PHENOLIC AND ANTIOXIDANT PROFILE OF GRAPES FROM WHITE GRAPEVINE VARIETIES GROWN IN THE REGION OF NORTHERN BULGARIA

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Abstract
A study to determine the phenolic and antioxidant profile of grapes from white introduced (Chardonnay, control), local (Dimyat) and hybrid (Druzhba) grape varieties grown in the region of northern Bulgaria was performed. The phenolic content of grapes, the content of phenolic compounds and antioxidant activity of its must were determined. The content of the varieties with regard to TPC, FPC and NPC in the structural elements of the cluster increased in the order whole berries < skins < bunches < seeds. The highest TPC contained the berries and seeds of the Chardonnay variety and the skins and bunches of the Dimyat variety. The content of FPC was the highest in the berries and skins of Druzhba variety, in the bunches of Dimyat variety and Chardonnay's seeds. The highest content of NPC was reported in the berries, skins and bunches of the Druzhba variety and in the seeds of the Chardonnay variety. The last variety dominated over the other varieties in the content of TPC (0.743 ± 0.005 g/dm^3) in the grape must too. According to the FPC indicator for the must, Druzhba shown the highest potential (1107.20 ± 7.60 mg/dm^3). According to the content of NPC, the highest concentration was again established in Chardonnay's must (565.50 ± 172.37 mg/dm^3). The grape must of the introduced control variety Chardonnay has the highest antioxidant activity (against DPPH radical) of all studied varieties. A close correlation between Chardonnay's TPC, NPC and antioxidant activity was found. This proved that it, as the richest of phenolic compounds, exhibited the highest anti-radical potential.

Key words: phenols, antioxidants, grapes, vine varieties, free radicals.

Received: 24.08.2021 Reviewed: 20.10.2021 Accepted: 21.10.2021

1. INTRODUCTION

The biological benefits of grapes and wine consumption are well known. They are basically related to the serious and significant potential of antioxidant effects, mainly due to the presence of phenolic compounds (especially in red grapes and wines) (Bub et al., 2003). Determining factors for their synthesis, species and quantitative presence in grapes are grapevine variety, soil and climatic zone of cultivation, temperature during the vine growing, features of the terrain, precipitation, direct sun exposure, relative humidity, application of agronomic and plant protection measures (Abrasheva et al., 2008; Nile et al., 2013).

The content of phenolic compounds in white varieties occupies a quantitative share from 200.00 to 500.00 mg/dm^3 and it is significantly lower than in red, where anthocyanins and polyphenols are added to the main phenolic profile (Velkov, 1996; Franco-Bañuelos et al., 2017). A number of studies have been performed determining both the total phenolic content and defining individual phenolic representatives in grapes from different regions of the world. Franco-Bañuelos et al. (2017) analyzed the total phenolic content in grapes of four white (Sauvignon Vert, Palomino, Furmint and Sémillon) grapevine varieties from the region of Mexico. The team found variation in total phenolic content from 112.70 mg GAE.100g^{-1}

(Sauvignon Vert) to 218.00 mg GAE.100g^{-1} (Furmint). Oprica et al. (2016) conducted a study to identify common phenols and falvonoids in grapes of three white varieties (Grasă de Cotnari, Fetească and Tămâioasă) grown in Romania. The team analyzed the content of phenols in the structural elements of the cluster and found the highest presence of common polyphenols and flavonoids in the seeds of the studied varieties. Margaryan et al. (2017) investigated the phenolic profile and antioxidant activity in 40 cultivated and wild varieties from Armenia. The team found noticeable differences in the concentration presence of total phenolic compounds and antioxidant activity between the groups and a decrease in these indicators in the order of wild species > aboriginal species > interspecific and intraspecific hybrids. A number of studies have reported a correlation between phenol content and antioxidant capacity (Arnous et al., 2002; Fillipe et al., 2001; Aviram and Fuhrman, 2002). Nile et al. (2013) conducted an extensive study to define the antioxidant activity (by DPPH method) of white and red grapes from 20 varieties of vines with different genetic origin - *Vitis vinifera*, *Vitis labrusca* and *Vitis hybrid* from the region of R. Korea. Their results indicate different variations in the antioxidant activity of the skins (12.50% to 60.20%) and the fleshy part (35.40% to 85.40%) of the grain. Based on the results obtained, they conclude that the freshly part has a higher antioxidant potential. Balík et al. (2009) investigated the relationship between polyphenols and grape antioxidant activity and statistically proved a significant correlation between the total polyphenol content and the higher antioxidant activity.

