

PHENOLIC AND ANTIOXIDANT PROFILE OF RED WINES FROM DIFFERENT REGIONS OF BULGARIA AGED WITH OAK CHIPS

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Abstract: *The phenolic and antioxidant profiles of four red wines (Cabernet Franc, Syrah, Cabernet Sauvignon/Merlot blend, Carménère/Cabernet Sauvignon/Cabernet Franc/Merlot blend) from regions of northern and southern Bulgaria, aged in oak, were determined. Oak chips with different origins and degrees of toasting, as well as different times of contact with the wines, was applied. Different phenolic fractions were determined – the total phenolic compounds (TPC), flavonoid phenolic compounds (FPC), non-flavonoid phenolic compounds (NPC), the total phenolic index (TPI), anthocyanins (ACNs). The spectral characteristics of the wines and their in vitro antioxidant activity were defined. The highest accumulation of phenols was found in the complex blend Carménère/Cabernet Sauvignon/Cabernet Franc/Merlot, harvest 2022, Harmanli, Southern Bulgaria. In direct relation to the phenolic profile, this wine also exhibited the highest radical scavenging capacity – with a potential to eliminate free DPPH radicals in vitro reaching 91.43±0.04%. High antioxidant activity (83.14±0.01%) was also displayed by the wine of the Syrah variety from the region surrounding the town of Varna in Northern Bulgaria. The differences in the phenolic composition of the studied wines from the commercial network were the result of varietal characteristics, the type and amount of oak wood used, as well as the contact time. The conducted study enriched the scientific literature with valuable data regarding the phenolic and antioxidant profile, determining the biological potential of wines obtained in contact with oak wood, from grapes of vine varieties from different regions of Bulgaria.*

Key words: *red wines, oak wood, phenols, antioxidants, free radicals.*

1. Introduction

The use of oak wood in various stages of obtaining, storing and aging of the wines is a technological practice with wide

application. The use of oak chips is an alternative method that differs from traditional aging in oak barrels, promoting an accelerated extraction process of oak-derived components from the composition

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of the oak, with the aim to improve the quality of aged red wines [3]. Contact with oak wood leads to significant changes in the physicochemical characteristics of red wines, affects their organoleptic properties – colour intensity, nuance, and increases the phenolic content [20]. One of the main effects of oak wood contact is the transfer of oak-specific phenols to the wine, which leads to an improvement of its phenolic profile and increases the biological potential, reflecting in increased antioxidant activity [1, 2, 19]. A number of authors confirm these effects. Zhao et al. [20] proved a positive correlation between the antioxidant activity and the content of total phenols, flavonoids and tannins in Cabernet Sauvignon wines aged with French oak chips with a high degree of toasting, establishing an antioxidant activity of 44% on the 60th day, and of 44.6% in the 90th day. Osorio-Alises et al. [16] investigated the influence of oak chips applied at doses of 3.00 and 6.00 g/L in three phases of Bobal red wine production – during alcoholic fermentation, during malolactic fermentation, and in young wines. A modification of the phenolic composition and antioxidant activity was found, while the wines in contact with oak chips during the malolactic fermentation showed a decrease in the anthocyanin and stilbene content. Jordão et al. [13] investigated the effect of contact time (30 and 60 days) of oak chips from different species (*Quercus petraea* (Matt.) Liebl. and *Quercus pubescens* Wild.) with low, medium and high degrees of toasting on the phenolic composition of Portuguese red wines from the Touriga Nacional variety. It was found that after 6 months of

storage in bottles, the wines showed a decrease in anthocyanin content, which was observed in all experimental variants. Del Fresno et al. [9] found significant differences in the content of anthocyanins and vitisin during the aging of red wines in 6 different unused oak barrels (with different degrees of toasting) produced from *Quercus petraea* oak. Gonçalves and Jordão [10] studied the effect of applied contact between Syrah wine and Portuguese and American oak chips at concentrations of 5.00 and 10.00 g/L over a period of 90 days. A growth in the antioxidant activity of the wine was identified during the first 52 days, followed by a slight decline. The team established a pronounced correlation between the content of monomeric proanthocyanidins and antioxidant activity. Silva et al. [17] applied French oak chips at a 6.00 g/L dose to Syrah red wine from the São Francisco Valley region of Brazil and found colour stabilization and an increase in the total polyphenolic index of the wine in contact with the chips.

