Content of phenolic substances in grapes and wine of the Vranec variety (*Vitis vilifera* L.) grown at different altitude

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Abstract

Introduction.The aim of this study was to determine the content of phenolic substances in grapes and wine of the Vranec variety grown at three different altitudes in the area of Macedonia.

Materials and methods. The total phenols content, antocyanins and flavan-3-ols in grape extracts and wines from the control (without application of summer pruning operations) and experimental variants (with application of three different pruning operations) were determined by spectrophotometric analysis.

Results and discussion. The highest levels of anthocyanins (10.74 mg/g FW) were found in the berries skin extract when the pruning operation "normation to 6 bunches per vine" was applied in the Veles region (280 m altitude). The concentration of anthocyanins in the other variants from the Veles region were lower and ranged from 7.26 mg/g FW (control) to 8.39 mg/g FW (variant 10 bunches per vine). The highest levels of total phenols were found in the berries skins and seeds of the "6 bunches per vine" variant in the Skopje region (595 m altitude), accumulated in amounts of 22.82 mg GAE/g FW (berries skin) and 101.04 mg GAE/g FW (berry seeds), respectively. The highest content of total flavan-3-ols was found in the berries skins and seeds of the "6 bunches per vine" variant from the Veles region, accumulated in amounts of 2.33 mg CE/g FW (berries skin) and 29.64 mg CE/g FW (berries seeds), respectively. In the wines from the experimental variants, the highest levels of anthocyanins were found in the wine of the variant "6 bunches per vine" from the Veles region (593.56 mg/L). In the experimental wines from this variant of the same Veles region, the highest amounts of total phenols were also found (2925.61 mg GAE/L). Flavan-3-ols were found in the highest amount (441.46 mg CE/L) in the wine of the variant "6 bunches per vine", but from the Skopje region.

Conclusions. The higher altitude showed to increase the amount only of total phenols in the berries skins and seeds.There were no significant differences in the content of phenolic substances – total anthocyanins and total phenols in the wine of the Vranec variety by variants and regions, as their quantities were higher in the variants with normation of grape bunches.

Introduction

Phenols are a significant group of substances found in the grapes and wine, which have great influence on their organoleptic characteristics, biological potential and quality (Zhang et al., 2023). They determine the colour of the berries skin and also the wine's colour (anthocyanins) and density (phenols increase the wine extract) and taste (Margaryan et al., 2017). These substances are transferred from grapes into the wine during the alcoholic fermentation and are directly involved in the formation of its quality (Gutiérrez-Escobar et al., 2021; Landrault et al., 2001). As biologically active substances, they have different effects on human health – antimicrobial, anticancer, cardioprotective, anti-inflamation, neuroprotective, antiaging, antidiabetic, and antioxidant (Milinčić et al., 2025; Neira-Opsina et al., 2024; Renaud and Lorgeril, 1992; Xia et al., 2010;). Their quantity in the grapes depends on many factors, which are divided into two groups: biological, including the ampelographic features of the variety; environmental – soil and climatic conditions of the region, applied agricultural techniques, plant protection measures, etc. Increased rainfall, as well as excessive irrigation, have a negative impact on the content of phenolic substances in grapes (Valdés et al., 2009). The lower content of total anthocyanins in grapes can be due to a later harvest and advanced technological maturity (Andjelkovic et al., 2013). The reduction of the anthocyanins in the skin of the berry provokes not only inhibition of the biosynthetic processes, but also a number of accompanying high temperatures factors such as the chemical and/or enzymatic degradation (Morri et al., 2007). At the beginning of the alcoholic fermentation, the extraction of anthocyanins from the berry skin in the must is slow, but gradually it increases and reaches its maximum when the alcohol content increases from 3 to 6 vol. %, after which their amount slowly decreases (Nagel and Wulf, 1979; Watson et al., 1995). At the end of the fermentation, the decrease in the concentration of anthocyanins in wine is due to adsorption by solid parts, the interaction of anthocyanins with tannins and the formation of polymer pigments (Singleton and Trousdale, 1992). The extraction of tannins from the skins and seeds increases even after free anthocyanins have reached their maximum concentrations (Ozmianski et al., 1986).

