# Biological potential (phenolic complex and antioxidant activity) of white grapes and wines from varieties with different genetic origin, grown in the region of Central Northern Bulgaria

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Abstract

#### **Keywords:**

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**Introduction**. The aim of the present study is to define the biological potential (based on phenolic complex and antioxidant activity) of white grapes (and their corresponding wines) grown under the influence of the terroir of Central Northern Bulgaria.

**Materials and methods**. The objects of the research were grapes and wine from the white varieties Chardonnay, Dimyat and Druzhba, harvest 2021, grown in the experimental plantations of the Institute of Viticulture and Enology (IVE), Pleven. Chemical analysis, analysis of phenolic compounds and antioxidant activities of grape musts and wines were made.

Results and discussion. The highest amount of total phenolic compounds (TPC) was found in the grape must of the local variety Dimyat. The study of the presence of flavonoid phenolic compounds (FPC) in the grape musts showed that Chardonnay has the highest potential for FPC accumulation. The highest amount of non-flavonoid phenolic compounds (NPC) was found in the grape must of the control introduced variety Chardonnay. At titratable acid (TE) 600.00 mg/dm<sup>3</sup>, the highest antioxidant activity was found in the grape must of the Druzhba hybrid. Dominance in the content of TPC was found in the wine form the local variety Dimyat  $(0.93\pm0.000 \text{ g/dm}^3)$ , while the Chardonnay showed the lowest amount of TPC (0.45±0.000 g/dm<sup>3</sup>). In terms of FPC content, Chardonnay wine dominated (696.46±0.37 mg/dm<sup>3</sup>), and Dimyat had the lowest content (439.38±3.35 mg/dm<sup>3</sup>). The highest concentration of NPC was found in the wine of the Druzhba hybrid  $(130.47\pm0.59 \text{ mg/dm}^3)$ . The lowest result according to this indicator was found in the wine of the control variety Chardonnay (84.13±0.43 mg/dm<sup>3</sup>). The Chardonnay wine showed significantly higher antioxidant activity compared to the other two studied varieties - 1.5 times higher radicaleliminating activity compared to Dimyat and compared to Druzhba – 2 times higher.

**Conclusions**. The research proved that the white varieties and wines grown in the region of Central Northern Bulgaria showed a balanced biological activity and potential, comparable to wines and grapes from other regions of the world.

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#### Introduction

Modern food science places serious emphasis on the biological potential of various foods and beverages. In-depth research is being carried out, aiming to define the components of the composition of food and drink products with high biological activity, functional and health effects, all of which reflect in an overall improvement in the functions of the human organism.

Antioxidants are substances capable to neutralize reactive oxygen species (free radicals) through direct binding, which leads to blocking the possibility of oxidative stress in the human organism (Lobo et al., 2010; Young and Woodside, 2019). Oxidative stress, determined by the activity of free radicals in the body and their binding to cellular structures, leads to the initiation of various damages associated with the disruption of a number of functions and the formation of many diseases: the initiation of cancer forms, atherosclerosis, cardiovascular diseases, arthritis, accelerated aging, neurodegenerative and autoimmune diseases (Lea, 1966; Harman, 1992; Pham-Huy et al., 2008).

Phenolic compounds are secondary metabolites that underlie the antioxidant capacity and activities of various plant species (Arvanitoyannis et al., 2006). Their accumulation in the grapes of *Vitis vinifera* L species is dependent on a number of factors: grapevine variety, soil and climatic conditions in the area of cultivation, irrigation, plant protection measures, sun exposure and others (Burin et al., 2014). Their accumulation in the wine includes some other factors: degree of grapes ripeness (Andrade et al., 2001), vinification technology (Salacha et al., 2007), aging of the wines (Zafrilla et al., 2003).

Numerous studies confirm the inhibitory ability of wine and grapes phenolics against free radicals, reducing the risks of cancer, cardiovascular diseases, diabetes and others (Palma-Duran et al., 2017; Kerry and Abbey, 1997). The direct relationship between phenolic compounds and antioxidant activity has been demonstrated in various studies regarding the influence of maceration in the accumulation of phenolics and the increase in the antioxidant activity of must and red Syrah wines from Brazil (Alencar et al., 2017), a study of the phenolic composition and antioxidant capacity of table grapes from the region of Italy and Portugal (Di Lorenzo et al., 2019), a study on antioxidant activity and polyphenolic compounds in white and red wines from North Macedonia (Mitrevska et al., 2020), it was proved a direct relationship between phenolic compounds, antioxidant activity and antihypertensive capacity in Argentinian red wines from Malbec and Merlot varieties (Rodriguez-Vaquero et al., 2020) and others.