Based on extensive studies concerning the potential of grapes as a source of biological components with antioxidant activity and the multicomponent influence of each region on the synthesis of such compounds, the aim of this study is to determine the phenolic and antioxidant profile of white grapes from the region of northern Bulgaria.

2. MATERIALS AND METHODS

Grapevine varieties

The study was conducted at the Institute of Viticulture and Enology (IVE) - Pleven (central northern Bulgaria). The object of the study was grapes, vintage 2020, from three white grapevine varieties - introduced, local and hybrid:

- *Chardonnay* - white grapevine variety, originating from Burgundy and Champagne, France (Sweet, 2007). For the region of Pleven it ripens around the middle of September. It has a very good fertility. It is not resistant to fungal diseases and gray rot. The variety is relatively resistant to low winter temperatures. It has the ability to accumulate sugars quickly, and at high sugar content (20-24%) retains a relatively high titratable acidity (7-9 g/dm^3). The grapes are used to produce quality white dry wines with a fruity aroma, harmonious taste and long aftertaste. When grapes are harvested at the appropriate technological maturity, wine materials for sparkling wines can also obtained (Radulov et al., 1992; Roychev, 2012).
- *Dimyat* - an old local, Bulgarian, white grapevine variety, also distributed in other wine-growing regions of the Balkan Peninsula. Late ripening variety, as for the region of Pleven it ripens in the second half of September. It has a high fertility and yield. It is sensitive to fungal diseases, weakly resistant to low winter temperatures. The grapes have good sugar accumulation (19-21%) at titratable acids of 6-7

g/dm^3. The wines have a delicate fruity aroma, fresh and harmonious taste. They are suitable for aging, where they develop a good bouquet and taste. When the variety is harvested at the appropriate technological maturity, the grapes can be used to obtain wine materials for sparkling wines and distillate wine materials (Radulov et al., 1992; Roychev, 2012).

● *Druzhba* - white grapevine variety, created by complex interspecific hybridization (Muscat Hamburg x Save Villar 12 375) x (Zarya Severa x Muscat Hamburg) and approved in 1983. Included in the Official Variety 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. The variety has increased resistance to fungal diseases and low winter temperatures. At technological maturity the sugar content is 19-21%, with titratable acids of 6.5-7.5 g/dm^3. The grapes are suitable for fresh consumption and for the production of dry wines with a pleasant, well-defined fruity and muscat aroma, soft and harmonious taste with sufficient freshness (Radulov et al., 1992; Roychev, 2012).

Experimental areas and pruning operations

The experimental work covers fruit-bearing vineyards of each studied variety and includes 20 experimental vines. All varieties are refined on the rootstock Berlandieri x Riparia SO4.

The vines of the Dimyat variety are planted at a distance of 2.20/1.30 m and are grown on a low-stem formation; Chardonnay - at a distance of 3.00/1.30 m and medium-stem formation; Druzhba - distance 3.20/1.30 m and medium stem formation.

The pruning and loading of the vines was done as follows:

● Chardonnay variety - 32 winter eyes on a vine;

● Dimyat variety - 18 winter eyes on a vine;

● Druzhba variety - 36 winter eyes on a vine.

Determination of the phenolic content of grapes and must from the studied varieties

To study the phenolic content of the grapes by varieties, at the onset of technological maturity and after harvesting, their content in the structural elements of the cluster - bunches, seeds, skins, berries were analyzed. For this purpose, an average sample of grapes of each variety (2 kg) was selected, from which 5 g of bunches (dried and cut into segments), 5 g of seeds (pre-ground in a mortar), 5 g of skins (pre-dried) and 10 g whole berries (pre-torn in a mortar and with crushed seeds) were weighed. The prepared and weighed quantities were transferred to Erlenmeyer flask. Extractant C_2H_5OH / HCl (1% v/v) was used for extraction (Stoyanov, 2007).