There are a number of studies in the scientific literature on the changes occurring in the phenolic profile of grapes grown at different altitudes and their corresponding wines. Jin et al. (2017) investigated the changes in the phenolic profile of red wines from Merlot and Cabernet Sauvignon varieties produced from grapes grown at three different altitudes – 2282 m, 2435 m and 2608 m in a mountainous region north of the Hengdnan Mountains, China. The team found that altitude had a significant effect, leading to an increase in the content of total phenolic compounds, total flavonoids and total anthocyanin content in the wines of both varieties. It was also reported that the tannin content of Cabernet Sauvignon wine increased proportionally with increasing altitude. Brighenti et al. (2017) determined the total polyphenol content of grapes from 13 red grapevine varieties grown in the São Joaquim region of Brazil at 1400 m above sea level. The team found a range of total polyphenol content from 523.87 mg/L to 4929.57 mg/L, with the highest levels in grapes from Ancelota, Uva di Troia and Croatina.

The aim of this study was to determine the content of phenolic substances in grapes and wine of the Vranec variety grown at three different altitudes in the area of Macedonia.

Materials and methods

Materials

The experimental work was conducted during the period 2012 – 2015, with vines of the local grape variety Vranec grown in three regions of the R. N. Macedonia with different altitudes: Gevgelija – 50 m, Veles – 280 m and Skopje – 595 m. The row and interlinear planting distances in the vineyard was 3.20/1.00 m in Gevgelija, while in Veles and Skopje – 3.20/1.20 m. The vines pruning system was double-Guyot loaded with 20 winter buds in total – two spurs with 2 buds and two fruit sticks with 8 buds.

In the study, 150 vines were included on each vineyard; four variants were formed with 35 vines of each variant:

- control – without application of summer pruning operations;
- with application of defoliation from the base of the fruiting shoots to the area of the bunches, carried out in the middle of August at 80% version of the berries;
- with normation of grape bunches by leaving 6 grape bunches per vine in the middle of July;
- with normation of grape bunches by leaving 10 grape bunches per vine in mid-July.

In order to determine the influence of altitude on the content of phenolic substances, the grapes from the three regions were harvested at the same time. The moment of harvesting was determined by visual control, measurement of sugar content with a refractometer and monitoring the appearance of wilting in 5 – 10 % of the berries bunch of the variant with normation of 6 bunches per vine.

For the analysis of the total phenols, total anthocyanins, total flavan-3-ols and the colour characteristics was used spectrophotometer Agilent 8453 UV/VIS (Agilent Technologies, California, USA)

Methods

Extraction of berries skins and seeds. Berries from couple of grape bunches were skinned with laboratory tweezers. The seeds were separated from the pulp, washed with distilled water and the water was removed with filter paper. Skins were blotted on filter paper to remove any residual pulp. The skins and seeds were ground in lab mortar. Approximately 1g of grape berry skins and 1g seeds were weight on analytical balance and were extracted twice for 15 min with10 mL acetone/water (80/20, v/v) containing HCl (0.1/10, v/v) to prevent oxidation of the polyphenolic substances in an ultrasonic bath at room temperature and then stirred for 30 min on a magnetic agitator. The samples were centrifugated (3000 rpm for 10 min) and the supernatants from both extractions were combined and made up to a final volume of 25 mL with distilled H_2O. All extracts were filtered before spectrophotometric determination of phenol compounds (Ivanova-Petropulos et al., 2010).

Total polyphenols content. The total polyphenols content in grape extracts and wines from the control and experimental variants were determined by spectrophotometric analysis at a wavelength of 765 nm by the Folin-Ciocalteu method (Singleton et al., 1999). The obtained results were expressed as mg/g FW (fresh weight) gallic acid equivalent (for grape extracts) and mg/L gallic acid equivalent (for wine). The obtained grape extracts or the tested wines were diluted (1:25). A quantity of 1 ml of this sample was transferred to a 10 ml flask, in which 5 ml of distilled water was previously poured. 5 ml of Folin-Ciocalteu reagent was added and 3 min were waited, then 1.5 ml of 20% Na_2CO_3 was added. At the next stage, the

flask was filled to the mark. The samples were placed in a water bath at a temperature of 50 ºC for 16 min. After that, they were cooled with cold water and the absorption of the spectrophotometer was established at a wavelength of 765 nm. In the control sample, 1 ml of distilled water was used instead of the tested solution. The result was established from a standard curve with gallic acid.

Total anthocyanins content. The grapes extracts or wines for analysis were centrifuged at 3000 rpm for 20 min. The centrifuged sample was diluted (1:100) and 0.1 ml of this sample was made up to 10 ml with acidified ethanol (96% ethanol: HCl). The absorbance of the sample was determined at 550 nm wavelength, and acidified ethanol was used as a control. Total anthocyanins content were determined by the formula: (15xD0) x R, where D0 – absorbance recorded at 550 nm wavelength; R = sample dilution. The content was expressed as mg/l (for wine sample) and mg/g FW (grapes extracts) (Di Stefano et al., 1989).

