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COMPARATIVE STUDY OF THE PHENOLIC COMPLEX, THE RESVERATROL CONTENT AND THE ORGANOLEPTIC PROFILE OF BULGARIAN WINES

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A comparative study of the phenolic complex, the resveratrol content, the antioxidant activity and the organoleptic profile of Bulgarian wines from the local vine varieties Dimyat, Pamid, Gamza and from the hybrid varieties Plevenska rosa, Storgozia, Kaylashki rubin was carried out. Differences in the chemical composition and tasting characteristics of the experimental wines were identified. As for the white wines, Dimyat had higher sugar-free extract and titratable acidity, while concerning the red ones, Storgozia and Kaylashki rubin showed higher rates. The *trans*-resveratrol amount in the red wines was significantly higher compared to the white ones, as the samples from the red local varieties had a higher content than the hybrid varieties. Difference in the phenolic composition of the wines was also found. Gamza wine had the highest concentration of total phenolic compounds, total flavonoids and catechin. Storgozia sample contained the highest rates of monomeric anthocyanins and epicatechin. Pamid revealed the lowest concentrations of all analyzed phenolic components except catechin. The red wines had better antioxidant features than the white ones. From the red wines, the highest and the lowest activity were reported in the local varieties – Gamza and Pamid, respectively. There was no strict correlation between the effects of the studied phenolic components on the wine organoleptic profile.

Keywords: wine, chemical composition, antioxidant activity, resveratrol, organoleptic profile

Chemical composition of grapes and wine is complex and varied. It is determined by a number of factors such as variety, soil and weather conditions, cultivation practices, maturity, winemaking technology, etc.

The phenolic compounds are important components of wines. They have a significant effect on their organoleptic profile, especially in terms of colour intensity and taste characteristics such as density, tartness, and bitterness. Most of them pass from the grapes, so their content in wines is determined by the variety, and its potential and phenolic reserves (Ghiselli et al., 1998; Lee and Koh, 2001). They are extracted from the solid particles during the alcoholic fermentation, influenced by the technological factors as well under which the process takes place – temperature, yeast strain, etc. (Monagas et al., 2005; Savova, 2013). White wines contain a minimum amount of phenolic substances (up to 50 $mg.l^{-1}$), mainly non-flavonoids. Red wines are characterized by a high phenolic content (up to 4 $g.l^{-1}$), predominantly flavonoid components and anthocyanins, due to the specificity of the production technology (Carvalho et al., 1998; Burns et al., 2003).

The phenols content in wine is usually associated with their antioxidant activity. Almost all groups of phenolic compounds have the ability to interact with free radicals and to disable active oxygen particles in human body (Joubert and Beer, 2006; Polovka, 2006). Antioxidant properties depend not only on the total amount of phenols, but also on individual and fractional composition of the polyphenol complex (Rivero-Perez, Muniz and Gonzales-Sanjose, 2008). Therefore, wines, especially the red ones, containing high ratios of catechins, procyanidins, anthocyanins, gallic acid and other phenolic matters, refer to beverages having high antioxidant activity (Valkova et al., 2004; Monagas et al., 2005; Savova, 2013).

In recent years there has been a growing interest in resveratrol (3,5,4-trihydroxystilbene) – a natural antioxidant contained in grape skins, that pass from there into red wines during the process of maceration and fermentation (Fartsov et al., 2012). It belongs to the group of polyphenol compounds produced by some plant species in response to stress, damage, bacterial or fungal infection. Its quantity in wine varies depending on variety and the area of cultivation (Mihaylova et al., 2012; Videnova and Fartsov, 2012). It exists both as a *cis* and a *trans* isomer as well as in a glycosidic form (Gu et al., 2000; Mark et al., 2005). The *trans* isomer predominates in wine.

The objective of this study was to determine and compare phenolic composition, antioxidant activity, resveratrol content and organoleptic profile of six Bulgarian wines, obtained from local and hybrid vine varieties.

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Material and methods

The study was carried out at the Institute of Viticulture and Enology (IVE) – Pleven, Bulgaria, and at the Viticulture Research Institute (VRI) – Tekirdag, Turkey. The investigation was focused on six samples of wine, vintage 2014 – two white and four red. The wines were made from the local wine grape varieties Dimyat (white), Pamid (red) and Gamza (red), distributed in Bulgaria and most of the Balkan region, and the varieties Plevenska rosa (white), Kaylashki rubin (red) and Storgozia (red), selected at IVE – Pleven through interspecies hybridization, characterized by increased resistance to diseases and low winter temperatures.

