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Study of phenolic compounds in red grapes and wines from Murfatlar wine center

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Abstract The latest research revealed that phenolic compounds play an important role in the quality of red wine, particularly on colour and astringency and also are responsible for the sanogenic or multiple benefic effects on human health after a moderate consumption of wine. This paper presents the ripening evolution of routine quality control parameters (sugars, acids, weight of 100 berries) and phenolic compounds (anthocyanins and polyphenolic index) during 2013 year for the most representative red grape varieties (Cabernet Sauvignon, Merlot, Feteasca Neagra, Pinot Noir and Mamaia) authorized to obtain wines with denomination of origin controlled in Murfatlar wine center. Also, the phenolic profile of obtained red wines was evaluated by reversed-phase high performance liquid chromatography. The reported results were useful to find the optimum moment for grape harvest ensuring the production of high quality wines.

Keywords: phenolic compounds, grape, wine, ripening, HPLC

1. Introduction

The importance of phenolic compounds in wine and winemaking is well known [1]. Vines synthesize in its organs important amounts of phenolic compounds, which accumulates in the solid parts of grapes (bunches, seeds and skins). By vinification of the grapes phenolic compounds are extracted into the wine and the alcohol formed during fermentation contributes to the solubilization and extraction of more phenolic compounds [2].

By their physico-chemical attributes the phenolic compounds are rightly considered the most important group of chemical compounds in grapes, after sugars and acids. They have an important contribution in defining the sensory attributes of wines as regards to visual, olfactory and taste aspects [3].

Grape phenolic content is variable, depending on the variety, degree of maturation of grapes, climate and culture system of the vine [4]. Monitoring the phenolic compounds during maturation is necessary and useful [5, 6], many winemaking decisions applied during processing of a particular variety being closely related with the phenolic substances [3].

Phenolic content of wines is smaller than grapes and is closely related the technological process of fermentation and maceration of must on marc, in which only a part of phenolic compounds is extracted (30-35%) [7].

Phenolic compounds in wines, mostly come from grape berries but some of them originate in chemical and biochemical reactions during the winemaking process [8].

The individual polyphenolic fingerprint, as reflected in the composition of phenolic acids (e.g. galic acid, syringic acid), as well as flavonoids (e.g. (+)-catechin, (-)-epicatechin, rutin) and derivatives of phenolic acids such as stilbenes (trans-resveratrol), is distinctive for any plant [9], and most probably for any grape variety and the resulted wines.

The maturation process has a great influence on the phenolic composition of grapes too, not only in the concentration of sugars and acids, which are

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usually the parameters mostly followed before harvesting. The purpose of this work consisted in establishing the optimal timing of harvest in order to obtain quality wines with denomination of controlled origin Murfatlar. Therefore was monitored besides the technological maturity (weight of 100 berries, sugars, total acidity) the phenolic maturity (anthocyanins and polyphenols indices). The evaluation of phenolic grapes ripening makes it possible to forecast the red wines quality and to model the technologies towards improving the wines phenolic structure.

2. Experimental

The research was conducted in Murfatlar wine center in the Research Station for Viticulture and Oenology Murfatlar on the most representative red grape varieties authorized for the obtainment of wines with denomination of origin controlled: Cabernet Sauvignon, Merlot, Feteasca neagra, Pinot noir and Mamaia.

The study is focused on the dynamics of grape ripening evaluated by periodic determinations during the last six weeks of technological maturation of grapes up to harvest, lasting from August to September, 2013.

2.1. Grape analysis

Following parameters have been investigated: the weight of 100 berries (g), sugars (g/L), total acidity expressed as tartaric acid (g/L) and phenolic maturity reflected in total anthocyanin (mg/L), polyphenols index.

Sugars content was determined using an electronic refractometer, total acidity was evaluated volumetrically, the weight of 100 berries was done gravimetrically. Total anthocyanin and polyphenols index were achieved according to ITV method [10] and is based on the extraction of phenolic compounds in acidic conditions (ethanol 95% and hydrochloric acid (HCl) 0.1% v/v), at room temperature, for two hours. Anthocyanin concentration was estimated by measuring the absorbance of the extract solution after dilution 1:20 with 1% HCl solution, at 520 nm, while polyphenol index was estimated by measuring the absorbance of the extract after a dilution 1:100 with distillate water, using a spectrophotometer with quartz cuvette of 1 cm.

