Dimitrov, D., Yoncheva, T., Iliev, A.. (2023): Study on the content of phenolic compounds, anthocyanins and antioxidant activity of red grapes and wines from Central Northern Bulgaria. Agriculture and Forestry, 69 (3): 21-34. doi:10.17707/AgricultForest.69.3.02

DOI: 10.17707/AgricultForest.69.3.02

Dimitar DIMITROV*[1], Tatyana YONCHEVA[1], Anatoli ILIEV[1]

STUDY ON THE CONTENT OF PHENOLIC COMPOUNDS, ANTHOCYANINS AND ANTIOXIDANT ACTIVITY OF RED GRAPES AND WINES FROM CENTRAL NORTHERN BULGARIA

SUMMARY

The content of phenolics and the antioxidant potential of grape must and wines from three varieties - Cabernet Sauvignon (introduced), Gamza (local) and Rubin (hybrid) grown in the terroir of the town of Pleven, Central Northern Bulgaria were investigated. The Rubin hybrid accumulated the most significant amount of phenols (TPC, FPC, NPC, and anthocyanins) identified in its grape must. This was reflected in the proved high antioxidant activity in the must of this variety. The highest content of TPC in the obtained wines from the studied varieties was found in Rubin. The results for the FPC of the wines tend to overlaped with the established anthocyanin content, namely in the order (Cabernet Sauvignon)>(Rubin)>(Gamza). It is well known that the anthocyanins are the part of FPC group. In this aspect our results were direct evidence that anthocyanins had a contribution to the formation of the total concentration of flavonoid phenolic compounds (FPC). The data on the established antioxidant activity in the red wines could be closely related to the NPC results of the wines. Gamza and Cabernet Sauvignon showed a close concentration presence of this group of phenols, which closely correlated with the values of the antioxidant activity found in their wines. The study proved high biological potential and good phenolic accumulation in grapes and wines of the studied varieties. The study enriches the field of viticultural and wine science with specific results on the biological potential of grapevine products from the *Vitis vinifera* L, under the conditions of a specific wine-growing region of Bulgaria.

Keywords: phenolic compounds, antioxidants, biological potential, grapes, wine.

[1]Dimitar Dimitrov (corresponding author: dimitar_robertov@abv.bg), Tatyana Yoncheva, Anatoli Iliev, Agricultural Academy, Institute of Viticulture and Enology, 1 “Kala Tepe” str., Pleven, BULGARIA

Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

Recieved:15/03/2023 *Accepted:05/07/2023*

INTRODUCTION

The defining of the phenolic composition of grapes and wines from different regions of the world is a field of diverse research in food science (Yildirim *et al*., 2005). The content of phenolic compounds can serve as an indicator of the terroir influence (the complex influence of the soil-climatic zone where the varieties are grown) (Pajović-Šćepanović *et al*., 2018; Pajović-Šćepanović *et al*., 2019). The correlation between phenolic accumulation in grapes and wines and their antioxidant activity has been proven and confirmed in different studies (Ginjom *et al.*, 2010; Di Lorenzo *et al*., 2019; Mitrevska *et al*., 2020; Rodriguez-Vaquero *et al.*, 2020).

Ghatak *et al*. (2011) investigated the phenolic composition and antioxidant activity of white, rosé and red wines from India. For the reds, the team found a variation in the total content of phenolic compounds from 3.10±0.02 mg/ml to 6.50±0.10 mg/ml and a high antioxidant activity of 84.60±1.10%. Mucaca *et al.* (2017) investigated the phenolic composition and antioxidant activity of tropical Brazilian Syrah and Cabernet Sauvignon red wines (harvests 2011, 2013 and 2014) from the São Francisco Valley. The team found a variation in total phenol compounds from 1528.90 mg/dm^3 to 4003.96 mg/dm^3. The anthocyanins ranged from 98.94 mg/dm^3 to 501.20 mg/dm^3, and the antioxidant activity was found to range from 58.02% to 95.70%. Osorio-Macias *et al.* (2017) studied total phenolic compounds in South American red wines and found a variation in their content from 1600.00 to 3500.00 mg/dm^3. Čeryova *et al.* (2021) investigated the phenolic content of Slovak wines from muscat varieties. The team found total phenolic content in the wines ranging from 262.10 mg/dm^3 to 568.00 mg/dm^3, total flavonoid content ranging from 24.80 mg/dm^3 to 169.10 mg/dm^3, and established antioxidant activity between wines ranging from 25.20% to 67.70%. Ismael (2018) determined the total phenolic compounds, flavonoid phenolic compounds and antioxidant activity of grapes from 7 red grapevine varieties grown in the Kurdistan Region, Iraq. They found a variation in the content of total phenolic compounds from 122.77 to 249.19 mg GAE/100g, the variation in the content of flavonoid phenolic compounds was from 584.23 to 288.55 mg/100 g, and the established antioxidant activity ranged from 41.79% to 92.30%. Büyüktuncel *et al.* (2014) determined by spectrophotometric method the total phenolic content of wines from the trade network and found a variation in the content of total phenols from 2599.90 to 4867.57 mg/dm^3.