To determine the phenolic content of the grapes (in the liquid phase, after the extraction) and the phenolic content of the must (separated immediately after crushing of the grapes) the following indicators were analyzed:

● Total phenolic compounds (TPC), g/dm^3 gallic acid - Singleton et Rossi method with Folin–Ciocalteu reagent;

● Flavonoid phenolic compounds (FPC), mg/dm^3 catechin equivalent - Sommers method;

● Non-flavonoid phenolic compounds (NPC), mg/dm^3 caffeic equivalent - Sommers method.

Determination of antioxidant (DPPH •) activity of grapes and must

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 values of the molecular light absorption (spectrophotometrically at a wavelength of 515 nm) of the control and experimental sample, noted by Ac and Ae, respectively, were measured. Measurements were made at reaction times of 5 and 15 minutes from the time of the reagents mixing. The antiradical activity was calculated by the formula:

$AAR = 10^2.(Ak - Ae).Ак^{-1}$, % (1)

Statistical processing

Statistical data processing was performed, including determination of standard deviation (± SD), with three repetitions for each analysis. The determination of the indicator was realized with the MS Excel 2007 from the Microsoft Package (Microsoft Corporation, USA).

3. RESULTS AND DISCUSSION

Phenolic content of grapes

The content of TPC, FPC and NPC in the structural elements of the cluster - berries, skins, bunches, seeds was studied.

The results of the grapes phenolic content of Chardonnay, Dimyat and Druzhba are presented in Table 1. The content of the studied components in all three varieties increased in the order of whole berries< skins < bunches < seeds. The phenolic content of structural elements of the grapes in relation to TPC in all three varieties was close. The highest TPC contained berries (0.24 ± 0.00 g/dm^3) and seeds (2.96 ± 0.02 g/dm^3) of Chardonnay variety and skins (0.55 ± 0.00 g/dm^3) and bunches (1.93 ± 0.00 g/dm^3) of Dimyat variety.

More significant differences were observed in the FPC content of the varieties. In the case of Druzhba the amount of FPC in the berries (1308.14 ± 7.40 mg/dm^3) and skins (1392.57 ± 1.17 mg/dm^3) was significantly higher compared to the other two varieties. The bunches of Dimyat variety had the highest content of FPC (3104.15 ± 3.70 mg/dm^3), followed by those of Druzhba variety. The concentration of FPC in Chardonnay's bunches was twice times lower than the other varieties. Chardonnay's seeds (6195.54 ± 23.65 mg/dm^3) contained the highest quantity of FPC, almost twice as much as Dimyat (3985.61 ± 29.87 mg/dm^3).

Differences were also found in the amount of NPC in the elements of the cluster of the investigated varieties. The results shown the highest content in the berries (76.82 ± 0.03 mg/dm^3), skins (129.78 ± 0.59 mg/dm^3) and bunches (266.05 ± 0.30 mg/dm^3) of Druzhba variety. Their amount was twice as large as in Dimyat's berries (46.16 ± 0.19 mg/dm^3) and Chardonnay's bunches (136.17 ± 0.40 mg/dm^3). The seeds of the Chardonnay variety (507.94 ± 1.07 mg/dm^3) had the highest content of NPC, followed by Druzhba. In Dimyat they were twice less (189.12 ± 1.90 mg/dm^3).

Phenolic compounds - TPC, FPC and NPC in grape must of the studied varieties

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

Analyzing the total phenolic compounds in the grape must of the introduced (Chardonnay), local (Dimyat) and hybrid (Druzhba) varieties, it was clear that Chardonnay shown the highest amount of TPC (0.743 ± 0.005 g/dm^3). The local variety Dimyat and the hybrid Druzhba had accumulated lower amounts of TPC, respectively 0.41 ± 0.00 g/dm^3 and 0.39 ± 0.00 g/dm^3.

