Polyphenolic Characterization of Vranec, Merlot and Cabernet Sauvignon Grapes and Wines from the Black River Basin in R. Macedonia

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Abstract - **In order to characterize polyphenols in tree grape varieties, Vranec, Merlot and Cabernet Sauvignon, grapes were sampled from representative vineyards from Black River Basin at harvest time during 2017 and 2018. On average, the highest content of low-molecular mass proanthocyanidins (LMP), high-molecular mass proanthocyanidins (HMP) and anthocyanins in the year 2017 was found in the Cabernet Sauvignon grapes. The highest content of anthocyanins in the year 2017 and the lowest content of LMP in years 2017 and 2018 was found in Vranac grapes. The highest content of extractable anthocyanins in the skin of grape berries was found in Vranec grapes in 2017 and 2018, followed by Cabernet Sauvignon and Merlot. On average, the Cabernet Sauvignon wines had a higher content of total polyphenols, anthocyanins in 2017 and 2018 respectively, LMP and HMP, compared to the Merlot and Vranec wines. The results obtained are important to better understand the polyphenolic potential of Macedonian red grape varieties in Black River Basin as possibilities and perspectives for the development of tourism.**

Keywords: Vranec, Merlot, Cabernet Sauvignon, grape varieties, polyphenols

I. INTRODUCTION

Wine production and growing grape vine in Macedonia goes back to ancient times due to the geographical position and warm climate of the area. Macedonian wines are unique amongst European wines for being made with very little, if any, additional sugar or sulphite preservatives. Preserved mostly by the grapes'own natural sugar, almost all Macedonian wines are dry rather than sweet. Now it is the second most important export agro-food product after the tobacco. In 2010 there were 86 registered wineries in Macedonia with a total capacity of ca. 2 million hL per year and the total capacity of bottling is around 0.65 million hL per year [1]. According to the climate characteristics and classification of the EU, Republic of Macedonia is classified as one geographic area for vine growing (zone III-C-b), that includes three viticultural regions divided into sixteen sub regions with specific favourable natural condition for production of quality wine. Red wine represents approx. 60% of the national production and includes both autochthonous and international grape varieties such as Vranec, Kratosija, Merlot and Cabernet Sauvignon. Vranec is the Macedonia`s best known red grape, that are also cultivated in Montenegro, Bosnia and Hercegovina, Croatia and Serbia and are considered as indigenous varieties of the Western Balkan countries [2]. Vranec grapes are considered to have a strong polyphenol potential [3] and high colour potential [4].

Wine is a complex product composed of various compounds. One of the most important components of red wines that influence their quality parameters are polyphenols. Some sensory attributes such as colour, body, astringency and bitterness are directly associated with the composition of anthocyanins and proanthocyanidins [5]. Anthocyanins are responsible for red colour of wines and proanthocianidins (also known as condensed tannins) for the colour stability, the taste of bitterness and mouthfeel of astringency [6]. Anthocyanins are only present in grape skins whereas proanthocyanidins are present in skins, seeds and stems [7]. The grape phenolic composition and content are affected by several factors such as grape variety, climate, soil, place of growing and vine cultivation, the winemaking techniques, etc. [8].

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The estimation of the polyphenolic potential allows the identification of differences in the polyphenol composition and provides factors with which to evaluate the oenological potential of the grape. There are many publications in which the phenolic composition of wines produced from the international grape varieties Merlot and Cabernet Sauvignon is studied [9, 10] but, there has been almost no detailed research on the phenolic profile of wines obtained from these grape varieties cultivated in Republic of Macedonia. There are also little data on the phenolic composition of Vranec wines which is indigenous Balkan variety [3, 11].

The aim of this research was to characterize Vranec, Merlot and Cabernet Sauvignon grapes from different wine regions in Macedonia according to the extractable polyphenol content of the grape berries. The content of extractable total polyphenols, total anthocyanins, low-molecular mass proanthocyanidins (LMP) and high-molecular mass proanthocyanidins (HMP) have been determined separately in the skin and seeds of grape berries, by using an extraction method that simulates the process of maceration [12]. The same polyphenol groups were evaluated in wines produced from the same grape.

II. EXPERIMENTAL

A. Chemicals and reagents

Ethanol, methanol, sodium hydroxide, sodium bisulphite, hydrochloric acid and L(+)-tartaric acid were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Merck (Darmstadt, Germany). Deionized water was obtained from an ultra pure water system TKA Lab micro (Niederelbert, Germany). The reagents Folin-Ciocalteu and vanillin were from Merck.