The diverse studies on the phenolic composition and antioxidant activity (biological potential) of grapes and wines from different regions of the world are the motivation for the aim of the present study – to characterize the phenolic composition and the antioxidant potential of grapes and wines obtained from red grapevine varieties grown in the area of the town of Pleven, Central Northern Bulgaria.

MATERIAL AND METHODS

Grapevine varieties

The object of the research were three red grapevine varieties (2021 harvest) - Cabernet Sauvignon (introduced), Gamza (local) and Rubin (hybrid), grown in the experimental plantations of the Institute of Viticulture and Enology (IVE), Pleven.

Cabernet Sauvignon – originating from the Bordeaux region of Southwestern France (Sweet, 2008). This variety has a control role in the research. For the Pleven region, it ripens in the second half of September. Its wines have intense ruby-red color, a variety-specific aroma and a dense taste. They have the aging potential (Radulov *et al.,* 1992; Roychev, 2012).

Gamza – it has a long history and popularity in Bulgaria and Romania. Its grapes ripen in the second half of September. The produced wines from it are characterized by bright red ruby color, delicate fruity aroma and don't have the aging potential (Radulov *et al*., 1992; Roychev, 2012).

Rubin – it was obtained through intraspecific hybridization by crossing Nebiolo x Shiraz (Petkov, 1977). For the Pleven region it ripens in the first half of September. The wines from it have intense dark red color, high extractability and harmonious taste (Radulov *et al*., 1992; Roychev, 2012).

Grape must chemical composition

The research of the chemical indicators was carried out according to the methods generally accepted in winemaking practice and includes: Determination of sugar content (g/dm^3) - hydrometrically, using a Dujardin hydrometer; Determination of the content of titratable acids (TA, g/dm^3) - by titration with 0.1n NaOH; Determination of pH - potentiometrically, using a pH meter.

Vinification

The studied varieties were harvested when they reached technological maturity. The grapes, in the amount of 30 kg, of each variety were processed in the Experimental Wine Cellar of IVE - Pleven, in the conditions of microvinification, according to the classic scheme for the production of red wines: Crushing - Destemming – Sulphitation (50 mg/kg SO_2) - Alcoholic fermentation (dry wine yeast Saccharomyces cerevisiae: 20 g/hl; temperature: 28°C) - Separation from solids - Additional sulphitation – Storage.

Wines chemical composition

The analyzes were carried out according to the methods generally accepted in the wine practice (Ivanov *et al*., 1979): The content of sugars (g/dm^3) - Schoorl's method; Alcohol content (vol. %) – distillation method using a Gibertini apparatus with a densimeter, by determining the density of a non-alcoholic sample; Titratable acids of the wine (TA, g/dm^3) – by titration with 0.1n NaOH; pH – potentiometrically with a pH meter; Total extract – by densimeter (Gibertini, Milan, Italy).

Grape must and wines phenolic content

Total phenolic compounds (TPC), g/dm^3 – determined according the method of Singleton et Rossi with Folin - Chiocalteu reagent (Ivanov *et al.,*

1979); Flavonoid phenolic compounds (FPC), mg/dm^3 - catechin equivalent - Sommers method (Chobanova, 2007); Non-flavonoid phenolic compounds (NPC), mg/dm^3 - coffee equivalent - Sommers method (Chobanova, 2007); Anthocyanins, mg/dm3 - method of Singleton et Rossi by pH changing (Ivanov *et al.*, 1979).

Grape must and wines antioxidant activity

The antioxidant activity was determined according to the method of Wang *et al.* (1996), as antiradical activity against the stable product DPPH (2,2 - diphenyl-1-picrylhydrazyl) (Sigma Aldrich, Germany). The studied grape musts and wines were diluted immediately before analysis with distilled water to a total extract (TE) content of 600.00 and 400.00 mg/dm^3, respectively. 0.5 cm^3 of the grape must or wine and 2.5 cm^3 of a freshly prepared solution (100μm) of DPPH• in ethanol were mixed in the test tubes. Thus, the TE:DPPH ratio in the reaction medium assumed values of 3:1 and 2:1, respectively. Similarly, a control sample was developed with distilled water instead of diluted grape must or wine. The values of the molecular absorption of light (spectrophotometrically at a wavelength of 515 nm) of the control and experimental samples, denoted by Ak and Ae, respectively, were measured. The measurements were performed at a reaction times of 5th and 15th min, counted from the moment of reagents mixing. The antiradical activity was calculated by the formula:

$$AAR = 10^2.(Ak - Ae).Ak^{-1}, \% \quad (1)$$

Statistical data processing

Statistical processing of the data was performed, including determination of standard deviation (±SD), with triplicate replication for each analysis. The statistical data processing was carried out using the Excel 2007 program (Microsoft Corporation, USA).

RESULTS AND DISCUSSION

The harvest for each variety was carried out at technological maturity. Three main technological indicators were defined: content of sugars, titratable acids (TA) and pH. The obtained results are presented in Table 1.

