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STATISTICAL ANALYSIS OF THE RESULTS OF *TRANS*-RESVERATROL CONTENT IN GRAPES OF HYBRID BULGARIAN GRAPE VARIETIES

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Abstract: *A study for statistical analysis of the results of the trans-resveratrol content in grapes of hybrid Bulgarian vine varieties was carried out. The chemical composition of the grapes was analyzed. Excellent technological grapes maturity (194.33 ± 19.13 g/dm³ - 244.00 ± 20.80 g/dm³ of accumulated sugars) was achieved. The detected ranges of titratable acids (5.66 ± 0.25 g/dm³ - 7.05 ± 0.33 g/dm³), tartaric acid (3.04 ± 0.15 g/dm³ - 4.66 ± 0.25 g/dm³), malic acid (4.42 ± 0.27 g/dm³ - 6.33 ± 0.15 g/dm³) and pH (3.24 ± 0.03 - 3.60 ± 0.32) were typical for grapes obtained from vines grown in the region of the Danube Plain, Central Northern Bulgaria. Trans-resveratrol was found in the highest amount (3.52 ± 0.59 mg/dm³) in the Kaylashki rubin grapes. Its content was close to this (3.46 ± 0.40 mg/dm³) of the control variety Pinot Noir. The lowest (1.78 ± 0.17 mg/dm³) was the amount of trans-resveratrol in the grapes of Storgozia variety. The statistical processing divided the varities into two groups (by trans-resveratrol content): the first group had higher trans-resveratrol content and included the varieties Kaylashki Rubin (3.52 ± 0.59 mg/dm³), Bouquet (3.18 ± 0.15mg/dm³) and Pinot Noir (3.46±0.40 mg/dm³); the second group consisting of the varieties Rubin (2.40±0.09 mg/dm³), Trapezitsa (2.30±0.80 mg/dm³) and Storgozia (1.78±0.17 mg/dm³) with lower trans-resveratrol content.*

Keywords: *grapes, wine, chemical composition, resveratrol, intraspecific and interspecific hybridization.*

1. Introduction

The *trans*-resveratrol as a chemical compound in grapes has been given attention by the scientific community since 1990 when the compound has been shown to have cardioprotective properties. *Trans*-resveratrol, as a gift of nature, has a wide range of health-promoting effects, including antibacterial, antifungal, antioxidant, anti-inflammatory, cardioprotective and anti-tumor activities. It protects vine from ultraviolet rays, viruses, bacteria, fungi and acts as a natural antibiotic [1].

The trans-resveratrol is an extremely rare substance. In addition to grapes and wine, certain amounts of it are contained only peanuts and some rare species of forest fruits such as blueberries, blackberries, strawberries, mulberries and cherries. Chemically, it is a type of natural polyphenol (stilben) [2]. It is produced naturally by some plants (including vine) when they are attacked by pathogens such as bacteria or fungi. The other factors for its accumulation include climate conditions (the big differences between daily and nightly temperatures), weather anomalies resulting in stress for the plant. Its

accumulation is the plant response to overcoming the unfavorable environmental conditions. The t*rans*-resveratrol is a powerful antioxidant. It has a high ability to capture and block the free oxidative radicals and prevents cells from malignant alterations. The substance has a number of benefits: anticancer and antiviral effect, it increases the energy and improves heart function, it normalizes blood glucose levels and stimulates the nervous system and significantly improves the sport achievements. The *trans*-resveratrol is an antidepressant that reduces "bad" LDL-chlesterol. It is also thought to block the formation of COX-2 enzyme that promotes the development of colon cancer [1].

2. Materials and methods

The study was carried out at the Institute of Viticulture and Enology – Pleven (IVE), during the period 2015-2017. Red grape varieties Storgozia, Kaylashki Rubin, Trapezitsa, Rubin and Bouquet, selected at IVE – Pleven, R. Bulgaria by the intraspecific and interspecific hybridization [3-4] were the object of the study. They were cultivated in certain micro-regions throughout the country (Pleven, Sadovets, Barkach, Suhindol, Pavlikeni, Brestovitsa, Karnobat, Burgas, Blagoevgrad and Sandanski).

The vine plantation was located at the Experimental Base of the IVE, on an area of 0.2 ha for each studied variety. The vineyards were fruit-bearing. The vines were grown on a medium stem training system. The planting distance was 3.00 m / 1.20 m. Berlandieri x Riparia SO4 was the used rootstock. During the vegetation period standard agricultural practices and plant protection measures were carried out.

Pleven is situated in Central Northern Bulgaria. This region is characterized by cold winters and hot summers. The sum of the average air temperatures during the vegetation period (from 16th April until 31st October) was approximately 4000 ˚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. The conditions were fovarable for obtaining of quality red grapes [5].

The brief climatic characterization of the years of the study was presented on table 1.

Table 1.