Total flavan-3-ols content. The quantification of total flavan-3-ols was performed with p-dimethylamino-cinnamaldehyde (p-DMACA) method and the obtained results were expressed as catechin equivalent (mg CE/L) (Di Stefano et al., 1989). In a 10 ml flask, 0.1 ml of red wine or extract was poured, three drops of glycerol were added, 5 ml of p-DMACA reagent flask was poured to the mark with distilled water. After 7 min, measuring of the absorbance at 640 nm wavelength was made. Methanol was used as a control sample. The result was determined from a standard curve of catechin hydrate.

Wine colur characteristics. The wine colour characteristics were determined according to the method of Ivanova-Petropolus (2013) and were expressed as Absolute Units (AU).

Colour intensity of the wines was determined by the level of the anthocyanins present in the wine and was defined as the sum of the absorbances at 420, 520 and 620 nm. In this study, the absorbance of wines was directly measured at 420, 520 and 620 nm using 2 mm cuvette. From the obtained results colour intensity (*CI*), hue or tint (*T*), proportion of red colour (% *Rd*), blue colour (% *Bl*), yellow colour (% *Ye*) were calculated.

Colour intensity (CI) is sum of $A420+A520+A620$

The hue or tint (T) is defined as the ratio of $A420/A520$.

The colour composition of the wines was expressed as percentage and it was calculated according to the following equations:

$$\% Ye \text{ or } \% Rd \text{ or} \% Bl = 100(A\lambda/CI)$$

where: % *Ye* is the percentage of yellow colour (λ = 420 nm), %*Rd* is thepercentage of red colour (λ = 620 nm) and % *Bl* is the percentage of blue colour (λ = 520 nm) in the overall wine colour.

Statistical analysis

The software package IBM SPSS Statistics 2015 was used for the statistical analysis. In order to verify if there is a statistical difference between the parameters, Duncan's multiple range test was used with significance level of 0.05

Results and discussion

Content of phenolic substances in the grapes of the Vranec variety for all investigated variants and regions, average for the period 2013–2015

The statistical comparative analysis of the content of phenolic substances in the grapes of the Vranec variety in all variants and regions, on average for the period 2013–2015 showed the lowest amount of total anthocyanins in the berries skins from the control variant which was statistically proven – 6.40 mg/g FW compared to the others (Table 1).

The highest anthocyanin amount was found in the variant with 10 bunches per vine – 9.05 mg/g FW in the berries skins of the Vranec variety for all investigated variants and regions, average for the period 2013–2015**.** The differences in the anthocyanin content in berries skins between the other studied variants (defoliation, 6 grape bunches and 10 grape bunches) were not statistically proven. The total phenols in the berries seeds were again statistically proven with the highest content in variant with 10 grape bunches per vine – 93.26 mg GAE/g FW, compared to the control – 77.21 mg GAE/g FW. The established increasing tendency for the total flavan-3-ols amount was the same, but statistically not proven, which again confirmed the positive impact of the yield normation on the quality of the obtained grapes.

Content of phenolic substances in the grapes of the Vranec variety by variants and regions, average for the period 2013–2015

The same trend was also established by comparing of the data for anthocyanins in the berries skins by variants and regions (Table 2). It should be noted that the vineyard in Gevgelija was characterized with higher temperature values than the vineyards of the other two regions, and respectively lower absolute values for the amount of total anthocyanins in all variants of the study, although statistically proven only in the variant with 6 grape bunches per vine.

The highest values for this indicator (total antocyanins content) in the studied variants (one control without pruning operations and the three variants with different pruning operations) were obtained in the berries skin from the vineyard located around Veles – control (7.26 mg/g), defoliation (8.35 mg/g), with 6 bunches per vine (10.74 mg/g) and with 10 bunches per vine (8.39 mg/g). The amount of total phenols was the highest in the grapes variants (one control without pruning operations and the three variants with different pruning operations) from the Skopje region. Their content varied from 14.84 mg GAE/g FW (defoliation) to 22.82 mg GAE/g FW (6 bunches per vine), and the lowest of total phenolic content was established in the variants from Gevgelija region with variation from 9.77 mg GAE/g FW (defoliation) to 12.39 mg GAE/g FW (6 bunches per vine). All established differences between the variants were statistically proven. The changes in the content of total flavan-3-ols were almost identical with those of the total anthocyanins, as the statistical differences between the variants were proved only in the variant with 10 bunches per vine compared to the grapes from Veles region, where the highest values for this indicator were reported. The total phenols in the berries seeds were statistically proven and had a higher values in the grapes from the Skopje region – 83.42 mg GAE/g FW (control), 88.62 mg GAE/g FW (defoliation), 101.04 mg GAE/g FW (6 bunches per vine) and 94.03 mg GAE/g FW (10 bunches per vine). The content of total flavan-3-ols was statistically proven in almost all variants, more in the berries seeds of grapes form Veles region – 23.42 mg CE/g FW (control), 20.82 mg CE/g FW (defoliation), 29.64 mg CE/g FW (6 bunches per vine) and 28.35 mg CE/g FW (10 bunches per vine). The higher altitude showed the influence to increase the amount only of total phenols in the berries skins and seeds, while the values of all other indicators were the highest in the grapes from the Veles region.