The varieties were grown at the Experimental vineyards of IVE – Pleven (Central Northern Bulgaria). The process of ripening was monitored, and upon reaching technological maturity the grapes were harvested. The chemical composition of grapes of the studied varieties is given in Table 1. The following methods were applied for determining the grapes composition from the studied varieties (Ivanov et al., 1979): sugars (g.l^{-1}) – airmeter of Dujardin; total acids (g.l^{-1}) – titration with NaOH; pH – pH-meter.

Table 1 Chemical composition of grapes of the studied varieties

Variety \ Indicator	Sugar (g.l^{-1})	Total acids (g.l^{-1})	pH
White varieties			
Dimyat	186.00	7.05	3.11
Plevenska rosa	198.00	5.30	3.27
Red varieties			
Pamid	174.00	5.13	3.35
Gamza	186.00	7.80	3.30
Storgozia	205.00	6.80	3.22
Kaylashki rubin	212.00	7.28	3.10

The grapes were processed at the Experimental Winery of IVE – Pleven under the conditions of micro-vinification. The classic white and red winemaking technology was applied (Amerine, Berg and Cruess, 1972):

- White wine – crushing, destemming, pressing, sulphuring (50 mg.dm^{-3} SO_2), must clarification, adding pure culture dry wine yeast *Saccharomyces cerevisiae Vitilevure* B + C (20 g.hl^{-1}), fermentation temperature 18 °C, racking, further sulphuring, storage.
- Red wine – destemming, crushing, sulphuring (50 mg.kg^{-1} SO_2), adding pure culture dry wine yeast *Saccharomyces cerevisiae Vitilevure* CSM (20 g.hl^{-1}), fermentation temperature 25 °C, separation of liquid part (young red wine) by pressing and racking, further sulphuring, storage.

After the completion of the process, the wines were decanted and further sulphured to 30 mg.dm^{-3} free SO_2.

The basic indicators of wine chemical composition were analyzed in the laboratories of IVE – Pleven by conventional methods used in the winemaking practice (Ivanov et al., 1979): sugars (g.dm^{-3}) – Schoorl's method; alcohol (vol. %) – distillation method, Gibertini apparatus with densitometry of the distillate density; total extract (TE) (g.dm^{-3}) – Gibertini apparatus with densitometry, density of alcohol-free sample; sugar-free extract (SFE) (g.dm^{-3}) – calculation method (the difference between TE and sugars); total acids (TA) (g.dm^{-3}) – titration with NaOH; colour intensity I [abs. units] – method of Somers; pH – pH meter.

The indicators concerning phenol complex of the wines, antioxidant activity and *trans*-resveratrol content were analyzed in the laboratories of VRI – Tekirdag. The following methods were used:

- total phenolic content was determined using the Folin-Ciocalteu's colorimetric assay (Waterhouse, 2002) and results were expressed as gallic acid equivalents (mg GAE.l^{-1});
- DPPH (1,1-diphenyl-2-picrylhydrazil) Radical Scavenging Activity assay was used based on the methods of Brand-Williams, Cuvelier and Berset (1995), as modified by Xu and Chang (2007). The free radical scavenging activity of wines was expressed as an equivalent of Trolox (µmol TEAC.ml^{-1}) using the calibration curve of Trolox. Linearity range of the calibration curve was 20 to 1,000 µM;
- ABTS [2,2-azino-di-(3-ethylbenzothialozine-sulphonic acid)] Radical Scavenging Activity was determined according to the method described by Re et al. (1999). The calibration curve between % inhibition and known solutions (0.5; 1.0; 1.5; 2.0 mM) of Trolox was then established. The radical-scavenging activity of the wines were expressed as trolox equivalent antioxidant capacity (µmol TEAC.ml^{-1});
- total monomeric anthocyanin content was determined by the pH differential method as described by Giusti and Wrolstad (2001) and the results were expressed as malvidin-3-glucoside equivalents (mg.l^{-1});
- total flavonoid content of the samples was determined according to the method described by Zhishen, Mengcheng and Jianming (1999). The results were calculated and expressed as catechin equivalents (mg CAE.l^{-1}) using the calibration curve of catechin;
- catechin, epicatechin, syringic acid,vanillic acid and *trans*-resveratrol levels (mg.l^{-1}) in wine samples were determined using based on the methods of Anonymous (2010), as modified by Gülcü (2016). HPLC system (Shimadzu LC 20 A) was combined with a fluorescence detector in an Inertsil ODS-3(C18) column (5 µm, 4.6 × 250 mm). Mobile phase A: 0.2% Formic acid in Water, mobile phase B: 0.2% Formic acid in Acetonitrile. For separation to following gradient; B Conc. 23% (5 min), 26% (12 min), 40% (14 min), 100% (14–18 min), 23% (22 min); the flow rate was 1.5 ml.min^{-1}. Column temperature was 30 °C. The fluorescence detector was set at λex 278 nm and λem 360 nm for catechin, epicatechin, syringic acid and vanillic acid, λex 300 nm and λem 386 nm for *trans*-resveratrol. Samples of 5 µl of standard or wine were directly injected. The wine samples, standard solutions were filtered by a 0.45 µm pore size PTFE syringe filter.

