

## DETERMINATION OF ANTIOXIDANT ACTIVITY AND RESVERATROL CONTENT IN BULGARIAN WINES FROM VARIETIES WITH INCREASED RESISTANCE TO DISEASES AND LOW WINTER TEMPERATURES

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### Abstract

Study was carried out about the antioxidant and antiradical properties, the phenolic components and trans-resveratrol content in three Bulgarian wines made from the wine varieties Plevenska Rosa, Kaylashki Rubin and Storgozia, selected by interspecies hybridization and grown in the region of the town of Pleven, Central Northern Bulgaria. The white wine Plevenska Rosa had significantly lower content of phenolic compounds, antiradical properties and resveratrol compared to the red wines Kaylashki Rubin and Storgozia. Kaylashki Rubin wine contained more total phenols, total flavonoids, syringic acid and vanillic acid than Storgozia wine. However it exceeded Kaylashki Rubin in the concentration of total monomeric anthocyanins, catechin and epicatechin. Higher content of epicatechin to catechin was found in these wines. Very well secured difference has been mathematically proven in the data of the analyzed phenolic components between the tested samples. It has not been observed correlation between the phenolic substances content and the antioxidant capacity of the experimental wines. According to ABTS test the increase in the antioxidant and antiradical properties has been in the order: Plevenska Rosa < Kaylashki Rubin < Storgozia. Although the lower content of total phenols, total flavonoids and phenolic acids, Storgozia sample had higher antiradical activity. It has not been found a direct correlation between the phenolic components content, the antioxidant properties of wines and resveratrol concentration in the experimental samples. Kaylashki Rubin and Storgozia wines differed in their phenolic composition, however they had close antiradical activity (according to ABTS method) and the difference in the amount of their resveratrol was insignificant.

**Keywords:** grape variety, wine, chemical composition, phenolic compounds, antioxidant activity, trans-resveratrol.

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### 1. INTRODUCTION

In the food industry there has been a tendency of growing interest in “natural antioxidants” of plant origin and food health qualities. That has determined the higher demands of consumers not only to the organoleptic characteristics of wine, but also to its antioxidant properties and the content of biologically active substances (Valkova et al., 2004).

Grapes and wine chemical composition is complex and diverse. It is determined by a number of factors such as the variety, soil and climatic conditions, the applied growing practices, degree of maturity and the winemaking technology.

The phenolic compounds are an important component of wines composition, determining their antioxidant properties. Most of them pass from grapes and have a significant effect on

their organoleptic characteristics (colour intensity, astringency, bitterness). White wines contain a minimum amount of phenolic substances (up to 50 mg/l), mainly non-flavonoids. Red wines are characterized by high phenolic content (up to 4 g/l), mainly flavonoid components and anthocyanins, due to the specificity of the winemaking technology (Sprangler et al., 1998; Burns et al., 2003). Therefore wines, especially red ones refer to beverages having a high concentration of natural antioxidants - catechins, procyanidins, anthocyanins, gallic acid, etc. (Valkova et al., 2004; Monagas et al., 2005; Savova, 2013). Wines antioxidant activity depends not only on the total amount of phenols, but also on the individual and fractional composition of polyphenol complex (Rivero-Perez et al., 2008). Almost all groups of phenolic substances have the ability to bind with the free

radicals and dispose the active oxygen particles in the human body. The amount of antioxidants is one of the most important factors determining health qualities of foods and beverages (Joubert and Beer, 2006).

Besides the phenols from grapes, wine also contains a number of other compounds exhibiting antioxidant properties - thiol-containing molecules, some amino acids (proline), glutathione, ascorbic acid, tocopherols, carotenoids, etc. (Kerchev et al., 2005).

In recent years there has been an increased interest in resveratrol (3,5,4-trihydroxystilbene) - a natural antioxidant contained in the grape berries skins and from there passing to wines during maceration and fermentation (Fartsov et al., 2012). It refers to the group of polyphenol compounds that some plant species produce in response to stress, injury, bacterial or fungal infection. Its amount in wine varies depending on the variety and the growing area (Videnova and Fartsov, 2012).

Wines vary widely in their antioxidant properties that depend on the varietal characteristics of the grapes, soil and climatic conditions in the area of cultivation, winemaking technology, etc. (Joubert and Beer, 2006).