The aim of the present study is to define the biological potential (based on phenolic complex and antioxidant activity) of white grapes (and their corresponding wines) grown under the influence of the terroir of Central Northern Bulgaria.

#### **Materials and methods**

The objects of the research were grapes and wines from the white varieties Chardonnay, Dimyat and Druzhba, harvest 2021, grown in the experimental plantations of the Institute of Viticulture and Enology (IVE), Pleven.

Grapevine varieties. Three white grapevine varieties were selected – introduced, local and hybrid.

*Introduced variety.* It had a control role in the study. Chardonnay is a white grapevine variety, originating from the area of Burgundy and Champagne, France (Sweet, 2007). For the region of Pleven, it ripens around the middle of September. It has very good fertility. It

 quickly accumulates sugars (20-24%) and retains relatively high titratable acidity (7.00-9.00 g/dm<sup>3</sup>) (Radulov et al., 1992; Roychev, 2012).

*Local variety.* Dimyat – old local, Bulgarian, white grapevine variety, distributed in the Balkan Peninsula. Late-ripening, for the region of Pleven it ripens in the second half of September. It has high fertility and yield. Grapes have good sugar accumulation (19-21%) with titratable acids of 6.00-7.00 g/dm<sup>3</sup> (Radulov et al., 1992; Roychev, 2012).

*Hybrid variety*. Druzhba is a white grapevine variety created by complex interspecies hybridization (Muscat hamburgski x Save Villar 12 375) x (Zarya Severa x Muskat hamburgski) and approved in 1983. Included in the Official Varietal List of Bulgaria in 2012. The variety is medium-ripening, the grapes ripen at the end of August and the beginning of September. The vines have very good fertility. At technological maturity, the content of sugars is 19-21%, with titratable acids of 6.50-7.50 g/dm<sup>3</sup> (Radulov et al., 1992; Roychev, 2012).

**Grape must chemical composition.** The research was carried out according to the methods generally accepted in winemaking practice and included: Determination of sugar content ( $g/dm^3$ ) using a Dujardin hydrometer; Determination of the content of titratable acids (TA,  $g/dm^3$ ) by titration with 0.1n NaOH; Determination of pH was done 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 dry white wines:

- Crushing the grapes
- Destamming
- Pressing
- Sulphitation  $(50 \text{ mg/dm}^3 \text{ SO}_2)$
- Clarification of the must and decanting
- Alcoholic fermentation (dry wine yeast *Saccharomyces cerevisiae* 20 g/hl; temperature 20°C)
- Racking
- Additional sulphitation
- Storage.

Chemical analysis of the obtained white wines. The analyzes were carried out according to the methods generally accepted in wine practice (Ivanov et al., 1979): The content of sugars (g/dm<sup>3</sup>) by 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<sup>3</sup>) by titration with 0.1n NaOH; Actual acidity (pH) potentiometrically with a pH meter; Total extract by densimeter (Gibertini).

**Determination of the phenolic content of grape must and wines.** Determination of total phenolic compounds (TPC) – according to the method of Singleton et Rossi; Determination of the content of flavonoid phenolic compounds (FPC); Determination of the content of non-flavonoid phenolic compounds (NPC);

**Determination of antioxidant (DPPH•) activity of grape must and wines.** 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 antiradical activity was calculated by the formula:  $AAR = 102(Ak - A0).Ak^{-1}$ , % (1)

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**Statistical analysis.** Statistical processing of the data was performed, including the determination of standard deviation ( $\pm$ SD), with triplicate replication for each analysis. The determination of the indicator was realized using the Excel 2007 program from the Microsoft Package (Microsoft Corporation, USA).

#### **Results and discussion**

The harvest for each variety was carried out at technological maturity. Analyzes of the grape must of each variety were carried out for the determination of three main technological indicators: sugar content, titratable acids (TA) and determination of pH of the must. The obtained results are presented in Table 1.