Table. 1. Phenolic content of grapes from the studied white varieties

Structural elements of the cluster	Indicators	Chardonnay	Dimyat	Druzhba
Whole berries	TPC, g/dm^3 g.a.	0.24±0.00	0.21±0.00	0.22±0.01
	FPC, mg/dm^3 cat. equiv.	1050.10±2.38	368.41±2.97	1308.14±7.40
	NPC, mg/dm^3 caf. equiv.	66.49±8.71	46.16±0.19	76.82±0.03
Skins	TPC, g/dm^3 g.a.	0.35±0.00	0.55±0.00	0.37±0.00
	FPC, mg/dm^3 cat. equiv.	1009.83±5.05	701.76±5.90	1392.57±1.17
	NPC, mg/dm^3 caf. equiv.	94.26±0.14	79.56±0.58	129.78±0.59
Bunches	TPC, g/dm^3 g.a.	0.99±0.00	1.93±0.00	1.62±0.00
	FPC, mg/dm^3 cat. equiv.	1600.49±3.18	3104.15±3.70	3065.87±5.36
	NPC, mg/dm^3 caf. equiv.	136.17±0.40	141.44±0.55	266.05±0.30
Seeds	TPC, g/dm^3 g.a.	2.96±0.02	2.04±0.05	2.87±0.07
	FPC, mg/dm^3 cat. equiv.	6195.54±23.65	3985.61±29.87	6083.97±5.74
	NPC, mg/dm^3 caf. equiv.	507.94±1.07	189.12±1.90	472.26±0.34

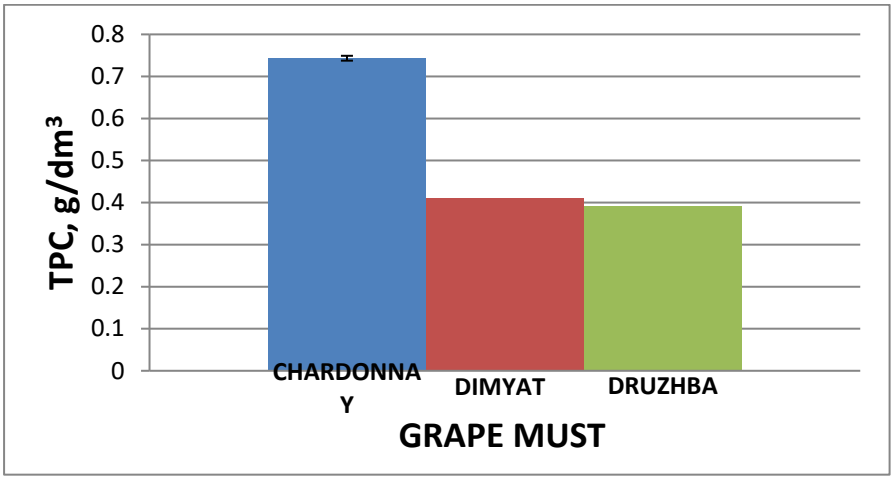

Fig. 1. Total phenolic compounds (TPC) in grape must of the studied varieties

The results indicated that the control introduced Chardonnay variety had a significantly higher potential to accumulate phenolic compounds, compared to the other two experimental white varieties, in the conditions of central northern Bulgaria.

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

The lowest concentration of FPC was found in the must of the control variety. Chardonnay's grape must shown a FPC content of 608.12 ±

9.92 mg/dm^3. The local variety Dimyat shown a higher concentration of FPC (785.55 ± 3.85 mg/dm^3) compared to the control. The highest concentration of FPC (almost twice higher than the control level) was found in the must of the hybrid variety Druzhba (1107.20 ± 7.66 mg/dm^3).

Druzhba variety is a complex interspecific hybrid. The results obtained for the 2020 harvest indicated that it had a better potential to accumulate FPC (under the soil and climatic conditions of central northern Bulgaria), which was reflected in higher levels of this component in its must.

