INTRODUCTION

The preventive biological effect of antioxidants associated with the elimination of the so-called reactive oxygen species (free radicals) capable of initiating mutations in cellular structures and inducing significant diseases is well known (Hertog et al., 1995; Srivastava and Kumar, 2015). Nowadays the attention of the scientific community in the field of food science becomes increasingly concentrated in defining the antioxidant capacity and activities of foods and beverages. The study of Renaud and Lorgeril (1992), known as the "French paradox", found a low incidence of cardiovascular diseases in men between the ages of 54 and 65, who moderate consume red wine, and despite the high consumption of fats from foods with animal origin. It gives the start and impetus for extensive and large-scale research on the wine healthy activities.

The antioxidant effect of wine is mainly due to its polyphenolic complex, consisting of natural polyphenolic substances (especially catechins), as well as some oxyacids and products of their metabolism, ascorbic and dioxyfurmaric acids (Velkov, 1996). The content of phenolic compounds in wine is determined by the phenolic stock of the particular grapevine variety and the applied technology (Ivanov, 1981; Chobanova, 2012). According to Waterhouse (2002), the total content of non-flavonoid phenolic compounds in red wines range from 1402.00 - 3130.00 mg/dm³ and significantly exceed that found in white wines (189.00 - 495.00 mg/dm³). Nistor et al. (2015) examined the content of anthocyanins and total phenolic compounds in wines of Cabernet Sauvignon and Pinot Noir varieties from three consecutive harvests (2011, 2012 and 2013) produced in two different wine-cellars. The team found a variation in the content of anthocyanins in the wines of the first group of 341.70 ± 34.32 mg/dm³ - 479.30 ± 46.64 mg/dm³ (Cabernet Sauvignon) and 311.40 ± 15.53 mg/dm³ - 346.50 ± 36.05 mg/dm³ (Pinot Noir). The content of anthocyanins in the analyzed wines of the second producer is in the range of 258.80 ± 10.29 mg/dm³ - 348.10 ± 6.43 mg/dm³ (Cabernet Sauvignon) and 214.20 ± 14.00 mg/dm³ - 296.30 ± 11.53 mg/dm³ (Pinot Noir). With regard to the found total phenolic compounds in the first group, a variation of 2072.00 ± 81.38 mg/dm³ - 2758.00 ± 149.90 mg/dm³ (Cabernet Sauvignon) and 1814.00 ± 92.51 mg/dm³ - 2695.00 ± 76.46 mg/dm³ (Pinot Noir) is found. For the red wines of the second group the total phenolic content is found in the range of 1986.00 ± 163.50 mg/dm³ - 2531.00 ± 77.76 mg/dm³ (Cabernet Sauvignon) and 1752.00 ± 94.90 mg/dm³ - 2214.00 ± 35.56 mg/dm³ (Pinot Noir). The phenolic composition of wines is mainly and closely related to their antioxidant activity.

Baydar et al. (2011) conducted a study to determine the radical-capturing activity of wines from white (Narince) and red varieties (Cabernet Sauvignon and Kalecik Karas), using the DPPH method. The study found a high percentage of antiradical activity in red wines (84.01% for Kalecik Karas and 81.34% for Cabernet Sauvignon), compared to the low values of this indicator in white wine - 19.10%. Kataliníč et al. (2004) investigated the antioxidant activity (via DPPH method) of 6 red wines (Dingač, Babič, Cabernet Sauvignon, Faros, Ezerov, and Carignano) from Croatia, GAE. 1t to 1366.21 mg. GAE. 1t (Baydar et al., 2011). Shadidi and Nazek (1995) found that the content of total phenolic compounds in red wines ranged from 1000.00 - 4000.00 mg/dm³, and in white from 50.00 - 2000.00 mg/dm³. Li et al. (2009) found that the content of total phenolic compounds in red wines ranged from 1402.00 - 3130.00 mg/dm³ and significantly exceed that found in white wines (189.00 - 495.00 mg/dm³). Nistor et al. (2015) examined the content of anthocyanins and total phenolic compounds in wines of Cabernet Sauvignon and Pinot Noir varieties from three consecutive harvests (2011, 2012 and 2013) produced in two different wine-cellars. The team found a variation in the content of anthocyanins in the wines of the first group of 341.70 ± 34.32 mg/dm³ - 479.30 ± 46.64 mg/dm³ (Cabernet Sauvignon) and 311.40 ± 15.53 mg/dm³ - 346.50 ± 36.05 mg/dm³ (Pinot Noir). The content of anthocyanins in the analyzed wines of the second producer is in the range of 258.80 ± 10.29 mg/dm³ - 348.10 ± 6.43 mg/dm³ (Cabernet Sauvignon) and 214.20 ± 14.00 mg/dm³ - 296.30 ± 11.53 mg/dm³ (Pinot Noir). With regard to the found total phenolic compounds in the first group, a variation of 2072.00 ± 81.38 mg/dm³ - 2758.00 ± 149.90 mg/dm³ (Cabernet Sauvignon) and 1814.00 ± 92.51 mg/dm³ - 2695.00 ± 76.46 mg/dm³ (Pinot Noir) is found. For the red wines of the second group the total phenolic content is found in the range of 1986.00 ± 163.50 mg/dm³ - 2531.00 ± 77.76 mg/dm³ (Cabernet Sauvignon) and 1752.00 ± 94.90 mg/dm³ - 2214.00 ± 35.56 mg/dm³ (Pinot Noir). The phenolic composition of wines is mainly and closely related to their antioxidant activity.