#### Table 1

Technological indicators	Grapevine varieties				
	Chardonnay	Dimyat	Druzhba		
Sugars, g/dm <sup>3</sup>	244.60±1.10	221.00±1.70	223.00±1.70		
TA, $g/dm^3$	7.25±0.22	$4.60 \pm 0.08$	5.97±0.04		
pH	3.26±0.005	$3.44 \pm 0.005$	$3.42 \pm 0.005$		

Main technological indicators of grape must from the investigated varieties (harvest 2021)

The highest sugar accumulation in the grape must of the white varieties was found in the control introduced variety Chardonnay ( $244.60\pm1.10 \text{ g/dm3}$ ). The must of Dimyat and Druzhba showed a very close, almost similar content of sugars ( $221.00\pm1.70 \text{ g/dm}^3$  and  $223.00\pm1.70 \text{ g/dm}^3$ , respectively).

According to Radulov et al. (1995) and Roychev (2012), the studied varieties in the conditions of Bulgaria should accumulate sugars as follows: Chardonnay -200.00 - 240.00 g/dm<sup>3</sup>; Dimyat -190.00 - 210.00 g/dm<sup>3</sup>; Druzhba -190.00 - 210.00 g/dm<sup>3</sup>.

It can be seen that the established sugar accumulation in the must for the specific harvest (2021) showed slightly higher levels than the data presented by Radulov et al. (1995) and Roychev (2012), namely 4.60 g/dm<sup>3</sup> more sugars for Chardonnay, 11.00 g/dm<sup>3</sup> more sugars for Dimyat and, respectively, 13.00 g/dm<sup>3</sup> more sugars for Druzhba. The resulting higher sugar accumulation could be explained as a consequence of the climatic conditions of the year. It was characterized by a very hot and dry summer, with a long period without precipitation, which led to an increased synthesis of sugars in the grapes.

Regarding the established titratable acidity (TA), the highest content of this indicator  $(7.25\pm0.22 \text{ g/dm}^3)$  was found in Chardonnay. Druzhba must showed higher content of titratable acids  $(5.97\pm0.04 \text{ g/dm}^3)$  than Dimyat  $(4.60\pm0.08 \text{ g/dm}^3)$ .

According to Radulov et al. (1995) and Roychev (2012) the content of titratable acids in the grape must of the investigated varieties ranges as follows: Chardonnay -7.00 - 9.00 g/dm<sup>3</sup>; Dimyat -6.00 - 7.00 g/dm<sup>3</sup>; Druzhba -6.50 - 7.50 g/dm<sup>3</sup>.

The results obtained for Chardonnay and Dimyat must correlated with the data presented by the cited authors. Druzhba must showed a slightly lower titratable acidity. The lower content of titratable acids is explained by the higher sugar accumulation in these varieties, which is 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.

 According to Abrasheva et al. (2008) in the conditions of the Republic of Bulgaria, the concentration presence of titratable acids in grapes varies in the wide range from 4.00 to 12.00 g/dm<sup>3</sup>. The results of the conducted research correlated with this range, which confirmed the good accumulation of acids in the grapes in the conditions of the town of Pleven, Central Northern Bulgaria, and confirmed also the reach of grapes technological maturity for carrying out the "vinification" process.

The pH represents the relationship between the amount and the strength of acids. The normal pH of grape must should be in the range of 2.80 - 3.80 (Chobanova, 2012). In the grape must of the studied varieties, the lowest pH was found in the control introduced Chardonnay variety ( $3.26\pm0.005$ ). Druzhba and Dimyat showed close values for this indicator. The obtained data were normal and correlated with those presented by Chobanova (2012).

The results for the three main technological indicators (sugars, TA, and pH) indicated that the grapes were harvested at the right time, at technological maturity, with a good balance between sugars and acids and good quality for undergoing the fermentation process.

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 investigated varieties, total phenolic compounds (TPC), flavonoid phenolic compounds (FPC) and non-flavonoid phenolic compounds (NPC) were determined.

The data for TPC in grape must of the studied varieties are presented in Figure 1.

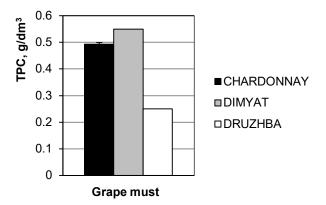


Figure 1. Total phenolic compounds (TPC) in grape must of the studied varieties

The research showed the lowest content of this indicator in the Druzhba hybrid  $(0.25\pm0.00 \text{ g/dm}^3)$ . It was two times lower, compared to the determined content of TPC in the introduced control variety Chardonnay  $(0.49\pm0.005 \text{ g/dm}^3)$  and the local Dimyat  $(0.55\pm0.00 \text{ g/dm}^3)$ . When analyzing the results, it could be seen that the must of Dimyat was the richest of TPC.