2.2. Winemaking procedure

Vinification was carried out in batches of 150-200 kg and was performed in the Laboratory of processing technology of grape and wine chemistry in microvinification department, applying traditional technology of obtaining quality dry red wines, without using any selected yeasts, nutrients or enzymes. Wines were analysed after application of specific operations: fill-up of the wine recipients, raking, sulfitation and resulfitation, as appropriate.

2.3. Wine analysis

Physico-chemical composition of obtained wines was evaluated based on general composition parameters such as alcohol concentration, total and volatile acidity, reducing sugar, total dry and unreduced extract, according to specific literature and international or state standards. Total anthocyanin was determined according to Ribereau Gayon-Stonestreet method, which is based on anthocyanin color change depending on pH. The variation of the absorbance of anthocyanin colour was measured at pH between 0.6 and 3.5, against distilled water, at a wavelength $\lambda = 520$ nm. Total polyphenols were determined by the Folin-Ciocâlteau, the resulting blue colour having a maximum absorbance at 765 nm, the absorbance being proportional to the amount of phenolic compounds in wine.

Chromatic characteristics (Ic, glass, L, C, a and b coordinates, the a/b ratio) and its colour (dA, d420, d520, d620) were determined by the CIE-Lab method and expressed as a percentage contribution of each of the three colour components (yellow, red and blue) in the colour intensity of the wine.

Another purpose of this study was to evaluate individual phenolic composition of red wines with denomination of protected origin Murfatlar by high performance liquid chromatography (HPLC).

For that, we used a Thermo Finnigan Surveyor Thermo Fisher Scientific Inc. (Whatman, USA) chromatographic system and the separation was made by Accuacore PFP column (2.6 µm, 100 x 2.1 mm) with elution gradient of two eluents: solvent A: water with 0.1% formic acid and solvent B: acetonitrile with 0.1% formic acid, with eluent B gradient increasing from 2% to 75% in 35 minutes, followed by returning to 2% and stabilization. The detection was spectrophotometric at a wavelength of 280 nm. The injection volume was 1 µL, the mobile phase flow rate was 0.4 mL/min., the column temperature was 30°C and the work duration 50 min.

All samples were filtered through 0.45 µm filters and analyzed immediately after the bottle opening. All the measurements were carried out in triplicat, at ambient temperature. Quantification was based on the peak area, using the external standard method. Blank solution and control standards were analyzed for each set of ten samples.

3. Results and Discussions

The ripening period of the grapes is different from one year to another and from one vineyard to another, depending mainly on the climate. For this reason it is necessary to follow the evolution of maturation of each variety, every year. Harvesting of the grapes is very important and it must be done timely because, generally, the quantity and quality of the harvest depends on it. Grapes full maturation is reached when the weights of grape berries achieve a maximum value and the evolution curve begins to decrease. At this moment, the sugar content of the grapes is also at its maximum. The evolution of sugar remains stationary for a few days and total acidity is reduced substantially and the evolution curve indicates a slow decrease of acidity. Reaching full maturity varied from variety to variety depending on the genetic traits. First varieties that reach ripeness in the Murfatlar region are Pinot noir and Feteasca Neagra, followed by Cabernet Sauvignon, Merlot and Mamaia.

2013 harvest year was noted as an year when the five varieties of grapes for red wines showed a high potential for accumulation of sugars with values above 214 g/L at harvest for Feteasca Neagra, Pinot Noir, Merlot and Cabernet Sauvignon varieties, while Mamaia variety shows a small accumulation, 197 g/L. For all investigated varieties, sugar accumulation rate was more intense at the beginning of ripening and then decreased gradually as approaching full maturity (**Fig 1**). Similar values for sugar contents at harvest were reported also in literature [11]. As regards the accumulation of sugars and anthocyanins in grapes there are certain correlations, the cultivars being differentiated quantitatively by their genetic traits, but decisively influenced by the specific conditions of the production year.

