

## STUDY ON THE ANTOCYANS PROFILE IN GRAPES OF RED WINE VARIETIES FROM DEALU BUJORULUI VINEYARD

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**ABSTRACT.** *The anthocyanins play an important role in the actual oenology research. They are glucosides of the anthocyanidins (cyanidin, delphinidin, petunidin, peonidin and malvidin), which in the case of Vitis vinifera are exclusively present as monoglucoside. Acetylated anthocyanins arise if the glucose part of the molecule is esterified with plant acids, such as acetic, coumaric, or caffeic acids. These compounds are summarized as acetylated anthocyanins. Wild wines and hybrids may also contain anthocyanidin-3,5-diglucoside. Analyses were performed by HPLC (high pressure liquid chromatography) and could be used to distinguish between different wine varieties. Data interpretation had to be performed by taking into account the possible changes in the ratio of the anthocyanidins and the relative composition following the different kind of wine varieties.*

**Key words:** Dealu Bujorului vineyard, red varieties, HPLC, ratio between anthocyanins

### INTRODUCTION

The purpose of our work was to get data on the anthocyan profile in technological mature grapes from main black varieties grown in Dealu Bujorului vineyard, Galați County. The results obtained were noticed according to anthocyanic (colour) potential of grapes and ratio between anthocyanins from grapes.

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## MATERIALS AND METHODS

In the first part of our work, we have studied the evolution of the weight of 100 berries and of main composition characteristics (sugars, acidity) during the grape maturation.

For the experiments, we have studied four black varieties, grown in Dealu Bujorului vineyard, in vine plantations of ProWINE INTERNATIONAL Trade Company of Cluj-Napoca, from the Viile Locality, Galați County, and Merlot (Farm 13, Piscul Hill), Cabernet Sauvignon, Fetească neagră and Băbească neagră (Farm 7, Gara Hill).

The grapes (1-1.5 kg, on the average) have been sampled periodically, at intervals of 10 days, between 30 August and 29 September 2005. This period has comprised, besides the end of ripeness stage, the full maturity, respectively, the technological maturity. Physico-chemical analysis of main composition characteristics of grapes (sugar, titrable acidity, anthocyanins) was carried out in the second day after sampling, within the Oenology Laboratory of the University of Agricultural Sciences and Veterinary Medicine of Iași. The determination of grape anthocyanic content was done by colorimetric method (Mazza, Miniati, 1993). For carrying out the investigations in the second part of the work, 2-3 kg grapes (under the shape of bunch fragments of 3-4 berries) were sampled on October 4 (Fetească neagră and Merlot) and October 7 (Cabernet Sauvignon and Băbească neagră) from 50-60 vine stocks of each variety.

After the grapes berries were removed from clusters, they were selected (numbered and weighed) according to data presented in *Table 1*.

Grape berries have been selected for obtaining mean samples, in order to determine the anthocyanins content from berries (mg/kg berries) and the anthocyanic extract necessary to estimate the anthocyan profile and the ratio between them.

According to data from *Table 1*, variant 2 from Cabernet Sauvignon (G=55.3g), variant 3 from Merlot (G = 62.2g), variant 2 from Fetească neagră (G = 68.4g) and variant 3 from Băbească neagră (G = 104.2g) have been processed according to the method for determining the anthocyanic content from grape berries.

For the determination of anthocyan profile from grape berries we have used variant 4 from Cabernet Sauvignon (G = 53.1g), variant 1 from Merlot (G = 62.2g), variant 3 from Fetească neagră (G = 68.0g) and variant 2 from Băbească neagră (G = 104.5g). The grape berries were first of all put into a freezer for 2-3 days (to be easily peeled), then skins were separated and introduced into 30 ml of p.a. methanol solution acidulated with 2 % HCl (5:1 v/v or in 100 ml methanol p.a. or 20 ml HCl concentration was added). Afterwards, the solutions were put again at freezer, so that the cell walls forming the skin could be broken by freezing and colour compounds (anthocyanins) could be released. Extracts were

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kept at freezer for 48 hours. After the maceration of extracts for 48 hours at cold, the macerate product was filtered through gauze and kept at room temperature. Afterwards, anthocyanic extracts have been centrifuged and filtered.

Table 1

Data on selecting grape berries

Variety	Selected grape berries				Studied grape berries		
	Category	No. berries	Percentage (%)	Selected	Variant	No. berries	Weight (g)
<b>Cabernet Sauvignon</b> (492 berries = 527 g)	I	121	24.59	12	1	50	55.2
	II	254	51.63	26	2	50	55.3
	III	117	23.78	12	3	50	53.8
	IV				4	50	53.1
<b>Merlot</b> (528 berries = 698 g)	I	71	13.15	7	1	50	62.2
	II	90	17.20	8	2	50	67.8
	III	51	10.10	5	3	50	62.2
	IV	316	59.55	30	4	50	67.5
<b>Fetească neagră</b> (530 berries = 716 g)	I	154	29.00	14	1	50	72.5
	II	126	23.70	12	2	50	68.4
	III	168	31.69	16	3	50	68.0
	IV	82	15.61	8	4	50	65.1
<b>Babească neagră</b> (325 berries = 685 g)	I	27	8.31	4	1	50	113.1
	II	46	14.16	7	2	50	104.5
	III	68	20.92	11	3	50	104.2
	IV	184	56.61	28	4	50	102.0