The obtained data regarding the established presence of TPC in the grape must were in absolute correlation with the ranges of their presence  $(200.00 - 500.00 \text{ mg/dm}^3)$ , presented by Velkov et al. (1996) as well as with the study of Franco-Bañuelos et al. (2017), who found a variation in TPC content of 112.70 mg GAE. 100 g<sup>-1</sup> to 218.00 mg GAE. 100 g<sup>-1</sup> in a study of grapes from four white grapevine varieties (Sauvignon Vert, Palomino, Furmint and Semillon) from the Mexico region.

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The obtained results regarding the content of FPC in grape must of the studied varieties are presented in figure 2.

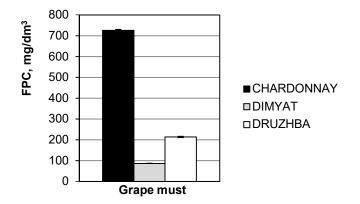


Figure 2. Flavonoid phenolic compounds (FPC) in grape must of the studied varieties

The grape must of the control introduced variety Chardonnay showed the highest quantitative presence of FPC ( $726.44\pm3.34 \text{ mg/dm}^3$ ), an amount that was more than eight times higher than that found in the grape must of the local variety Dimyat ( $87.07\pm1.16 \text{ mg/dm}^3$ ) and over three times higher than that found in the white hybrid variety Druzhba ( $213.97\pm3.04 \text{ mg/dm}^3$ ). It could be seen that the must of Dimyat showed the lowest levels on this indicator, and Chardonnay dominated the other two varieties.

The data on the content of NPC in grape must of the studied varieties are presented in Figure 3.

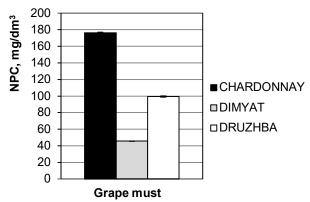


Figure 3. Non-flavonoid phenolic compounds (NPC) in grape must of the studied varieties

The highest NPC content was found in the control introduced variety Chardonnay  $(176.33\pm0.51 \text{ mg/dm}^3)$ . It was almost four times higher compared to that of the local variety Dimyat  $(45.65\pm0.11 \text{ mg/dm}^3)$  and over one and a half times from that found in the must of the Druzhba hybrid  $(99.26\pm0.72 \text{ mg/dm}^3)$ . From the obtained results for NPC, it could be seen that Dimyat showed the lowest concentrations of the investigated indicator.

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

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antihypercholesterolemic effect. The NPC group includes the so-called stilbenes characterized by high antioxidant activity, preventive effect against cardiovascular diseases, anticarcinogenic effect (Jang et al., 1997; Lu and Serrero, 1999).

The antioxidant activity is one of the main factors determining the biological potential of grapes and wines.

The obtained data on the antioxidant activity (DPPH•) of grape must from the introduced (Chardonnay), local (Dimyat) and hybrid (Druzhba) white grapevine varieties are presented in Figures 4, 5 and 6.

The data on the antioxidant activity of the must from the three investigated white varieties at  $TE = 600.00 \text{ mg/dm}^3$  revealed that the highest activity showed the must of the hybrid Druzhba variety. With a reaction time of 5 min, it registered a free radical elimination activity of 43.14±0.09 %. When the reaction time was increased to 15 min, an increased elimination of DPPH radicals was registered up to  $48.73\pm0.15$  %.

The grape must of the other two studied varieties – the introduced control Chardonnay and the local Dimyat at  $TE = 600.00 \text{ mg/dm}^3$  showed almost similar results with a very small difference in favor of Chardonnay. Its must at a reaction time of 5 min showed  $34.72\pm0.28$  % elimination of free radicals. When the reaction time increased to 15 min, an increased in the antiradical activity was recorded and it reached  $37.22\pm0.14$  %.

The grape must of the local Dimyat variety showed the lowest antiradical activity, but its difference with Chardonnay was small (2–3% higher activity in Chardonnay). Dimyat at TE=600.00 mg/dm<sup>3</sup> and reaction time 5 min demonstrated an antioxidant activity of  $32.43\pm0.03$  %. At 15 min of the reaction, in the same total extract, the activity increased slightly to  $34.29\pm0.24$  %.

