Volatile compounds identification was based on comparison of GC retention indices (RI), mass spectra (comparison with MS spectra database and internal library of the laboratory) and odor properties (Tranchant J. et al., 1995; Le Guen S., 2000; Campeanu et all., 2001).

Gas Chromatography/Olfactometry (GC/O)

The GC/O system consisted of a 3300 Varian GC, equipped with a flame ionization detector (FID) and a sniffing port. Each extract (2 µL) was injected in the spitless mode (250°C injector temperature) into a capillary column. The flow rate of helium (carrier gas) was 1mL/min. The oven temperature was programmed from 50 to 200°C at a rate of 5°C/min (Serot Th., 2001).

Aroma Extract Dilution Analysis

The AEDA method is commonly applied in characterization of aromatic compounds from foods, juices, grapes and wine must. Serial dilutions (1:3) were prepared using as thinner dichloromethane. From each dilution was injected 1 µL in a FSOT column, conditions being the same as for GC/MS (Rapp A., 1998; Guth H., 1993; Ullrich F. and Grosch W., 1988). Odor description as perceived by panelists during olfactometry analysis.

RESULTS AND DISCUSSIONS

Identification of the compound

Volatile compound identification was based on comparison of GC retention indices (RI), mass spectra (comparison with MS spectra database and internal library of the laboratory) and odor properties. By using the chromatographic analysis, 23 compounds were identified and their quantity was evaluated by the method of the internal standard. The results of olfactometric analysis are summarized in table 1.

In the must of *labrusca* grapes were detected a number of 29 volatile compounds, from which 23 aromatic compounds were identified and dosed (table 2).

Aldehydes and acids

Were identified 4 aldehydes, with characterized flavors as green apples, sour (acetaldehyde), grass, green (hexanal), rose, floral aroma (phenyl acetaldehyde).

Compounds with 6 atoms of carbon, as hexenal and hexanal, are responsible for that raw, greenery smell; these compounds are formed during production and processing of the must

by the enzymatic action of lipases, lipoxygenase and alcohol dehydrogenase on the unsaturated fatty acids.

In the analyzed must were identified both compounds, but in the highest concentration was found the hexenal, which was characterized by a rough flavor, of raw, greenery, grass, cucumber.

The aroma of phenyl acetaldehyde (pure substance) can be described as honey-like, rose, green, grassy and is added to fragrances to impart hyacinth, narcissi, or rose nuances (Chisholm M. G. et al., 1994).

In the analyzed must, compound was characterized as having a sweet aroma, floral, rose (figure 1). Between acids were identified: 3-methylbutanoic acid, with a characterized aroma as dried fruits, hay and caprylic acid, with an unpleasant aroma of wax, tallow, fatty flavor (Guedes de Pinho P., 1995).

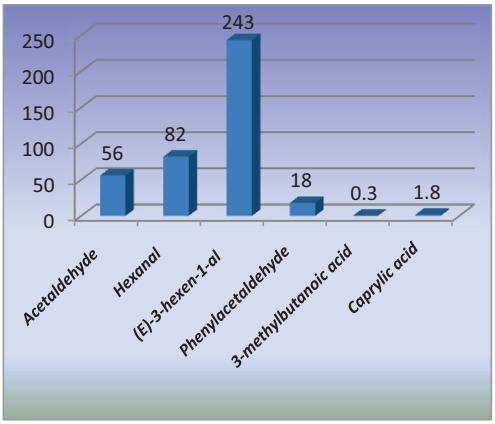

Figure 1 Aldehydes and acids identified in must of *labrusca* grapes (ppm)

Esters

In *labrusca* grapes were identified 9 esters, represents 40% from the identified compounds. This were characterized by having pleasant smells, fruitiness and floral, as Ethyl butyrate, Ethyl 2-methyl butanoate, Ethyl hexanoate, Ethyl decanoate, ethyl 3-hidroxy butanoate and Phenyl acetate, but heavy odor, unpleasant: Ethyl acetate, Ethyl caproate (pungent, sour, cheese), and Ethyl-2-hydroxy-3-phenylpropanoate. The hydroxyl esters contributed to the "fruity" character of *labrusca* grapes (Schreier P., 1980). In the analyzed must the hydroxy esters were identified in high concentrations, way beyond threshold (figure 2).

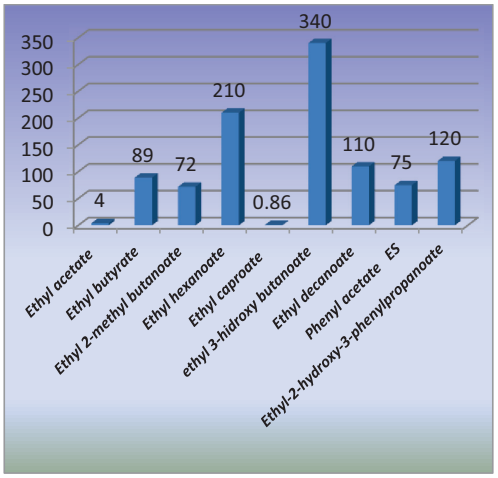

Figure 2 Esters identified in must of *labrusca* grapes (ppm)

Alcohols

Alcohols identified: 2-methyl 3-buten-2-ol with sweet, oily, fruity, herbal aroma and 2-phenylethanol (rose, floral).

2.5-dimethyl tetrahydro (2H)-3-furanone

In *Vitis vinifera* wines, furaneol is found in reduced concentrations. In contrary in must and wines of American hybrids have caramel flavor or strawberries and is found in big quantity, above his sensory perception threshold, of 30-300 µg/l (Rapp et al., 1980).