Based on obtained results we can say that the optimum time for full maturity of grapes was reached at 12th September for Cabernet Sauvignon and Feteasca neagra varieties, 13th September for Pinot noir, 14th for Mamaia and 17th September for Merlot variety, being in concordance with other similar studies [11]. Accumulation of phenolic compounds in grapes evaluated by the anthocyanin content and polyphenols index ranged between 312.1-589.3 mg/L for total antocyanins, with lower values for Mamaia variety and higher values for Cabernet Sauvignon variety. Concerning polyphenols index, the obtained values ranged between 44.2-69.6, with lower values for Mamaia variety and higher values for Feteasca neagra variety (**Table 1**). Quality of raw material has a decisive role for the production of quality wines. Red wines obtained in 2013 harvest are dry wines with a high alcohol level, more than 13.0% vol., except for Mamaia variety, which has only 11.6% vol. Alcoholic degree. The total acidity, expressed as tartaric acid, had medium to high values with lowest value (4.27 g/L) for Mamaia and highest value (7.72 g/L) for Fetească neagra variety; low volatile acidity indicate a correct fermentation processes in terms of alcoholic fermentation and malolactic fermentation. Furthermore, the unreduced extract with values between 25.7 g/L and 28.9 g/L certify the quality of obtained wines and their qualification as wines with denomination of protected origin Murfatlar.

The anthocyanins and polyphenols content complete the organoleptic qualities of wines. Quality of anthocyanins material in red wines, with considerable influence on aspect, is given by the proportions of the three types of colour components (yellow, red and blue) and colour intensity levels in wines ranging between 1.690 and 11.400.

The most intense colour was shown by Cabernet Sauvignon and Feteasca neagra wines (**Table 1**), these two being also the varieties with the highest content of anthocyanins (466.37 mg/L, respectively 325.92 mg/L); middle colour attributes presented Merlot wine variety and lower intensity was found for Pinot noir and Mamaia varieties which presented also the smallest values of anthocyanins (147.44, respectively 173.43 mg/L).