B. Grape sampling

Grapes were sampled at the time of their technological maturity, at the end of September in 2017 and 2018. Grape samples of Vranec (n = 5), Merlot (n = 6) and Cabernet Sauvignon (n = 6) were collected from vineyards located in the Black River Basin in R. Macedonia. The characteristics of the vineyards from which grapes were sampled are presented in Table 1. Approximately 50 kg of grapes from each vineyard were representively sampled.

Table 1. The characteristics of the vineyards from which grapes were sampled

Locality	Rootstock	Planting year	Plant density	Row orientation
		Vranec (n = 5)		
1	Kober 5bb	2003	2.5 x 1.0 m	north - south
2	Kober 5bb	2005	2.5 x 0.9	north - south
3	Kober 5bb	1995	2.6 x 0.8	north - south
4	Kober 5bb	2007	2.4 x 1.0	north - south
5	Kober 5bb	1989	2.5 x 0.9	north - south
		Merlot (n = 6)		
2	Kober 5bb	2005	2.5 x 0.9	north - south
4	Kober 5bb	2007	2.4 x 1.0	north - south
5	Kober 5bb	1989	2.5 x 0.9	north - south
7	Kober 5bb	2000	2.5 x 0.7	north - south
9	Teleki SO4	2006	2.6 x 0.7	north - south
10	Kober 5bb	1996	2.6 x 0.8	east - west
		Cabernet Sauvignon (n = 6)		
2	Kober 5bb	2005	2.5 x 0.9	north - south
4	Kober 5bb	2007	2.4 x 1.0	north - south
5	Kober 5bb	1989	2.5 x 0.9	north - south
6	140 Ruggeri	2006	2.5 x 0.9	north - south
7	Kober 5bb	2000	2.5 x 0.7	north - south
8	Kober 5bb	2006	2.6 x 0.8	north - south

C. *Determination of grape physicochemical characteristics*

Grape samples of 100 berries each were representatively collected from the 50 kg sample and weighed. Total soluble solids (TSS), titratable acidity (TA) and the pH of the berry juice were determined by International Organisation of Vine and Wine procedures [13].

D. Polyphenol extraction from grapes

A selective extraction of polyphenols from the skins and seeds of grape berries that simulates the maceration process of red wines was used [14, 15]. Skins and seeds of 200 g of randomly samled grape berries were manually separated and separately extracted for five days at 30°C in a 200 mL solution consisting of ethanol:water (12:88 v/v), 100 mg/L of SO2, 5 g/L tartaric acid and a pH value adjusted to 3.2 with NaOH. Skins and seeds were removed from the hydro-alcoholic solution after five days and the skin extract was centrifuged for 10 min at 3500 $\times$ g. Extracts were poured into dark glass bottles, flushed with nitrogen and stored at 4°C until required for spectrophotometric analyses. Analyses were conducted three months later.

E. Wine samples. Micro-vinification

Wines were prepared with using a standardised microvinification procedure. The harvestet grapes (50 kg), which originated from different localities, were vinified in the experimental laboratory of IMACO Winery Stip, R. Macedonia, during the 2017 and 2018 seasons. Grapes were processed using commercial grape destemmer/crusher, homogeneously and transferred into stainless-steel fermentation tanks for the maceration and fermentation. The obtained mash was treated with 60 mg/L SO2 (added in a form of 5% sulphurous acid) before inoculation with 200 mg/kg selected yeast (*Saccharomyces cerevisiae*) NEUTRE SC (Lallemand). Maceration lasted for five days at a temperature between 25 and 28°C. The cap was punched down twice a day until completion of fermentation. Wines were decanted at the end of alcoholic fermentation and were stored at room temperature. The new wines were inoculated with selected malolactic bacteria (Lalvin) and after malolactic fermentation, the wines were decanted again and treated with 30 mg/L SO_2. Polyphenols in wines were analysed three months after the fermentation.

F. Spectrophotometric measurements

Analyses of polyphenols were performed using a spectrophotometer (Cary 50 SCAN, Varian, Inc., USA). All measurements were performed in triplicates.