Brief climatic characterization of study years

Probability (P %) of precipitation and average air temperature (*T*°) for the years of the study				
Years		2015	2016	2017
$N_{(V-IX)}$	%	17 (Very wet)	35 (Wet)	6 (Very wet)
$T^{o}_{(V-IX)}$		29 (Average hot)	56 (Average cool)	33 (Average hot)

P – probability, N – (precipitations, T^{o} - 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.

The soil in the region was leached chernozem on loess foundation. It was suitable for vine cultivation.

The intraspecific varieties Storgozia, Kaylashki Rubin and Trapezitsa and the interspecific variety Bouquet were actually resistant to stress factors (lower winter temperatures and mildew) [3-4].

The parental forms of the studied varieties were as follows:

Storgozia – Bouquet x Villard Blanc12375

Kaylashki Rubin – Pamid x Hybrid VI-2-15 x Gamay Noir x *Vitis amurensis*

Trapezitsa – Dunavska Gamza x Marseilles Early

Dunavska Gamza – Bouquet X Villard Blanc 12375

Rubin – Nebbiolo x Syrah

Bouquet – Mavrud x Pinot Noir

The control variety was **Pinot Noir** of *Vitis vinifera*, which according to the available literature data had the highest content of *trans*-resveratrol [6].

Upon reaching of the technological maturity (20-23% sugars), the grapes were harvested and analyzed.

The main chemical indicators of the grape pulp from the studied varieties were determined according to the methods generally accepted [7,2,8].

For determining of the *trans*-resveratrol content in the grapes for each sample, two-fold extraction of the grape pulp with methanol was carried out. High pressure liquid chromatography (HPLC), according to the modified method of Anli et al. [9], was used for determining the *trans*-resveratrol content in the wine samples.

The results were statistically processed by one-factor analysis of variance (ANOVA) [10].

The aim of the present study was to analyze statistically the results of *trans*-resveratrol content in grapes of hybrid Bulgarian grape varities.

3. Results and discussion

Table 2 presents the grapes chemical composition and the date of harvest for each variety studied.

Table 2.

Grapes chemical composition of the studied varieties (average with ±SD) for the period 2015 - 2017

Variety	Storgozia	Kaylashki Rubin	Trapezitsa	Rubin	Bouquet	Pinot Noir
Harvest date	06.10.2015 10.09.2016 18.09.2017	16.09.2015 11.09.2016 12.09.2017	02.09.2015 30.08.2016 28.08.2017	03.09.2015 31.08.2016 12.09.2017	20.09.2015 12.09.2016 26.09.2017	03.09.2015 30.08.2016 30.08.2017
Reducing sugars g/dm^3	232.33±9.45	212.33±10.50	194.33±19.13	244.00±20.80	221.00±11.53	234.00±13.52
Titratable acids g/dm^3	5.97±0.36	7.05±0.33	5.66±0.25	6.81±0.55	6.67±0.59	6.80±0.33
Tartaric acid g/dm^3	3.30±0.42	4.50±0.32	4.66±0.25	3.04±0.15	3.32±0.12	3.56±0.17
Malic acid g/dm^3	5.52±0.21	5.84±0.19	4.95±0.10	6.33±0.15	5.49±0.25	4.42±0.27
pH	3.60±0.32	3.40±0.28	3.45±0.22	3.58±0.26	3.39±0.31	3.24±0.03
Trans-resveratrol mg/dm^3	1.78±0.17	3.52±0.59	2.30±0.80	2.40±0.09	3.18±0.15	3.46±0.40

From the indicators presented in Table 2, it was evident that grapes have accumulated sugars from 194.33±19.13 g/dm^3 (Trapezitsa) to 244.00±20.80 g/dm^3 (Rubin). The sugars in must of ripe and healthy grapes are in the range of 150.00 - 250.00 g/dm^3 [2]. The results of this study correlated with this finding. The grapes were reached good technological maturity. The titratable acids were within the range from 5.66±0.25 g/dm^3 (Trapezitsa) to 7.05±0.33 g/dm^3 (Kaylashki Rubin). The

acid content of the grape juice is dependent of the grape variety, geographic location and climatic conditions of the region in which it is grown [11]. At the conditions of Bulgaria, the grapes acids are in the range of 4.00 - 12.00 g/dm^3 [11]. The results for the "titratable acidity" indicator in the present study fully correlated with the range of acidity variations in mature grapes.

The tartaric acid is essential for grapes. Its quantitative accumulations are influenced exclusively by the location of the area - to the south (2.00 - 3.00 g/dm^3); in the northern regions (6.00 g/dm^3 and above) [11]. The highest quantitative presence (4.66 ± 0.25 g/dm^3) of tartaric acid was found in the grapes of the Kaylashki rubin variety. The lowest (3.04 ± 0.15 g/dm^3) was its amount in the Rubin grapes. The region where the grapes were grown was the Danube Plain, Central Northern Bulgaria. All established tartaric acid concentrations in the grapes of the varieties studied exceed 3.00 g/dm^3, which was typical for this northern region.