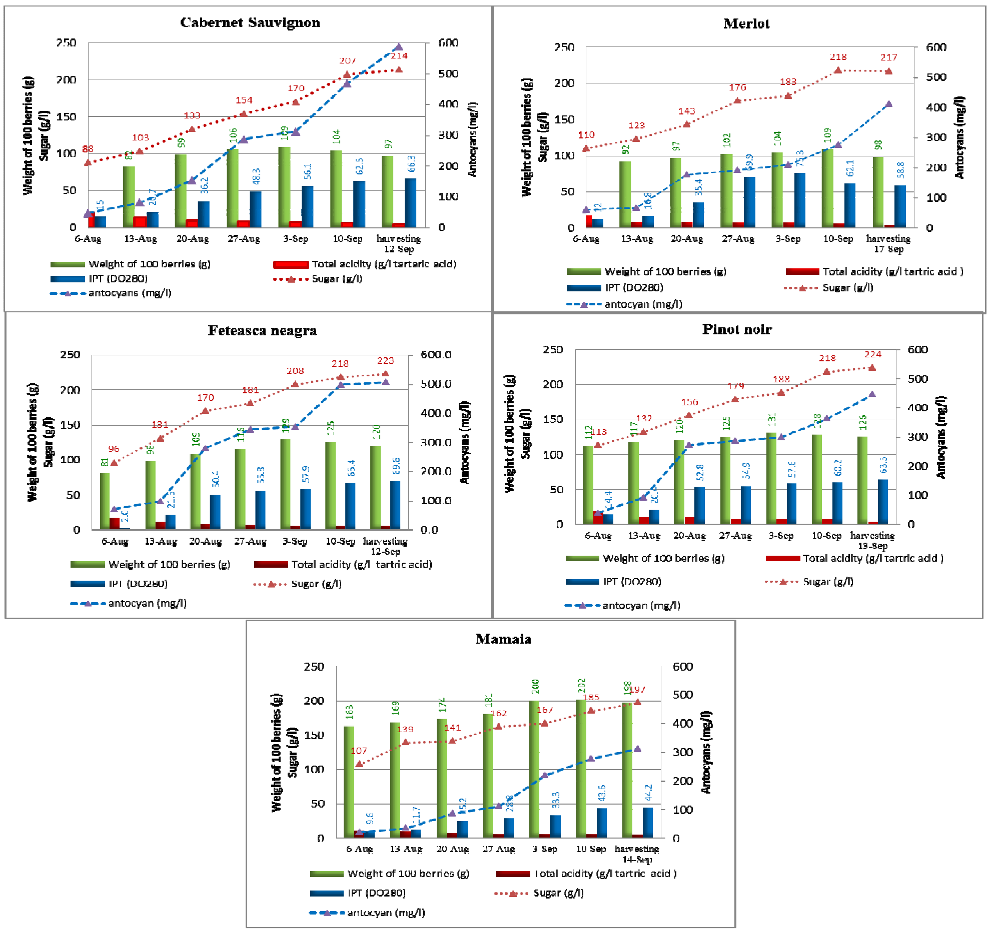

Fig 1. Dynamic of grapes ripening for the investigated black varieties in 2013

In colour constitution it has been found a high percentage of red pigments with 62.89%, 61.61% and 58.67% values for Cabernet Sauvignon, Merlot and Feteasca neagra respectively, which gave to the wines a nice red colour due to a high proportion of anthocyanins in the form of flaviliu ion, dA ranging between 70.50 and 64.78%.

In the case of Pinot Noir and Merlot varieties, prevail yellow pigments with 46.69% and 48.52% values, reducing in this way the red colour intensity and also anthocyanins as flaviliu present lower values, respectively 33.33% and 30.99%. Higher levels of total polyphenols were reported for Cabernet Sauvignon, followed by Feteasca neagra variety. Pinot noir and Merlot varieties show similar values, while Mamaia wine variety presents the lowest value.

The type and concentration of phenolic compounds in wine depends on grape variety, ripening, atmospheric conditions, viticultural and vinification techniques [12]. In the studied wines, phenolic acids represented by galic and syringic acids were reported in relative lower amounts, between 0.10 and 1.04 mg/L for galic acid and 0.10 and 1.33 mg/L with important amounts in Feteasca neagra wine variety.

Table 1 Physico-chemical composition of red wines from Murfatlar wine center, 2013 harvest

No.	Parameter	Unit	Cabernet Sauvignon	Merlot	Feteasca neagra	Pinot noir	Mamaia
General composition							
	Alcoholic degree	% vol	13.35	13.05	13.55	13.45	11.4
	Total acidity	g/L tartaric acid	7.64	7.34	7.72	4.27	6.14
	Volatile acidity	g/L acetic acid	0.44	0.35	0.31	0.39	0.69
	Unreduced sugar	g/L	2.6	2.3	2.6	2.6	2.8
	Total dry extract	g/L	28.7	28.0	31.5	29.7	29.1
	Unreduced extract	g/L	26.1	25.7	28.9	27.1	26.3
Phenolic composition							
	Antocyanins	mg/L	466.37	259.96	325.92	147.44	173.43
	Polyphenols	mg/L	1280	1025	1155	1080	670
Colour							
	Colour intensity	1 cm cuvette	11.400	6.590	10.840	2.870	1.690
	Hue	-	0.463	0.510	0.536	1.089	1.155
	dA	%	70.50	68.84	64.78	33.33	30.99
	% DO420	%	29.12	31.41	31.46	46.69	48.52
	% DO520	%	62.89	61.61	58.67	42.86	42.01
	% DO620	%	7.98	6.98	9.87	10.54	9.47
	Luminosity	-	20.99	33.97	16.97	49.15	64.59
	Chromaticity	-	70.22	82.15	62.35	56.19	38.59
	a component	-	46.12	62.58	41.11	44.70	31.38
	b component	-	52.95	53.23	46.87	34.04	22.45
	b/a ratio	-	1.15	0.85	1.14	0.76	0.72

Pinot noir variety show higher amounts of catechin and epicatechin, followed by Merlot and Feteasca neagra variety and lower value were reported for Mamaia variety. Important amounts of rutin were quantified in Feteasca neagra and Cabernet Sauvignon. *trans*-Resveratrol, a stilbenic compound with great interest due to his potential valuable health effects, occurs in relative low concentrations as compared with other detected phenolic compounds, ranging between 0.10-0.51 mg/L, with higher amount in Mamaia red wines. The values of individual phenolic compounds reported in this study were similar with the results in Croatian wines [13, 14].

