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Research Article

Changes of some groups of phenolic compounds, color characteristics and antioxidant activity of red wine from Bulgaria as a result of bentonite and cold treatments

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Abstract

The influence of bentonite and cold treatments on some groups of phenolic compounds, color characteristics and antioxidant activity of young red Cabernet Sauvignon wine from Bulgaria was studied. Bentonite was administered in increasing doses in the range of 0.2-1.6 $g.L^{-1}$. The wine was chilled at -5°C for 24 hours. The influence of bentonite on the index 280, phenolic complex and color characteristics was stronger when it was applied in doses of above 0.6 $g.L^{-1}$, whereas antioxidant activity was more substantially affected at doses of above 1 $g.L^{-1}$. Cold treatment led to greater changes in the studied indices of the composition and antioxidant activity of the wine in comparison to adding bentonite. It led to a decrease in the index 280 by 19%, and the concentration of the studied phenolic complex groups decreased by about 6-7%. Color intensity decreased the most of all studied indices. This index was reduced by about 32 %, without a significant change in the composition of its color and shade. The decrease in the content of phenolic compounds is probably the reason for the 23% decrease in the antioxidant activity of the studied wine.

Keywords: red wine, phenolic compounds, wine color, antioxidant activity, bentonite, cold treatment

Abbreviations: AOA – antioxidant activity, CI – color intensity, dA – brilliance of red color, DPPH – 2,2-Diphenyl-1-picrylhydrazyl, FP – flavonoids phenols, I_{280} – index 280, N – shade, NP – nonflavonoids phenols, TE – trolox equivalent, TP – total phenols

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Introduction

Food industry and preventive medicine have demonstrated considerable interest in natural antioxidants of plant origin. Plant tissues are the main biological systems synthesizing α-tocopherol, ascorbic acid, carotenoids and a wide range of phenolic compounds (Kanner et al. 1994). Phenolic compounds are among the most common secondary plant metabolites and are found in many plants used as foods and food supplements (Hagerman et al. 1998; Pintać 2022). Studies proving the properties of flavonoids to act as free radical traps, as well as their ability to bind metals in complexes catalyzing oxidative processes, allow them to be called natural plant antioxidants (Kharadze et al. 2018; Basaran et al. 2022). Studies have shown that many polyphenols of plant origin are more effective *in vitro* antioxidants than the vitamins E and C and can make a significant contribution to preventing unwanted *in vivo* oxidation of lipids and proteins (Catherine et al. 1996; Gris et al. 2013; Macedo et al. 2013).

Grapes and products derived from them-juices, extracts and wines-are a valuable source of antioxidants. Red wine is a significant natural source of monomeric phenolic compounds and if consumed in moderation supplies more than 1 g per day, which is why it is often the subject of research. Amongst the wide range of phenolic compounds isolated from red wine, (+)-catechin, (-)-epicatechin, trans-resveratrol and quercetin have proven to be more effective than α-tocopherol *in vitro* oxidation inhibition of low-density lipoprotein (LDL) in human blood. High antioxidant activity of wines, especially red ones, has been proven in many studies. It has been found that this is mainly due to the catechins, anthocyans, gallic acid and resveratrol they contain (Garaguso et al. 2015; Ivanova-Petropulos et al. 2015a; Ivanova-Petropulos et al. 2015b; Chobanova 2016; Lingua et al. 2016).

On the other hand, wines on the market should preserve their clarity over time. This requires that they are subjected to various treatments to ensure their stability against colloidal, metallic, crystalline and biological haziness (Chobanova 2016; Ribereau-Gayon et al. 2006; Jackson 2014; Boulton et al. 1996; Zoecklein et al. 1999 et al.). As a result of the treatments, the phenolic composition of wines is affected and, respectively, a change in antioxidant activity and color characteristics can also be expected.

Scientific literature on wine renders relatively scarce information on the change in antioxidant activity of red wines subjected to various stabilizing treatments. This is the main motive for the present work, which aims to study the change of some groups of compounds of phenolic profile, color characteristics and antioxidant activity of red wine as a result of treatments with bentonite and cold application.

Materials and Methods

Young, unprocessed and colloidally unstable red Cabernet Sauvignon wine from the Danube Plain region, Bulgaria was used in this study. It was treated with increasing doses of activated calcium bentonite (0.2–1.6 $g.L^{-1}$) introduced as a 5% bentonite suspension as described by Chobanova (2007). Five days after adding bentonite, the examined wine samples were decanted separating sediment from liquid and filtered through K5 filter sheet.