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obtained from different harvests. For red wines, the team found a variation from 54.60% (Faros) to 82.60% for Dingač wine. The Cabernet Sauvignon wine showed close antioxidant activity (82.20%) to that of Dingač. In the analysis of the four white wines with the highest DPPH• radical-capturing activity is characterized the wine of the variety Maršinka (16.16%), and with the lowest of the variety Traminak (10.30%). Marković et al. (2015) conducted a study on the total phenolic composition and antioxidant activity (via DPPH•) of 11 white wines from the trade market, obtained from the local variety Žilavka from Bosnia and Herzegovina. The team found antioxidant capacity from 28.80% to 70.20%.

The aim of the present study was to determine the phenolic composition and antioxidant activity of white and red wines obtained from introduced, local and hybrid grapevine varieties from the region of Plevn, Central Northern Bulgaria.

**MATERIAL AND METHODS**

**Grapevine varieties**

The study was conducted at the Institute of Viticulture and Enology (IVE) - Plevn (Central Northern Bulgaria). The subject of the study were white and red wines, harvested in 2020, of three white and three red grapevine varieties - introduced, local and hybrid:

**Introduced varieties**

These varieties had a control role in research.

- **Chardonnay** - white grapevine variety originating from Burgundy and Champagne, France (Sweet, 2007). For the region of Plevn it ripens around the middle of September. It accumulates sugars quickly, maintaining a relatively high titratable acidity (7.0-9.0 g/dm³) at high sugar content (20-24%) (Radulov et al., 1992; Roychev, 2012).

- **Cabernet Sauvignon** – red grapevine variety originating from the Bordeaux region in southwestern France (Sweet, 2008). For the region of Plevn it ripens in the second half of September. At technological maturity, grapes can accumulate sugars up to 21-24%, and its titratable acidity is relatively high (6.5-9.0 g/dm³) (Radulov et al., 1992; Roychev, 2012).

**Local varieties**

- **Dimyat** - old local, Bulgarian, white grapevine variety. Late ripening, as for the region of Plevn it ripens in the second half of September. Grapes accumulate sugars of 19-21% at titratable acids 6.0-7.0 g/dm³ (Radulov et al., 1992; Roychev, 2012).

- **Gamza** - local white grapevine variety. Its grapes ripen in the second half of September. Sugar accumulation is in the range of 19-21% at titratable acids 5.9-8.9 g/dm³ (Radulov et al., 1992; Roychev, 2012).

**Hybrid varieties**

- **Druzhba** - white variety, created by an international team of IVE - Plevn and VNIIH - Novocherkassk (Russia), through complex interspecific hybridization (Muscat Hamburg x Seiv Villar 12 375) x (Zarya Severa x Muscat Hamburg) and approved in 1983. Included in the Official Variety List of Bulgaria in 2012. At technological maturity the sugar content is 19-21%, with titratable acids 6.5-7.5 g/dm³ (Radulov et al., 1992; Roychev, 2012).