Content of phenolic substances in the grapes of the Vranec variety by variants and years, average for the period 2013–2015

The statistical comparative analysis of the content of phenolic substances in the grapes of the Vranec variety by variants showed that during the year with the most precipitation – 2014, the total anthocyanins in the berries skins were the lowest and this was statistically proven (control – 4.85 mg/g, defoliation – 4.75 mg/g, 6 grape bunches per vine – 7.64 mg/g and 10 grape bunches per vine – 4.77 mg/g) (Table 3). Only in the variant with 6 grape bunches per vine there was no statistically significant difference with the others.

In the case of total phenols, the emerging trend related to the influence of humidity on their accumulation was maintained, and again in 2014 their content was the lowest. There was diversity in this indicator, which was expressed by proven higher amounts in the berries skins from the Gevgelija region – 19.11 mg GAE/g FW (control), 23.75 mg GAE/g (6 grapes bunches per vine) and 22.11 mg GAE/g FW (10 grapes bunches per vine). Only in the variant with defoliation the value of this indicator was the highest in Skopje region – 15.28 mg GAE/g FW. In general, the flavan-3-ols also had the highest content in the grapes from Gevgelija region, with exception again for the variant with defoliation. Their accumulation starts at the beginning with the berry formation and continues until the phenophase verison. Then, in the period from verison to reaching the phenolic maturity, the synthesis of the monomeric flavan-3-ols – (+) catechin, (-) epicatechin and (-) epicatechin gallate are slowed or in some cases stopped, leading to a decrease in their amount (Kennedy et al., 2000). This process in the present study was caused by yield normation of the vine. There was a statistically significant difference in the total phenols and flavan-3-ols content in the seeds between the vineyard located in Veles region and the other two. Yield normation definitely increased the proven average values of these two groups of phenolic substances. The total flavan-3-ols content in 2014 reached the highest and statistically proven levels in the seeds, and the reason of this result was related with the bad weather conditions that caused difficulties in the proper course of the grapes ripening process. These results showed that the studied indicators in the grapes skins and seeds from all three studied regions were strongly depended of the external climatic conditions for the individual years and it was not possible to identify clearly any trend related to the importance of the altitude for their formation.

Content of phenolic substances in the wine of Vranec variety by variants, average for the period 2013–2015

The statistical comparative analysis of the phenolic substances content in the wine of the Vranec variety by variants and regions showed that there were no significant differences between the variants for the total anthocyanins and total phenols indicators (Table 4). It was clearly noticed that in the variants with grape bunches normation, higher quantities were achieved compared to the control and defoliation variants. The wines obtained from the variants with normalized yield with 6 bunches and 10 bunches per vine had superior values compared to the others variants, but had no statistically proven differences.

The same analysis applied for the content of total flavan-3-ols, showed differences in the values between the studied variants. The yield normation variants had statistically proven difference with the others. The highest values of all three indicators were reported for the variant with 6 grape bunches per vine – total anthocyanins (571.44 mg/L), total phenols (2610.04 mg GAE/L) and total flavan-3-ols (364.24 mg CE/L), compared to the control – 425.74 mg/L, 2035.07 mg GAE/L and 277.36 mg CE/L, respectively. The defoliation, as a summer pruning operation, also showed higher values compared to the control, but there was no statistically significant difference. The highest colour intensity value was determined in the variant with 6 grape bunches per vine. It had statistically proven differences from the others. The wine from the control variant had the lowest colour intensity. The levels of the other indicators – hue, yellow colour, red colour and blue colour had very similar values that were not statistically proven.