At TE = 400.00 mg/dm<sup>3</sup>, however, the grape must of the introduced control variety Chardonnay showed an increase in its antioxidant activity. Thus, 5 min from the moment of mixing the radical and the sample, an antioxidant activity of  $43.50\pm0.47$  % was recorded. At 15 min of the reaction, it marked a jump and reached  $51.02\pm0.12$  %.

Second according to this indicator at TE =  $400.00 \text{ mg/dm}^3$  was the grape must of the Druzhba hybrid. At a reaction time of 5 min, it showed a free radical elimination capacity of  $32.79\pm0.16$  %. At 15 min of the reaction, a growth in the antioxidant activity was reported, and  $36.12\pm0.58$  % elimination of the DPPH radical was found.

The grape must of the local variety Dimyat showed the lowest antioxidant activity in this extract as well (400.00 mg/dm<sup>3</sup>). At 5 min of the reaction, an antioxidant activity of  $28.01\pm0.07$  % was found. At 15 min, it rose and occupied a value of  $34.99\pm0.04$  %.

The grapes were vinified by microvinification. 30 kilograms of grapes, for each variety, were processed according to the classic scheme for the production of white dry wines (Yankov, 1992). The data regarding the main chemical parameters of the obtained wines are presented in Table 2.

Table 2

Wines	Alcohol content, vol. %	Total extract, g/dm <sup>3</sup>	Sugars, g/dm <sup>3</sup>	Titratable acids, g/dm <sup>3</sup>	рН
Chardonnay	$14.14 \pm 0.05$	22.43±0.05	$1.76 \pm 0.15$	6.98±0.07	$3.52 \pm 0.000$
Dimyat	9.80±0.04	68.50±2.15	47.33±2.30	5.49±0.42	3.71±0.000
Druzhba	13.33±0.05	22.02±0.31	5.07±0.61	5.40±0.15	3.71±0.000

Chemical parameters of the experimental wines, harvest 2021

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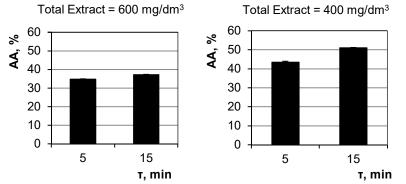


Figure 4. Antioxidant activity of grape must from white introduced variety – Chardonnay at  $TE = 600 \text{ mg/dm}^3$  and  $TE = 400 \text{ mg/dm}^3$ 

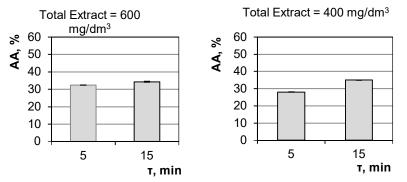


Figure 5. Antioxidant activity of grape must from white local variety – Dimyat at TE =  $600.00 \text{ mg/dm}^3$  and TE =  $400.00 \text{ mg/dm}^3$ 

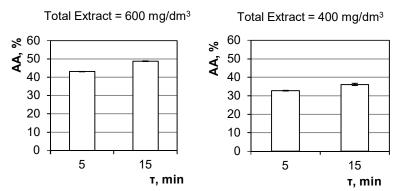


Figure 6. Antioxidant activity of grape must from white hybrid variety – Druzhba at  $TE = 600.00 \text{ mg/dm}^3$  and  $TE = 400.00 \text{ mg/dm}^3$ 

The highest alcohol content was found in Chardonnay ( $14.14\pm0.05$  vol. %). Low levels of alcohol were found in the wine of the Dimyat variety ( $9.80\pm0.04$  vol. %). Ethyl alcohol varies normally in wines in the range of 7.00 vol. % – 17.00 vol. % and accordingly it the analyzed wines could be categorized (by alcohol content) in the following order: Dimyat and Druzhba as dry (table), whose category (Chobanova, 2012) covers an alcohol range from 9.00 vol. % to 13.00 vol.%; Chardonnay – as strong, whose category is characterized by an ethanol content above 14.00 vol. %.