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

The results indicated the highest concentration of NPC in the must of the control Chardonnay variety (556.50 ± 172.37 mg/dm^3). The local variety Dimyat shown the lowest content of NPC (148.29 ± 0.54 mg/dm^3) of the three studied varieties. The content of NPC in Druzhba grape must ranked second, after the control, with the presence of NPC of 295.70 ± 0.39 mg/dm^3.

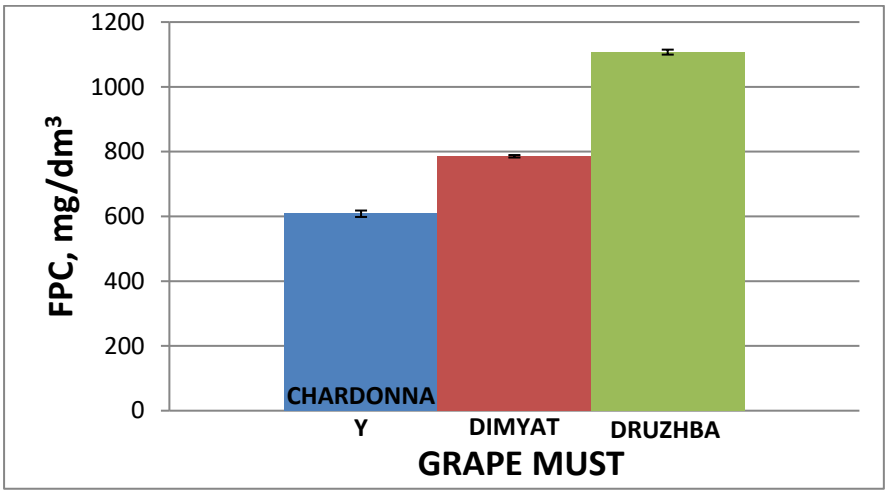

Fig. 2. Content of FPC in grape must of the studied varieties

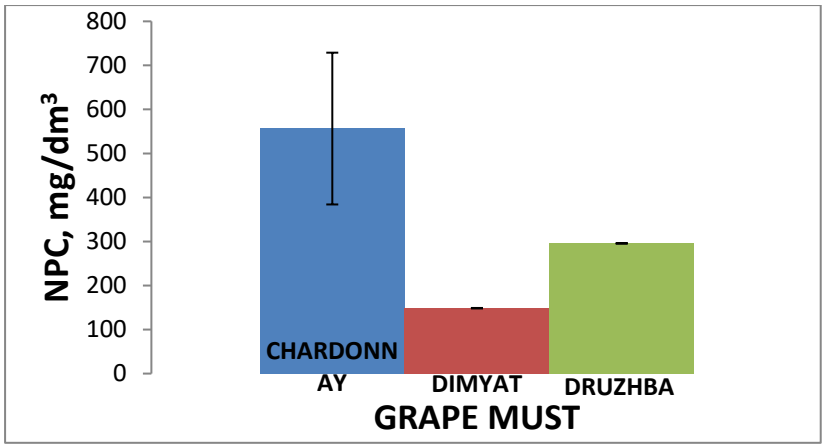

Fig. 3. Content of NPC in grape must of the studied varieties

From the obtained results it is clear that the control variety Chardonnay shown a significant advantage in terms of the concentration presence of NPC, compared to the other two studied varieties.

Antioxidant activity (as anti-radical activity against a stable DPPH radical) in grape must of the studied white varieties

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

From the obtained results it was clear that the grape must of the introduced white variety - Chardonnay shown the highest antioxidant activity, compared to the other two experimental varieties. At TE = 600.00 mg/dm^3 and a reaction time of 5 min, Chardonnay grape must shown 94.09 ± 0.20% antiradical activity. It increased with increasing of the reaction time and reached a value of 96.62 ± 0.32% at 15 minutes.

At a lower amount of extract (TE = 400.00 mg/dm^3), respectively, at a reaction time of 5 min Chardonnay shown antioxidant activity of 69.74 ± 0.06% capture of the free DPPH radical. At 15 min of the same extract an increase in antioxidant activity was found and it reached 70.38 ± 0.11%.