Table 1

Content of phenolic substances in the grapes of the Vranec variety for all investigated variants and regions, average for the period 2013–2015

Variants	Phenolic fractions				
	Total anthocyanins, mg/g FW	Total phenolics, mg GAE /g FW	Total flavan-3-ols, mg CE/g FW	Total phenolics, mg GAE/g FW	Total flavan-3-ols, mg CE/g F
	Berry skins			Berry seeds	
Control	6.40 ^{b}	14.01 ^{a}	1.45 ^{a}	77.21 ^{b}	16.63 ^{a}
Defoliation	6.82 ^{ab}	12.52 ^{a}	0.83 ^{a}	80.16 ^{b}	17.58 ^{a}
6 grape bunches	7.89 ^{ab}	15.96 ^{a}	1.37 ^{a}	86.33 ^{ab}	21.78 ^{a}
10 grape bunches	9.05 ^{a}	17.93 ^{a}	1.65 ^{a}	93.26 ^{a}	23.28 ^{a}

Note: a, b, c – Duncan significance level of 0.05, GAE – gallic acid equivalent; CE – catechin equivalent; FW – fresh weight

Table 2

Content of phenolic substances in the grapes of the Vranec variety by variants and regions, average for the period 2013–2015

Vineyard location		Variants and phenolic fractions				
		Total anthocyanins, mg/g FW	Total phenolics, mg GAE /g FW	Total flavan-3-ols, mg CE/g FW	Total phenolics, mg GAE /g FW	Total flavan-3-ols, mg CE/g FW
		Berry skins			Berry seeds	
Control	Gevgelija	5.79 ^{a}	10.49 ^{b}	1.02 ^{a}	66.19 ^{b}	12.53 ^{b}
	Veles	7.26 ^{a}	14.15 ^{ab}	1.73 ^{a}	82.02 ^{a}	23.42 ^{a}
	Skopje	6.14 ^{a}	17.36 ^{a}	1.61 ^{a}	83.42 ^{a}	13.94 ^{b}
Defoliation	Gevgelija	5.22 ^{a}	9.77 ^{b}	0.64 ^{a}	69.82 ^{b}	17.11 ^{a}
	Veles	8.35 ^{a}	12.95 ^{ab}	0.96 ^{a}	82.06 ^{a}	20.82 ^{a}
	Skopje	6.91 ^{a}	14.84 ^{a}	0.88 ^{a}	88.62 ^{a}	14.81 ^{a}
6 bunches per vine	Gevgelija	8.13 ^{b}	12.39 ^{b}	1.24 ^{a}	86.43 ^{b}	20.95 ^{b}
	Veles	10.74 ^{a}	18.57 ^{ab}	2.33 ^{a}	92.32 ^{ab}	29.64 ^{a}
	Skopje	8.29 ^{b}	22.82 ^{a}	1.37 ^{a}	101.04 ^{a}	19.28 ^{b}
10 bunches per vine	Gevgelija	7.23 ^{a}	11.01 ^{b}	1.03 ^{b}	80.47 ^{b}	18.79 ^{b}
	Veles	8.39 ^{a}	16.23 ^{ab}	2.14 ^{a}	84.48 ^{b}	28.35 ^{a}
	Skopje	8.07 ^{a}	20.64 ^{a}	0.95 ^{b}	94.03 ^{a}	18.22 ^{b}

Note: *a, b, c – Duncan significance level of 0.05;* GAE – gallic acid equivalent; CE – catechin equivalent; FW – fresh weight

Table 3

Statistical comparative analysis of the content of phenolic substances in the grapes of the Vranec variety by variants and years, average for the period 2013–2015

Years	Variants and phenolic fractions				
	Total anthocyanins, mg/g FW	Total phenolics, mg GAE/g FW	Total flavan-3-ols, mg CE/g FW	Total phenolics, mg GAE/g FW	Total flavan-3-ols mg CE/g FW
	Berry skins			Berry seeds	
Control					
2013	6.29 ^{b}	19.11 ^{a}	2.53 ^{a}	83.68 ^{a}	19.62 ^{a}
2014	4.85 ^{c}	8.88 ^{c}	1.07 ^{b}	76.36 ^{ab}	18.41 ^{a}
2015	8.01 ^{a}	13.44 ^{b}	0.44 ^{b}	71.02 ^{b}	9.95 ^{b}
Defoliation					
2013	6.83 ^{b}	12.52 ^{b}	0.83 ^{b}	80.16 ^{a}	17.58 ^{b}
2014	4.75 ^{c}	9.76 ^{c}	1.13 ^{a}	81.89 ^{a}	23.75 ^{a}
2015	8.89 ^{a}	15.28 ^{a}	0.52 ^{c}	78.43 ^{a}	11.41 ^{c}
6 bunches per vine					
2013	8.81 ^{a}	23.75 ^{a}	2.32 ^{a}	89.35 ^{b}	22.69 ^{b}
2014	7.64 ^{a}	14.46 ^{b}	1.94 ^{a}	101.42 ^{a}	30.45 ^{a}
2015	9.83 ^{a}	13.44 b	0.44 ^{b}	86.57 ^{b}	13.81 ^{c}
10 bunches per vine					
2013	8.30 ^{b}	22.11 ^{a}	2.14 ^{a}	88.59 ^{a}	22.28 ^{a}
2014	4.77 c	9.24 ^{c}	1.30 ^{b}	84.19 ^{a}	27.43 ^{a}
2015	9.89 a	14.94 ^{b}	0.49 ^{b}	84.01 ^{a}	12.87 ^{b}