The objective of the present study is to define the phenolic and antioxidant profile of red wines from different regions of Bulgaria aged with oak chips.

2. Materials and Methods

2.1. Researched Wines

The study was conducted in 2023. Four red wines provided by the commercial network were analysed. A different oak aging technology was applied. Information about the studied wines is presented in Table 1.

Researched variants of wines from the commercial network

Table 1

Variety	Region	Harvest	Oak chips dose [g/L]	Oak origin and degree of toasting	Contact time [months]
Cabernet Franc	Varna, Northern Bulgaria	2021	1.00	French oak medium toasted chips	8.00
Syrah	Varna, Northern Bulgaria	2021	1.00	French oak medium toasted chips	2.00 – 2.50
Cabernet Sauvignon/Merlot	Asenovgrad, Southern Bulgaria	2019	1.00	American oak high toasted chips	3.00 – 4.00
Carménère (50%)/Cabernet Sauvignon (30%)/Cabernet Franc (20%)/Merlot (10%)	Harmanli, Southern Bulgaria	2022	0.30	American oak high toasted chips	3.00

2.2. Determination of the Phenolic Composition of Wines in Contact with Oak Chips

The phenolic composition of wines was determined in accordance with methods presented in the methodological manuals of Chobanova [7] and Ivanov et al. [12], respectively:

- Total phenolic compounds (*TPC*, g/L), gallic acid – were determined using the Singleton and Rossi method with Folin-Ciocalteu reagent and spectrophotometric measurement of light absorbance at λ 750 nm;
- Flavonoid phenolic compounds (*FPC*, mg/L) catechin equivalent and non-flavonoid phenolic compounds (*NPC*, mg/L) caffeic acid equivalent – were determined using the Somers method. For the analytical and calculation part, 0.2 ml of the studied sample was added to 10 ml of 1n HCl, and the light

absorbance of the solution was measured spectrophotometrically between the 3rd and 4th hour at λ 280 and 320 nm;

- Total phenols index (*TPI*, absolute units) was determined spectrophotometrically by measuring the light absorbance at λ 280 nm;
- Total monomeric anthocyanins (*ACNs*, mg/L) were determined using the Gayon and Stonestreet method by pH adjustment using buffer solutions of pH 0.6 and 3.5, followed by spectrophotometric measurement of light absorbance at λ 520 nm.

2.3 Determination of the Wines Spectral Characteristics

They were determined by measuring the light absorbance of the samples in a 0.1 cm cuvette at λ 420, 520 and 620 nm and equating to a 1 cm cuvette [7]:

- Colour intensity (IC', absolute units) – was determined using the Glories method, as the sum of absorbance measurements at the three wavelengths (420, 520 and 620 nm);
- Colour nuance (T) – was determined using the Glories method, as the ratio of absorbance at 420 to that at 520 nm;
- dA [%] – was determined using the Glories method, the indicator expressed the participation of free and bound flavylc forms of anthocyanins in the overall colour of the wine;
- Colour composition – was determined using the Glories method by showing the percentage participation of each of the three colour components in the overall colour of the wine:
 - Percent of yellow colour - percentage participation of yellow colour in the wine total colour – was determined using the Glories method by measurement of absorbance at 420 nm;
 - Percent of red colour (percentage of red colour in the total wine colour) – was determined using the Glories method by measuring absorbance at 520 nm;
 - Percent of blue colour (percentage of blue colour in the total wine colour) – was determined using the Glories method by measuring of absorbance at 620 nm.

2.4. Determination of the Wines Antioxidant Activity

The antioxidant activity was determined according to the method of Wang et al. [18], as antiradical activity against the stable product DPPH• (2,2–diphenyl-1-picrylhydrazyl) (Sigma Aldrich, Germany). It was expressed as a

percentage elimination of the DPPH radical by the wine samples. The studied wines were diluted immediately before the analysis with distilled water to a total extract (TE) content equal to 600.00 and 400.00 mg/dm³, respectively and the analysis was performed for those two wine extracts.