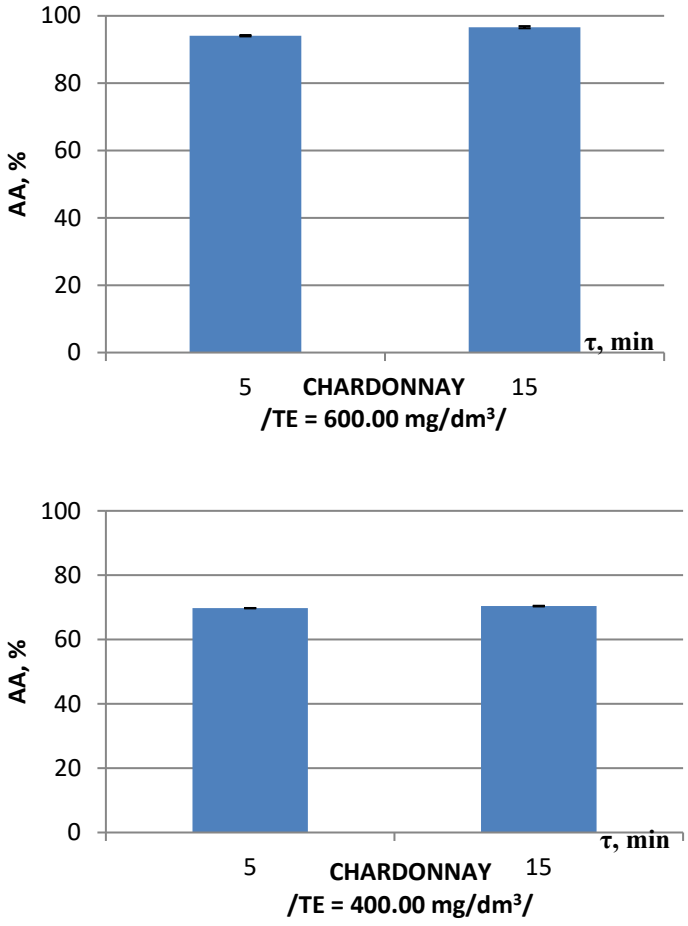

Fig. 4. Antioxidant activity of white introduced variety - Chardonnay at TE = 600.00 mg/dm^3 and TE = 400.00 mg/dm^3

The difference between the detected antioxidant activity in the two total extracts was significant, more than 20% higher at TE = 600.00 mg/dm^3. The result was clear evidence that higher must extractivity led to higher levels of antioxidant activity. When the extractivity of Chardonnay must was reduced, a significant decrease in its anti-radical properties was registered.

The local variety Dimyat shown the lowest antioxidant activity (figure 5) of the three studied varieties. At TE = 600.00 mg/dm^3 and a reaction time of 5 min, 57.12 ± 0.07% antioxidant activity was observed in its must. At a reaction time of 15 minutes the activity increased and reached 59.72 ± 0.04%.

At the lower amount of extract (TE = 400.00 mg/dm^3) the antioxidant activity at a reaction time of 5 min was 58.64 ± 0.05%. At a reaction time of 15 minutes, it increased slightly and reached 59.68 ± 0.01%.

As can be seen from the obtained results, the Dimyat variety was not changed its antiradical activity when the total extract was reduced. The results obtained for both extracts shown close percentages of antioxidant activity, almost identical. In Dimyat, in contrast to the Chardonnay control, a smooth activity was preserved, almost independent of the total extract of the grape must.