To investigate the effect of cold application, a sample of the same wine was kept at a constant temperature of -5°C for 24 hours followed by filtering through K5 filter sheet at the same temperature.

The obtained experimental wines were analyzed according to the following indices and methods:

- Index 280 (I_{280}) - spectrophotometrically (Ribereau-Gayon et al. 2006)

- Total phenols (TP, like catechin equivalent), flavonoids phenols (FP, like catechin equivalent) and nonflavonoids phenols (NP, like caffeic equivalent) - spectrophotometrically by method of Somers (Chobanova 2007)

- Color intensity (CI), shade (N) – spectrophotometrically, OIV-MA-AS2-07B (OIV 2022)

- Color composition, brilliance of red color (dA)-spectrophotometrically by Y. Glories (Chobanova 2007; Ribereau-Gayon et al. 2006)

- Antioxidant activity (AOA) – spectrophotometrically by the DPPH assay, described by Brand-Williams et al. (1995). The wine was diluted 50 times with distilled water. After mixing the components of the working sample (diluted wine and 60 μM DPPH solution in methanol) and an incubation period of 30 min in the dark, the light absorption at 517 nm (A_2) was measured against a blank (distilled water and methanol). Under the same conditions, the absorption (A_1) of the control, filled with distilled water instead of wine, was identified. The percentage of inhibition (I) was calculated using the equation (1).

$$I = \frac{A_1 - A_2}{A_1} . 100, \% \qquad (1)$$

Using a standard line constructed in advance, the percentage of DPPH inhibition was reduced to antiradical activity in Trolox equivalents per liter of wine (μmol TE.L^{-1} wine).

The analyzes via the described methods were performed using a Shimadzu UV-1800 UV-VIS spectrophotometer. All analyzes were performed thrice and the present work contains the mean values and standard deviations of their results. EXCEL software was used for the statistical processing of the results.

Results and Discussion

The values of the studied indices of raw wine (control) are given in Table 1. The obtained values of the studied parameters correspond well with previous studies of the phenolic profile of Bulgarian wines (Stoyanov 2007; Nikolov 2015) and the reported values of antiradical activity in world scientific literature (Šeruga et al. 2011).

Table 1. Values of the studied indicators in the control (untreated) wine

Wine	**I_{280}, au**	**TP, mg.L^{-1} catechin equivalent**	**NP, mg.L^{-1} caffeic equivalent**	**FP, mg.L^{-1} catechin equivalent**	**CI, au**	**dA, %**	**N, 1**	**AOA, μmol TE.L^{-1} wine**
Cabernet Sauvignon	57.60 ±0.01	3765.0 ±14.2	2593.3±9.8	273.2±27.8	17.84±0.01	59.0±0.1	0.6±0.1	11 516.0±308.1

Change of some indices describing the phenolic profile of red wine

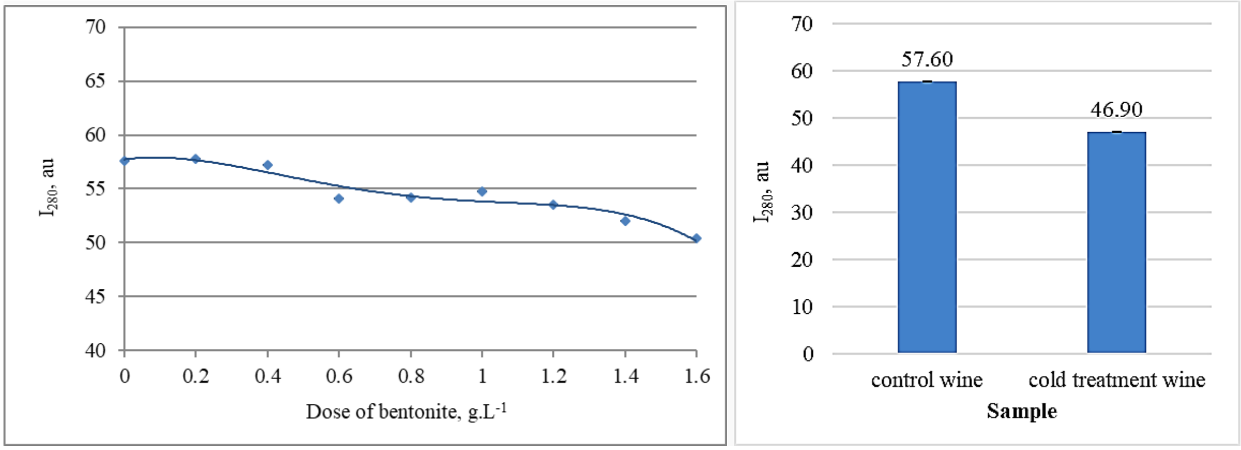
In Fig. 1-A it can be seen that adding low doses of bentonite (0.2-0.4 g.L^{-1}) did not result in a substantial change in Index 280 (I_{280} or total phenolic index).