G. Total polyphenols

The total polyphenols (TP) were determined by the reduction of Folin-Ciocalteu reagent to blue pigments caused by polyphenols in alkaline solution. Other compounds such as sugars, organic acids, amino acids and free SO_2, were removed by clean-up of samples (grape extracts and wine) using classic (0.35 g) C-18 columns, that interfere with the assay [16]. When the absorbance was between 0.3 and 0.6 AU (the linear response range), results were expressed against the corresponding blank as (+)-catechin: 186.5 $\times$ A $\times$ d in mg/kg grape fresh mass (FM) or in mg/L of wine; A = absorbance and d = sample dilution.

H. High-molecular mass proanthocyanidins

The content of high-molecular mass proanthocyanidins (HMP) were evaluated by transformation into cyanidin [17]. When the absorbance was between 0.3 and 0.6 AU, results were expressed against the corresponding blank as cyaniding chloride: 1162.5 $\times$ ΔA $\times$ d in mg/kg grape FM or in mg/L of wine; ΔA = difference in absorbance between sample and blank, and d = sample dilution. This method provides a good estimation for the evaluation of HMP [18].

I. Low-molecular mass proanthocyanidins – index of vanillin

The content of low-molecular mass proanthocyanidins (LMP) were analysed according to the optimized and controlled vanillin-HCl method of Broadhurst and Jones [19], following the conditions described by Di Stefano *et al.* [17]. This method provides an estimation of the free carbon 6 and carbon 8 of the A-ring of both catechins and proanthocyanidins. This index decreases with the increase in polymerization, because mainly carbon 6 and carbon 8 are involved in polymerization bonds. When the absorbance was between 0.2 and 0.4 AU, the LMP were evaluated as (+)-catechin = 290.8 $\times$ ΔA $\times$ d in mg/kg grape FM or in mg/L of wine; ΔA = difference in absorbance between sample and blank, and d = sample dilution. The method provides a good estimation of free flavanols and a low degree of polymerised flavanols.

J. Total anthocyanins

Determination of total anthocyanins (TA) was performed using the method described by Di Stefano *et al.* [17]. Samples were diluted with a solution consisting of ethanol:water:HCl = 69:30:1 and the absorbance was measured at 540 nm. Because of the lack of malvidin-3-glucoside, the total anthocyanins content was calculated using the following equation proposed by Di Stefano *et al*. [17]: TA = A × 16.7 × d in mg/kg grape FM or in mg/L of wine as malvidin-3-glucoside equivalents.; A = absorbance at 540 nm, and d = sample dilution.

K. Statistical analysis

For comparison of the means, ANOVA and Tukey`s honest significant difference (HSD) test were applied at the 95% significance level ($p < 0.05$). Statistical analysis was performed using the Statgraphics Centurion XVI program (Manugistics Inc., USA).

III. RESULTS AND DISCUSSION

A. Physicochemical characteristics of Vranec, Merlot and Cabernet Sauvignon grapes at the time of harvest

The physicochemical characteristics of Vranec, Merlot and Cabernet Sauvignon grapes at the time of harvest from different vineyard locations in the Black River Basin in Republic Macedonia in 2017 and 2018 are listed in Table 2.

Table 2. Phisicochemical characteristics (SKIN/BERRY - percent of skin by berry weight, MB – Mass of 100 berries, TSS – Total soluble solids, TA – Titratable acidity, as tartaric acid) of Vranec, Merlot and Cabernet Sauvignon grapes at the time of harvest from different vineyard locations (L) in the Black River Basin in Republic Macedonia