Table 1. Main technological indicators of grape must from the studied varieties (harvest 2021)

Technological indicators	**Grapevine varieties**		
	Cabernet Sauvignon	***Gamza***	***Rubin***
Sugars, g/dm^3	235.60±2.500	242.00±1.700	265.00±0.000
TA, g/dm^3	9.54±0.190	4.90±0.100	6.20±0.110
pH	2.97±0.000	3.44±0.005	3.48±0.005

Rubin's sugar content was the highest (265.00±0.00 g/dm^3), followed by Gamza (242.00±1.70 g/dm^3). The lowest sugar accumulation was found in Cabernet Sauvignon (235.60±2.50 g/dm^3). According to Radulov *et al*. (1992) and Roychev (2012), the studied varieties in the conditions of Bulgaria should accumulate sugars as follows:

- Cabernet Sauvignon – 210.00 – 240.00 g/dm^3
- Gamza – 190.00 – 210.00 g/dm^3
- Rubin – 220.00 – 240.00 g/dm^3

It was clear from the data that only the Cabernet Sauvignon must felt within the range of variation proposed by the above authors. Gamza's must showed 32.00 g/dm^3 more sugars, and Rubin's 25.00 g/dm^3 more than the suggested range. The reasons for this were related to the influence of weather conditions during the year, as well as the characteristics of the growing area. The particular year was characterized by a very hot and dry summer, with a long period without precipitation, which led to an increased sugars synthesis in the grapes. Regarding the established titratable acidity (TA), Cabernet Sauvignon must was characterized by the highest content of it (9.54±0.19 g/dm^3). Rubin's must contained more titratable acids (6.20±0.119 g/dm^3) compared to Gamza (4.90±0.19 g/dm^3). According to Radulov *et al.* (1992) and Roychev (2012) the content of titratable acids in the must of the investigated varieties ranges as follows:

- Cabernet Sauvignon – 6.50 – 9.00 g/dm^3;
- Gamza – 5.90 – 8.90 g/dm^3;
- Rubin – 5.50 – 6.00 g/dm^3

The Cabernet Sauvignon's and Rubin's musts showed a slightly higher content of titratable acids, while Gamza was characterized by a lower content of this indicator, compared to the suggested by the cited authors. The lower content of titratable acids was explained by the higher sugar accumulation in these varieties, which was reflected in a slight decrease in their titratable acidity. In addition, the content of titratable acids is a highly variable factor depending on variety, geographical area and climate.

pH represents the relationship between the amount and strength of the acids. The pH of the grape must should vary in the range 2.80 – 3.80 (Chobanova, 2012). The must of the control (Cabernet Sauvignon) showed the lowest pH (2.97±0.000). The obtained data for this indicator in the must of all investigated varieties were normal and correlated with those presented by Chobanova (2012).

The results of the three main technological indicators (sugars, TA and pH) indicated that the grapes were harvested and processed at technological maturity. The phenolic complex is a major factor for the biological value of grapes and wine, determining their antioxidant activities.

In the grape must of the studied varieties, total phenolic compounds (TPC), flavonoid phenolic compounds (FPC), non-flavonoid phenolic compounds (NPC) and anthocyanins were determined. The obtained results for the phenolics are presented in table 2.

Table 2. Content of total phenolic compounds (TPC), flavonoid phenolic compounds (FPC), non-flavonoid phenolic compounds and anthocyanins in grape must of the studied varieties

Grapevine varieties	**TPC g/dm^3**	**FPC mg/dm^3**	**NPC g/dm^3**	**Anthocyanins, g/dm^3**
Cabernet Sauvignon	0.79±0.00	55.09±12.98	48.80±1.58	12.14±0.27
Gamza	0.45±0.00	284.71±4.15	134.40±0.25	3.76±0.50
Rubin	1.18±0.00	1412.05±5.95	177.97±0.37	35.01±0.32

When analyzing the results for TPC content in the must of the studied red varieties, it could be saw that Rubin showed a significantly high content. It exceeded more than twice the content of TPC found in Gamza (the lowest of the three studied varieties) and more than one time that found in the control introduced variety Cabernet Sauvignon. The hybrid red grapevine variety Rubin at the growing conditions of the area showed a good ability to accumulate large amounts of phenolic compounds, which suggested a high biological potential. The data on the determined content of TPC in the studied must of the red variety Cabernet Sauvignon were in absolute correlation with the studies of Franco-Bañuelos *et al.* (2017) who found TPC content of 321.90 mg GAE. 100 g^{-1} to 607.60 mg GAE. 100 g^{-1} in a study of grapes from four red wine varieties (Cabernet Sauvignon, Merlot, Rubired and Petite Syrah) from the region of Mexico. The data in the present study were also correlated with the range of variation (1000.00 – 5000.00 mg/dm^3) of TPC in red grape varieties presented by Velkov (1996).