Increasing the dose of bentonite led to a gradual decrease in Index 280. The highest dose of the added dose ranges (1.6 g.L^{-1}) reduced the value of the index by almost 13% to 50.40±0.01 au. The observed change in the I_{280} is probably the result of the sorption of some of the phenolic compounds by the added bentonite.

Cold application led to a more significant reduction in this index. The value reported for the wine kept at a constant low temperature was 46.90±0.01 au, which is equivalent to a reduction of about 19% (Fig. 1-B).

Index 280 provides quick information about the presence of phenolic compounds in the sample due to the fact that benzene nucleus has the ability to absorb electromagnetic radiation at a wavelength of 280 nm (Zoecklein et al. 1999). It is known that under low temperature conditions, colloidal coloring matter comes out of the dissolved state and precipitates (Chobanova 2016; Ribereau-Gayon et al. 2006).

It has a complex composition whose main component are phenolic compounds with varying degrees of polymerization. The decrease in solubility of this colloidal complex is, in all



A B

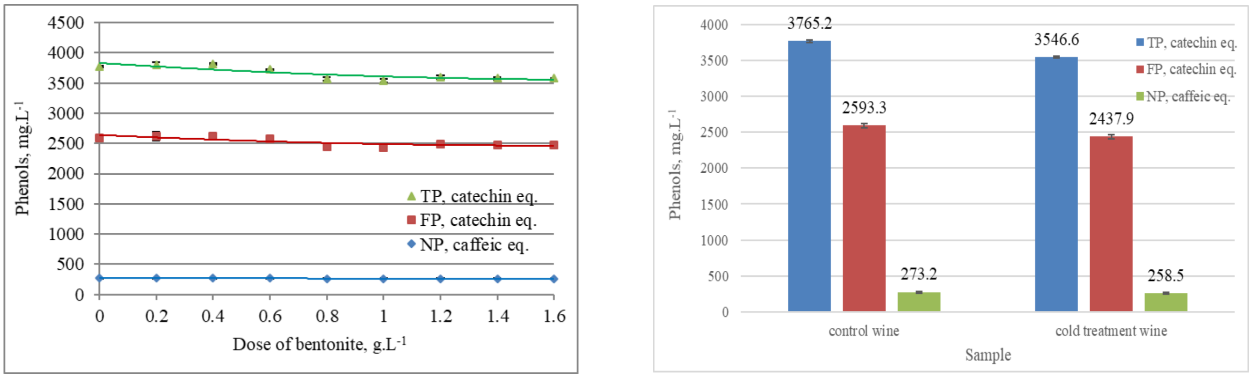
Figure 1. Change of Index 280
A - influence of bentonite dosage; B - influence of cold treatment

likelihood, the main reason for the decrease in I_{280}, too. Given the results obtained, it can be assumed that reducing the concentration of phenolic compounds in the test samples will lead to a reduction of their antioxidant activity (Vermerris and Nicholson 2006).

The Index 280 (also called index total phenols) is a very convenient comparative index of the impact of different treatments on the phenolic composition of red wines, but does not provide information on which components change. Therefore, changes in the concentrations of the different groups of phenolic compounds were tested (total phenols - TP, flavonoids phenols - FP и nonflavonoids phenols - NP). The obtained results are shown in Fig. 2-A.

Bentonite, applied in doses of 0.2-0.8 g.L^{-1}, resulted in a more significant reduction in TP and FP content. The administration of higher doses (above 0.8 g.L^{-1}) did not change the content of these substances. Adding bentonite did not significantly affect the amount of NP.

Cold application (Fig. 2-B) resulted in more significant changes in the concentrations of the different groups of phenolic compounds in comparison to adding bentonite. There was a decrease of TP by 6.8%, of NP by about 6.0%, and FP decreased the most-by 7.2%. The probable reasons for these results have already been mentioned.