	2017				2018			
Locality	Skin/ Berry (%)	MB (g)	TSS (°Brix)	TA (g/L)	Skin/ Berry (%)	MB (g)	TSS (°Brix)	TA (g/L)
Vranec (n = 5)								
1	9.1	272	22.8	5.2	10.4	234	20.8	5.1
2	9.4	253	22.3	5.3	10.7	226	21.9	4.9
3	9.7	234	23.6	5.1	10.9	219	22.4	5.3
4	9.3	263	19.9	5.5	10.3	243	18.9	4.5
5	10.1	223	20.4	5.4	10.7	223	22.1	4.7
Mean	**9.5 b**	**249 a**	**21.8**	**5.3 b**	**10.6 b**	**229 a**	**21.2 b**	**4.9 b**
Merlot (n = 6)								
2	14.9	104	23.7	6.8	14.6	112	23.2	6.2
4	13.5	124	24.8	6.7	13.1	125	24.3	6.5
5	14.1	118	24.1	5.8	14.1	121	24.1	5.9
7	13.2	135	23.4	5.6	13.1	139	23.2	5.7
9	12.8	139	23.1	5.4	12.5	141	23.6	5.6
10	14.3	106	24.3	5.9	14.2	118	22.6	5.5
Mean	**13.8 a**	**121 b**	**23.9**	**6.0 a**	**13.6 a**	**126 b**	**23.5 a**	**5.9 ab**
Cabernet Sauvignon (n = 6)								
2	14.5	98	22.8	6.5	14.2	112	22.6	6.7
4	13.1	117	23.9	5.9	13.1	123	23.1	6.4
5	13.7	114	23.5	5.8	13.4	119	23.6	5.9
6	12.9	130	23.1	5.5	12.7	133	22.3	5.8
7	12.5	133	22.8	6.2	12.1	136	22.7	6.5
8	13.7	128	23.7	5.5	13.7	115	23.1	5.9
Mean	**13.4 a**	**120 b**	**23.3**	**5.9 a**	**13.2 a**	**123 b**	**22.9 ab**	**6.2 a**
Sign. F	***	***	n.s.	***	***	***	*	*

ANOVA was used to compare data ($^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$, n.s. not significant). Different lower-case letters indicate significant differences of means between varieties using Tukey`s HSD test ($p \leq 0.05$).

The average mass of 100 berries did not differ significantly between the Merlot and Cabernet Sauvignon varieties in 2017 (121 g and 120 g, respectively) and 2018 (126 g and 123 g, respectively) (Table 2). The average berry mass of Vranec was significantly higher (249 g and 229 g in 2017 and 2018 respectively) than that of the Merlot and Cabernet Sauvignon in both years. Grape berries of Vranec are almost twice as heavy as those of Merlot and Cabernet Sauvignon and have a significantly lower percentage of skins by berry weight (9.5% and 10.6% in 2017 and 2018 respectively) compared to Merlot (13.8% and 13.6%) and Cabernet Sauvignon grapes

(13.4% and 13.2% in 2017 and 2018 respectively) (Table 2). Average TSS content (Table 2) at the time of sampling was the highest in the grape juice of Merlot (23.9°Brix and 23.5°Brix in 2017 and 2018 respectively), followed by Cabernet Sauvignon (23.3°Brix and 22.9°Brix in 2017 and 2018 respectively). Vranec grape juice reported the lowest TSS values (21.8°Brix and 21.2°Brix in 2017 and 2018 respectively).

The acidity of the grape juice was low, as is typical for warm climates such as the Macedonian region. Significant lower titratable acidity (TA) was assessed in the Vranec grapes (5.3 g/L and 4.9 g/L in 2017 and 2018, respectively) compared to the Merlot grapes (6.0 g/L and 5.9 g/L), even if not significantly different from Cabernet Sauvignon (5.9 g/L and 6.2 g/L).

B. Contents of extractable total polyphenols, low- and high-molecular mass proanthocyanidins and total anthocyanins in grapes of Vranec, Merlot and Cabernet Sauvignon

The content of extractable polyphenols in Vranec, Merlot and Cabernet Sauvignon in the skin and seeds of grape berries at the time of harvest from different vineyard locations in the Black River Basin in Republic Macedonia in 2017 and 2018 is shown in Table 3.

Table 3. Content of extractable polyphenols (TP – total polyphenols, TA – total anthocyanins, LMP – low-molecular mass proanthocyanidins, HMP – high-molecular mass proanthocyanidins) in Vranec, Merlot and Cabernet Sauvignon fresh grape berries from different vineyard locations (L) in the Black River Basin in Republic Macedonia

