STATISTICAL ANALYSIS ON THE CLIMATE – ORGANOLEPTIC PROFILE – CONTENT OF TRANS-RESVERATROL RELATION IN RED WINES FROM THE REGION OF NORTHERN BULGARIA

Vanyo **Haygarov**[1], Tatyana **Yoncheva**[1], Dimitar **Dimitrov**[1*], Emil **Tsvetanov**[2]
[1]Department of Enology and Chemistry, Institute of Viticulture and Enology, 1 Kala Tepe str., 5800 Pleven, Bulgaria
[2]Department of Technologies, Institute of Viticulture and Enology, 1 Kala Tepe str., 5800 Pleven, Bulgaria
*e-mail: dimitar_robertov@abv.bg

Abstract
The study was carried out at the Institute of Viticulture and Enology (IVE) – Pleven, during the period 2015 - 2017. The object of the study was the red wines from Storgozia, Kaylashki Rubin, Trapezitsa, Rubin and Bouquet varieties, selected at IVE – Pleven by means of intraspecific and interspecific hybridization. A statistical analysis of the results obtained from the wine organoleptic evaluation, the weather conditions of the region and the content of the stilbene trans-resveratrol has been made. On average, for the three years of the investigation, the studied varieties could be divided into two groups. The first group had higher rates of trans-resveratrol and comprised Kaylashki Rubin (3.52 m mg/dm^3), Bouquet (3.18 mg/dm^3) at Pinot Noir as a control (3.46 mg/dm^3). The difference between them was insignificant and statistically unproven. The second group of varieties included Rubin (2.39 mg/dm^3), Trapezitsa (2.3 mg/dm^3) and Storgozia (1.77 mg/dm^3). It had significantly lower rates of trans-resveratrol compared to the first one. The difference between the varieties in this group was also statistically unproven. There was no significant difference in the tasting evaluations during the years of the study. The weather conditions throughout the vegetation period did not have a significant impact on wine quality.

Keywords: grape, wine, chemical composition, trans-resveratrol, organoleptic evaluation, statistical analysis.

Received: 23.08.2018 Reviewed: 08.11.2018 Accepted: 15.11.2018

1. INTRODUCTION

Wine, as a tasting product, has deep historical roots in its wide assortment range and organoleptic diversity. It has always gone along with the history of human civilization.

No other agricultural product is as varied and delicate as grapes, and no other commercial product can compete with wine in its enigmatic components.

The organoleptic evaluation of wine allows the quality of the drink to be assessed based on sensory impressions: taste, olfactory, visual and auditory. It aims at: controlling the quality of beverages in the process of their production and realization, checking the results of scientific research, competitive assessment, comparing different technological methods, comparing different vintages and vintage wines, diagnosing different imperfections and wine diseases (Prodanova, 2007; Marni, 2014). According to these authors, even the most precise chemical analysis nowadays cannot identify these subtle flavours and nuances in taste that make one wine better than another. Due to this "small" peculiarity, wine tasting, the language of the wine can not be replaced by another means for determining wine quality and for now it remains the only possible method.

There has been a growing research interest in the role of the particular compounds of grapes and wine, on improving human health and disease prevention, in recent years. The interest of the scientific community in the health promotion action of wine was enhanced by a study known as the "French Paradox" (Renaud and Lorgeril, 1992). It has found a low mortality rate and frequency of cardiovascular diseases among French male, age 54 to 65 (a risk group for cardiovascular disorders), regardless of the consumption of significant amounts of animal fats. The study has concluded that the moderate consumption of

red wines had the major impact on this fact. The antioxidant effect of wine was mainly due to its phenolic complex consisting of natural polyphenol substances (Chobanova, 2012). An important compound assumed to play a significant role in the protective and healthy effects of wine was trans-resveratrol (Corder et al., 2001). It was contended that this compound showed a protective effect against cardiovascular diseases (Abril et al., 2005). Gehm et al. (1997) have reported that resveratrol has an inhibitory effect against the oxidation of low density lipoproteins (LDL). Trans-resveratrol blocks platelet aggregation (Bartelli et al., 1995) and thus it could prevent the progression of atherosclerosis, as well as myocardial infarction. It reduces the triacylglycerols levels and the lipid peroxidation processes in the liver (Melzoch, 2001). Recent studies have shown that trans-resveratrol stimulates the metabolic reprogramming of human CD4+ T-cells, and the authors have found that small doses of the substance lead to stimulation of CD4+ T-cells to produce elevated levels of the inflammatory cytokine γ-interferon (Craveiro et al., 2017).
Studies on the resveratrol content in wines have also been carried out in Bulgaria. Wines from Pinot Noir, Mavrud, Merlot, Cabernet Sauvignon, Melnik, Chardonnay, Sauvignon Blanc and Muscat Ottonel varieties (Farzov et al., 2013) have been investigated.
Results of the resveratrol content testing in grapes and wine of the studied varieties were published (Haygarov et al., 2017).
The objective of this study was to find out a statistical relation between the organoleptic evaluation of the produced wines, the weather conditions of the region and the content of the stilbene trans-resveratrol.