2.5. Statistical Analysis

Statistical processing was performed on the data obtained from triplicate performance of the chemical analyses, represented by mean value and standard deviation (\pm SD). The Excel 2007 program (Microsoft Office) was used for the determination.

3. Results and Discussion

The results on the phenolic profile of the studied wines are presented in Table 2.

All investigated red wines were produced in contact with oak wood inserted in the form of chips, in different doses and degrees of toasting. Along with varietal characteristics, this had a strong influence on their phenolic composition. The blend Carménère/Cabernet Sauvignon/Cabernet Franc/Merlot, harvest 2022, had the highest values of TPC, NPC, TPI and ACNs indicators. In this wine, the content of TPC was 3.96 ± 0.00 g GAE/L, followed by Cabernet Sauvignon/ Merlot, harvest 2019 – 3.71 ± 0.00 g GAE/L. In the wine samples from the town of Varna, the content of TPC was almost similar between them – 1.29 ± 0.01 for Syrah wine and 1.26 ± 0.01 for Cabernet Franc wine. The highest FPC content was found in Cabernet Sauvignon/Merlot, harvest 2019 (1842.94 ± 1.77 mg CE/L) and Cabernet Franc, harvest 2021 (1818.81 ± 0.64 mg

CE/L). NPC were almost double in concentration in the samples Carménère/Cabernet Sauvignon/Cabernet Franc/Merlot, harvest 2022 (553.09±0.02 mg CA/L) and Cabernet Sauvignon/Merlot, harvest 2019 (516.14±0.09 mg CA/L) compared to Syrah, harvest 2021 and Cabernet Franc, harvest 2021. The same trend was observed in TPI. The values of this indicator were the highest in the blend of Carménère/Cabernet Sauvignon/Cabernet Franc/Merlot (98.03±0.01 abs. units), which was twice as high as that of the Cabernet Sauvignon/Merlot wine and three times higher than that of the Syrah - Varna samples. The blend Carménère/Cabernet

Sauvignon/Cabernet Franc/Merlot, harvest 2022 (584.20±1.19 mg/L), followed by Syrah, harvest 2021 (412.84±0.04 mg/L) and Cabernet Franc, harvest 2021 (344.18±0.19 mg/L) had the highest content of total monomeric anthocyanins (ACNs). Cabernet Sauvignon/Merlot blend, harvest 2019, had the lowest ACNs (250.73±0.03 mg/L). Probably, the influence of the harvest on this indicator was stronger than that of the oak wood. The low values in the wine from the oldest harvest (2019) were a consequence of the anthocyanins' chemical transformations as a result of aging and changes in its colour characteristics [8].

Phenolic composition of wines in contact with oak wood

Table 2

Wines	Indicators					
	Harvest	TPC [g GAE/L]	FPC [mg CE/L]	NPC [mg CA/L]	TPI [absolute units]	ACNs [mg/L]
Cabernet Sauvignon/Merlot	2019	3.71±0.00	1842.94±1.77	516.14±0.09	42.04±0.01	250.73±0.03
Syrah	2021	1.29±0.01	1732.10±1.32	205.91±0.00	36.99±0.01	36.99±0.01
Cabernet Franc	2021	1.26±0.01	1818.81±0.64	170.25±0.10	34.99±0.01	34.99±0.01
Carménère/Cabernet Sauvignon/Cabernet Franc/Merlot	2022	3.96±0.00	1700.48±0.07	553.09±0.02	98.03±0.01	98.03±0.01

Note: *TPC – Total phenolic compounds; FPC – Flavonoid phenolic compounds; NPC – Non-flavonoid phenolic compounds; TPI – Total phenols index; ACNs – Total monomeric anthocyanins.