	2017				2018			
L	TP (mg/kg (+) catechin)	TA (mg/kg)	LMP (mg/kg (+) catechin)	HMP (mg/kg cyanidin chloride)	TP (mg/kg (+) catechin)	TA (mg/kg)	LMP (mg/kg (+) catechin)	HMP (mg/kg cyanidin chloride)
				Vranec (n = 5)				
1	1 816	1 161	857	1 189	1 676	985	756	1 526
2	2 458	1 329	1 397	1 456	1 958	1 136	1 287	1 713
3	2 479	1 256	1 489	1 512	2 159	1 073	1 046	1 803
4	1 785	1 073	1 125	1 275	1 612	992	1 153	1 329
5	2 497	1 196	1 317	1 503	2 075	1 169	1 053	1 139
Mean	**2 207 ab**	**1 203 a**	**1 237 b**	**1 387 b**	**1 896 ab**	**1 071 a**	**1 059 b**	**1 502 b**
				Merlot (n = 6)				
2	1 531	723	1 329	1 285	1 113	579	974	1 578
4	1 812	851	1 573	1 491	1 385	673	1 379	1 735
5	1 785	983	1 498	1 672	1 347	739	1 278	1 812
7	1 516	792	1 297	1 351	1 176	814	1 082	1 429
9	1 604	812	1 325	1 579	1 258	548	1 496	1 588
10	1 286	573	1 726	1 556	963	997	1 525	1 560
Mean	**1 589 b**	**789 b**	**1 458 ab**	**1 489 b**	**1 207 b**	**725 b**	**1 289 b**	**1 617 b**
				Cabernet Sauvignon (n = 6)				
2	1 956	924	2 065	2 285	1 873	689	1 896	3 219
4	1 985	1 057	1 579	1 983	1 984	788	1 659	3 087
5	3 149	1 378	2 572	3 491	2 471	1 186	2 051	2 643
6	3 457	1 294	2 248	3 186	2 753	1 093	1 799	3 157
7	2 889	1 217	1 779	2 251	1 752	964	1 589	2 042
8	2 254	748	1 307	1 876	1 809	1 262	1 578	1 722
Mean	**2 615 a**	**1 103 a**	**1 925 a**	**2 512 a**	**2 107 a**	**997 ab**	**1 762 a**	**2 645 a**
Sign. F	*	***	*	***	**	***	***	**

ANOVA was used to compare data ($^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$). Different lower-case letters indicate significant differences of means between varieties using Tukey`s HSD test ($p \leq 0.05$).

The mean content of total extractable polyphenols of grape berries was the highest in Cabernet Sauvignon in both years (2 615 mg/kg and 2 107 mg/kg in 2017 and 2018, respectively) and was significantly higher compared to Merlot grapes (1 589 mg/kg and 1 207 mg/kg). The mean content of total extractable polyphenols in Vranec grapes was intermediate (2 207 mg/kg and 1 896 mg/kg in 2017 and 2018, respectively). The results presented here are in compliance with the extractable total polyphenol content in red grape varieties grown in

Montenegro and Slovenia. Pajovic *et al.* [20] showed that the mean content of total extractable polyphenols in the skin and seeds of grape berries from different vineyard locations in Montenegro in a two-year study was the highest in Cabernet Sauvignon (2 705 mg/kg and 2 017 mg/kg). Vrhovsek *et al.* [21] showed that the average extractable total polyphenol content in grapes of red *Vitis vinifera* varieties grown in Slovenia in a two-year study was between 1 100 and 2 100 mg/kg grape fresh mass (FM), and the highest content was reported in Cabernet Sauvignon (2 000 mg/kg to 2 100 mg/kg). Furthermore, Vranec and Merlot showed a higher amount of total polyphenols compared to some Australian grape cultivars [22].

The mean content of total extractable anthocyanin of the grapes was the highest in Vranec in both years (1 203 mg/kg and 1 071 mg/kg in 2017 and 2018, respectively) and slightly lower in Cabernet Sauvignon (1 103 mg/kg and 997 mg/kg in 2017 and 2018, respectively). Merlot grapes proved to have a significantly lower content of extractable anthocyanins compared to Vranec and Cabernet Sauvignon in both years (789 mg/kg and 725 mg/kg in 2017 and 2018, respectively). The extractable anthocyanin content in Montenegrin red grapes was 960 to 1 113 mg/kg in Vranec, 861 to 1035 mg/kg in Cabernet Sauvignon and 456 to 517 mg/kg in Kratosija [20], whereas in 14 Sardinian red grape varieties the total extractable anthocyanin content ranged from 800 to 2 000 mg/kg [15]. In *Vitis vinifera* grape varieties, anthocyanins are located mainly in the skin of the grape berries. The highest content of extractable anthocyanins in the skin of grapes (Fig. 1) was found in Vranec grapes (9 895 mg/kg and 8 278 mg/kg skin FM in 2017 and 2018, respectively), followed by Cabernet Sauvignon (7 126 mg/kg and 6 054 mg/kg skin FM) and Merlot (5 982 mg/kg and 5 273 mg/kg skin FM) (Fig. 1). Furthermore, Vranec and Merlot showed also a similar amount of anthocyanins in skins compared to some Italian Merlot grapes analyzed in different seasons [23]. The Macedonian Vranec and Merlot grapes contained a lower amount of anthocyanins, compared to Cabernet Sauvignon and Merlot skin and seed extracts from Bordeaux grapes [24].