2. MATERIALS AND METHODS

The study was carried out at the Institute of Viticulture and Enology (IVE) – Pleven, during the period 2015 - 2017. The object of the study was the red wines from Storgozia, Kaylashki Rubin, Trapezitsa, Rubin and Bouquet varieties, selected at IVE – Pleven by means of intraspecific and interspecific hybridization (Petkov, 1977; Roychev, 2012; Ivanov, 2016). They were grown in certain micro-regions throughout the country (Pleven, Sadovets, Barkach, Suhindol, Pavlikeni, Brestovitsa, Karnobat, Burgas, Blagoevgrad and Sandanski). The control was Pinot Noir variety of *Vitis vinifera*, which according to the available data had the highest rates of trans-resveratrol (Teissedre et al., 1996; Videnova, 2017).
The vine plantation was located in the experimental base of the IVE, on an area of 0.2 ha with each studied variety. The vineyards were fruit-bearing, grown on a medium stem training system, at a planting distance of 3.00 m / 1.20 m, Berlandieri x Riparia SO4 rootstock. During the vegetation season standard agricultural practices and plant protection measures were carried out.
The climate is continental, characterized by cold winters and hot summers. The sum of the average air temperatures during the vegetation varied from 3535 to 4500°C. The average air temperature of the warmest month was always above 20°C. Temperatures below 20°C and over 30°C were not recorded, that would impede grapevine physiological processes (Haygarov, 2013).
The study has covered three consecutive years (2015, 2016 and 2017).
The weather characterization over the years has been determined by a mathematical and statistical method (Sirakov, 1981).

Table 1. Probability (P%) of precipitation and average air temperature (T°) for the years of the study

Years		2015	2016	2017
$N_{(V-IX)}$	P %	17 (Very wet)	35 (Wet)	6 (Very wet)
$T^o_{(V-IX)}$		29 (Hot)	56 (Average cool)	33 (Hot

P – probability, N –(precipitations, T - average air temperature

With regard to the average air temperatures, the years 2015 and 2017, could be defined as average hot and 2016 as an average cool. As for the precipitation 2015 and 2017 were very wet and 2016 – wet (Sirakov, 1981).
The soil is leached chernozem on loess foundation suitable for vine growing.
These data show that the Pleven region provides suitable conditions for obtaining quality red grapes as a raw material for red wines production.
The main chemical indicators of the grape pulp and the experimental wines made from the studied varieties were determined according to the methods generally accepted in wine-making (Ivanov et al., 1979; Chobanova, 2007; Pandeliev et al., 2010).
Upon reaching the technological maturity of the grapes (20-23%), the grapes were harvested and vinification according to the classic technology for red wines was carried out for each variety (Marinov, 1990; Yankov, 1992). After the clarification of the wine a chemical and organoleptic analysis was made.
High pressure liquid chromatography (HPLC), according to the modified method of Anli et al. (2006), was used for determining the resveratrol content in the wine samples.
For the organoleptic evaluation, a 100-score scale (Prodanova, 2008, Marni Old, 2016) was applied using Microsoft Excel from the MS Office (Microsoft Corporation) and the standard deviations were pointed.
The results were statistically processed by two-factor analysis of variance (ANOVA, Dimova and Marinkov, 1999).