For the determination of anthocyan profile, chromatograms were registered by HPLC (high-pressure liquid chromatography), using Hewlett-Packard 1100 chromatogram, C18 column and UV-VIS detector. Reading was done at 519 nm. As A eluent, we have used water:formic acid:acetonitrile 87:10:3, and as B eluent water:formic acid:acetonitrile 40:10:50, with B eluent increasing gradient from 6% to 60%. Because the quantitative determination of each anthocyan from the anthocyanic extract is difficult and expensive, the results concerning these products are easily interpreted from the point of view of area percentage ratios. Malvidin-3-monoglucoside has the highest peak area, therefore, we referred to it. A differentiation parameter in case of black grapes may be used as in case of wines, the sum of esterificated anthocyan (acetylated and coumarilated) and the ratio between them (acetylated/coumarilated anthocyan).

These last parameters present a clear differentiation between the varieties of high anthocianic potential and the less coloured varieties.

Phisico-chemical analyses were carried out by using the methods indicated by present State and international standards or by the speciality literature (\*\* 1988, \*\*\* 2005).

## RESULTS AND DISCUSSION

Data concerning grape maturation (weight of 100 berries, total sugars, titrable acidity and anthocyanins) were presented in *Table 2*.

Table 2

## Data on grape maturation

Main composition characteristics	Variety			
	Date of analysis			
	30 August	9 September	19 September	29 September
<b>Cabernet Sauvignon</b>				
Weight of 100 berries (g)	78	92	101	109
Total sugars (g/l)	122	144	170	194
Titrable acidity (g/l C <sub>4</sub> H <sub>6</sub> O <sub>6</sub> )	8.34	7.80	6.73	5.66
Anthocyanins (mg/kg berries)	524	811	1080	1336
<b>Merlot</b>				
Weight of 100 berries (g)	125	136	145	136
Total sugars (g/l)	136	157	178	205
Titrable acidity (g/l C <sub>4</sub> H <sub>6</sub> O <sub>6</sub> )	9.25	8.01	7.12	6.56
Anthocyanins (mg/kg berries)	604	785	952	1186
<b>Fetească neagră</b>				
Weight of 100 berries (g)	133	144	155	141
Total sugars (g/l)	138	154	177	208
Titrable acidity (g/l C <sub>4</sub> H <sub>6</sub> O <sub>6</sub> )	8.84	7.62	7.01	6.36
Anthocyanins (mg/kg berries)	580	807	934	1102
<b>Băbească neagră</b>				
Weight of 100 berries (g)	186	206	223	218
Total sugars (g/l)	112	135	169	198
Titrable acidity (g/l C <sub>4</sub> H <sub>6</sub> O <sub>6</sub> )	9.98	8.45	7.78	6.25
Anthocyanins (mg/kg berries)	221	342	505	634

Data on composition characteristics of grapes at vintage, for each variety, were presented in *Table 3*.

Grape yield in each variety, correlated to main composition characteristics (total sugars, titrable acidity, pH, anthocyanins) had typical values for obtaining quality wines with Denomination of controlled origin (DOC): Denomination of controlled origin DOC-Harvesting at full maturity (HFM) (Cabernet Sauvignon,

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Merlot, Băbească neagră); Denomination of controlled origin and late harvest, LH, (Fetească neagră).

The anthocyan content, expressed in mg/kg berries, had values between 689 in Băbească neagră and 1353 in Cabernet Sauvignon. These values, correlated to the other composition characteristics, pointed out the specific data for a good yield year, both from the point of view of anthocyan (colour) potential and quality characteristics. Afterwards, we have presented the anthocyan profile from the anthocyanic extracts obtained from the skin of berries in the four grape varieties Cabernet Sauvignon, Merlot, Fetească neagră and Băbească neagră.

**Table 3**

### Main composition characteristics of grapes at vintage

Composition characteristics	Variety				
	Cabernet Sauvignon	Merlot	Fetească neagră	Băbească neagră	
Date of harvesting	7.10	4.10	4.10	7.10	
Weight of 100 berries (g)	107	132	135	211	
Total sugars (g/l)	200	210	214	205	
Titrate acidity (g/l C <sub>4</sub> H <sub>6</sub> O <sub>6</sub> )	5.52	6.40	6.28	6.17	
pH	3.33	3.33	3.38	3.34	
Anthocyan (mg/kg berries)	1353	1156	1048	689	
Yield	vinestock (kg)	1.16	2.14	2.01	2.50
	ha (t)	3.82	7.05	6.64	8.25
Quality category	DOC-HFM	DOC-HFM	DOC-LH	DOC-HFM	

For each chromatogram we have identified and calculated the relative proportions of the following anthocyan: delphinidin-3-monoglucoside (Dp), cyanidin-3-monoglucoside (Cy), petunidin-3-monoglucoside (Pt), peonidin-3-monoglucoside (Po) malvidin-3-monoglucoside (Mv), peonidin-3-monoglucoside acetilate (Po-a), malvidin-3- monoglucoside acetilate (M-a), peonidin-3-monoglucoside coumarilated (Po-cm), malvidin-3- monoglucoside coumarilated (M-cm).