The presented test results were the average of three independent replicates from the measurement of each analyzed indicator. The organoleptic features of the experimental samples were determined according to 100-score scale for the indicators: colour, aroma, taste and general impression (Prodanova, 2008) by a nine-member tasting committee.

The data were subjected to correlation analysis using the statistical software package JMP (version 7, SAS Institute, Cary, NC, USA).

Results and discussion

Two white and four red Bulgarian wines obtained from local and hybrid varieties were selected for the study, which made it possible to determine and compare their chemical composition, phenolic complex, antioxidant activity, resveratrol content and organoleptic profile.

The chemical composition and the tasting assessments of the experimental wines are presented in Table 2. The results showed that the wines had no deviations from the normal rates of the investigated indicators. They were within the typical ranges for each variety, according to its specificity and varietal potential. The alcoholic fermentation in the samples occurred completely, with a full sugar fermentation and maximum alcohol accumulation, evidenced by the residual sugars content.

The amount of sugars in the samples varied from 1.06 to 1.98 $g.l^{-1}$. Plevenska rosa (12.57% vol.) and Kaylashki rubin (12.69% vol.) were distinguished with higher alcohol content. The differences in the sugar-free extract (SFE) were the result of the varietal features. That indicator had an influence on the taste properties of wine and determined its density. From the white wines, Dimyat had higher SFE, as for the red ones – Storgozia and Kaylashki rubin. However, the higher content of SFE was not related to the better wine tasting assessment. The total acids of the samples were within the typical ranges for the variety. Lower rates were recorded in the wine from the aromatic variety Plevenska rosa (5.15 $g.l^{-1}$) and Pamid (4.71 $g.l^{-1}$), characteristic for their variety, and the highest rates – in Kaylashki rubin (6.87 $g.l^{-1}$). The acids in the wine had an influence on taste freshness, but the results did not reveal any correlation with the wine tasting assessment. The samples from the hybrid varieties Storgozia and Kaylashki rubin showed higher SFE and titratable acidity in comparison with the samples from the local red varieties Pamid and Gamza. The *trans*-resveratrol content determined in the experimental wines showed that its amount in the red wines was considerably higher than in the white wines. Higher resveratrol rate was in the samples from the local red varieties, compared to the samples from the red hybrid varieties. Its concentration was the highest in Pamid wine (1.26 $mg.l^{-1}$) and in Gamza (1.23 $mg.l^{-1}$), followed by Kaylashki rubin and Storgozia (Table 2).

The phenolic compounds contained in wines also significantly influenced their sensory properties and were determining to their antioxidant properties.