In the analyzed must, the 2,5-dimethyl tetra hydro (2H)-3-furanone is found in large quantity, the compound being characterized as having a sweet candy and candy floss aroma (Acree T. and Lavin E, 1990). Furaneol is one of the compounds responsible with the specific character of *labrusca*.

Among ketones were identified o-Aminoacetophenone, 2-pentanone, with a rough aroma, of solvent; 1-octene-3-ona with a mushroom, earth, oxidized metal aroma; 6-methyl-1-heptenone with green grass aroma;

o-Aminoacetophenone

Rough smell of foxy compound, cat urine, were identified in higher concentration, being considered together with 2,5-dimethyl tetra hydro (2H)-3-furanone aromatic compounds, predominantly in hybrid must, responsible for the foxy odour of *Vitis lambrusca* (Rogers I.M. and C.J. van Wyk, 2000).

Table 1. Odor-Active Compounds detected in the grape must by Olfactometric Method

No. peak	Compound	RI DB-Wax column	Odor description
1	Acetaldehyde	705	green apples, sour
2	Ethyl acetate	851	sweet unpleasant smell
3	unknown	915	caramel, pleasant
4	2-pentanone	982	acetone
5	Ethyl butyrate	1045	fruity, pineapple
6	Ethyl 2-methyl butanoate	1062	green apples, fruity
7	2-methyl 3-buten-2-ol	1070	sweet, oily, fruity, herbal
8	Hexanal	1084	grass, green
9	unknown	1112	nnpleasant, mice urine
10	(E)-3-hexen-1-al	1145	grass, green, cucumber
11	unknown	1198	bitter, greenery
12	Ethyl hexanoate	1250	green apples, fruity
13	Ethyl caproate	1259	cheese, oil, pungent, sour
14	1-octene-3-ona	1315	mushroom, earth, metal
15	6-methyl-1-heptenone	1368	grass, green
16	ethyl 3-hidroxy butanoate	1520	sweaty
17	Phenylacetaldehyde	1652	rose, floral, sweet
18	Ethyl decanoate	1672	brandy, grape, pear
19	3-methylbutanoic acid	1681	dried fruits, hay
20	unknown	1695	green, foxy
21	unknown	1752	dark chocolate
22	Phenyl acetate	1832	sweet, floral, honey, spice
23	Ethyl-2-hydroxy-3-phenylpropanoate	1995	fat, fruit, pungent
24	2-phenylethanol	2011	rose, floral
25	4-hydroxy-2,5-dimethyl-3(2H) -furanone	2052	sweet candy, candy-floss
26	Caprylic acid	2072	wax, tallow, fatty flavor
27	o-Aminoacetophenone	2195	foxy, cat urine
28	unknown	2235	smell channel
29	β-mircen	2241	floral, pleasant

Table 2. Concentrations of volatile compounds identified in *labrusca* must

No. peak	Compound		ppm
1	Acetaldehyde	25	56
2	Ethyl acetate	-	4
3	2-pentanone	-	0.045
4	Ethyl butyrate	-	89
5	Ethyl 2-methyl butanoate	1.12	72
6	2-methyl 3-buten-2-ol	300-330	147
7	Hexanal	10	82
8	(E)-3-hexen-1-al	-	243
9	Ethyl hexanoate	76	210
10	Ethyl caproate	240	0.86
11	1-octene-3-ona	0.03–1.12	0.8
12	6-methyl-1-heptenone	10	trace
13	ethyl 3-hidroxy butanoate	-	340
14	Phenylacetaldehyde	1.1	18
15	Ethyl decanoate	-	110
16	3-methylbutanoic acid	0.7	0.3
17	Phenyl acetate	-	75
18	Ethyl-2-hydroxy-3-phenylpropanoate	-	120
19	2-phenylethanol	25-105	59.8
20	4-hydroxy-2,5-dimethyl-3(2H) -furanone	25	292
21	Caprylic acid	10	1.8
22	o-Aminoacetophenone	0.5	0.7
23	β-mircen	36-461	2.4

CONCLUSIONS

In musts from *labrusca* grapes were identified 29 volatile compounds, from which 23 are aromatic compounds.

The identified aldehydes were hexenal, in higher concentration, characterized with a rough aroma, of raw, greenery, grass, cucumber, hexanal, acetaldehyde and phenyl acetaldehyde with a sweet aroma, floral, of roses.

Among acids were identified 3-methylbutanoic acid, with a characterized aroma as dried fruits, hay and caprylic acid with an unpleasant aroma of wax, tallow, fatty flavor.

In the *labrusca* grapes were identified 9 esters characterized as having pleasant smells, floral and fruitiness (Ethyl butyrate, Ethyl 2-methyl butanoate, Ethyl hexanoate, Ethyl decanoate, ethyl 3-hidroxy butanoate and Phenyl acetate) and even havy scents, unpleasant: Ethyl acetate, Ethyl caproate (pungent, sour, cheese), and Ethyl-2-hydroxy-3-phenyl propanoate.

In analyzed must, the hydroxy esters were identified in higher concentrations, far over threshold.

In must of *labrusca* grapes the 2.5-dimethyl tetra hydro (2H)-3-furanone is found in higher quantity, compound being characterized as having aroma of sweet candy, candy floss, one of the compounds responsible of specific character of *labrusca*.

o-Aminoacetophenone characterized as having a heavy smell of foxy, cat urine, were identified in higher concentration being together with 2,5-dimethyl tetra hydro (2H)-3-furanone aromatic compounds responsible for the foxy character of *Vitis lambrusca*.

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