- **Rubin** - a hybrid red grapevine variety. It was obtained by intraspecific hybridization by crossing Nebiolo x Syrah (Petkov, 1977) and approved in 1961. Included in the Official Variety List of Bulgaria in 2012. The variety is medium ripening, and for the region of Plevn ripens in the first half of September. The variety has a high sugar accumulation capacity - 22-24% and more, with titratable acids 5.5-6.0 g/dm³ (Radulov et al., 1992; Roychev, 2012).

**Vinification**

The grape harvest of the studied varieties is carried out upon reaching technological maturity. The grapes, in the amount of 30 kg, of each variety were processed in the Experimental Wine Cellar of IVE - Plevn, in the conditions of microvinification, according to the classical schemes for production of white and red wines (Yankov, 1992).

- **Production of white wines**
  - The technological operations include: Crushing the grapes ► Destamming ► Pressing ► Sulphitation (50 mg/dm³ SO₂) ► Clarification of the must and decanting ► Alcoholic fermentation (dry wine yeast Saccharomyces cerevisiae 20 g / hl; temperature 20° C) ► Racking ► Additional sulphitation ► Storage
  - **Production of red wines**
    - The technological operations include: Crushing ► Destemming ► Sulphitation (50 mg/kg SO₂) ► Alcoholic fermentation (dry wine yeast Saccharomyces cerevisiae 20 g/hl; temperature 28° C) ► Separation from solids ► Additional sulphitation ► Storage

**Chemical analysis of the obtained white and red wines**

The analyzes were performed according to the generally accepted methods in wine practice (Ivanov et al., 1979).

- **Determination of sugar content (g/dm³)** - Shooil method;
- **Determination of the alcohol content (vol. %)** - distillation method using a Gibertini apparatus with a densimeter, by determining the density of a non-alcoholic sample;
- **Determination of titratable acids of wine (TA, g/dm³) - by titration with NaOH;**
- **Determination of the pH - with pH meter;**
- **Determination of total extract - by densimeter (Gibertini).**

**Determination of the phenolic content of the wines**

- **Determination of total phenolic compounds (TPC)** - according to the method of Singleton et Rossi (Chobanova, 2012);
- **Determination of the content of flavonoid phenolic compounds (FPC);**
- **Determination of the content of non-flavonoid phenolic compounds (NPC);**
- **Determination of anthocyanin content - method of Singleton et Rossi by changing the pH (Chobanova, 2012) * the analysis was performed only for red wines;**

**Determination of antioxidant (DPPH) activity of the wines**

The antioxidant activity was determined according to the method of Wang et al., 1996 as antiradical activity against a stable product DPPH (2,2-diphenyl-1-picrylhydrazyl) (Sigma Aldrich, Germany). The wine samples were diluted to a total extract of 600.00 mg/dm³ and 400.00 mg/dm³ and the analysis was carried out on the samples thus diluted, respectively marked as TE = 600.00 mg/dm³ and TE = 400.00 mg/dm³.

**Statistical data processing**

The statistical analysis of the data included the determination of the standard deviation (± SD), with three repetitions for each analysis. The determination of the indicator was realized by the program Excel 2007 from the Microsoft Office Package (Microsoft Corporation, USA).

**RESULTS AND DISCUSSION**

The data on the main chemical indicators of the wines obtained are presented in Table 1.