The objective of the study was to determine the phenolic composition, antioxidant activity and resveratrol content in three Bulgarian wines made from hybrid varieties with increased resistance to diseases and low winter temperatures.

## 2. MATERIALS AND METHODS

The study was carried out at the Institute of Viticulture and Enology (IVE) – Pleven, Bulgaria and at the Institute of Viticulture (IV) - Tekirdag, Turkey. The study was focused on wines, vintage 2014, made from the varieties Plevenska Rosa, Kaylashki Rubin and Storgozia, selected at IVE – Pleven by interspecies hybridization. They are distinguished for their increased resistance to diseases and low winter temperatures.

The varieties were grown at the Experimental base of IVE – Pleven (Central Northern Bulgaria). Upon reaching technological maturity, grapes were picked up and processed at the Experimental winery under the conditions of micro-vinification. The classical technology for making white and red dry wines were applied (Amerine et al., 1972):

➤ White wine making - crushing, draining, pressing, sulphating ( $50 \text{ mg/dm}^3 \text{ SO}_2$ ), must clarification, adding pure culture lyophilized wine yeast *Saccharomyces cerevisiae* (*Vitilevure B+C*) in the amount of 20 g/hl, fermentation temperature 20°C.

➤ Red wine making - removing the berries, crushing, sulphating ( $50 \text{ mg/kg SO}_2$ ), adding pure culture lyophilized wine yeasts *Saccharomyces cerevisiae* (*Vitilevure CSM*) in the amount of 20 g/hl, fermentation temperature 28°C.

The course of the alcoholic fermentation was monitored by the change in the dry matter, measured daily with Abbe refractometer to a constant value. After the completion of the process the samples were decanted (white wines) or separated from solid particles, decanted (red wines) and further sulphated to  $30 \text{ mg/dm}^3$  free  $\text{SO}_2$ .

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; Chobanova, 2007):

- sugars,  $\text{g/dm}^3$  – Schoorl's method;
- alcohol, vol. % - distillation method, Gibertini apparatus with densitometry of the distillate density;
- total extract (TE),  $\text{g/dm}^3$  - Gibertini apparatus with densitometry, density of alcohol-free sample;
- sugar-free extract (SFE),  $\text{g/dm}^3$  - calculation method (the difference between TE and sugars);
- titratable acids (TA),  $\text{g/dm}^3$  - titration with NaOH;
- color intensity I [abs. units] - method of Somers;
- pH - pH meter.

The indicators concerning the phenol complex of the wines, the antioxidant activity and trans-resveratrol content were analyzed in the laboratories of IV - 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);

- DPPH (1,1-diphenyl-2-picrylhydrazil) Radical Scavenging Activity assay was used based on the methods of Brand-Williams et al. (1995), as modified by Xu and Chang (2002). The free radical scavenging activity of wines was expressed as an equivalent of Trolox ( $\mu\text{mol TEAC/ml}$ ) using the calibration curve of Trolox. Linearity range of the calibration curve was 20 to 1000  $\mu\text{M}$ ;

- ABTS [2,2-azino-di-(3-ethylbenzothiazolone-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 ( $\mu\text{mol TEAC/ml}$ );

- total monomeric anthocyanin content was determined by the pH differential method as described by Giusti and Wrolstad (2001) and results were expressed as malvidin-3-glucoside equivalents (mg/l);

- total flavonoid content of the samples was determined according to the method described by Zhishen et al. (1999). The results were calculated and expressed as catechin equivalents (mg CAE/l) using the calibration curve of catechin;

- catechin, epicatechin, syringic acid, vanillic acid and trans-resveratrol levels (mg/l) in wine samples were measured by a HPLC system (Shimadzu LC 20 A). This system combined with a fluorescence detector in an Inertsil ODS-3(C18) column ( $5\mu\text{m}$ ,  $4.6 \times 250$  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%

(14.01-18 min), 23 % (22 min); the flow rate was 1,5 ml/min. Column temperature was  $30^{\circ}\text{C}$ . The fluorescence detector was set at  $\lambda_{\text{ex}}$  278 nm and  $\lambda_{\text{em}}$  360 nm for catechin, epicatechin, syringic acid and vanillic acid,  $\lambda_{\text{ex}}$  300 nm and  $\lambda_{\text{em}}$  386 nm for trans-resveratrol. Samples of 5  $\mu\text{l}$  of standard or wine were directly injected. The wine samples, standard solutions were filtered by a 0.45  $\mu\text{m}$  pore size PTFE syringe filter.