Figure 2 presents the phenolic profile of investigated red wines with Pinot noir, Merlot and Cabernet Sauvignon showing a significant phenolic composition.

4. Conclusions

The year of 2013 was noted as an year in which the grape varieties for red wines showed a high potential for accumulation of sugars, with values above 214 g/L at harvest for Feteasca neagra, Pinot noir, Merlot and Cabernet Sauvignon and with a lower accumulation in Mamaia variety with 197 g/L. Accumulation of phenolic compounds in grape expressed as anthocyanins content shows values between 411.2 and 589.3 mg/L, the Mamaia variety presented the lowest content of anthocyanins, 312.1 mg/L; total polyphenol index had values between 44.2-69.6, the highest value reached in Feteasca neagra variety. The sugar and anthocyanins accumulation in grapes showed certain similarities, these being related to genetic nature of each variety, but also decisively influenced by the specific conditions of production year.

Red wines produced in 2013 were dry wines with a high alcohol level, of more than 13.0% vol, with the exeption of Mamaia wine which only had 11.6% vol. Also, unreduced extract was of excellent values, certifying that the wines qualify as wines with denomination of protected origin Murfatlar. The most intense colour was shown by Cabernet Sauvignon and Feteasca neagra wines, these being also the varieties with the highest content of anthocyanins; middle colour presented Merlot wine variety and lower intensity was found for Pinot noir and Mamaia varieties, which presented also the smallest values of anthocyanins.

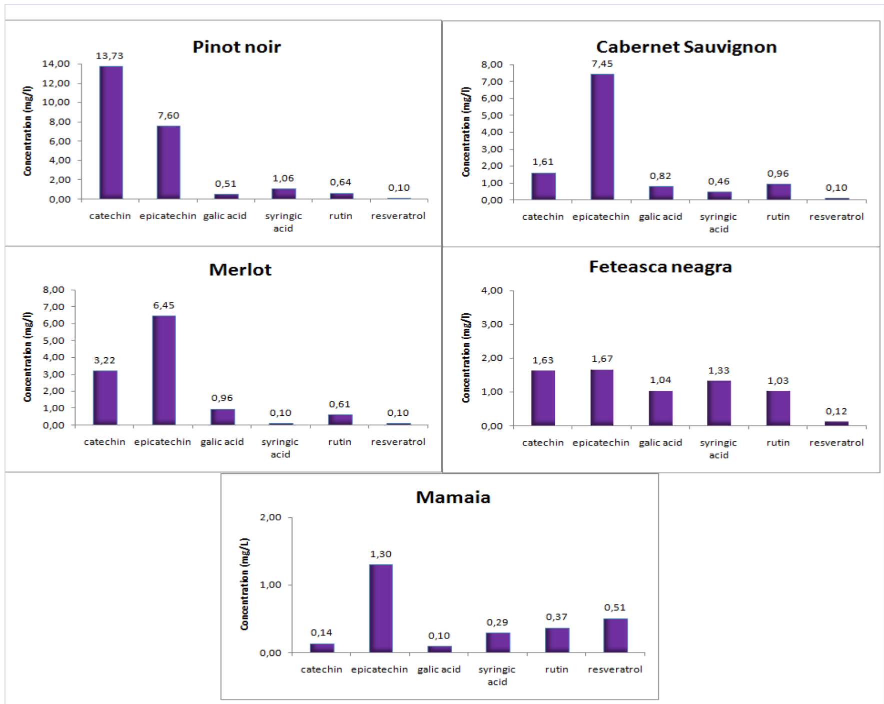

Fig. 2. Individual phenolic profile of red wines varieties from Murfatlar vineyard

The phenolic profiles determined for Pinot noir, Merlot and Cabernet Sauvignon wines, reflected too the differences in the phenolic composition of each variety, each showing different contents of catechin, epicatechin and gallic acid.

5. References

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