Almost all analyzed wines showed a normal total extract. An exception, however, was observed in the wine of the Dimyat variety. Its extract content was extremely high  $(68.50\pm2.15 \text{ g/dm}^3)$ . The reason for this was the high residual sugar of this wine  $(47.33\pm2.30 \text{ g/dm}^3)$ . The yeast microflora, only in this variant, did not complete the fermentation, which reflected in a lower alcohol content and directly on its higher total extract.

Examining the content of residual sugars in the analyzed white wines, their high presence in Dimyat wine were visible  $(47.33\pm2.30 \text{ g/dm}^3)$ . As interpreted above, this content, linked to the alcohol content, indicated incomplete fermentation biotransformation of grape sugars. According to the content of residual sugars, Chardonnay wine  $(1.76\pm0.15 \text{ g/dm}^3)$  was categorized as dry (up to 4.00 g/dm<sup>3</sup> residual sugars), and that of the Druzhba hybrid  $(5.07\pm0.61 \text{ g/dm}^3)$  as semi-dry (from 4.00 to 12.00 g/dm<sup>3</sup> residual sugars).

The highest titratable acidity was found in Chardonnay ( $6.98\pm0.07 \text{ g/dm}^3$ ). Dimyat ( $5.49\pm0.42 \text{ g/dm}^3$ ) and Druzhba ( $5.40\pm0.15 \text{ g/dm}^3$ ) showed close values for this indicator with a slight advantage for Dimyat. All established levels of titratable acids were in the optimum ( $5.00 \text{ to } 9.00 \text{ g/dm}^3$ ) for this indicator (Chobanova, 2012).

Regarding the actual acidity (pH), a variation was found from  $3.52\pm0.000$  (Chardonnay) to  $3.71\pm0.000$  (Druzhba and Dimyat).

The data on the content of total phenolic compounds (TPC) in the investigated wines are presented in figure 7.

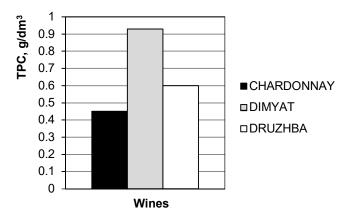


Figure 7. Content of total phenolic compounds (TPC) in white wines of the studied varieties

In the analysis of the TPC of white wines, the highest concentration presence was found in Dimyat  $(0.93\pm0.000 \text{ g/dm}^3)$ . The wine of the Druzhba hybrid variety also showed a high concentration of TPC  $(0.60\pm0.000 \text{ g/dm}^3)$ . The lowest phenolic accumulation for the specific harvest (2021) was identified in the wine of the control introduced variety Chardonnay

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 $(0.45\pm0.000 \text{ g/dm}^3)$ . In terms of concentration, the quantitative presence of TPC correlated with the results of other studies, which determined a range in the content of TPC in white wines from 50.00 to 2000.00 mg/dm<sup>3</sup> (0.05–2.00 g/dm<sup>3</sup>) (Shadidi and Nazck, 1995). The data also correlated with the study of Radeka et al. (2022), who found total phenolic content of white wines from Croatian Malvasia and Pošip varieties ranged from 226.20 to 505.40 mg/dm<sup>3</sup> (0.26 to 0.50 g/dm<sup>3</sup>).

The data on the detected flavonoid phenolic compounds (FPC) in white wines are presented in Figure 8.

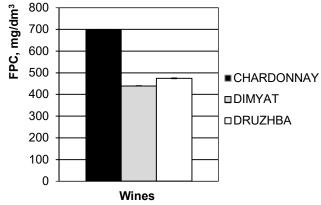


Figure 8. Content of flavonoid phenolic compounds (FPC) in white wines of the studied varieties

The highest content of FPC was found in the wine of the control introduced variety Chardonnay (696.46 $\pm$ 0.37 mg/dm<sup>3</sup>). The wines of the other two studied varieties demonstrated close values for this indicator – the wine of the Druzhba hybrid showed a slightly higher concentration of FPC (473.54 $\pm$ 3.03 mg/dm<sup>3</sup>), compared to the wine of the local Dimyat variety (439.38 $\pm$ 3.35 mg/dm<sup>3</sup>).

The main representatives of the flavonoid group of phenolic compounds in grapes and wine 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 white wines ranged from 49.00±0.97 mg/dm<sup>3</sup> to 296.05±5.92 mg/dm<sup>3</sup>. The FPC content found in our study was higher.

The data on the determined content of NPC in the investigated wines are presented in figure 9.