The presented experimental results are the average values of three independent repetitions from the measurement of each analyzed indicator.

Data were mathematically processed by analysis of variance at confidence levels of the differences (Student criteria)  $p=5.0$  %,  $p=1.0$  %,  $p=0.1$  % (Dimova and Marinkov, 1999).

### 3. RESULTS AND DISCUSSION

The study was carried out with three Bulgarian wines, made from varieties with increased resistance to diseases and low winter temperatures. That allowed the chemical composition, phenolic complex, antioxidant activity and resveratrol content in wines from hybrid varieties obtained through interspecies hybridization to be determined.

The chemical composition of the experimental samples is presented in Table 1. The results did not show any deviations of the wines from the normal rates of the studied indicators. They were within the specific range for each variety, according to its varietal characteristics and potential. The alcoholic fermentation was complete, with sugars fermented in full and maximum alcohol accumulation, as evidenced by the residual sugars content.

The amount of sugar-free extract and titratable acids, respectively determining the density and freshness of taste are of particular importance for the wine tasting features. Plevenska Rosa had the lowest rates of these indicators, but within typical range for white wines. From the red wine samples Kaylashki Rubin had higher acidity and colour intensity, while Storgozia contained more SFE.

**Table 1. Chemical composition of the studied experimental wines**

Indicators Wine	Alcohol vol. %	Sugar g/l	Total extract g/l	Sugar-free extract (SFE) g/l	Total acids g/l	Colour intensity [abs. un.]	pH
White wine							
Plevenska Rosa	12.57	1.47	19.83	18.36	5.15	0.29	3.11
Red wine							
Kaylashki Rubin	12.69	1.98	26.03	24.05	6.87	10.16	3.28
Storgozia	12.41	1.49	26.57	25.08	5.57	9.34	3.19

**Table 2. Content of total phenolic compounds, anthocyanins and phenolic components in the studied experimental wines. Analysis of variance.**

Indicators Wine	Total Phenolic, mg GAE/L	Total Monomeric Anthocyanin Content, mg/l	Total Flavonoid Content mg CAE/l	Catechin, mg/l	Epicatechin, mg/l	Syringic acid, mg/l	Vanillic acid, mg/l
White wine							
Plevenska Rosa	538.40 +++	-	81.80 +++	2.29 +++	1.69 +++	N.D.	0.02 +++
Red wine							
Kaylashki Rubin	1567.00 +++	103.50 ++	552.32 +++	11.10 +++	11.35 +++	10.53 +++	4.64 +++
Storgozia	1144.50 +++	160.53 ++	396.83 +++	14.58 +++	29.49 +++	6.37 +++	3.18 +++
	GD(5.0%) =116.267 GD(1.0%) =192.828 GD(0.1%) =360.611	GD(5.0%) =15.776 GD(1.0%) =36.387 GD(0.1%) =115.845	GD(5.0%) =31.100 GD(1.0%) =51.580 GD(0.1%) =96.460	GD(5.0%) =0.259 GD(1.0%) =0.429 GD(0.1%) =0.802	GD(5.0%) =0.620 GD(1.0%) =1.028 GD(0.1%) =1.922	GD(5.0%) =0.075 GD(1.0%) =0.172 GD(0.1%) =0.547	GD(5.0%) =0.085 GD(1.0%) =0.141 GD(0.1%) =0.264

N.D. – not detected; (+) – the difference is significant; (++) – the differences is well secured; (+++) – the difference is very well secured; N.S. – the difference is not significant

The phenolic compounds contained in wine had also a significant effect on the sensory characteristics - colour, aroma, taste, astringency. As a result of their interaction with proteins, polysaccharides or other phenolic compounds, they had an important role in the aging process and the physicochemical stability of wines (Frankel et al., 1995). They were also determining with regard to the wine antioxidant properties. Data on the content of total phenolic compounds, anthocyanins and phenolic fractions in the studied wines are presented in Table 2.