Note: a, b, c – Duncan significance level of 0.05; GAE – gallic acid equivalent; CE – catechin equivalent; FW – fresh weight

Table 4

Content of phenolic substances in the wine of Vranec variety by variants, average for the period 2013–2015

Variants	Phenolic fractions							
	Total anthocyanins, mg/L	Total phenolics, mg GAE/L	Total flavan-3-ols, mg CE/L	Color intensity, AU	Hue, AU	Yellow color, %	Red color, %	Blue color, %
Control	425.74 [a]	2035.07 [a]	277.36 [b]	1.85 [c]	0.443 [a]	30.48 [a]	57.36 [a]	12.15 [a]
Defoliation	463.89 [a]	2042.16 [a]	293.36 [ab]	2.08 [bc]	0.435 [a]	30.06 [a]	59.16 [a]	10.78 [a]
6 grape bunches	571.44 [a]	2610.04 [a]	364.24 [a]	2.87 [a]	0.443 [a]	30.45 [a]	57.17 [a]	12.37 [a]
10 grape bunches	531.88 [a]	2470.01 [a]	373.86 [a]	2.52 [ab]	0.430 [a]	30.21 [a]	57.93 [a]	11.82 [a]

Note: a, b, c – Duncan significance level of 0.05, GAE – gallic acid equivalent; CE – catechin equivalent;

Table 5

Content of phenolic substances in the wines of Vranec variety by variants and regions, average for the period 2013–2015

Vineyard location		Phenolic fractions			Colour characteristics				
		Total anthocyanins, (mg/L)	Total phenolics, mg GAE/L	Total flavan-3-ols, mg CE/L	Color intensity, AU	Hue, AU	Yellow color, %	Red color, %	Blue color, %
Control	Gevgelija	405.44[a]	1702.13 [a]	230.51 [a]	1.71[ab]	0.423[a]	30.05 [a]	57.36 [a]	11.84 [a]
	Veles	446.44 [a]	2141.04 [a]	308.78 [a]	2.49 [a]	0.417[a]	29.10 [a]	58.51 [a]	11.77 [a]
	Skopje	425.33 [a]	2262.07 [a]	292.77 [a]	1.35 [b]	0.484[a]	31.71 [a]	55.44 [a]	12.85 [a]
Defoliation	Gevgelija	444.66 [a]	1628.57 [a]	226.12 [a]	1.73 [a]	0.415 [a]	29.46 [a]	59.97 [a]	10.56 0[a]
	Veles	483.50 [a]	2332.90 [a]	321.77 [a]	2.48 [a]	0.410[a]	29.03 [a]	60.95 [a]	10.020 [a]
	Skopje	463.50 [a]	2165.00 [a]	332.21 [a]	2.06 [a]	0.480[a]	31.68 [a]	56.56 [a]	11.750 [a]
6 bunches per vine	Gevgelija	537.44 [a]	2216.67 [a]	281.66 [b]	2.49 [b]	0.410	29.71 [a]	58.64 [a]	11.64 [a]
	Veles	593.56 [a]	2925.61 [a]	369.58 [ab]	3.44 [a]	0.487	31.19 [a]	56.15 [a]	12.66 [a]
	Skopje	583.33 [a]	2687.84 [a]	441.46 [a]	2.67 [ab]	0,430[a]	30.46 [a]	56.73 [a]	12.81 [a]
10 bunches per vine	Gevgelija	503.44 [a]	2075.55 [a]	295.52 [b]	2.16 [a]	0.430[a]	30.36 [a]	57.96 [a]	11.67 [a]
	Veles	555.66 [a]	2656.67 [a]	359.90 [ab]	2.98 [a]	0.415[a]	29.24 [a]	60.59 [a]	10.17 [a]
	Skopje	541.33 [a]	2672.53 [a]	439.91 [a]	2.55 [a]	0.433[a]	30.58 [a]	56.57 [a]	12.77 [a]

Note: a, b, c – Duncan significance level of 0.05; GAE – gallic acid equivalent; CE – catechin equivalent.