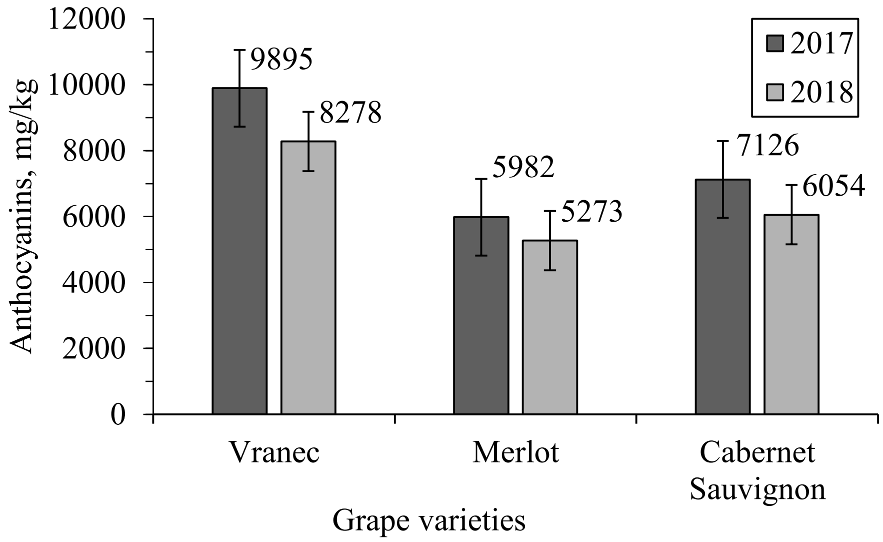

Figure 1. Content of extractable anthocyanins in the skin of grape berries (mg/kg skin fresh mass) of different varieties: Vranec (n = 5), Merlot (n = 6) and Cabernet Sauvignon (n = 6). The error bars represent the standard deviation of the content from different locations from the Black River Basin in Republic Macedonia

The average extractable LMP content was the highest in Cabernet Sauvignon grapes (1 925 mg/kg and 1 762 mg/kg in 2017 and 2018, respectively) and significantly lower contents were found in Merlot grapes (1 458 mg/kg and 1 289 mg/kg in 2017 and 2018, respectively) and in Vranec grapes (1 237 mg/kg and 1 059 mg/kg in 2017 and 2018, respectively). Similarly, the average extractable HMP content was significantly higher in the Cabernet Sauvignon grapes (2 512 mg/kg and 2 645 mg/kg in 2017 and 2018, respectively) compared to Merlot and Vranec. The average extractable HMP content did not differ significantly between the Merlot grapes (1489 mg/kg and 1617 mg/kg in 2017 and 2018, respectively) and Vranec grapes (1387 mg/kg and 1502 mg/kg in 2017 and 2018, respectively). The average extractable HMP contents in Cabernet Sauvignon grapes were similar with results obtained by Vrhovsek *et al.*[21] and Mattivi *et al.* [12], who reported contents of HMP ranging between 2 300 to 2 700 mg/kg and 2 500 and 2 800 mg/kg of grape FM, respectively.

C. Contents of total polyphenols, low- and high-molecular mass proanthocyanidins and total anthocyanins in Vranec, Merlot and Cabernet Sauvignon wines

Contents of total polyphenols, low- and high-molecular mass proanthocyanidins and total anthocyanins in three-months-old Vranec, Merlot and Cabernet Sauvignon wines from different vineyard locations in Macedonian region in 2017 and 2018 are shown in Table 4.