The analysis of the data showed that the hybrid Rubin had the highest quantitative presence of FPC. Compared to the control introduced variety Cabernet Sauvignon, it was twenty-five times more. Cabernet Sauvignon showed the lowest quantitative value for this indicator. In the must of the local Gamza variety FPC were nearly five times lower than that of the Rubin hybrid.

The Rubin hybrid had the highest content of NPC, followed by the local variety Gamza (the difference between them was not very large). It was found that the concentration presence of NPC in the must of the control introduced variety Cabernet Sauvignon was more than three times lower, compared to the NPC of the hybrid variety Rubin.

Some representatives of the NPC groups exhibit different biological activities (Chobanova, 2012), related to the manifestation of antiseptic properties, bactericidal activity, anticholesterolemic effect.

The content of anthocyanins in fresh grape must (immediately after grapes crushing) of the three studied varieties was also determined. Due to the weaker contact during grapes crushing (as the first phase of technological processing), the amounts of these phenols in the fresh grape must were low. The results showed that the must of the red hybrid variety Rubin was characterized by the highest content of anthocyanins. Their amount exceeded nearly three times that

found in the control Cabernet Sauvignon and 11 times that found in the must of the local Gamza variety.

It could be saw from the results for the overall phenolic composition of the must (table 2) from the studied varieties that Rubin excelled not only in the "anthocyanin content" indicator, but also demonstrated significantly higher levels of all studied phenolic compounds groups (TPC, FPC, NPC). The obtained results were evidence for the potential of this variety, in the conditions of the town of Pleven (Central Northern Bulgaria), to accumulate high amounts of phenols, which reflected in a direct probability of the biological benefits manifestation.

The antioxidant activity is one of the main factors determining the biological potential of grapes and wines. The data regarding the antioxidant activity of the must from the studied varieties are presented in table 3.

Table 3. Antioxidant activity (in %) of grape must from the studied varieties

Grapevine varieties	**Antioxidant activity (in %) of grape must with different total extracts and reaction times**			
	Total extract of 600.00 mg/dm^3		**Total extract of 400.00 mg/dm^3**	
	5th min	**15th min**	**5th min**	**15th min**
Cabernet Sauvignon	40.86±0.16	42.29±0.005	41.24±0.22	49.96±0.03
Gamza	44.49±0.21	48.73±0.06	33.70±0.29	42.49±0.09
Rubin	55.79±0.17	54.73±0.02	51.30±0.03	52.17±0.02

The results for the antioxidant activity at TE = 600.00 mg/dm^3 showed that the highest potential for free radicals capturing was found in the must of the red hybrid variety Rubin. After Rubin came Gamza. The lowest antioxidant activity for the same extract was recorded in the must of the control introduced variety Cabernet Sauvignon. Thus, Cabernet Sauvignon must showed significantly lower antioxidant activity than that of the Rubin hybrid. In 5 min of the reaction at TE=600.00 mg/dm^3 it was nearly 15% lower, and in 15 min of the reaction Cabernet Sauvignon showed 12% lower ability to eliminate the free DPPH• radicals.

The obtained data for the antioxidant activity of the must from the studied red varieties, at TE=600.00 mg/dm^3, correlated with the obtained data for the amount of FPC and NPC. The following correlation dependence was obtained: FPC+NPC+AA (hybrid variety)>FPC+NPC+AA (local variety)> FPC+NPC+AA (introduced variety). The two groups of phenolic compounds consist of subgroups with representatives showing significant free radical scavenging potential - catechins, proanthocyanidins, anthocyanins, flavones, flavonones and falvonols (FPC) (Harbone, 1980), phenolic acids, coumarins and stilbenes (NPC) (Chobanova, 2012). The study proved a direct correlation between the two groups of phenolic compounds (FPC and NPC) and the established antioxidant activity in

the must of the studied red grapevine varieties grown in the conditions of the town of Pleven, Central Northern Bulgaria.

From the results for the obtained antioxidant activity of the must from the studied red varieties at the lower total extract (400.00 mg/dm^3) Rubin again showed the highest activity recorded. Second by the antioxidant capacity was the must of Cabernet Sauvignon variety. The Gamza must by the studied sample extract (400.00 mg/dm^3) showed the lowest antioxidant activity.

The study related to the antioxidant potential of the must from the studied red varieties in both extracts showed a dominance of Rubin. The hybrid grapevine variety demonstrated high antioxidant activity, potentially exceeding that found in both the control introduced and the local varieties. The relationship was also confirmed through the established correlation between FPC, NPC and the antioxidant activity of the red grapevine varieties.

The grapes were vinified according to the classic scheme for the production of dry red wines (Yankov, 1992). The data on the main chemical indicators of the obtained wines are presented in table 4.