3. RESULTS AND DISCUSSION

From the testing of the red wines obtained from the above varieties, it was found as follows:
Table 2 presents the phenolic composition (TPS – Total Phenolic Substances, FPS – Flavonoid Phenolic Substances, NFB – Non-flavonoid Phenolic Substances, Anthocyanins content) and wine trans-resveratrol content per variety. The values are average for the years of the study. The phenolic compounds amount is within the normal range for quality red wines.
Data on the statistical processing of the results are presented in Table 3. On the average over the three years of the research, the wines from the studied varieties could be divided into two groups. The first group had higher rates of trans-resveratrol. It included the varieties Kailashki Rubin (3.52 mg/dm^3), Bouquet (3.18 mg/dm^3) and the control variety Pinot Noir (3.46 mg/dm^3). The difference between them was insignificant and statistically unproven.
The second group of varieties – Rubin (2.39 mg/dm^3), Trapezitsa (2.30 mg/dm^3) and Storgozia (1.77 mg/dm^3) had significantly lower trans-resveratrol than the first one and the difference between the varieties in the group was also statistically unproven.

Table 2. Phenolic composition of the experimental wines and trans-resveratrol on the average for the period 2015 – 2017

№	**Indicators**	**Varieties**					
		Trapezitsa	**Pinot Noir**	**Kaylashki Rubin**	**Bouquet**	**Rubin**	**Storgozia**
1.	**TPS, g/dm^3**	1.65	3.54	1.58	2.19	4.52	1.80
2.	**FPS, mg/dm^3**	1636	3170	2972	2359	4519	2669
3.	**NPS, mg/dm^3**	210	209	414	265	301	236
4.	**Anthocyanins, mg/dm^3**	168	196	342	317	359	224
5.	**Resveratrol, mg/dm^3**	2.30	3.46	3.52	3.18	2.39	1.77

Table 3. Statistical data on the trans-resveratrol content in wine from the studied varieties

Variants	Average value	Trapezitsa		Pinot Noir		Kaylashki Rubin		Bouquet		Storgozia	
		Difference	Significant	Difference	Significant	Difference	Significant	Difference	Significant	Difference	Significant
Trapezitsa	2.30	x	x								
Pinot Noir	3.46	1.16	++	x	x						
Kaylashki Rubin	3.52	1.22	++	0.07	n.s	x	x				
Bouquet	3.18	0.88	+	-0.28	n.s	-0.34	n.s	x	x		
Storgozia	1.77	-0.52	n.s	-1.68	---	-1.75	---	-1.40	--	x	x
Рубин	2.39	0.10	n.s	-1.06	--	-1.13	--	-0.78	-	0.62	n.s

5% - (+) (-); 1% - (++) (--); 0.1% - (+++) (---); <5% - (n.s)

Table. 4. Overall tasting evaluation

Variants		Repetitions					
Year	**Variety**	**1**	**2**	**3**	**4**	**5**	**6**
2015	**Trapezitsa**	74	75	72	74	76	72
	Pinot Noir	83	81	76	80	84	80
	Kaylashki Rubin	77	66	75	81	79	77
	Bouquet	78	70	69	81	81	76
	Storgozia	74	74	72	72	77	71
	Rubin	77	72	70	79	77	72
2016	**Trapezitsa**	76	78	75	77	79	75
	Pinot Noir	80	80	79	81	80	77
	Kaylashki Rubin	79	77	80	78	79	83
	Bouquet	81	79	80	84	81	76
	Storgozia	81	75	78	81	85	76
	Rubin	83	85	81	85	84	85
2017	**Trapezitsa**	65	78	73	71	74	74
	Pinot Noir	70	72	79	77	78	74
	Kaylashki Rubin	82	83	83	75	79	79
	Bouquet	72	79	78	74	76	75
	Storgozia	69	78	78	73	71	76
	Rubin	85	81	81	75	76	79

Table 4 a. Statistical analysis of Trapezitsa organoleptic evaluation

Variants	Average value	2015		2016	
		Difference	Significant	Difference	Significant
2015	73.83	x	x		
2016	76.67	2.83	n.s	x	x
2017	72.50	-1.33	n.s	-4.17	-

5% - (+) (-); 1% - (++) (--); 0.1% - (+++) (---); <5% - (n.s)

Table 4 b. Statistical analysis of Pinot Noir organoleptic evaluation

Variants	Average value	2015		2016	
		Difference	Significant	Difference	Significant
2015	80.67	x	x		
2016	79.50	-1.17	n.s	x	x
2017	75.00	-5.67	--	-4.50	-

5% - (+) (-); 1% - (++) (--); 0.1% - (+++) (---); <5% - (n.s)