Table 4. Content of polyphenols (TP – total polyphenols, TA – total anthocyanins, LMP – low-molecular mass proanthocyanidins, HMP – high-molecular mass proanthocyanidins) in three-month-old wines from Vranec, Merlot and Cabernet Sauvignon from the 2017 and 2018 vintages from different vineyard locations (L) in the Black River Basin in Republic Macedonia

	2017				2018			
L	TP (mg/L (+) catechin)	TA (mg/L)	LMP (mg/L (+) catechin)	HMP (mg/L cyanidin chloride)	TP (mg/L (+)catechin)	TA (mg/L)	LMP (mg/L (+) catechin)	HMP (mg/kg cyanidin chloride)
Vranec (n = 5)								
1	1 003	993	341	852	996	829	326	954
2	1 110	1 176	559	894	1 135	1 013	521	1 012
3	1 488	1 089	606	1 052	1 403	924	427	1 078
4	1 149	895	438	973	978	792	378	752
5	1 290	737	316	994	1 273	822	338	684
Mean	**1 208**	**978 a**	**452**	**953 b**	**1 157**	**876 a**	**398**	**896 b**
Merlot (n = 6)								
2	935	612	479	893	893	498	423	1 025
4	1 025	751	563	995	927	583	578	1 235
5	1 307	908	512	1 471	1 209	692	534	1 301
7	1 024	712	475	1 219	1 006	734	429	954
9	1 219	802	598	1 075	990	588	635	1 023
10	958	433	739	1 139	963	841	473	798
Mean	**1 078**	**703 c**	**561**	**1 132 b**	**998**	**656 ab**	**512**	**1 056 ab**
Cabernet Sauvignon (n = 6)								
2	1 159	769	629	1 264	1 058	632	487	1615
4	1 271	915	572	1 332	1 173	593	598	1 784
5	1 527	1 107	823	2 127	1 417	742	732	978
6	1 432	852	764	1 895	1 389	683	659	1452
7	1 329	901	598	1 413	1 187	794	512	1 076
8	1 352	538	652	705	1 444	1 080	480	853
Mean	**1 345**	**847 b**	**673**	**1 456 a**	**1 278**	**754 a**	**578**	**1 293 a**
Sign. F	n.s.	***	n.s.	**	n.s.	***	n.s.	*

ANOVA was used to compare data ($^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$, n.s. not significant). Different lower-case letters indicate significant differences of means between varieties using Tukey`s HSD test ($p \leq 0.05$).

The average content of total polyphenols in the Cabernet Sauvignon wines was higher in both years (1 345 mg/L and 1 278 mg/L in 2017 and 2018, respectively) compared to the Vranec wines (1 208 mg/L and 1 157 mg/L) and Merlot wines (1 078 mg/L and 998 mg/L), although the differences were not statistically significant.

The anthocyanin content was the highest in Vranec wines in both years (978 mg/L and 876 mg/L in 2017 and 2018, respectively) and slightly lower in Cabernet Sauvignon wines (847 mg/L and 754 mg/L in 2017 and 2018, respectively) and Merlot wines (703 mg/L and 656 mg/L in 2017 and 2018, respectively).

The average LMP content was the highest in the Cabernet Sauvignon wines in both years (673 mg/L and 578 mg/L in 2017 and 2018, respectively) and slightly lower in Merlot wines (561 mg/L and 512 mg/L in 2017 and 2018, respectively). The lowest LMP content, although not statistically significant, was found in Vranec wines (452 mg/L and 398 mg/L in 2017 and 2018, respectively).

The average HMP content was the highest in the Cabernet Sauvignon wines in both years (1 456 mg/L and 1 293 mg/L in 2017 and 2018, respectively), followed by Merlot wines (1 132 mg/L and 1 056 mg/L in 2017 and

2018, respectively), and Vranec wines (953 mg/L and 896 mg/L in 2017 and 2018, respectively). The trend in the polyphenol contents in both years determined in wines was correlated well with the contents found in the grapes (Table 3). Lower contents of LMP were found in wines than in grape extracts, thus concluding that the extraction of LMP was less efficient in vinification than in the grape extraction process.

IV. CONCLUSIONS

Polyphenols play an important role in the organoleptic characteristics of wine. The evaluation of polyphenol compounds in grapes and wines of Macedonian red grape varieties provided a technological characterization that can be used by winegrowers and winemakers. Cabernet Sauvignon and Vranec grapes proved to be highest in extractable total polyphenols during 2017 and 2018. The same trend was also found in wines. Merlot grapes showed lowest polyphenol potential in comparison to Cabernet Sauvignon and Vranec. Vranec grapes and wines showed the highest anthocyanin content that gave the wines an intense red colour. The average LMP and HMP contents in the grape skins and wines were the highest in the Cabernet Sauvignon. The results obtained are important to better understand the polyphenolic potential of Macedonian red grape varieties. All these scientific results will be used as a basis for opportunities and perspectives for tourism development in the Black River Basin.

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