The highest content of NPC in white wines was found in the Druzhba hybrid variety  $(130.47\pm0.59 \text{ mg/dm}^3)$ . The wine of the local variety Dimyat ranked immediately after it  $(93.51\pm0.34 \text{ mg/dm}^3)$ , and the lowest content was recorded in the wine of the control introduced variety Chardonnay (84.13±0.43 mg/dm<sup>3</sup>), as the difference between the wines of the local and the introduced variety according to this indicator was not great.

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 white wines from 159.60 $\pm$ 68.40 mg/dm<sup>3</sup> to 275.20 $\pm$ 35.40 mg/dm<sup>3</sup>. The data in our study correlated with those established by the cited team.

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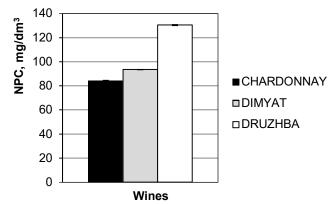


Figure 9. Content of non-flavonoid phenolic compounds (NPC) in white wines of the studied varieties

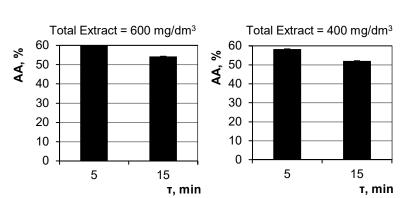
The data on the antioxidant activity of the white wines from the studied varieties are presented in Figures 10, 11 and 12.

The highest antioxidant activity in these wines was found in the wine of the control introduced variety Chardonnay. At TE =  $600.00 \text{ mg/dm}^3$  at 5 min of the reaction with added DPPH radical, the elimination of free radicals was found to be  $62.37\pm0.20$  %. After 15 min of interaction with the radical, a slight decrease in activity was recorded, reaching  $54.26\pm0.11$  %. At TE =  $400.00 \text{ mg/dm}^3$  at 5 min of the reaction in the wine of this variety, a radical-scavenging activity of  $58.30\pm0.04$  % was found. A slight decrease in this extract was observed after 15 min of the reaction with a generated antioxidant activity of  $52.00\pm0.13$  %.

Second, in terms of its ability to capture free radicals, was the wine of the local Dimyat variety. At TE = 600.00 mg/dm<sup>3</sup> and a reaction time of 5 min, a radical-scavenging activity of  $38.47\pm0.31$  % was generated. A slight decrease was observed at 15 min of the reaction reaching  $35.15\pm0.19$  % DPPH capture. At TE = 400.00 mg/dm<sup>3</sup>, after 5 minutes of the reaction, an activity of  $41.55\pm0.45$  % was found. At 15 min in the same extract, a decrease in antioxidant activity was found, reaching  $30.79\pm0.00$  %.

Among the investigated white wines, the Druzhba hybrid wine has the lowest antioxidant activity. At TE =  $600.00 \text{ mg/dm}^3$  and at the 5 min from the moment of mixing of the radical with the wine, a radical-scavenging activity of  $31.05\pm0.24$  % was found. At 15 minutes of reaction, the antioxidant activity of the wine of this variety had low change – a negligible decrease was observed with a reported antioxidant activity of  $30.44\pm0.03$  %. At TE =  $400.00 \text{ mg/dm}^3$ , a decrease in activity was observed, and at 5 min it was  $25.40\pm0.20$  %. At 15 min, in this extract, as with the previous one, a very slight decrease was found with generated antioxidant activity of  $24.51\pm0.02$  %.

The trend in the established antioxidant activity of white wines could be directly related to the accumulation of FPC. In terms of both indicators (antioxidant activity and FPC), the Chardonnay wine is significantly different from the wines of the other two studied varieties, which showed similar concentrations of FPC and, accordingly, similar percentages in the capture of free radicals. Compared to the wine of the Dimyat variety, Chardonnay showed more than 1.5 times higher radical-eliminating activity, and compared to Druzhba – 2 times higher. This, for the specific harvest (2021), proved that the control introduced Chardonnay variety was characterized by the highest biological potential.