The analysis data of the wine phenolic complex had confirmed the results obtained by other authors, namely that white wines were

distinguished for significantly lower concentration of phenolic compounds compared to the red wines (Paixao et al., 2007; Stasko et al., 2008). The difference in the total phenolic content of the samples was determined by the influence of a number of factors, but mainly from the grapes varietal specificity and its phenol contents (Ghiselli et al., 1998). In the red wines, the sample of Kaylashki Rubin variety contained 70% more total phenols and total flavonoids. The wine from Storgozia variety, however, exceeded Kaylashki Rubin with respect to the concentration of total monomeric anthocyanins (by 64%), catechin (by 76%) and epicatechin (by 38%). In these samples it was found higher

content of epicatechin to catechin, in contrast to Plevenska Rosa. With respect to the analyzed phenolic acids all experimental wines contained vanillic acid at various concentrations depending on the variety. Kaylashki Rubin variety had a greater amount of syringic acid and vanillic acid, compared to Storgozia. Syringic acid in white wine Plevenska Rosa was not found.

The mathematical processing of the test results proved well (total monomeric anthocyanins) and very well secured difference in the data of the rest analyzed indicators among the studied wines from the different varieties (Table 2).

The phenolic compounds content in the tested red wines determined their antioxidant and antiradical activity. Therefore, it was determined their antioxidant effect through the use of two analytical tests DPPH and ABTS. The results are presented in Table 3. The data show significant differences between the two used methods. The reason was probably due to the availability of other components in wine composition exhibiting antiradical properties (Kerchev et al., 2005).

According to DPPH test the ranking was as follows: Storgozia<Plevenska Rosa<Kaylashki Rubin. Despite the high content of phenolic compounds in the Storgozia sample, it was not found any difference in the antioxidant and antiradical capacity of the wines from this variety and Plevenska Rosa variety. That was also confirmed by the mathematical processing of the results, that proved well secured difference between radical scavenging activity rates of the Kaylashki Rubin sample compared to Plevenska Rosa and Storgozia, however the difference between Storgozia and Plevenska Rosa was not proven.

According to ABTS test the increase in antioxidant and antiradical properties of the studied wines was in the following order: Plevenska Rosa<Kaylashki Rubin<Storgozia. Despite the lower content of total phenols, total flavonoids and phenolic acids the Storgozia sample had higher antiradical activity.

These results indicated that there had not always been a correlation between the phenolic components content in wines and their

antioxidant capacity. In red wines that was due to the different degree of polymerization of procyanidins and the different ratio of individual catechins in the polymer phenols molecule (Valkova et al., 2004).

The white wine Plevenska Rosa had twice lower antiradical activity compared to the red wine Kaylashki Rubin. The highest antioxidant properties were reported in the Storgozia sample. Very well secured difference was mathematically proven in the data for the antiradical capacity of the red wines in comparison with Plevenska Rosa. The difference in the antioxidant properties of Kaylashki Rubin and Storgozia, was not proven (Table 3).

Resveratrol refers to the group of polyphenolic compounds and in wines it passes from the berries skins during maceration and fermentation (Fartsov et al., 2012). It exists simultaneously as cis and trans isomer, as well as in glycosidic form (Mark et al., 2005). The trans isomer is prevailing in wine. The results of trans-resveratrol content in the tested experimental wines are presented in Figure 1.

The data had confirmed the findings of other authors that the amount of resveratrol in red wine was significantly more than in white wines (Souto et al., 2001; Ratola et al., 2004). The obtained results did not reveal a direct correlation between the content of phenolic components, the antioxidant properties of wines and the concentration of resveratrol in the studied experimental samples. Although Kaylashki Rubin wine was characterized by higher rates of total phenols, total flavonoids and phenolic acids than Storgozia, the antiradical activity of both wines was close (according to ABTS method) and the difference in the amount of resveratrol was insignificant.

The mathematical processing of the results proved very well secured difference in resveratrol content between the white wine and the red wines while the difference was well secured between the samples of Kaylashki Rubin and Storgozia at  $GD(5.0\%)=0.023$ ,  $GD(1.0\%)=0.038$ ,  $GD(0.1\%)=0.70$ .