Table 4 d. Statistical analysis of Bouquet organoleptic evaluation

Variants	Average value	2015		2016	
		Difference	Significant	Difference	Significant
2015	75.83	x	x		
2016	80.17	4.33	n.s	x	x
2017	75.67	-0.17	n.s	-4.50	n.s

5% - (+) (-); 1% - (++) (--); 0.1% - (+++) (---); <5% - (n.s)

Table 4 e. Statistical analysis of Storgozia organoleptic evaluation

Variants	Average value	2015		2016	
		Difference	Significant	Difference	Significant
2015	73.33	x	x		
2016	79.33	6.00	+	x	x
2017	74.17	0.83	n.s	-5.17	-

5% - (+) (-); 1% - (++) (--); 0.1% - (+++) (---); <5% - (n.s)

Table 4 f. Statistical analysis of Rubin organoleptic evaluation

Variants	Average value	2015		2016	
		Difference	Significant	Difference	Significant
2015	74.50	x	x		
2016	83.83	9.33	+++	x	x
2017	79.50	5.00	+	-4.33	n.s

5% - (+) (-); 1% - (++) (--); 0.1% - (+++) (---); <5% - (n.s)

Table 4 shows the overall tasting evaluation of the wines for the three years of the study.
In the case of Trapezitsa wine, the lowest tasting score was obtained in 2017, with the difference between it and 2016 being statistically proven. There is no statistically proven difference in the overall tasting score in 2015 compared to the other two years.
It was proven that the overall tasting evaluation of Pinot Noir in 2017 was lower compared to the other two years of the study. The difference between the years 2015 and 2016 was statistically not substantiated.
For Kaylashki Rubin wines there was no proven difference between the tasting score during the years of the study, revealing that the type of the year did not have a significant impact for this variety.
For Bouquet wines there wasn't either proven difference in the overall tasting score during the three years of the study.
In Storgozia wines, the overall tasting score was higher in 2016, while the difference in the tasting scores in 2015 and 2017 was insignificant and statistically unproven.
In the case of Rubin wine, the lowest tasting score was obtained in 2015, while the tasting scores in 2016 and 2017 were higher but the difference between them was statistically unproven

4. CONCLUSION

From the organoleptic evaluation of the studied wines, it could be argued that there was no significant difference between the tasting scores in the years of the research, showing that the weather conditions during the vegetation of the investigated varieties have had a significant impact on the wine quality.
It was found that the trans-resveratrol rates in Kaylashki Rubin red wine (3.52 mg/dm^3) were the highest, followed by Bouquet variety wine (3.18 mg/dm^3), Rubin (2.39 mg/dm^3), Trapezitsa (2.30 mg/dm^3) and Storgozia (1.77 mg/dm^3) for the three studied years.
Obviously, Pinot Noir had retained its genetic superiority and was found to have the highest trans-resveratrol rates. Close to it in trans-resveratrol rate was Kaylashki Rubin variety (3.52 mg/dm^3 interspecific hybrid), Bouquet

(3.18 mg/dm^3 and Rubin 2.39 mg/dm^3 (interspecific hybrid, created at IVE – Pleven).
Based on these data, it could be stated that Bulgarian varieties and wines have a strong potential to accumulate and store in sufficient quantities this powerful antioxidant.

5. REFERENCES

[1]. Abril, M., Negueruela, I., Perez, C., Juan, T., Estapanan, G. Preliminary study of resveratrol content in Aragon red and rose wines, Food Chemistry 92, 2005, p: 729-736.

[2]. Anli, E., Vursal, N., Demiray, S., Ozkan, M. Trans-resveratrol and other phenolic compounds in Turkish red wines with HPLC. Journal of Wine Research 17, 2007, p: 117-125.

[3]. Bartelli, E., Giovaninni, L., Giannessi, D., Migliori, M., Bernini, W., Fregoni, M. Bertelli, A. Antiplatelet activity of synthetic and natural resveratrol in red wine, International Journal of Tissue Reactions 17, 1995, p: 1-3.

[4]. Chobanova, D. Enology. Part I: Composition of wine. Academic Press of University of Food Technologies, Plovdiv, 2012. (Bg)

[5]. Chobanova, D. Manual for enology exercises, Academic Press of University of Food Technologies, Plovdiv, Bulgaria, 2007. (Bg)

[6]. Corder, R., Douthwaite, J.A., Lees, D.M. Endothelin-1 synthesis reduced by red wine. Nature 414, 2001, p: 863–864.