Table 6

Statistical comparative analysis of the content of phenolic substances in the grapes of the Vranec variety by variants and years, average for the period 2013–2015

Years	Phenolic fractions			Colour characteristics				
	Total anthocyanins, mg/L	Total phenolics, mg GAE/L	Total flavan-3-ols, mg CE/L	Color intensity, AU	Hue, AU	Yellow color, %	Red color, %	Blue color, %
Control								
2013	435.44 ^{ab}	2414.44 ^{a}	313.83 ^{a}	2.09 ^{a}	0.406 ^{a}	30.55 ^{ab}	54.92 ^{ab}	14.53 ^{a}
2014	365.66 ^{b}	1625.96 ^{b}	224.94 ^{a}	1.57 ^{a}	0.463 ^{a}	32.64 ^{a}	54.46 ^{b}	12.89 ^{b}
2015	476.11 ^{a}	2064.82 ^{ab}	293.29 ^{a}	1.89 ^{a}	0.453 ^{a}	28.27 ^{b}	62.69 ^{a}	9.04 ^{c}
Defoliation								
2013	463.88 ^{ab}	2042.16 ^{a}	293.36 ^{a}	2.08 ^{a}	0.435 ^{a}	30.06 ^{ab}	59.16 ^{ab}	10.78 ^{ab}
2014	397.66 ^{b}	1883.49 ^{a}	271.62 ^{a}	2.13 ^{a}	0.423 ^{a}	31.75 ^{a}	55.78 ^{b}	12.46 ^{a}
2015	530.11 ^{a}	2200.82 ^{a}	315.11 ^{a}	2.04 ^{a}	0.447 ^{a}	28.36 ^{b}	62.55 ^{a}	9.09 ^{b}
6 bunches per vine								
2013	557.33 ^{b}	3033.66 ^{a}	400.76 ^{a}	3.17 ^{a}	0.467 ^{a}	32.41 ^{a}	54.00 ^{b}	13.59 ^{a}
2014	487.33 ^{c}	2188.46 ^{b}	390.44 ^{a}	2.76 ^{a}	0.393 ^{a}	30.12 ^{a}	55.77 ^{b}	14.11 ^{a}
2015	669.66 ^{a}	2607.98 ^{ab}	301.50 ^{a}	2.67 ^{a}	0.467 ^{a}	28.84 ^{a}	61.75 ^{a}	9.41 ^{b}
10 bunches per vine								
2013	494.00 ^{b}	2749.66 ^{a}	401.28 ^{a}	2.79 ^{a}	0.430 ^{a}	31.27 ^{a}	54.83 ^{b}	13.90 ^{a}
2014	466.33 ^{b}	2179.60 ^{a}	346.01 ^{a}	2.38 ^{a}	0.403 ^{a}	31.14 ^{a}	56.22 ^{b}	12.65 ^{a}
2015	635.33 ^{a}	2480.76 ^{a}	374.31 ^{a}	2.36 ^{a}	0.450 ^{a}	28.22 ^{a}	62.74 ^{a}	8.93 ^{b}

Note: a, b, c – Duncan significance level of 0.05; GAE – gallic acid equivalent; CE – catechin equivalent.

Content of phenolic substances in the wines of Vranec variety by variants and regions, average for the period 2013–2015

The statistical comparative analysis of the content of phenolic substances in the wine of the Vranec variety analysed by regions showed that for most of the indicators the established differences were not statistically significant (Table 5).

The values for the total anthocyanins content was higher in the variants with yield normation, 6 and 10 grape bunches per vine. In the variants, the highest anthocyanins content were found in the wine obtained from grapes grown in the Veles region – control (446.44 mg/L), defoliation (483.50 mg/L), with 6 grapes bunches per vine (593.56 mg/L) and with 10 grape bunches per vine (555.66 mg/L). In the Veles region the highest anthocyanins accumulation was achieved in the normation variant with 6 grape bunches per vine. The total phenols content in the control and the variant with 10 grapes bunches per vine was the highest in the wine from the Skopje region (2262.07 mg GAE/L and 2672.53 mg GAE/L), and in the variants with defoliation and 6 grapes bunches per vine in Veles (2332.90 mg GAE/L and 2925.61 mg GAE/L). The wine variant (Veles region) with 6 grapes bunches per vine had the highest total phenols and total flavan-3-ols content, more than all the other normation variants. All the differences were statistically proven. In the control variant, their quantity was the highest in Veles region – 308.78 mg CE/L and in the other variants in Skopje region (defoliation – 332.21 mg CE/L, with 6 bunches per vine – 441.46 mg CE/L and with 10 bunches per vine – 439.91 mg CE/L). The highest values for the colour intensity was determined in the wines from Veles region in all studied variants. The proven differences were determined only in the control and 6 grapes bunches per vine variants. The obvious trends in the change of the quantities of the analyzed studied indicators were significantly weaker in the others – hue, yellow colour, red colour, blue colour, but since the differences between the variants and the regions were unproven and as absolute values very similar so it was assumed that they did not change the wine quality.