Table 4. Chemical parameters of the experimental wines, harvest2021

Wines	**Alcohol content vol. %**	**Total extract g/dm^3**	**Sugars g/dm^3**	**Titratable acids g/dm^3**	**pH**
Cabernet Sauvignon	13.24±0.08	26.30±0.17	3.35±0.08	6.79±0.11	3.92±0.011
Gamza	15.01±0.10	24.96±0.51	3.53±1.67	5.08±0.14	4.24±0.005
Rubin	14.58±0.07	36.33±0.05	6.57±0.02	6.77±0.11	4.10±0.000

The amount of ethyl alcohol in Gamza was the highest (15.01±0.10 vol. %) but its difference with Rubin (14.58±0.07 vol. %) was very small. The ethanol content in Cabernet Sauvignon wine was the lowest (13.24±0.08 vol. %). Ethyl alcohol normally varies in wines in the range 7.00 vol.% - 17.00 vol.% (Chobanova, 2012).

Almost all analyzed wines showed a normal total extract. The highest extractability was showed by Rubin (36.33±0.05 g/dm^3). The wines of Cabernet Sauvignon (26.30±0.17 g/dm^3) and Gamza (24.96±0.51 g/dm^3) did not show a big difference by this indicator, but a slight preponderance was observed for Cabernet Sauvignon.

According to the established content of residual sugars, Cabernet Sauvignon (3.35±0.08 g/dm^3) and Gamza (3.53±1.67 g/dm^3) belonged to the dry wines category. The Rubin wine (6.57±0.02 g/dm^3) had a higher content, as a result of the higher sugars in the grapes, the accumulation of the maximum amount of alcohol and the incomplete course of the alcoholic fermentation.

An almost similar content of titratable acids between Cabernet Sauvignon (6.79±0.11 g/dm^3) and Rubin (6.77±0.11 g/dm^3) was found. The titratable acids content (5.08±0.14 g/dm^3) was lower in Gamza's wine. All established levels of

titratable acids were in the optimum (5.00 to 9.00 g/dm^3) for this indicator (Chobanova, 2012).

Regarding the actual acidity (pH) a variation from 3.92±0.011 (Cabernet Sauvignon) to 4.24±0.005 (Gamza) was found.

The data on the content of total phenolic compounds (TPC), flavonoid phenolic compounds (FPC), non-flavonoid phenolic compounds (NPC) and anthocyanins in the studied wines are presented in table 5.

Table 5. Content of total phenolic compounds (TPC), flavonoid phenolic compounds (FPC), non-flavonoid phenolic compounds and anthocyanins in wines of the studied varieties

Wines	**TPC g/dm^3**	**FPC mg/dm^3**	**NPC g/dm^3**	**Anthocyanins, g/dm^3**
Cabernet Sauvignon	1.55±0.00	6709.18±5.40	410.51±0.56	303.11±0.16
Gamza	1.05±0.005	3341.01±11.06	417.77±0.66	253.02±5.90
Rubin	4.23±0.005	4223.41±20.90	270.84±0.29	287.26±0.13

The wines showed very significant differences in the concentration of TPC. The content of TPC in the wine of the hybrid variety Rubin was significant and very high. The result was in correlation with the established higher levels of TPC found in its grape must (table 2). Second in quantitative presence of phenols was the wine of the Cabernet Sauvignon variety. The lowest phenolic content was found in Gamza. The data were in correlation with the results of this indicator for the grape must of the studied varieties (table 2). The data regarding the amount of TPC detected correlated with the study of Shahidi and Naczk (1995), in which a variation for the presence of TPC in red wines from 1000 to 4000 mg/dm^3 was found. Li *et al*. (2009) found a range of variation in TPC of studied red wines from 1402.00 to 3130.00 mg/dm^3. Our data were also in agreement with the study of Radeka *et al.* (2022) who investigated red wines from the Croatian varieties Teran and Plavac and found a TPC variation from 1527.12 mg/dm^3 to 3936.21 mg/dm^3. The data also correlated with the results of Nistor *et al*., (2015), who investigated the content of total phenolic compounds in Cabernet Sauvignon and Pinot Noir wines from three consecutive harvests (2011, 2012 and 2013) produced by two different wineries. A variation of 2072.00±81.38–2758.00±149.90 mg/dm^3 (Cabernet Sauvignon) and 1814.00±92.51–2695.00±76.46 mg/dm^3 (Pinot Noir) was found for the established total phenolic compounds from the first group of red wines. In the case of red wines from the second group, the total phenolic content was found within the limits of 1986.00±163.50–2531.00±77.76 mg/dm^3 (Cabernet Sauvignon) and 1752.00±94.90–2214.00±35.56 mg/dm^3 (Pinot Noir) (Nistor *et al*., 2015).

The data on the established flavonoid phenolic compounds (FPC) showed that the control variant - the wine of the introduced variety Cabernet Sauvignon, had the highest content of FPC. Second, according to the concentration presence

of this group of compounds, was the wine of the Rubin hybrid, and the lowest FPC concentration was found in the red wine of the local Gamza variety.

Main representatives of the flavonoid group of phenolic compounds in grapes and wines of *Vitis vinifera* L. varieties are anthocyanins, flavan-3-ols, tannins and their reaction products (Casassa, 2017).