A B

Figure 2. Change of some groups phenols
A - influence of bentonite dosage; B - influence of cold treatment

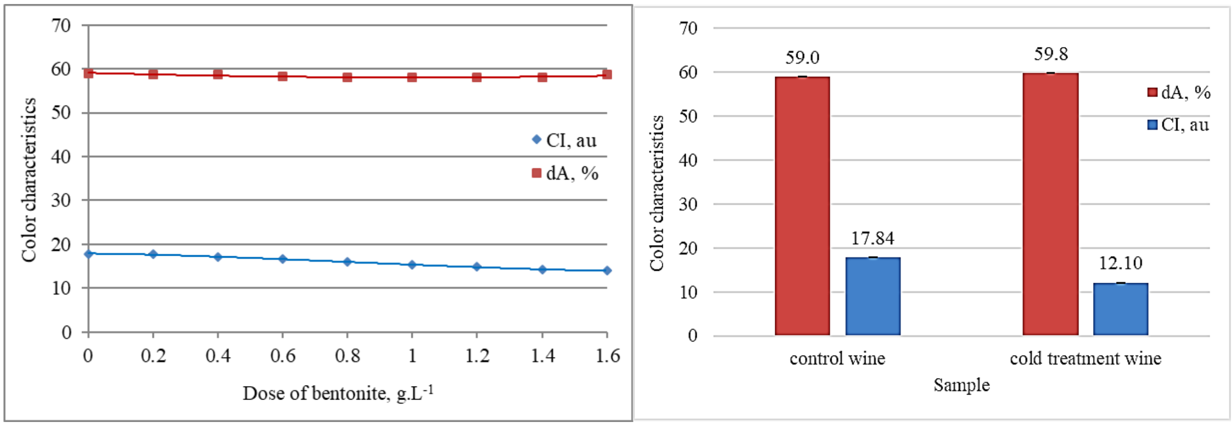
Change of the color characteristics of red wine

Since phenolic compounds are responsible for the intensity and color perception of wine, the effect of bentonite and cold treatments on the color characteristics of wine was studied (Fig. 3-A).

Color intensity decreased gradually with increasing dose of bentonite. The highest dose of bentonite (1.6 g.L^{-1}) resulted in a decrease of 17.84±0.01 au (in the control) to 13.94±0.01 au, which is equivalent to a decrease of about 22%. This reduction may be due to the sorption of monomeric anthocyans by bentonite. Bentonite is a type of mineral clay. It is composed of multiple layers of montmorillonite. It has very strong swelling properties, thanks to which it is capable of absorbing up to 10-12 times more water than its own mass (Ribereau-Gayon et al. 2006; Jackson 2014). Moreover, individual layers of montmorillonite move apart from each other (Savage 2005). This leads to a strong increase in its sorption properties, probably in relation to the coloring matter of the wines as well. On the other hand, bentonite in wine conditions has a negative electrostatic charge. It can interact with other substances that are positively charged and precipitate them on the basis of mutual flocculation of opposite charges. Such charges, in the conditions of red wines, likewise possess anthocyans which are the main carriers of color (Morata 2019). The two abovementioned properties of bentonite explain to a great extent the decrease in color intensity.

Despite the decrease in color intensity, the way it is perceived did change significantly. This is evident from the almost constant values of the dA index, i.e., the color remains brilliant and vivid. These results are very interesting. On the one hand, the color intensity decreases, and on the other hand, the nature of prominence of the red color of the wine is preserved, since this index is directly related to the degree of expression of the sparkling, bright, lively color of young red wines. The color intensity of red wines is formed by light absorption values at 420, 520 and 620 nm, while dA indicates the nature of the absorption curve at the peak at 520 nm (Jacobson 2006; Chobanova 2016; Ribereau-Gayon et al. 2006; OIV 2022). Absorption at 420 nm indicates the presence of the yellow component, the one at 520 nm - of the red component, and at 620 nm - of the blue component in the red color of the wine. It is likely that the introduction of bentonite into the wine resulted in a proportional reduction of the substances that absorb at these wavelengths, which explains to a large extent the reduction in color intensity, but the nature of red color appearance of the experimental wine samples is preserved. In order to test this assumption, we conducted an analysis of the percentage participation of these three-color components forming the overall red color of the wine. The results are shown below.