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Figure 10. Antioxidant activity of white wine from control introduced variety Chardonnay at  $TE = 600 \text{ mg/dm}^3$  and  $TE = 400 \text{ mg/dm}^3$ 

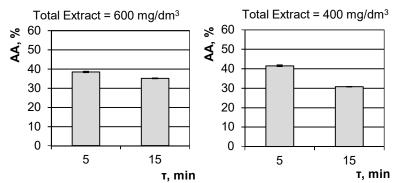


Figure 11. Antioxidant activity of white wine from the local variety Dimyat at TE = 600 mg/dm<sup>3</sup> and TE = 400 mg/dm<sup>3</sup>

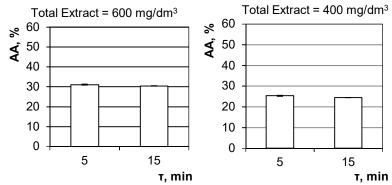


Figure 12. Antioxidant activity of white wine from Druzhba hybrid variety at TE = 600 mg/dm<sup>3</sup> and TE = 400 mg/dm<sup>3</sup>

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Paixão et al. (2007) studied white wines from the region of the island of Madeira and found a variation in the percentage of eliminated DPPH radicals from  $13.47\pm2.10$  % to  $68.69\pm5.40$  %. The results of our research were in complete agreement with the cited team. The results were also in agreement with the study of Marković et al. (2015), who found antioxidant activity in white wines of the local Croatian white variety Žilavka, which varied between the eighteen investigated samples from different regions of Croatia in the range of  $23.40\pm1.40$  % to  $83.10\pm1.60$  %. Our data also correlated with the study by Kharadze et al. (2018), who determined the antioxidant activity of white wines from five varieties grown in the region of western Georgia. The team examined 14 white wines and found a variation in their antioxidant activity from 27.00 % to 45.25

# Conclusions

The following conclusions could be made from the conducted research:

- The results obtained regarding the three main technological indicators (sugars, titratable acids and pH) of the grape must indicated that the grapes were harvested at the right time, at technological maturity, with a good balance between sugars and acids and good quality for undergoing fermentation process.
- The highest presence of TPC was proved in the grape must of the local variety Dimyat.
- The study of the presence of FPC in the grape must of the varieties showed that Chardonnay has the highest potential for FPC accumulation, reflecting in a significantly higher amount of this component of the phenolic composition, compared to the other two varieties.
- The highest presence of NPC was found in the grape must of the control introduced variety Chardonnay. It was almost four times higher, compared to that of the local variety Dimyat and more than one and a half times, compared to the Druzhba hybrid.
- At TE=600.00 mg/dm<sup>3</sup>, the highest antioxidant activity was found in the grape must of the Druzhba hybrid variety. The musts of the introduced Chardonnay and local Dimyat showed similar antioxidant activity in this extract.
- When the extract was reduced (400.00 mg/dm<sup>3</sup>), however, Chardonnay demonstrated the highest antioxidant activity, followed by Druzhba, and it was lowest in Dimyat.
- The chemical parameters of the analyzed wines were optimal. The only exception was the wine of Dimyat, in which low alcohol (9.80±0.04 vol. %), very high extract (68.50±2.15 g/dm<sup>3</sup>) and high residual sugars (47.33±2.30 g/dm<sup>3</sup>) were found. The reason was a disturbance in the alcoholic fermentation, which led to its incomplete progress.
- Dominance in the content of TPC was found in the wine form the local variety Dimyat (0.93±0.000 g/dm<sup>3</sup>), while the Chardonnay control showed the lowest presence of TPC (0.45±0.000 g/dm<sup>3</sup>).
- In terms of FPC content, Chardonnay wine dominated (696.46±0.37 mg/dm<sup>3</sup>), and Dimyat had the lowest content (439.38±3.35 mg/dm<sup>3</sup>).
- The highest concentration of NPC was found in the wine of the Druzhba hybrid variety (130.47±0.59 mg/dm<sup>3</sup>). The lowest result according to this indicator was found in the wine of the control introduced variety Chardonnay (84.13±0.43 mg/dm<sup>3</sup>).
- The trend in the established antioxidant activity of the white wines was directly related to the accumulation of FPC. In terms of both indicators (antioxidant activity and FPC), the Chardonnay wine was significantly different from the wines of the other two

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studied varieties, which showed similar concentrations of FPC and, accordingly, similar percentages in the capture of free radicals. Compared to the wine of the Dimyat variety, Chardonnay showed more than 1.5 times higher radical-eliminating activity, and compared to Druzhba - 2 times higher.

The research proved that the white varieties and wines grown in the region of Central Northern Bulgaria showed a balanced biological capacity and potential, comparable to wines and grapes from other regions of the world.

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