Mitrevska *et al.* (2020) investigated commercial Macedonian red and white wines and found that total flavonoid content in red wines ranged from 547.00±10.00 mg/dm^3 to 1732.00±7.00 mg/dm^3. The data in our study related to the content of FPC was higher.

The wine of Gamza variety showed the highest content of NPC, which was very close to that found in the control introduced Cabernet Sauvignon variety. The lowest NPC content for the red wines was found in Rubin. The non-flavonoid phenolic compounds (NPC) present in wines include representatives of phenolic acids and stilbenes (Fernandes *et al.*, 2017). Woraratphoka *et al*. (2007) investigated the content of phenolic compounds in selected wines from northeastern Thailand. The team found a variation of NPC in the studied red wines from 195.30±3.70 mg/dm^3 to 575.60±341.60 mg/dm^3. The data in our study correlated with those established by the cited team.

The anthocyanins are the red pigments of the varieties and are mainly responsible for the wine color. They are available in wines in amounts from 200.00 to 500.00 mg/dm^3 (Chobanova, 2012). In the studied harvest, the highest anthocyanin content was found in the wine of the control introduced variety Cabernet Sauvignon, followed by the wine of Rubin, and their content was the lowest in Gamza. The anthocyanins are part of the FPC group. The established results for FPC as a trend overlaped with the established anthocyanin content, namely in the order: (Cabernet Sauvignon) > (Rubin) > (Gamza). This was direct evidence that anthocyanins had a significant contribution to the formation of the total concentration of flavonoid phenolic compounds. Our results correlated with data of Tsiakkas *et al*. (2020) who found anthocyanin content of red wines from Cyprus ranged from 24.44±2.61 mg/dm^3 to 509.18±8.18 mg/dm^3. Slightly higher levels of anthocyanins were found by Kharadze *et al.* (2018) in a study of wines obtained from Georgian endemic varieties. The team found a variation in the anthocyanin content of the studied wines from 327.10 mg/dm^3 to 871.70 mg/dm^3. The data on the established antioxidant activity in the red wines of the studied varieties are presented in table 6.

Table 6. Antioxidant activity (in %) of wines from the studied varieties

Wines	**Antioxidant activity (in %) of wines with different total extracts and reaction times**			
	Total extract of 600.00 mg/dm^3		**Total extract of 400.00 mg/dm^3**	
	5th min	**15th min**	**5th min**	**15th min**
Cabernet Sauvignon	55.91±0.37	75.31±0.07	24.03±0.47	41.48±0.14
Gamza	62.19±0.54	71.93±0.06	27.69±0.27	33.06±0.08
Rubin	59.60±0.21	60.05±0.22	54.75±0.29	55.21±0.15

At TE = 600.00 mg/dm^3, high values of antioxidant activity in the wines of the Cabernet Sauvignon and Gamza varieties were observed. In 5 min of reaction with this extract, the wine of Gamza variety showed a higher radical-capturing capacity, compared to Cabernet Sauvignon. In 15 min of the reaction, an increase in radical-elimination activity was observed in both varieties, being more significant in the wine of the introduced Cabernet Sauvignon variety, comparing it to the local Gamza.

When the concentration of TE=400.00 mg/dm^3 was reduced, a significant drop in the wines antioxidant activity of both varieties was observed, but the trend for the strength of the activity at 5 and 15 min was preserved. On the other hand, the wine of the hybrid variety Rubin at TE=600.00 mg/dm^3 in 5 min of the reaction showed an antioxidant activity, which was slightly higher than that of the control introduced variety Cabernet Sauvignon and slightly lower than the established for the wine of the local Gamza variety. At 15 min of the reaction with this extract, Rubin showed a lower antioxidant activity than the wines of the other two investigated varieties.

The situation with Rubin based on TE=400.00 mg/dm^3 was interesting. Compared to the previous extract, a decrease in antioxidant activity was observed, but it was much smaller compared to the same in the wines of the other two varieties. At both 5 min and 15 min, Rubin's wine showed higher antioxidant activity, comparing it to the analogous results of the other two varieties. From these results, it could be concluded that Rubin's wine showed a smoother and more stable manifestation of antioxidant activity, regardless of the extract.

The data on the established antioxidant activity in red wines can be closely related to NPC results. Gamza and Cabernet Sauvignon showed a close concentration presence of this group of phenols, which correlated with the close values of antioxidant activity found in the wines of these two varieties.

The data obtained from our study were in correlation with the research of Bajčan *et al.* (2016) who studied 28 Cabernet Sauvignon red wines from different regions of Slovakia and found an average DPPH antioxidant activity in the range 69.00–84.20%. Our data were also in agreement with the research of Banc *et al.* (2020) who studied red wines from different varieties and regions of Romania and found antioxidant activity ranging from 63% to 95%.

CONCLUSIONS

The must of the Rubin variety demonstrated significantly higher levels of all investigated groups of phenolic compounds (TPC, FPC, NPC and anthocyanins) in comparison with the other two investigated grapevine varieties. The obtained results were evidence for the potential of this variety, in the conditions of the town of Pleven (Central Northern Bulgaria), to accumulated high amounts of phenols, which reflected in a direct probability of the manifestation of biological benefits.