The Carménère/Cabernet Sauvignon/Cabernet Franc/Merlot blend, harvest 2022, was the youngest of the wines studied. Its rich phenolic composition was the result of the characteristics and phenolic content of individual varieties, as well as the influence of the type of oak wood used. The wine was stored for 3 months in contact with medium-toasted French oak chips. The high levels of phenolic components in the 2019 Cabernet Sauvignon/Merlot sample

were due to the inclusion of American oak chips with a strong degree of toasting. The Syrah and Cabernet Franc samples from the 2021 harvest were produced with medium-toasted French oak chips, but with different contact times. They had a similar phenolic composition in terms of TPC and TPI. The Cabernet Franc wine, harvest 2021, produced with 8 months of contact with the wood contained more FPC, but less NPC and ACNs. The reason for this could be the phenolic potential of the

respective variety.

The spectral characteristics of the studied wines, as a result of their contact with oak wood, are presented in Table 3.

The highest colour nuance was determined for Cabernet Franc, harvest 2021 (0.60 ± 0.00), and the lowest for Cabernet Sauvignon/Merlot, harvest 2019 (0.44 ± 0.01). A dependence was observed in the values of the colour intensity and the

indicator dA%, expressing the participation of the free and bound flavyl forms of anthocyanins in the overall wine colour. Values were highest in Carménère/Cabernet Sauvignon/Cabernet Franc/Merlot, 2022 harvest (16.24 ± 0.04 abs. units and 76.50 ± 0.16 , respectively) and Syrah, 2021 harvest (15.10 ± 0.01 abs. units, respectively and 75.50 ± 0.12).

Spectral characteristics of wines in contact with oak wood

Table 3

Wines	Indicators					
	Nuance	Intensity [abs. units]	dA [%]	% yellow colour	% red colour	% blue colour
Cabernet Sauvignon /Merlot	0.44 ± 0.01	13.89 ± 0.01	74.40 ± 0.08	24.20 ± 0.10	65.10 ± 0.11	10.70 ± 0.06
Syrah	0.52 ± 0.00	15.10 ± 0.01	75.50 ± 0.12	23.80 ± 0.15	67.70 ± 0.22	8.50 ± 0.08
Cabernet Franc	0.60 ± 0.00	13.88 ± 0.00	74.60 ± 0.21	23.40 ± 0.10	66.30 ± 0.12	10.30 ± 0.03
Carménère /Cabernet Sauvignon/ Cabernet Franc/Merlot	0.52 ± 0.03	16.24 ± 0.04	76.50 ± 0.16	24.40 ± 0.17	67.50 ± 0.16	8.10 ± 0.05

For the other two wines (Cabernet Sauvignon/Merlot and Cabernet Franc), the colour intensity (13.89 ± 0.01 abs. units and 13.88 ± 0.00 abs. units, respectively) and the indicator dA% ($74.40\pm 0.08\%$ and $74.60\pm 0.21\%$, respectively) had very similar values between those two investigated wines. The ratio between the three main colours forming the colour of red wines was also determined in the analysed samples. The results showed a higher percentage of yellow colour in the two blend samples - $24.40\pm 0.17\%$ in Carménère/Cabernet Sauvignon/Cabernet Franc/Merlot, 2022 harvest and $24.20\pm 0.10\%$ in Cabernet Sauvignon/Merlot, 2019 harvest. This was due to the inclusion of more grapevine