Table 2 Chemical composition and tasting score of the experimental wines

Indicator / Wine	Alcohol (vol. %)	Sugar ($g.l^{-1}$)	Total extract ($g.l^{-1}$)	SFE ($g.l^{-1}$)	Total acids ($g.l^{-1}$)	Colour intensity (abs. un.)	pH	*Trans* -resveratrol ($mg.l^{-1}$)	Tasting score
White wines									
Dimyat	12.44	1.45	21.00	19.55	6.70	0.16	3.26	0.09	76.60
Plevenska rosa	12.57	1.47	19.83	18.36	5.15	0.29	3.11	0.02	80.80
Red wines									
Pamid	12.17	1.06	21.80	20.74	4.71	7.75	3.10	1.26	79.60
Gamza	12.32	1.47	23.73	22.26	5.51	9.66	3.17	1.23	78.30
Storgozia	12.41	1.49	26.57	25.08	5.57	9.34	3.28	1.00	78.70
Kaylashki rubin	12.69	1.98	26.03	24.05	6.87	10.16	3.19	1.06	80.00

Table 3 Phenolic composition of the experimental wines.

Indicator / Wine	Total phenolic compounds (mg $GAE.l^{-1}$)	Total monomeric anthocyanins content ($mg.l^{-1}$)	Total flavonoids content (mg $CAE.l^{-1}$)	Catechin ($mg.l^{-1}$)	Epicatechin ($mg.l^{-1}$)	Syringic acid ($mg.l^{-1}$)	Vanillic acid ($mg.l^{-1}$)
White wines							
Dimyat	315.90	–	54.24	3.02	0.70	N.D.*	0.09
Plevenska rosa	538.40	–	81.80	2.29	1.69	N.D.*	0.24
Red wines							
Pamid	819.50	23.63	301.71	20.42	9.47	0.74	2.95
Gamza	2402.00	61.86	902.32	34.12	17.60	5.20	2.37
Storgozia	1144.50	160.53	396.83	14.58	29.49	6.37	3.18
Kaylashki rubin	1567.00	103.50	552.32	11.10	11.35	10.53	4.64

*N.D. – not detected

The data on the content of total phenols, total monomer anthocyanins, total flavonoids, catechin, epicatechin, syringic and vanillic acids in the studied wines are presented in Table 3. The red wines were distinguished by a higher rate of phenolic compounds. The difference was determined by the influence of a number of factors, mainly the grape variety specificity. The results demonstrated a significant difference in the phenolic composition of the wines obtained from the local varieties Dimyat, Pamid and Gamza and those made from the hybrid varieties of Plevenska rosa, Storgozia and Kaylashki rubin.

The sample from Plevenska rosa exceeded that of Dimyat in the amount of total phenols, total flavonoids, epicatechin and vanillic acid. Dimyat wines contained more catechin. In the white wine samples, no syringic acid was found.

The wines from the local varieties Pamid and Gamza had a lower content of monomeric anthocyanins, syringic and vanillic acids, but a significantly higher rate of catechins. The sample of Pamid showed the lowest concentrations of all analyzed phenolic components except catechin. The wine from Gamza variety contained approximately circa 3 times more total phenols, total monomeric anthocyanins and total flavonoids compared to Pamid. The rate of catechin and epicatechin was also higher. Gamza wine had a greater amount of syringic acid and less vanillic acid compared to Pamid. In the case of red wines from the hybrid varieties, the sample of Kaylashki rubin contained more total phenols and total flavonoids. Storgozia wine, however, surpassed Kaylashki rubin in terms of the concentration of total monomeric anthocyanins, catechin and epicatechin. Gamza wine was characterized by the highest rate of total phenolic compounds, total flavonoids and catechin, followed by Kaylashki rubin in terms of total phenolic compounds and total flavonoids. Storgozia sample had the highest content of monomeric anthocyanins and epicatechin. As for the analyzed phenolic acids all red wines contained different rates of syringic and vanillic acid. Their amount was significantly higher in the samples from the hybrid varieties Storgozia and Kaylashki rubin. The highest content of both phenolic acids was found in Kaylashki rubin wine. In all samples, except for Pamid, the syringic acid content exceeded that of vanillic acid.

Table 4 Antioxidant activity of the experimental wines.

Wine	Radical scavenging activity (µmol TEAC.ml^{-1})	
	DPPH	**ABTS**
White wines		
Dimyat	0.41	3.52
Plevenska rosa	1.16	7.69
Red wines		
Pamid	0.98	6.56
Gamza	1.91	23.70
Storgozia	1.14	15.63
Kaylashki rubin	1.27	14.68

The phenolic compounds content in wine was associated with their antioxidant capacity. Therefore, their antioxidant activity was determined using the two analytical tests DPPH and ABTS (Table 4). The data from the two analytical tests used revealed the better antioxidant properties of the red wines compared to the white ones. This was due to both the higher phenolic content and to the different degree of polymerization of the procyanidins in white and red wines, and to the different ratio of the individual catechins in the polymer phenolic molecule (Valkova et al., 2004). The probable cause for the differences between both methods was the presence of other components in the wine composition exhibiting antiradical properties (Kerchev, Yoncheva and Ivanov, 2005).