The malic acid is the second by quantitaty. In the grapes of the northern region it is present in quantities of 4.00 - 7.00 g/dm^3 and in the southern - 1.00 - 3.00 g/dm^3 [11]. In the present study, it was found in the highest amount (6.33 ± 0.15 g/dm^3) in Rubin grapes and in the lowest (4.42 ± 0.27 g/dm^3) in Pinot Noir. Its variations were typical for grapes grown in the northern region of Bulgaria.

The pH of grapes must be within 2.8 - 3.8 (Chobanova, 2012). In the present study, the pH was recorded in the range of 3.24 ± 0.03 (Pinot Noir) - 3.60 ± 0.32 (Storgozia). The data was in agreement with the presented by [2].

The data showed that the amount of *trans*-resveratrol in the grapes from the studied varieties in the region of Pleven was almost equal and comparable to that of Pinot Noir control of *Vitis vinifera*.

The results for *trans*-resveratrol (average for 2015, 2016 and 2017 harvests) and its statistical analysis are presented in Table 3. The quantitative characterization distinguished the hybrid Kaylaski rubin variety as the quntitavely dominated of *trans*-resveratrol (3.52 ± 0.59 mg/dm^3). The difference between it and the Pinot Noir control (3.46 ± 0.40 mg/dm^3) was negligible. In all other studied varieties, lower amounts of *trans*-resveratrol were observed in comparison with Pinot Noir. The control variety showed its genetic superiority for increased synthesis of resveratrol. The lowest amount of the stilbene (1.78 ± 0.17 mg/dm^3) was found in the must of Storgozia variety.

Table 3 presents the statistical processing of the obtained results. On the average, for the three years of the research, the studied varieties could be divided into two groups. The first group had higher trans-resveratrol content and included the varieties Kaylashki Rubin (3.52±0.59 mg/dm^3), Bouquet (3.18±0.15 mg/dm^3) with Pinot Noir control (3.46±0.40 mg/dm^3). The difference between them was insignificant and statistically unproven. The second group consisting of the varieties Rubin (2.40±0.09), Trapezitsa (2.30±0.80) and Storgozia (1.78±0.17) had proven lower trans-resveratrol content compared to the first one and the difference between the varieties in the group was also statistically unproven.

Table 3.
Statistical analysis of the results for the content of *trans*-resveratrol in grapes of studied grape varieties

Variants	Average value, mg/dm^3	Trapezitsa		Pinot Noir		Kaylashki Rubin		Bouquet		Storgozia	
		differ.	sign.	differ.	sign.	differ.	sign.	differ.	sign.	differ	sign.
Trapezitsa	2.30±0.80	x	x								
Pinot Noir	3.46±0.40	1.16	++	x	x						
Kaylashki Rubin	3.52±0.59	1.22	++	0.07	n.s	x	x				
Bouquet	3.18±0.15	0.88	+	-0.28	n.s	-0.34	n.s	x	x		
Storgozia	1.78±0.17	-0.52	n.s	-1.68	---	-1.75	---	-1.40	--	x	x
Rubin	2.40±0.09	0.10	n.s	-1.06	--	-1.13	--	-0.78	-	0.62	n.s

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

4. Conclusion

From the results obtained, the following conclusions can be made:

• The studied grapes reached an excellent technological maturity with accumulated sugars of 194.33 g/dm^3 (Trapezitsa) to 244.00 ± 20.80 g/dm^3 (Rubin).

• The presence of titratable acids (5.66 ± 0.25 g/dm^3 - 7.05 ± 0.33 g/dm^3) was typical for grapes grown in the region of Central Northern Bulgaria.

• The tartaric and malic acids were found in concentrations typical for grapes of northern Bulgaria.

• The quantitative characteristic for the presence of the stilbene *trans*-resveratrol distinguished the grapes of the Kaylashki rubin variety. The grapes from this variety contained 3.52 ± 0.59 mg/dm^3 *trans*-resveratrol. The established quantity was close to the amount found in the control (3.46 ± 0.40 g/dm^3) Pinot Noir.

• The chemical indicators of the studied varieties selected through the intraspecific and interspecific hybridization were not different from those of *Vitis vinifera* control. These results indicate that the grapes of the varieties studied are suitable for the production of quality red wines.

• The statistical processing divided the varieties into two groups: First witn the highest content of resveratrol - Kaylashki Rubin (3.52±0.59), Bouquet (3.18±0.15) and Rubin (2.40±0.09); the second with the lowest resveratrol content - Storgozia (1.78±0.17), Trapezitsa (2.30±0.80) and Pinot Noir control (3.46±0.40).The statistical difference between the two groups of varieties was insignificant and statistically unproven. The amount of resveratrol in the grapes from the studied varieties cultivated in the region of Pleven, Central Northern Bulgaria was almost equal and comparable to those of Pinot Noir control from *Vitis vinifera*.

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