Content of phenolic substances in the grapes of the Vranec variety by variants and years, average for the period 2013–2015

The experimental data results, related with the content of phenolic substances in the wine of the Vranec variety presented by years and variants, for all indicators (total anthocycnins, total phenols and total flavan-3-ols) had the lowest obtained values in 2014 (Table 6). Again, over the years, the largest amounts of these indicators were achieved in the wine samples obtained from the variants with grape normation per vine.

Total content of anthocyanins was the highest in wines from 2015 harvest in all variants – control (476.11 mg/L), defoliation (530.11 mg/L), with 6 bunches per vine (669.66 mg/L) and with 10 bunches per vine (635.33 mg/L). The total phenols content was more in 2013 harvest, except for the defoliation variant – 2015 harvest. For this indicator, the differences in the obtained results between the years were statistically proven for the control and 6 bunches per vine variants. The same trend was observed of the total flavan-3-ols amount. The data for the colour intensity and hue of the wine had very close values. The statistical analysis showed unproven difference in any of the variants. Over the years, there were some differences in the percentage of the wine colours – yellow, red and blue, which were not statistically proven only for yellow colour in the variants with 6 and 10 bunches per vine. Red predominated, followed by yellow and blue colour. Depending on the year (harvest), the highest percentage of red colour was achieved in 2015 in all variants, percentage of yellow

colour in 2014 for control and defoliation, and percentage of blue colour in 2013 for control and 10 bunches per vine.

The data in the present study were in correlation with the study of Mateus et al. (2001), who found that grapes grown at higher altitudes synthesized higher levels of anthocyanins in grape skins, while procyanidins in skins and seeds were accumulated in higher amounts at lower altitudes. Coklar (2017) also found that as altitude increased from 1000 m to 1500 m, the accumulation of phenolics and antioxidant activity increased in berries, seeds and skins of Ekşikara grape (*Vitis vinifera* L) from the Konya region, Turkey. The data of our study correlate also with the research of Nascimento et al. (2016), who investigated the phenolic profile of tropical wines produced from grapes grown in northeastern Brazil at an altitude of 1100 m. Red wines from four varieties (Cabernet Franc, Pinot Noir, Malbec and Cabernet Sauvignon) were studied and it was found a range of total phenols from 1024.21 mg GAE/L to 2958.96 mg GAE/L. The total anthocyanins ranged from 28.47 mg/L to 260.92 mg/L. Osorio-Macias et al. (2018) studied the phenolic profile of 34 red wines from different countries and found a variation in the total phenolic content from 1600.00 to 3500.00 mg GAE/L, with results confirming that wines obtained from grapes grown at altitudes above 1500 m showed higher levels of total phenolic compounds. De Oliveira et al. (2019) found that Syrah grapes grown at an altitude of 1100 m in the Bahia region of Brazil showed high levels of anthocyanins and condensed tannins in the skins.

Conclusions

Such conclusions from the conducted research, the following conclusions could be made:

- The content of phenolic substances in the grape berries skins and seeds of the Vranec variety in all studied variants and regions, expressed as total anthocyanins, total phenols and flavan-3-ols had statistically proven the highest content when the vine had normated yield with 10 grapes bunches per vine. The higher altitude showed to increase the amount only of total phenols in the berries skins and seeds, while the content of all other indicators were the highest in the grapes from the Veles region. The studied indicators in the berries skins and seeds of the grapes from all three regions were strongly depended on the external climatic factors in the individual years.
- There were no significant differences in the content of phenolic substances – total anthocyanins and total phenols in the wine of the Vranec variety by variants and regions, as their quantities were higher in the variants with normation of grape bunches. The wine colour intensity was characterized by the highest value in the variant with 6 grape bunches per vine. In the different years in the levels of the indicators hue, yellow colour, red colour and blue colour some differences were noticed. The wines colour intensity and hue indicators had very close values and the differences between them were not statistically proven in any variant. The average altitude in the Veles region provided the best soil and climatic conditions for the Vranec variety growing and for obtaining of the highest quality grapes and wine.

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