The study related to the antioxidant potential of the must in both extracts showed a dominance of Rubin. The hybrid variety demonstrated high antioxidant

activity, potentially exceeding that found in both the control introduced and the local varieties. The relationship was also confirmed by the established correlation between FPC, NPC and antioxidant activity.

A high presence of TPC in the analyzed wines was identified in the wine of the Rubin hybrid. It exceeded almost three times that found in the wine of the control introduced variety Cabernet Sauvignon and four times that found in the wine of the local variety Gamza.

Gamza and Cabernet Sauvignon wines showed very close concentrations in their NPC contents, while Rubin showed a lower content.

The established results for FPC in the wines as a trend overlap with the established anthocyanin content, namely in the order: Control introduced variety (Cabernet Sauvignon) > Hybrid variety (Rubin) > Local variety (Gamza). This was direct evidence that anthocyanins have a significant contribution to the formation of the total flavonoid phenolic compounds concentration.

The data on the established antioxidant activity in red wines could be closely related to the NPC results of the wines. Gamza and Cabernet Sauvignon showed a close concentration presence of this group of phenols, which correlated with the close values of antioxidant activity found in the wines of these two varieties.

The study proved high biological potential and good phenolic accumulation in grapes and wines of the studied varieties, a result that was closely related to the characteristics of the Central Northern Bulgaria terroir.

REFERENCES

Bajčan, D., Vollmannová, A., Šimanský, V., Bystrická, J., Trebichalský, P., Árvay, J. & Czako, P. (2016). Antioxidant activity, phenolic content and colour of the Slovak cabernet sauvignon wines. Potravinarstvo, 10: 89-94.

Banc, R., Loghin, F., Miere, D., Ranga, F. & Socaciu, C. (2020). Phenolic composition and antioxidant activity of red, rosé and white wines originating from Romanian grape cultivars. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 48(2): 716–734.

Büyüktuncel, E., Porgali, E. & Çolak, C. (2014). Comparison of total phenolic content and total antioxidant activity in local red wines determined by spectrophotometric methods. Food and Nutrition Science, 5: 1660-1667.

Casassa, L. (2017). Flavonoid phenolics in red winemaking. Chapter 6 from the book "Phenolic compounds – natural sources, importance and applications. Ed. "Intech Open", 153-189, ISBN 978-953-51-2958-5.

Čeryova, N., Bajčan, D., Lidikova, J., Musilova, J., Šnirc, M., Jančo, I., Fanková, H. & Bláhová, M. (2021). Phenolic content and antioxidant activity of Slovak varietal wines of muscat type. Journal of Microbiology, Biotechnology and Food Sciences, 10(5): e492.

Chobanova D. (2007). Guide to Exercises in Oenology. Academic Press of University of Food Technologies, Plovdiv, Bulgaria. [In Bulgarian]

Chobanova D. (2012). Enology. Part I: Composition of wine. Academic Press of University of Food Technologies, Plovdiv, Bulgaria, 264 p. [In Bulgarian]

Di Lorenzo, C., Colombo, F., Biella, S., Orgin, F., Frigerio, G., Regazzoni, L., de Sousa, L., Bavarescu, L., Bosso, A., Aldini, G. & Restani, P. (2019). Phenolic profile and antioxidant activity of different grape (*Vitis vinifera* L.) varieties. Bio Web Conference, 12, 04005.

Fernandes, I., Pérez-Gregorio, R., Soares, S., Mateus, N. & de Freitas, V. (2017). Wine flavonoids in health and disease prevention. Molecules, 22(2): 292.

Franco-Bañuelos, A., Galicia-Hernánde, M.E., Contreras-Martínez, C.S., Carranza-Télle, J. & Carranza Concha, J. (2017). Total phenolic content and antioxidant capacity of wine grapes grown in Zacatecas, Mexico. Investigación y Desarrollo en Ciencia y Tecnología de Alimentos, 3: 477-482.

Ghatak, A., Chaturved, P. & Desai, N. (2011). Indian grape wines: A potential source of phenols, polyphenols, and antioxidants. International Journal of Food Properties, 17(4): 818- 828.

Ginjom, I., D'Arcy, B., Caffin, N. & Gidley M. (2010). Phenolic contents and antioxidant activities of major Australian red wines throughout the winemaking process. Journal of Agricultural and Food Chemistry, 58 (18): 10133 – 10142.

Harborne, J. B. (1980). "Plant phenolics". In Bell, E. A.; Charlwood, B. V. (eds.). Encyclopedia of Plant Physiology, volume 8 Secondary Plant Products. Berlin Heidelberg New York: Springer-Verlag. pp. 329–395.

Ismael, D. (2018). Phenolic content and antioxidant activity of variety grapes from Kutdistan Iraq. Slovak Journal of Food Science, 12(1): 680-686.