The obtained experimental data did not allow a specific comparison between the antioxidant properties of the wines made from the local and the hybrid varieties. In the case of the white wines, the sample of Plevenska rosa had a better antioxidant potential than Dimyat, but for the red wines, with both analytical methods, the highest and the lowest activity was recorded for the local varieties. The highest rate was found in Gamza, and the lowest – in Pamid which contain the highest and the lowest content of total phenols and total flavonoids, respectively. According to the DPPH method, Kaylashki rubin wine had better antioxidant

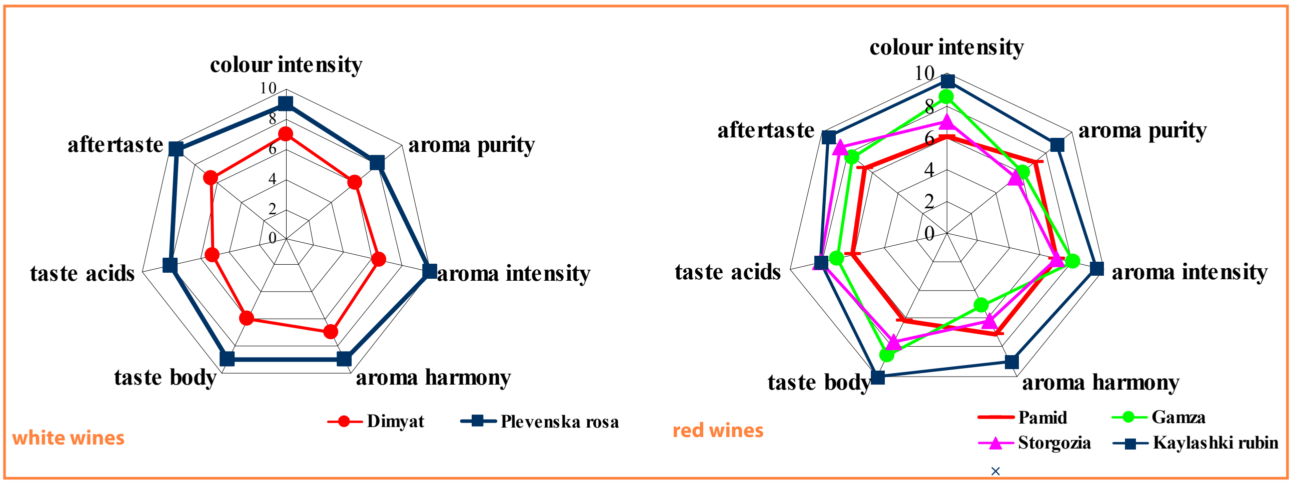

Figure 1 Organoleptic profile of the studied experimental wines

Table 5 Correlation analysis between the data determined with all experimental wine samples (*n* = 18)