Again, cold application resulted in a more significant reduction in color intensity compared to bentonite treatment (Fig. 3-B).



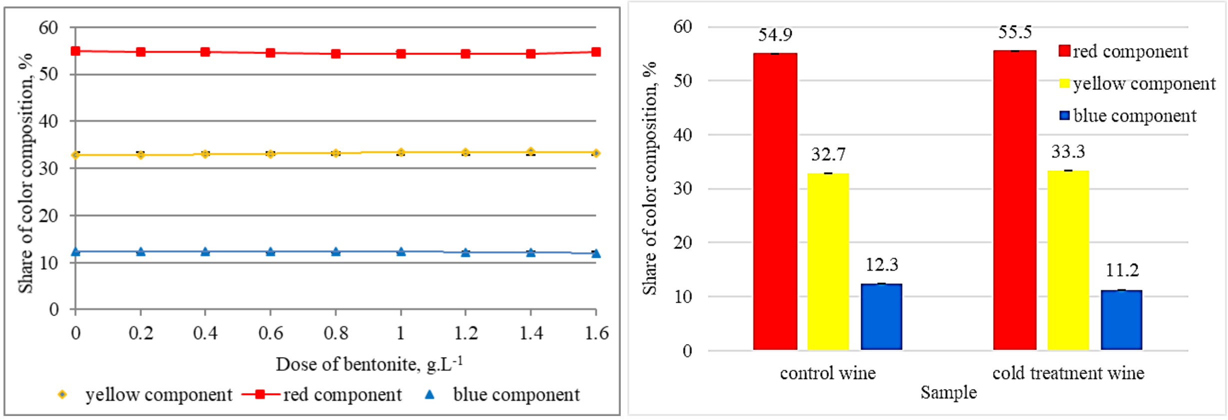
A

B

Figure 3. Change of color intensity (CI) and brilliance of red color (dA)
A-influence of bentonite dosage; B-influence of cold treatment

The value of this index in the wine after chilling was 12.10±0.01 au, which is equal to about 32% reduction compared to the control. In other words, cold application resulted in about 10% greater reduction in color intensity than bentonite. The dA value did not change significantly compared to the control sample. A possible reason for these results is perhaps precipitation of substances from the dye complex of the wine, which are part of the composition of the colloidal dye matter under the influence of low temperature (Morata 2019). These have already been discussed above.

Despite the changes in the color intensity, the ratio between the red, yellow and blue components in the average color of the wine did not change significantly compared to the control (55% : 33% : 12% resp.) regardless of the applied treatment (Fig. 4-A and 4-B). These results prove the earlier made assumption about proportional decrease of the three-color components within the red color of wine while preserving almost the same percentage composition thereof.



A B

Figure 4. Change of color composition
A - influence of bentonite dosage; B - influence of cold treatment

But yet, with increasing bentonite dose, there was a minimal decrease in the percentage of the red component (about 1%) and at the same time a minimal increase in the yellow component (just over 2%). This is probably due to the sorption of monomeric anthocyans by bentonite. To verify this assumption, it is necessary to conduct additional analyzes for anthocyan content in treated wines.

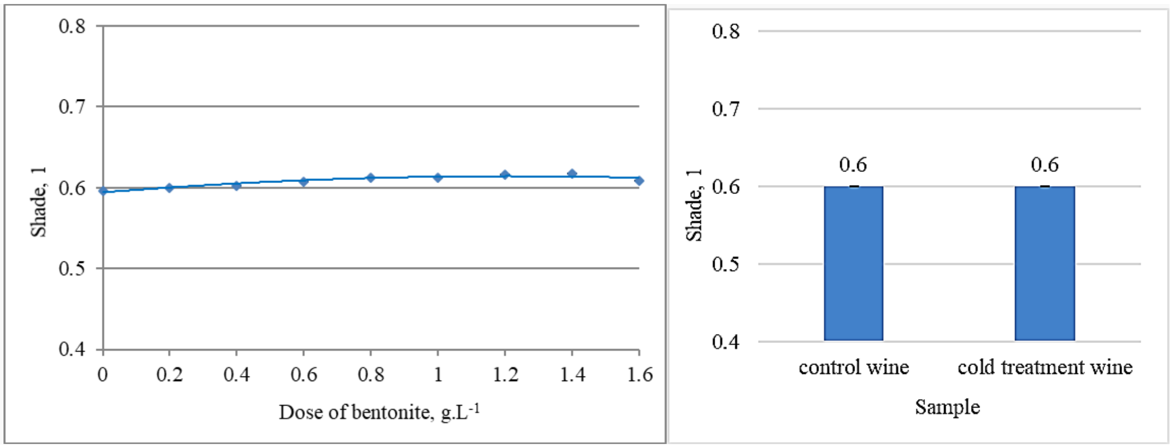
Increasing the bentonite dose led to a very slight gradual increase in the color shade values to 0.62±0.01 in wines treated with the highest doses of bentonite (Fig. 5-A). This tendency for however slight increase, is an indirect confirmation of the above-mentioned assumption of sorption of some of the anthocyans by bentonite.