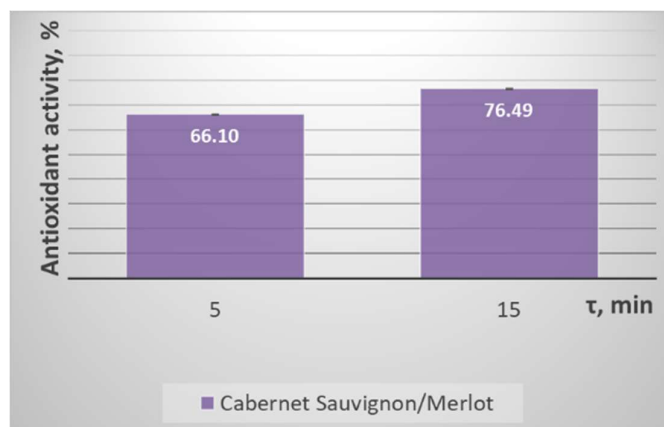
varieties in the first sample, as well as the development of colour associated with an older harvest in the second sample. The percentage of red colour in the studied wines varied from $65.10\pm 0.11\%$ to $67.70\pm 0.22\%$. Higher values were reported for Syrah, 2021 harvest and Carménère/Cabernet Sauvignon/Cabernet Franc/Merlot, 2022 harvest. The proportion of red colour was the lowest in the Cabernet Sauvignon/Merlot sample from the oldest harvest, i.e. 2019. The proportion of blue colour ranged from $8.10\pm 0.05\%$ to $10.70\pm 0.06\%$. In the Carménère/Cabernet Sauvignon/Cabernet Franc/Merlot blend, the percentages of yellow and red colour were the highest, whereas the percentage of blue colour was

the lowest. The opposite was observed in the case of the Cabernet Sauvignon/Merlot blend. The percentage of blue colour was the highest in the Cabernet Sauvignon/Merlot blend, harvest 2019, and in the Cabernet Franc, harvest 2021.

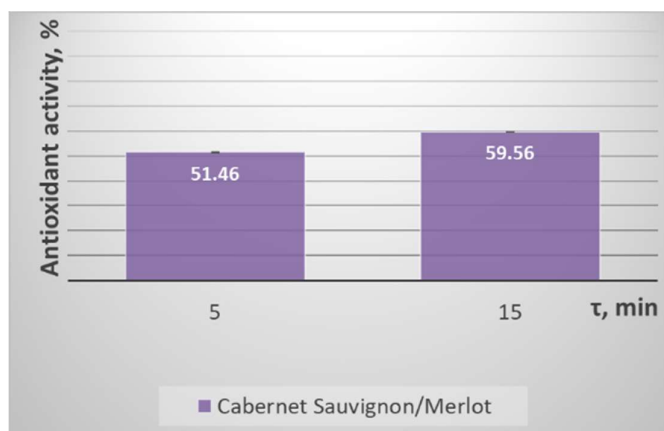
The data on the established antioxidant

activity of different total extracts (TE), obtained after dilution of the studied red wines with distilled water, presented in Figures 1 to 4.

The lowest levels of antioxidant activity were found in the Cabernet Sauvignon/Merlot wine (Figure 1).



a.



b.

Fig. 1. Antioxidant activity of red wine /blend/ from the Cabernet Sauvignon/Merlot varieties: a. TE = 600.00 mg/L; b. TE = 400.00 mg/L

At TE = 600.00 mg/L and a reaction time of 5 min, an antioxidant activity of $66.10 \pm 0.21\%$ was found. When increasing the reaction time to 15 min, a significant growth in the antioxidant potential was

observed, with a percentage of free radical elimination of $76.49 \pm 0.07\%$. Lower activities were found when the TE was reduced to 400.00 mg/L. Thus, at the 15th min of the reaction with this extract, a

percentage elimination of free radicals reaching $59.56 \pm 0.13\%$ was found.

The other two wines - Cabernet Franc and Cabernet Sauvignon/Merlot showed lower antioxidant activity, and between

them they showed close values, with a slight dominance of Cabernet Franc. At $TE = 600.00 \text{ mg/L}$ at the 5th min of reaction, Cabernet Franc (Figure 2) showed an antioxidant activity of $68.66 \pm 0.11\%$.