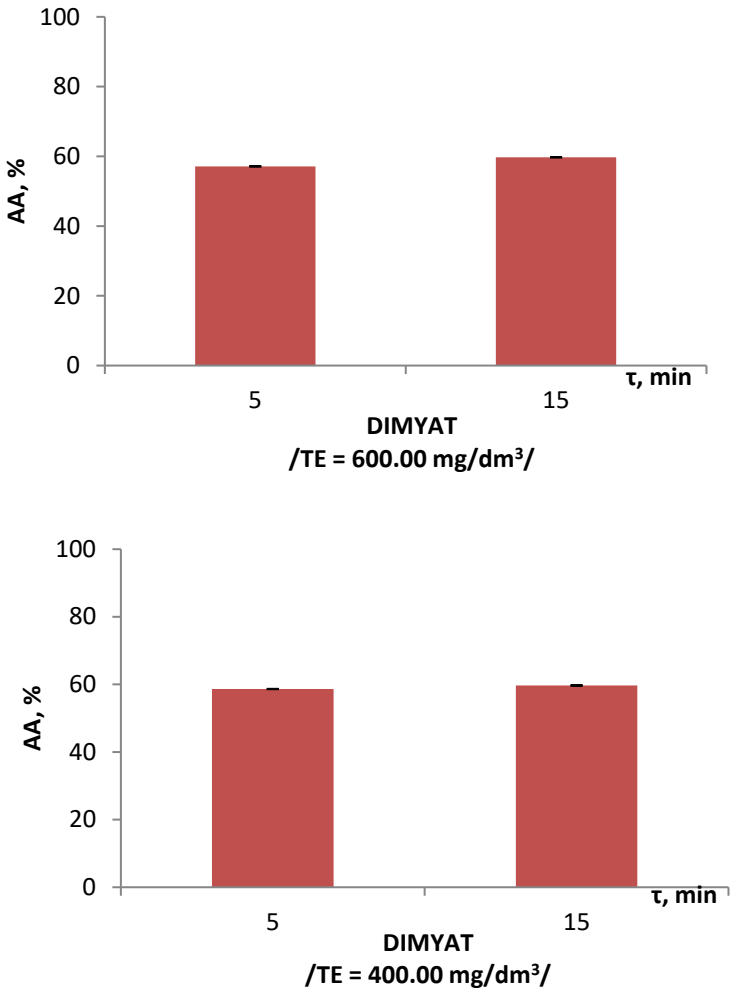

Fig. 5. Antioxidant activity of white local variety - Dimyat at TE = 600.00 mg/dm^3 and TE = 400.00 mg/dm^3

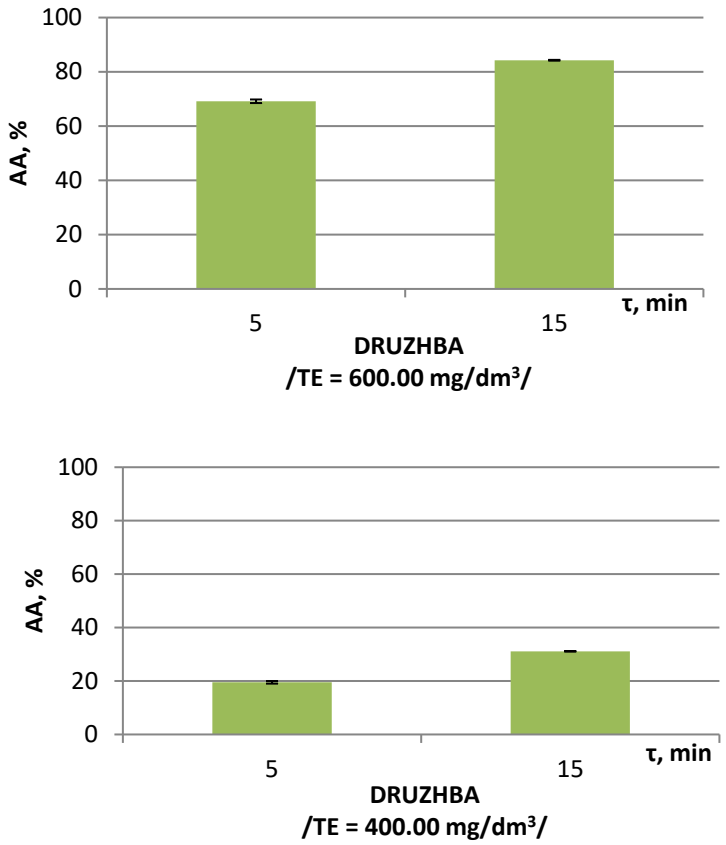

Fig. 6. Antioxidant activity of grape must of hybrid variety - Druzhba at TE = 600.00 mg/dm^3 and TE = 400.00 mg/dm^3

The third studied white variety - Druzhba hybrid, ranked second in antioxidant capacity, after Chardonnay (figure 6).