The wine, kept at a constant low temperature, also had a color shade value of 0.60±0.01, i.e., this type of processing did not significantly affect the commented index either (Fig. 5-B).

Change of the antioxidant activity of red wine

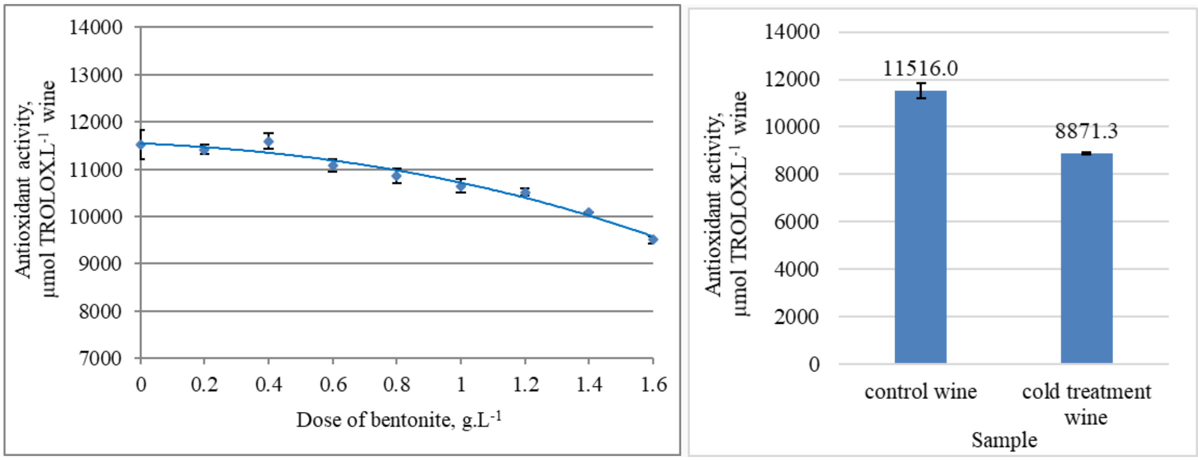
Adding bentonite at doses of up to 1.0 g.L^{-1} did not result in a significant change in antiradical activity. With increasing the dose above that, the values of this index began to decrease significantly (Fig. 6-A).

The highest applied dose of bentonite (1.6 g.L^{-1}) resulted in a nearly 20% reduction in antioxidant activity. A probable reason for these observations is the sorption capacity of bentonite when compared to some of the phenolic compounds. This assumption is supported by the already commented data on the change of I_{280}, TP and FP.



A B

Figure 5. Change of color shade
A - influence of bentonite dosage; B - influence of cold treatment



A B

Figure 6. Change of antioxidant activity
A - influence of bentonite dosage; B - influence of cold treatment

The wine treated with cold demonstrated the lowest antiradical activity amongst all experimental wines, equal to 8871.3±48.0 µmol Trolox equivalents per liter of wine (Fig. 6-B). The reduction is by 23%, i.e., 3% stronger than that in the wine treated with the highest dose of bentonite. The probable reasons for this decrease are also related to the reduction of some of the components of the phenolic complex, discussed above (Jackson 2014).

Conclusions

As the dose of bentonite increases, I_{280}, TP and FP decrease, whereas NP do not change significantly. The cold application led to more significant changes in the phenolic profile of the studied wine than treatment with bentonite.

Amongst the studied color characteristics, the color intensity turned out to be the most influenced by the treatments. Once again, cold application caused a more significant reduction compared to bentonite. The other indices (dA, shade, percentage of color components) did not change significantly.

Adding of bentonite at a dose above 1.0 $g.L^{-1}$ reduced the antioxidant activity of wine. The effect of cold application on antioxidant activity is even stronger than that of bentonite.

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