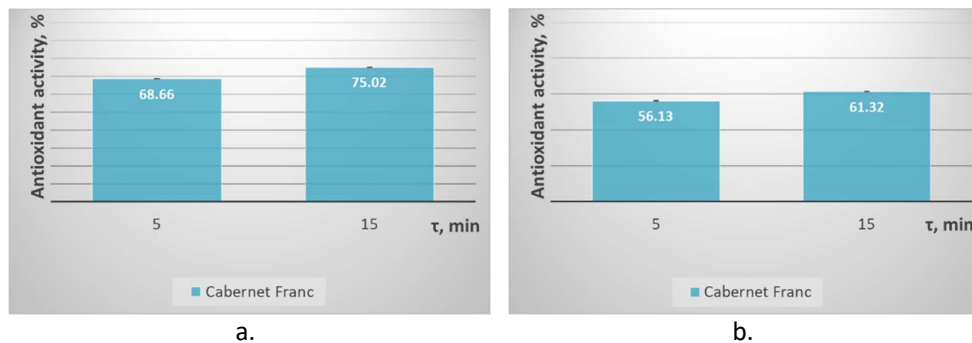


Fig. 2. Antioxidant activity of red wine from the Cabernet Franc variety: a. $TE = 600.00 \text{ mg/L}$; b. $TE = 400.00 \text{ mg/L}$

At the 15th min with the same extract, a growth in the antiradical capacity was observed, and a DPPH elimination of $75.02 \pm 0.02\%$ was reached. At $TE = 400.00 \text{ mg/L}$, lower antioxidant activities were observed. Thus, at the 5th min of the reaction, a percentage elimination of free radicals of $56.13 \pm 0.10\%$ was achieved. At

the 15th min, with the same extract, an increase in antioxidant activity was reported and it reached $61.32 \pm 0.02\%$.

Comparing the four wines, the Carménère/Cabernet Sauvignon/Cabernet Franc/Merlot blend showed the highest antioxidant potential (Figure 3).

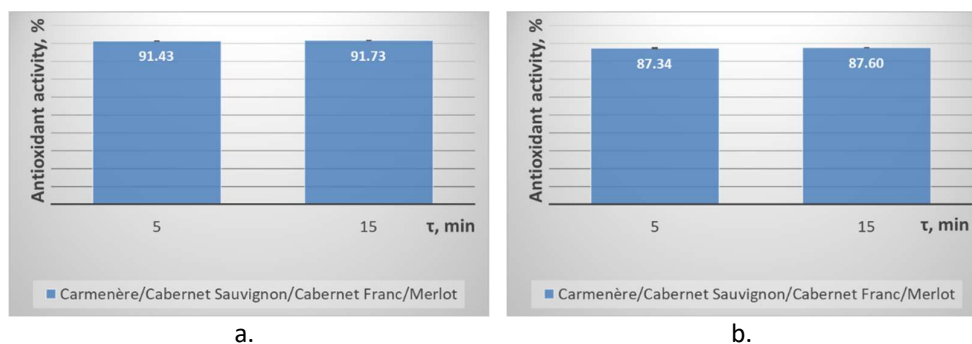


Fig. 3. Antioxidant activity of red wine /blend/ from the Carménère/Cabernet Sauvignon/Cabernet Franc/Merlot varieties: a. $TE = 600.00 \text{ mg/L}$; b. $TE = 400.00 \text{ mg/L}$

At the 5th min of the reaction with the radical, a very high ability to eliminate

DPPH was found in it - $91.43 \pm 0.04\%$. At the 15th min of the reaction, a slight increase in

the activity was detected as it reached $91.73 \pm 0.01\%$. At $TE = 400.00$ mg/L, a slight drop in the antioxidant activity was observed and at the 5th min of the reaction it reached $87.34 \pm 0.23\%$. At the 15th min, with the same extract as with the previous one, a slight increase in the antioxidant activity was observed as it reached $87.60 \pm 0.01\%$.

The wine of the Syrah variety (Figure 4) also demonstrated high antioxidant potential, ranking second. At $TE = 600.00$ mg/L and the 5th min after the reaction, an antioxidant activity of $87.12 \pm 0.03\%$ was achieved. When the reaction time increased to 15 min, an increase in the radical-elimination capacity was observed as it reached $91.28 \pm 0.02\%$. At $TE = 400.00$ mg/L, a decrease in antioxidant activity was observed, reaching $79.40 \pm 0.06\%$ in the 5th min after the reaction with the DPPH radical. When the reaction time increased to 15 min, an increase in the antiradical activity was observed and it reached $83.14 \pm 0.01\%$.