From obtained chromatographs we have extracted peak areas (in mAU\*s) of anthocyan (from berries skin) for the four studied varieties (*Table 4*). In case of Dp (delfinidin-3-monoglucoside) the peak area had values comprised between 3825.2 and 16094.3, the highest one being found in Cabernet Sauvignon (the most coloured variety) and the lowest one, in Băbească neagră (the less coloured variety). A similar case was found in Mv (malvidin-3-monoglucoside). The other main anthocyan (cyanidin-3-monoglucoside (Cy), petunidin-3-monoglucoside (Pt), peonidin-3-monoglucoside (Po)) had the peaks area in Merlot and decreasing values in Cabernet Sauvignon, Fetească neagră and Băbească neagră.

A differentiation parameter in case of black grapes may be, as in case of wines, the sum of esterified anthocyanins (acetylated and coumarilated) and the ratio between them (acetylated/coumarilated anthocyanins), the sum of peak area of acetylated and coumarilated anthocyanins (peonidin-3-monoglucoside acetilate (Po-a), malvidin-3-monoglucoside acetilate (M-a), peonidin-3-monoglucoside coumarilated Po-cm), malvidin-3-monoglucoside coumarilated (M-cm), had the following values: 15600.2 in Merlot, 12513.1 in Fetească neagră, 11416.0 in Cabernet Sauvignon and 17217.7 in Băbească neagră. The ratio between the peak areas between acetylated anthocyanins and coumarilated has decreased in the following order: 0.28 in Merlot, 0.26 in Fetească neagră, 0.22 in Cabernet Sauvignon and 0.18 in Băbească neagră.

**Table 4**  
**Peak area (in mAU\*s) of studied anthocyanins (from berries skin)**

Anthocyanins	Grape variety			
	Cabernet Sauvignon	Merlot	Fetească neagră	Băbească neagră
Dp	16094.3	14235.4	10174.7	3825.2
Cy	3128.2	3989.8	1568.8	972.10
Pt	11927.5	12968.5	10390.5	5614.9
Po	10414.2	14733.6	8839.0	7565.5
Mv	68622.9	53516.9	51334.4	49915.4
Po-a	979.1	1601.8	1026.1	199.8
Mv-a	1103.8	1794.8	1530.8	2374.6
Po-cm	1420.9	3432.1	1419.2	1507.9
Mv-cm	7912.2	8771.5	8537.0	13135.2
Σ(first 4 anthocyanins)	41564.2	45927.3	30973.0	17977.7
Σ(first 5 anthocyanins)	110187.1	99444.2	82307.4	67893.1
Σ(acetylated anthocyanins)	2082.9	3396.8	2556.9	2574.4
Σ(coumarilated anthocyanins)	9333.1	12203.9	8956.2	14643.1
Σ(all the anthocyanins)	121603.1	115044.4	94820.5	85110.6
Σ(acetylated anthocyanins+ coumarilated anthocyanins)	11416.0	15600.2	12513.1	17217.5
Acetylated anthocyanins/coumarilated anthocyanins	0.22	0.28	0.26	0.18

An assessment of each anthocyanin from the obtained extract, according to peak areas, being difficult to achieve, the interpretation of results was done easily by means of percentage ratios of the areas of main anthocyanins from grapes.

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Malvidin-3-monoglucoside having the highest peak area was considered as reference point.

**Table 5**

**Values of ratios between main anthocyanins (%) related to malvidin concentration from the skin of studied grape berries**

Ratios between anthocyanins	Grape variety			
	Cabernet Sauvignon	Merlot	Fetească neagră	Babească neagră
Dp/Mv	23.45	26.60	19.82	7.66
Cy/Mv	4.56	7.46	3.06	1.95
Pt/Mv	17.38	24.23	20.24	11.25
Po/Mv	15.18	27.53	17.22	15.16
Po-a/Mv	1.43	2.99	2.00	0.40
Mv-a/Mv	1.61	3.35	2.98	4.76
Po-cm/Mv	2.07	6.41	2.76	3.02
Mv-cm/Mv	11.53	16.39	16.63	26.31

According to data from *Table 5*, we calculated for each variety the values of ratios between main anthocyanins (%), related to the concentration of malvidin from the skin of studied berries.

## CONCLUSIONS

The obtained results concerning the quantitative aspect of grapes harvest and the qualitative aspect of grapes reflect a year favourable to high quality red wines with denomination of controlled origin, harmonious wines from the point of view of colour and other composition characteristics.

The sums of peak areas point out the colour potential of studied black varieties, being in direct correlation to the amount of anthocyanins (mg/kg berries) from grapes.

The percentage ratios of areas of main anthocyanins from grapes could be used for establishing the identity and authenticity of black varieties cultivated in the Moldavian vineyards.

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