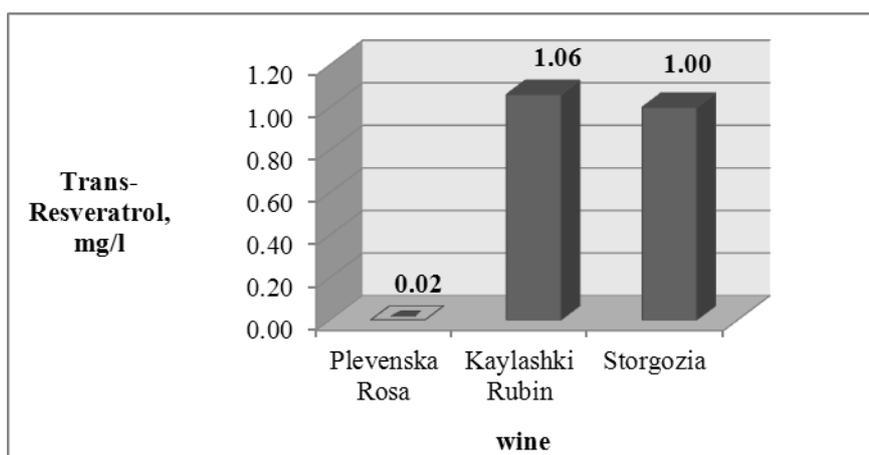
That showed that resveratrol synthesis in vine, passing respectively in grapes and wine, was determined by a number of factors, the main of

which was the variety, its potential and specificity.

**Table 3. Radical scavenging activity (RSA) of the experimental wines. Analysis of variance**

Wines		Plevenska Rosa <i>white wine</i>		Kaylashki Rubin <i>red wine</i>		Storgozia <i>red wine</i>		
Wines	RSA μmol TEAC/m l  x~	Difference	Verification	Difference	Verification	Difference	Verification	
<b>DPPH</b>								
Plevenska Rosa <i>white wine</i>	<b>1.16</b>	x	x	- 0.113	--	0.020	n.s	GD(5.0%)=0.064 GD(1.0%)=0.106 GD(0.1%)=0.198
Kaylashki Rubin <i>red wine</i>	<b>1.27</b>	0.113	++	x	x	0.133	++	
Storgozia <i>red wine</i>	<b>1.14</b>	-0.020	n.s	- 0.133	--	x	x	
<b>ABTS</b>								
Plevenska Rosa <i>white wine</i>	<b>7.69</b>	x	x	- 6.990	---	- 7.943	---	GD(5.0%)=1.374 GD(1.0%)=2.278 GD(0.1%)=4.260
Kaylashki Rubin <i>red wine</i>	<b>14.68</b>	6.990	+++	x	x	- 0.953	n.s	
Storgozia <i>red wine</i>	<b>15.63</b>	7.943	+++	0.953	n.s	x	x	

N.D. – not detected; (+) – the difference is significant; (++) – the differences is well secured; (+++) – the difference is very well secured; N.S. – the difference is not significant



**Fig. 1. Trans-resveratrol content in the studied experimental wines**

#### 4. CONCLUSIONS

On the basis of the obtained data from the study it could be summarized that:

➤ The white wine Plevenska Rosa had significantly lower content of phenolic compounds, antiradical activity and resveratrol

compared to the red wines Kaylashki Rubin and Storgozia.

➤ Kaylashki Rubin wine contained 70% more total phenols and total flavonoids, more syringic acid and vanillic acid compared to Storgozia wine. However, Storgozia exceeded Kaylashki Rubin in the concentration of total monomeric anthocyanins (by 64%), catechin (by 76%) and epicatechin (by 38%). It was found higher content of epicatechin to catechin.

➤ According to ABTS test the increase in antioxidant and antiradical properties of the studied wines was in the following order: Plevenska Rosa < Kaylashki Rubin < Storgozia. Despite the lower content of total phenols, total flavonoids and phenolic acids the Storgozia sample had higher antiradical activity.

➤ It has not been found a direct correlation between the phenolic components content, the antioxidant properties of wines and resveratrol concentration in the experimental samples. Although Kaylashki Rubin wine was characterized by higher rates of total phenols, total flavonoids and phenolic acids than Storgozia, the antiradical activity of both wines was close (according to ABTS method) and the difference in the amount of resveratrol was insignificant.

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