**Table 1.** Chemical indicators of the studied wines

<table>
<thead>
<tr>
<th>WINES</th>
<th>Alcohol content, vol. %</th>
<th>Total extract, g/dm³</th>
<th>Sugars, g/dm³</th>
<th>Titratable acids, g/dm³</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHARDONNAY</td>
<td>12.83±0.030</td>
<td>23.53±0.115</td>
<td>2.03±0.080</td>
<td>8.32±0.075</td>
<td>3.06±0.010</td>
</tr>
<tr>
<td>Introduced white grapevine variety</td>
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<tr>
<td>DIMYAT</td>
<td>12.17±0.005</td>
<td>23.93±0.665</td>
<td>7.35±0.130</td>
<td>6.87±0.040</td>
<td>3.33±0.005</td>
</tr>
<tr>
<td>local white grapevine variety</td>
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</tr>
<tr>
<td>DRUZHBA</td>
<td>14.86±0.055</td>
<td>20.96±0.349</td>
<td>1.56±0.161</td>
<td>5.72±0.046</td>
<td>3.44±0.005</td>
</tr>
<tr>
<td>hybrid red grapevine variety</td>
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<tr>
<td>CABERNET SAUVIGNON</td>
<td>13.35±0.020</td>
<td>26.56±0.057</td>
<td>0.96±0.057</td>
<td>5.35±0.116</td>
<td>3.88±0.000</td>
</tr>
<tr>
<td>introduced red grapevine variety</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAMZA</td>
<td>14.29±0.025</td>
<td>25.63±0.057</td>
<td>1.46±0.277</td>
<td>4.83±0.229</td>
<td>3.82±0.000</td>
</tr>
<tr>
<td>local red grapevine variety</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>RUBIN</td>
<td>13.80±0.017</td>
<td>24.23±0.115</td>
<td>1.30±0.196</td>
<td>6.37±0.075</td>
<td>3.55±0.005</td>
</tr>
<tr>
<td>hybrid red grapevine variety</td>
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</tr>
</tbody>
</table>

The highest alcohol content in white wines was found in the wine from Druzhba variety (14.86 ± 0.025 vol. %), while in red wines this indicator was the highest in Gamza (14.29 ± 0.025 vol. %). The wines had good extractivity, as a rule the total extract of the reds was higher than that of the whites. In the latter group Druzhba...
showed the highest extractivity (20.96 ± 0.349 g/dm³). The other two white wines showed similar values on this indicator. Red wine extract was the highest in Cabernet Sauvignon (26.56 ± 0.057 g/dm³). Regarding the presence of residual sugars, almost all wines were categorized as dry - with a sugar content up to 4.0 g/dm³ (Chobanova, 2012). The only exception was Dimyat wine, which had a higher residual sugar (7.35 ± 0.130 g/dm³), defined as semi-dry.

According to Chobanova (2012), the optimal titratable acidity for dry wines varies from 5.00 to 9.00 g/dm³. In the present study, a variation in this indicator for white wines from 5.72 ± 0.046 g/dm³ (Druzhba) to 8.32 ± 0.075 g/dm³ (Chardonnay) was found. For red wines it was lower and occupied the range from 4.83 ± 0.029 g/dm³ (Gamza) to 6.37 ± 0.075 g/dm³ (Rubin). The titratable acidity found in the studied wines was typical for quality dry wines.

The normal pH wine values range from 2.8 to 3.8 (Chobanova, 2012). The study of the three white wines showed a variation on this indicator from 3.06 ± 0.010 (Chardonnay) to 3.44 ± 0.005 (Druzhba), for the red ones it was from 3.55 ± 0.005 (Rubin) to 3.88 ± 0.000 (Cabernet Sauvignon). All wines showed normal pH values.

The data for the content of total phenolic compounds (TPC) in the analyzed wines are presented in Figure 1.

The trend in the amount of FPC was the same as in TPC - higher total amount in red wines compared to white ones. In white wines, the highest presence of FPC was found in the wine of the introduced control Chardonnay variety (769.84 ± 1.833 mg/dm³), followed by the local Dimyat (506.85 ± 1.044 mg/dm³) and the lowest was the content of FPC in the wine of the hybrid Druzhba (278.61 ± 1.251 mg/dm³).

In the analysis of the FPC results in red wines, the quantitative dominance of the Cabernet Sauvignon control (2314.44 ± 2.045 mg/dm³) was absent. It has been replaced by the Rubin hybrid (2532.40 ± 49.938 mg/dm³), which made this wine the richest of FPC from the red group. The wine of the local variety Gamza (1765.53 ± 4.015 mg/dm³) was characterized by the lowest FPC content.

The results regarding the presence of NPC in the studied wines are presented in Figure 3.

The trend in the content of NPC in white wines was the same as that observed for FPC. The wine of the control introduced Chardonnay variety (115.78 ± 0.325 mg/dm³) was characterized by the highest content of NPC. The wine of the local variety Dimyat showed an NPC content of 102.78 ± 0.177 mg/dm³, slightly lower than the control. The lowest content according to this indicator (78.37 ± 0.344 mg/dm³) was found in the wine of the hybrid variety Druzhba. It was lower than the other two wines studied.