At TE = 600.00 mg/dm^3 and a reaction time of 5 min, Druzhba grape must shown 69.15 ± 0.65% antioxidant activity. As the reaction time increased to 15 min, it increased significantly and reached 84.26 ± 0.14%.

At TE = 400.00 mg/dm^3, Druzhba's antioxidant capacity was decreased. At a reaction time of 5 minutes, 19.51 ± 0.48% antioxidant activity was found. As the reaction time increased to 15 minutes, it increased by just over 10% and reached 31.11 ± 0.09%.

Visible from the obtained results for the Druzhba variety was the tendency of high antioxidant activity at higher extract. At TE = 600.00 mg/dm^3 in the grape must of this variety were found at a reaction time of 5 min - over three times higher antioxidant activity, and at a reaction time of 15 min - over twice as high antioxidant capacity, compared to the data, obtained at TE = 400.00 mg/dm^3.

The established highest antioxidant activity in the introduced Chardonnay variety was closely correlated with the established content of TPC and NPC. According to these two indicators, it shown the highest values (figure 1 and figure 3). Chardonnay's grape must, due to the highest established total phenolic composition and higher amount of NPC, shown the best antiradical effect (DPPH •) from the studied varieties.

The grape must of the Druzhba variety was ranked after Chardonnay in terms of its antioxidant capacity. It was evident from the data for NPC in Druzhba (figure 3) that it was ranked on second place by the quantitative presence of this group of compounds. A correlation between Druzhba TPC and antioxidant activity was not observed, but considering the confirmed correlation between NPC and antioxidant activity in grape must of this variety, it was clear that this group of compounds had the highest effect on the manifestation of its antioxidant activity. The same correlation was observed for the local variety Dimyat. It shown the lowest antioxidant activity, correlating with the lowest content of NPC from the three studied varieties.

4. CONCLUSIONS

As a result of the conducted research the following main conclusions could be made:

- The phenolic content of the varieties with regard to TPC, FPC and NPC in the structural elements of the cluster increased in the order of whole berries < skins < bunches < seeds.
- The highest TPC contained the berries and seeds of the Chardonnay variety and the skins and bunches of the Dimyat variety. The content of FPC was highest in berries and skins of Druzhba variety, in the bunches of Dimyat variety and Chardonnay's seeds. The highest content of NPC was reported in the berries, skins and bunches of the Druzhba variety and in the seeds of the Chardonnay variety.
- The highest content of TPC in the grape must of investigated white varieties was found in the control introduced variety Chardonnay (0.743 ± 0.005 g/dm^3), followed by the local Dimyat and the hybrid Druzhba. Chardonnay had a significant potential to accumulate phenols in the conditions of Pleven, central northern Bulgaria.
- The lowest concentration of FPC was found in the grape must of the Chardonnay control (608.12 ± 9.92 mg/dm^3). According to this indicator, the must of the hybrid white variety Druzhba shown the highest potential (1107.20 ± 7.66 mg/dm^3).
- The Chardonnay control shown a significant advantage in accumulating of NPC (565.50 ± 172.37 mg/dm^3) compared to the other two varieties. The following order of NPC accumulation was observed: NPC (grape must of introduced variety, control) > NPC (grape must of hybrid variety) > NPC (grape must of local variety).
- The grape must of the introduced control Chardonnay variety had the highest antioxidant activity (against DPPH radical) of all studied varieties. A close correlation was found between TPC, NPC and Chardonnay's antioxidant activity. This proved that it, as the richest in phenolic compounds, exhibited the highest antiradical potential.

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