Ivanov, T., Gerov, S., Yankov, A., Bambalov, G., Tonchev, T., Nachkov, D. & Marinov, M. (1979). Guide to wine technology. Publishing House G. Danov ", 530 p. [In Bulgarian]

Kharadze, M., Djaparidze, I., Shalashvili, A., Vanidze, M. & Kalandia A. (2018). Phenolic compounds and antioxidant properties of some white varieties of grape wines spread in western Georgia. Bulletin of the Georgian National Academy of Sciences, 12(3): 103-109.

Li, H., Wang, X., Li, Y., Li, P. & Wang, H. (2009). Polyphenolic compounds and antioxidant properties of selected China wines. Food Chemistry, 112: 454- 460.

Mitrevska, K., Grigorakis, S., Loupassaki, S. & Calokerinos, C. (2020). Antioxidant activity and polyphenolic content of North Macedonian wines. Applied Sciences, 10: 1-11.

Mucaca, C., Filho, J., Nascimento, E. & Arruda, L. (2017). Phenolic composition, chromatic parameters and antioxidant activity "in vitro" in Tropical Brazilian red wines. Journal of Food and Nutrition Research, 5(10): 754-762.

Nistor, E., Dobrei, A., Dobrei, A., Camen, D., Mălăescu, M. & Prundeanu, H. (2015). Anthocyanins and phenolics in Cabernet Sauvignon and Pinot noir wines. Journal of Horticulture, Forestry and Biotechnology, 19(1): 226- 229.

Osorio-Marcias, D.E. Vásquez, P., Carrasco, C., Bergenstahl, B. & Peñarrieta, J.M. (2017). Resveratrol, phenolic antioxidants, and saccharides in South American red wines. International Journal of Wine Research, 10: 1-11.

Pajović-Šćepanovć, R., Wendelin, S., Forneck., A. & Eder, R. (2019). Suitability of flavan-3-ol analysis to differentiate grapes from Vranac, Kratošija and Cabernet Sauvignon (*Vitis vinifera* L.) grown in Montenegro. Australian journal of grape and wine research, 25: 376-383.

Pajović-Šćepanović, R., Wendelin, S. & Eder, R. (2018). Phenolic composition and varietal discrimination of Montenegrin red wines (*Vitis vinifera* var. Vranac, Kratošija, and Cabernet Sauvignon). European Food Research and Technology, 244: 2243–2254.

Petkov, G. (1977). Biochemical and technological study of the varieties Bouquet,Rouen and Rubin for the production of red wines. Dissertation. Institute of Viticulture and Enology, Pleven. [In Bulgarian]

Radeka, S., Rossi, S., Bestulić, E., Budić-Leto, I., Ganić, K., Horvat, I., Lukić, I., Orbanić, F., Jurjević, T. & Dvornik Š. (2022). Bioactive compounds and antioxidant activity of red and white wines produced from autochthonous Croatian varieties: Effect of moderate consumption on human health. Foods, 11(2): 1804.

Radulov, L., Babrikov, D. & Georgiev, S. (1992). Ampelography with basics of winemaking. Sofia, Zemizdat, p. 187. [In Bulgarian]

Rodriguez-Vaquero, M., Valleijo, C. & Aredes-Fernández, A. (2020). Antibacterial, antioxidant and antihypertensive properties of polyphenols from argentinean red wines varieties. Open Journal of Pharmacology and Pharmacotherapevtics, 5(1): 001-006.

Roychev, V. (2012). Ampelography. Plovdiv, Academic Publishing House of the University of Plovdiv, p. 576. [In Bulgarian]

Shahidi, F. & Naczk, M. (1995). Food Phenols: Source, Chemistry, Effects and Applications. Technomic Publishing Company (USA), pp. 331.

Sweet, N. (2008). Cabernet Sauvignon at FPS. FPS Grape Program Newsletter, 16-32.

Tsiakkas, O., Escott, C., Loira, I., Morata, A., Rauhut, D. &, Suarez-Lepe, J. (2020). Determination of anthocyanin and volatile profile of wines from varieties Yiannoudi and Maratheftiko from the island of Cyprus. Beverages, 6(1): 4.

Velkov E. (1996). Encyclopedia of alcoholic beverages. "Poligrafia" Ltd., Plovdiv, Bulgaria, 1996, ISBN 954-698-002-1. [In Bulgarian]

Wang, H., Cao, G. & Prior R.L. (1996). Total antioxidant capacity of fruits. Journal of Agriculture and Food Chemistry, 44(3), 701-705.

Woraratphoka, J., Intarapichet, K. & Indarapichate, K. (2007). Phenolic compounds and antioxidative properties of selected wines from the northeast of Thailand. Food Chemistry, 104: 1485-1490.

Yankov, A. (1992). Winemaking Technology. Sofia, Zemizdat, 355 p. [In Bulgarian]

Yildirim, H., Akçay, Y., Güvenç, U., Altindisli, A. & Sözmen, E. (2005). Antioxidant activities of organic grape pomace, juice, must, wine and their correlation with phenolic content. International Journal of Food Science and Technology, 40: 133-142.