As can be seen from the results presented in the figure, that red wines showed higher content of TPC than white wines. This is a normal trend due to purely technological reasons - fermentation of grape juice (for white wines) and fermentation with solid parts (for red wines).

The data regarding the established content of TPC in white wines showed almost identical concentration of phenols in the wine of the introduced Chardonnay variety and the local Dimyat (700.00 ± 0.000 mg/dm³ and 703.33 ± 5.773 mg/dm³, respectively). The wine of the Druzhba hybrid showed lower levels of TPC (400.00 ± 0.000 mg/dm³) compared to the wines of the other two varieties. In the red wines the content of TPC was the highest in the wine of the control introduced variety Cabernet Sauvignon (1666.66 ± 5.773 mg/dm³). It was followed by the wine of the hybrid variety Rubin (1526.66 ± 5.773 mg/dm³), and the lowest content of TPC was found in Gamza (1153.33 ± 5.773 mg/dm³). The data obtained in the present study on the content of TPC in white and red wines correlated with the results reported for this indicator in other studies (Shadidi and Nazck, 1995; Li et al., 2009; Nistor et al., 2015). The content of anthocyanins in red wines is presented in Figure 4.

The content of anthocyanins in red wines is presented in Figure 4.

The anthocyanins are red pigments. After vinification in young red wines, they are accumulated in the amount of 200.00 - 500.00 mg/dm³ (Chobanova, 2012). In the present study, the highest content of anthocyanins was found in the wine of the control introduced Cabernet Sauvignon (390.85 ± 0.374 mg/dm³). It was lower in Rubin (235.60 ± 0.636 mg/dm³) and the lowest in the wine of the local variety Gamza (178.88 ± 0.425 mg/dm³). The established anthocyanin content followed the order: wine-introduced variety > wine-hybrid variety > wine-local variety. The anthocyanin content data obtained in the present study correlated with the results from the study of Nistor et al. (2015).

Figures 5, 6 and 7 show the results of the established antioxidant activity of the studied white wines.
The wine of the control introduced Chardonnay variety (Fig. 5) showed the highest antioxidant activity of the three studied wines. At TE = 600.00 mg/dm³ activity at 5th min from 25.98 ± 0.011 % was reported and at 15th min from the moment of radical addition it slightly increased to 27.00 ± 0.005 %. When the concentration of the extract was reduced to 400.00 mg/dm³ in Chardonnay wine, lower antiradical activity was reported, respectively at 5th min from 16.98 ± 0.080 % and at 15th min from 17.68 ± 0.017 %.

The difference in the established antiradical activity of the wines from the local and hybrid variety was small. Slightly higher activity showed the wine of the hybrid variety Druzhba. At TE = 600.00 mg/dm³ in 5th min of reaction, Druzhba wine showed elimination of the DPPH radicals of 18.62 ± 0.000 %. At 15th min of reaction it increased very slightly to 19.79 ± 0.017 %. At TE = 400.00 mg/dm³, a decrease was observed, as at 5th min antiradical activity of 9.45 ± 0.061 % was registered, and at 15th min it was slightly higher (11.32 ± 0.005 %).

The established results correlated with the studies of Katalinić et al. (2004) and Marković et al. (2015), which analyzed white wines and found variation in their antioxidant activity, respectively from 10.30% - 16.16% and from 28.80% - 70.20%.

The wine of the local variety Dimyat showed the lowest antioxidant activity, but it was only approximately 2% different from that of Druzhba. Thus, at TE = 600.00 mg/dm³ at 5th minutes of reaction in Dimyat, an antiradical activity of 16.15 ± 0.010 % was found. At 15th min it increased to 18.31 ± 0.011 %. At TE = 400.00 mg/dm³ a decrease was observed, as at 5th min antioxidant activity of 9.45 ± 0.061 % was registered, and at 15th min it was slightly higher (11.32 ± 0.005 %).