Bajčan et al. [4] conducted a study to determine the antioxidant activity of 28 Cabernet Sauvignon red wines from different regions of Slovakia, finding an

average DPPH antioxidant activity between them ranging from 69.00% to 84.20%. The results were consistent with these data and correlated with the studies of Banc et al. [5] who found antioxidant activity of Cabernet Sauvignon red wines ranging from 63.00% to 95.00%. Caridi et al. [6] investigated the antioxidant activity of 87 red wines of the Gaglioppo variety (harvest 2009), from the Calabria region, Italy, using different yeast strains and aging the wines from 4 to 120 months. After four months of storage, they found a variation in the antioxidant potential from $29.00 \pm 2.76\%$ to $51.12 \pm 0.24\%$, which was lower than the values found in our study. Granato et al. [11] investigated the relationship between phenolic composition and antioxidant activity of 26 Brazilian red wines from Merlot, Malbec, Pinot Noir, Cabernet Sauvignon and Syrah varieties from different regions of Brazil and different harvests. The team found a variation between the antioxidant activities of the wines by variety, as follows: Merlot – from 51.57 to 66.04%; Malbec – from 31.95 to 34.47%; Pinot Noir – from 47.84 to 80.61%; Cabernet Sauvignon – from 52.96 to 76.39%; Syrah – from 64.75 to 75.90%.

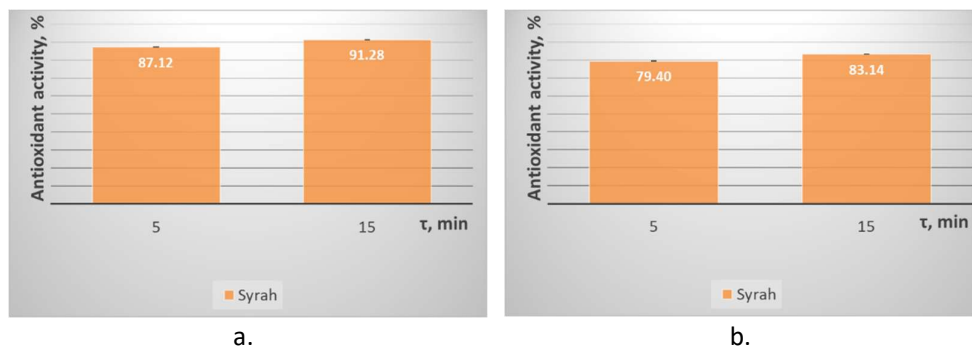


Fig. 4. Antioxidant activity of red wine from Syrah variety:
a. $TE = 600.00$ mg/L; b. $TE = 400.00$ mg/L

The data in the study also correlated with these results as well as with those obtained by Mitić et al. [14], who determined the radical scavenging activity (by DPPH method) of Serbian red wines from different regions of the country. The team found a variation in antioxidant activity between the wines from 74.80 to 87.90%. Ofoedu et al. [15] investigated the antioxidant properties of red wines from the commercial network of Nigeria and found a variation in antioxidant activity from $28.16 \pm 0.16\%$ to $57.04 \pm 0.22\%$, and these results were consistent with some of those obtained in our study.

4. Conclusions

The differences in the phenolic composition of the studied red wines from the commercial network are the result of the varietal characteristics, the type and amount of oak wood used, as well as the contact time. The blend Carménère/Cabernet Sauvignon/Cabernet Franc/Merlot, harvest 2022, had the highest values of the studied phenolic indicators, anthocyanins and colour intensity.

As expected and directly related to the higher phenolic profile, the highest radical scavenging capacity was found in the complex blend Carménère/Cabernet Sauvignon/Cabernet Franc/Merlot - its potential to eliminate DPPH free radicals *in vitro* at the higher total extract (600.00 mg/L) at the 5th min of the reaction reached the high percentage of $91.43 \pm 0.04\%$.

The conducted study enriched the scientific literature with valuable data regarding the phenolic and antioxidant profile, determining the biological potential of red wines obtained in contact

with oak wood, from grapes of vine varieties from different regions of the Republic of Bulgaria.

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