[7]. Craveiro, M., Cretenet, G., Cedric, M., Matias, M., Caron, O., Pedroso de Lima, M., Zimmermann, V., Solary, E., Dardalhon, V., Dulic, V., Taylor, N. Resveratrol stimulates the metabolic reprogramming of human CD4+ T cells to enhance effector function. Science Signaling 10, 2017, Issue 501.

[8]. Dimova, D., Marinkov, E. Experimental work and biometry. Academic Publishing House of Higher Institute of Agriculture Plovdiv, 1999, p. 263.(Bg)

[9]. Fartzov, K., Alyakov, M., Onisiforou, D. Pargov, M., Videnova, R., Toshev, D. Trans - resveratrol in red wines and its physiological effects. Editor: Limasol, 2013

[10]. Gehm, D., Mcandrews, M., Chien, P.Y., Jameson, J.L. Resveratrol a polyphenolic compound found in grapes and wine, is an agonist for the estrogen receptor, Proceedings of the National Academy of Sciences of the United States of America 94, 1997, p: 14138-14143.

[11]. Haygarov, V., Yoncheva, T., Dimitrov, D. (2017). Study of resveratrol content in grapes and wine of the varieties Storgozia, Kaylashki Rubin, Trapezitsa, Rubin, Bouquet and Pinot Noir. Journal of Mountain Agriculture on the Balkans 20(3), 2017, p: 300 – 311.

[12]. Haygarov, V. Study the Possibilities for Production of Quality White Wines in the Region of Pleven. Dissertation thesis. Plovdiv, Bulgaria: University of Food Technologies. 2012, 246 p. (Bg)

[13]. Ivanov, M. Hybridization in the vine selection. Monography. Academic Publishing of Agricultural University, Plovdiv, 2016. (Bg).

[14]. Ivanov, M., Simeonov, I., Nakov, Z. 2012. Trapezitsa – new red wine grapevine variety. Agricultural Science 45(1), 2012, p: 57-62.

[15]. Ivanov, T., Gerov, S., Yankov, A., Bambalov, G., Tonchev, T., Nachkov, D., Marinov, M. Practicum in Wine Technology, Plovdiv, Bulgaria: Publ. House Hristo G. Danov, 1979, 530 p. (Bg)

[16]. Marinov, M. Technology of wine and higher alcohol drinks, Zemizdat, Sofia, Bulgaria, 1990. (Bg).

[17]. Melzoch, K. Resveratrol in parts of vine and wine originating from Bohemian and Moravian vineyard regions. Agriculturae Conspectus Scientificus 66(1), 2001, p: 53-57.

[18]. Marni, Old. Wine – Tasting course, First published in Great Britain, 2014.

[19]. Petkov, G. Biochemical and technological study of Bouchet, Ruen and Rubin grapevine varieties for production of red wines. PhD Thesis. Institute of Viticulture and Enology, Pleven, 1977. (Bg).

[20]. Pandeliev, S., Harizanov, A., Botyanski, P., Roychev, V., Kemilev, S. Practical advices of vine and wine, Dionysus, Sofia, Bulgaria, 2010. (Bg).

[21]. Prodanova, N. 2008. Wine Tasting or How to Know Wine. Sofia, Bulgaria: Gourmet. 2008, pp. 115–118. ISBN 9789542917205. (Bg)

[22]. Renaud, S., de Lorgeril, M. Wine, alcohol, platelets, and the French paradox for coronary heart disease. Lancet 339 , 1992, p: 1523–1526.

[23]. Roychev, V. Ampelography. Academic Publishing House of Agricultural University, Plovdiv, 2012. (Bg).

[24]. Sirakov, D. Statistical methods in metrology, University Publishing House St. Kliment Ohridski – Sofia, 1981, p. 269 (Bg)

[25]. Teissedre, P. L., Waterhouse, A. L., Walzem, R. L., German, J. B., Frankel, E. N., Ebeler, S. E., Cliford, A. J. Composes phenoliques du raisin et du vin et sante. Bulletin O.I.V. 69, 1996, p: 252-297.

[26]. Videnova, R., Investigation of changes in resveratrol content in the processing of berries, Thesis for PhD, 2017 (Bg).

[27]. [27] Yankov, A. Wine Making Technology. Sofia, Bulgaria: Zemizdat. 1992, 355 p. (Bg)