The data on the established antioxidant activity in the studied red wines are presented in figures 8, 9 and 10.
Figure 8: Antioxidant activity of red wine of the introduced Cabernet Sauvignon variety at TE = 600.00 mg/dm$^3$ and TE = 400.00 mg/dm$^3$

Figure 9: Antioxidant activity of red wine of the local variety Gamza at TE = 600.00 mg/dm$^3$ and TE = 400.00 mg/dm$^3$

Figure 10: Antioxidant activity of red wine of the hybrid variety Rubin at TE = 600.00 mg/dm$^3$ and TE = 400.00 mg/dm$^3$

The highest antioxidant activity in red wines was found in Gamza. At TE = 600.00 mg/dm$^3$ and a reaction time of 5 min it was 34.63 ± 0.158 %. When the reaction time was increased to 15 min, an increase to 37.37 ± 0.036 % was reported. At TE = 400.00 mg/dm$^3$ at 5th min of the reaction anti-radical activity of 29.02 ± 0.011 % was found, and at 15th min it was higher and reached a value of 32.39 ± 0.020 %.

The wine of the introduced control variety Cabernet Sauvignon showed lower antioxidant activity than Gamza, but the difference between them was very small. At TE = 600.00 mg/dm$^3$ and a reaction time of 5 min, Cabernet Sauvignon wine showed an antioxidant activity of 32.58 ± 0.100 %. At 15 minutes of the reaction, a higher activity was registered, namely 38.34 ± 0.025 %. At TE = 400.00 mg/dm$^3$, an antioxidant activity of 15.59 ± 0.107 % was found at 5 minutes of the reaction, and an activity of 20.04 ± 0.020 % was recorded at 15 minutes for this extract. Gamza and Cabernet Sauvignon wines showed very similar radical-capturing activity.

The wine of the hybrid variety Rubin showed the lowest antioxidant activity. At TE = 600.00 mg/dm$^3$, at 5th min of the reaction it was 28.38 ± 0.150 %. At 15th min it increased to 35.42 ± 0.055 %. At TE = 400.00 mg/dm$^3$, a normal decrease in activity was observed, recorded as 18.09 ± 0.080 % in 5th min of reaction and increasing to 24.44 ± 0.047 % in 15th min.

There was a correlation between the antioxidant activity of red wines and the content of NPC in them, respectively: NPC (AA) Gamza > NPC (AA) Cabernet Sauvignon > NPC (AA) Rubin.

**CONCLUSION**

The following conclusions could be made from the study regarding the phenolic composition and antioxidant activity of white and red wines from introduced, local and hybrid grapevine varieties from the region of Central Northern Bulgaria:

- The chemical parameters of the wines were in optimum.
- The white wines of the introduced Chardonnay variety and the local Dimyat showed almost identical TPC content (700.00 ± 0.000 mg/dm$^3$ and 703.33 ± 5.773 mg/dm$^3$), respectively, higher than that of the Druzhba hybrid. In the red wines this indicator dominated in the wine of the control introduced variety Cabernet Sauvignon (1666.66 ± 5.773 mg/dm$^3$).
- The highest content of FPC in white wines, in the conditions of Central Northern Bulgaria (harvest 2020), was found in the Chardonnay control wine (769.84 ± 1.833 mg/dm$^3$). It was lower in Druzhba wine (278.61 ± 1.251 mg/dm$^3$). The FPC content of red wines dominated in the Rubin hybrid (2532.40 ± 49.938 mg/dm$^3$).
• The highest accumulation of NPC in white wines was found in Chardonnay (115.78 ± 0.325 mg/dm³), while in reds this indicator was dominated in the wine of the local variety Gamza (235.63 ± 0.498 mg/dm³).
• The established content of anthocyanins in the studied red wines followed the order wine-introduced variety > wine - hybrid variety > wine - local variety.
• The highest antioxidant activity in white wines was found in Chardonnay, and the difference in the percentage of free radical elimination between wines of the local and hybrid variety was small.
• The red wines of Gamza and Cabernet Sauvignon showed a close percentage of radical-scavenging activity, but it was slightly higher in the wine of the local variety Gamza.
• A correlation between the antioxidant activity of red wines and the content of NPC in them was found, respectively: NPC (AA) Gamza > NPS (AA) Cabernet Sauvignon > NPC (AA) Rubin.
• White and red wines from introduced, local and hybrid grapevine varieties from the region of Pleven, Central Northern Bulgaria showed good and balanced phenolic accumulation capacity, resulting in optimal ability for in vitro elimination of free DPPH radicals.

REFERENCES