Alcohol												
Total extract	0.191 ^{NS}											
Total acids	0.658 **	0.324 ^{NS}										
Colour intensity	-0.163 ^{NS}	0.843 **	-0.038 ^{NS}									
pH	0.197 ^{NS}	0.521 *	0.615 **	0.071 ^{NS}								
Total phenolic compounds	-0.057 ^{NS}	0.587 *	0.023 ^{NS}	0.784 **	-0.023 ^{NS}							
DPPH	-0.028 ^{NS}	0.382 ^{NS}	-0.275 ^{NS}	0.631 **	-0.295 ^{NS}	0.900 **						
ABTS	0.009 ^{NS}	0.652 **	-0.009 ^{NS}	0.743 **	0.120 ^{NS}	0.956 **	0.904**					
Total flavonoids	-0.130 ^{NS}	0.608 **	0.011 ^{NS}	0.821 **	-0.007 ^{NS}	0.993 **	0.868 **	0.938 **				
Catechin	-0.587 *	0.360 ^{NS}	-0.356 ^{NS}	0.729 **	-0.169 ^{NS}	0.840 **	0.753 **	0.761 **	0.875 **			
Epicatechin	-0.206 ^{NS}	0.833 **	-0.155 ^{NS}	0.777 **	0.428 ^{NS}	0.565 *	0.486 *	0.702 **	0.590 *	0.552 *		
***Trans*-resveratrol**	-0.422 ^{NS}	0.662 **	-0.219 ^{NS}	0.952 **	-0.095 ^{NS}	0.724 **	0.578 *	0.632 **	0.780 **	0.826 **	0.675 **	
Tasting score	0.306 ^{NS}	-0.009 ^{NS}	-0.415 ^{NS}	0.154 ^{NS}	-0.687 **	0.044 ^{NS}	0.358 ^{NS}	0.048 ^{NS}	-0.001 ^{NS}	-0.091 ^{NS}	-0.035 ^{NS}	0.111 ^{NS}
	Alcohol	**Total extract**	**Total acids**	**Colour intensity**	**pH**	**Total phenolic compounds**	**DPPH**	**ABTS**	**Total flavonoids**	**Catechin**	**Epicatechin**	***Trans*-resveratrol**

NS: Non significant
** Correlation is significant at the 0.01 level (2-tailed)
* Correlation is significant at the 0.05 level (2-tailed)

activity compared to Storgozia, while according to the ABTS test it was just the opposite. Regardless the lower content of total phenols, total flavonoids and phenolic acids, Storgozia sample had a higher antiradical activity. These results show that there is not always a correlation between the content of phenolic components in wines and their antioxidant capacity.

The organoleptic profiles of the studied experimental wines are presented in Figure 1.

The wines from the hybrid varieties Plevenska rosa (white) and Kaylashki rubin (from the red ones) were determined to have the best tasting features in terms of colour, aroma, taste and aftertaste. They had a pure, distinctive varietal aroma, harmony and balance of the tasting indicators. The data did not reveal a strict correlation between the influence of the studied phenolic components and wine organoleptic profile. The sample of Gamza variety, despite its higher ratio of total phenols and total flavonoids, had lower tasting assessment compared to Storgozia and Kaylashki rubin. Storgozia wine contained most monomeric anthocyanins, but received a lower tasting score than Kaylashki rubin. The reason was due to the difference in the total impact of the studied indicators of the wine composition on its sensory characteristics.

The relations between the determined parameters are statistically evaluated in Table 5. The significant correlations among total phenols, total flavonoids, catechin, resveratrol and different radical scavenging activity assays (DPPH and ABTS) were found in wine samples. The colour intensity of wines showed significant correlation with total extract, phenolic composition and radical scavenging activity. Tasting scores exhibited a significant correlation only with pH value, while it had no statistically significant correlations with all other analysis parameters.

Conclusions

- From the white wines, Dimyat had higher sugar-free extract and titratable acidity. From the red wines, the samples of the hybrid varieties Storgozia and Kaylashki rubin showed higher rates compared to the samples from the local varieties Pamid and Gamza.
- The *trans*-resveratrol amount in the red wines was significantly higher compared to the white ones, as the samples from the red local varieties had a higher content than the samples from the hybrid varieties. The highest rates of resveratrol concentration showed the wines Pamid (1.26 mg.l^{-1}) and Gamza (1.23 mg.l^{-1}).
- The red wines had more phenolic compounds. Difference in the phenolic composition of the samples obtained from the local varieties and those from the hybrid varieties was also found. Gamza wine had the highest concentration of total phenolic compounds, total flavonoids and catechin. Storgozia sample contained the highest rates

of monomeric anthocyanins and epicatechin. Pamid revealed the lowest concentrations of all analyzed phenolic components except of catechin. The content of phenolic acids in the wines from the hybrid varieties Storgozia and Kaylashki rubin was significantly higher. Syringic acid was not found in the white wines.

- The red wines had better antioxidant features than the white ones. From the red wines, the highest and the lowest activity was reported in the local varieties – Gamza and Pamid, respectively, which contain the highest and lowest content of total phenols and total flavonoids.
- Strict correlation between the effects of the studied phenolic components on the wine organoleptic profile was not found. The wines with the best tasting characteristics in terms of color, aroma, taste and aftertaste were the wines from the hybrid varieties Plevenska rosa and Kaylashki rubin.

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