Original scientific paper
DOI: /10.5513/JCEA01/26.4.4662
Journal of Central European Agriculture, 2025, 26(4), p.1090-1100

# Study on the phenolic composition and antioxidant potential of grape juices from white grapevine varieties

# Изследване на фенолния състав и антиоксидантен потенциал на гроздови сокове от бели сортове лози

Dimitar DIMITROV[1] (✉), Dushko NEDELKOVSKI[2], Marija GJOSHEVA KOVACHEVIKJ[3], Teodora YANEVA[4], Anatoli ILIEV[1], Miroslav IVANOV[1], Petya RAEVA[5]

[1] Agricultural Academy, Institute of Viticulture and Enology, Department of Vine Selection, Enology and Chemistry, 1 Kala Tepe Str., 5800 Pleven, Bulgaria
[2] University Ss. Cyril and Methodius in Skopje, Institute of Agriculture, Department of Viticulture, Skopje, 16 Makedonska Brigada 3A Str., N. Macedonia
[3] University Ss. Cyril and Methodius in Skopje, Institute of Agriculture, Department for Agricultural Business, Economy and Rural Development, Skopje, 16 Makedonska Brigada 3A Str., N. Macedonia
[4] Agricultural Academy, Institute of Food Preservation and Quality, Department of Food Technologies, Plovdiv, 154 Vasil Aprilov Blvd., Bulgaria
[5] University of Food Technologies, Faculty of Economics, Department of Catering and Nutrition, Plovdiv, 26 Maritza Blvd., Bulgaria

✉ Corresponding author: dimitar_robertov@abv.bg

Received: January 9, 2025; accepted: May 15, 2025

## ABSTRACT

The study aimed to evaluate the influence of different treatments on the phenolic composition and antioxidant potential of four types of grape juice. The four variants of treatments (of the juices) were: pasteurization with added ascorbic acid (V1), pasteurization only (V2), a combination of grape and apple juice with subsequent pasteurization (V3), and grape juice with added preservative agent potassium sorbate (V4). In all investigated variants, a correlation ($P$<0.001) between the concentration of sugars and titratable acids was proven. The influence of the three groups of phenolic compounds (TPC, FPC, and NPC) on the pronounced antioxidant activity in all investigated juices was significant ($P$<0.0001). Grape juices exhibited strong bioactivity under the applied treatments and proved to be a valuable source of phenolic compounds. The study defined the influence of different groups of phenolic compounds on the *in vitro* antioxidant potential of grape juices, proved a substantial reserve of these bio-composites, and manifested a high antioxidant capacity, which could serve as an important factor in the reduction of oxidative stress in the human organism.

**Keywords:** antioxidants, phenols, oxidative stress, grape juice

## РЕЗЮМЕ

Целта на изследването е да се определи влиянието на различни обработки върху фенолния състав и антиоксидантен потенциал на четири вида гроздови сокове. Приложени са четири варианта на обработка на соковете: пастьоризация с добавена аскорбинова киселина (V1), пастьоризация (V2), комбинация на гроздов и ябълков сок с последваща пастьоризация (V3) и гроздов сок с добавен консервант – калиев сорбат (V4). Във всички изследвани варианти е доказана корелация ($P$<0.001) между концентрацията на захари и титруеми киселини. Влиянието на трите групи фенолни съединения (ОФС, ФФС и НФС) върху изявената антиоксидантна активност е значително ($P$<0.0001) във всички изследвани варианти. Гроздовите сокове проявяват силна биоактивност при приложените обработки и се потвърждава, че са ценен източник на фенолни съединения. Изследването дефинира влиянието на различни групи фенолни съединения върху *in vitro* антиоксидантния потенциал на гроздови сокове, доказвайки значителен резерв на тези биокомпозити и изява на висок антиоксидантен капацитет, който може да послужи като важен фактор за редукция на оксидативния стрес в човешкия организъм.

**Ключови думи:** антиоксиданти, феноли, оксидативен стрес, гроздов сок

## INTRODUCTION

The food science is becoming increasingly oriented towards research related to the potential of foods as a source of biologically active substances. The effects of the phenolic compounds on the maintenance of good health status and their capacity for prevention of numerous serious diseases are undeniable and widely studied (Ivanović et al., 2016; Beara et al., 2017). Grapes, especially red grapes, are an extremely rich source of species-diverse phenolics (Pastrana-Bonilla et al., 2003). They are located in their skins, seeds, rachis and fleshy part (Chobanova, 2012). A number of grapes phenols bioeffects were studied and confirmed: anti-carcinogenic properties, anti-inflammatory activity, potential preventive effect against diabetes, positive influence on stimulating the growth of probiotic strains of bacteria from the intestinal microbiome in a mouse model, antioxidant activity - high reactive oxygen species (ROS) elimination, reduced risk of cardiovascular diseases, neuroprotective effects, preventive action against neurodegenerative diseases, preventing the initiation of a chain reaction of the oxidation of LDL-cholesterol action and increase in serum antioxidant capacity in vivo (Day et al., 1997; Yi et al., 2005; Trifunović et al., 2015; Almulaiky et al., 2017; Chacar et al., 2018; Rasines-Perea and Teissedre, 2017; Akter et al., 2021; Chiavaroli et al., 2021; Krivokapić et al., 2022; Ghanem et al., 2023). The accumulation of phenols in grapes is a value that is highly dependent on a number of factors: genetic characteristics of the grapevine variety, soil and climatic conditions of the region of cultivation, plant-protection measures, and applied agrotechniques (Fang et al., 2008).

The grape juice has significant potential as a source of biologically active composites. It has been revealed in a number of studies. Beara et al. (2017) demonstrated a high antioxidant activity, an inhibitory effect on lipid peroxidation, and a neuroprotective effect (by inhibiting acetylcholinesterase) in red grape juices from the region of Fruška Gora, Serbia. Burin et al. (2010) studied commercial, domestic and organic grape juices in Santa Catarina, Brazil, and demonstrated a significant correlation between antioxidant activity and total phenolic compounds content. The same dependence was confirmed by da Silva et al. (2015) in the grape juice of the Brazilian cultivars BRS-Cora and Isabela, and they also defined a correlation in the antioxidant capacity influenced by anthocyanins. Cazarin et al. (2013) reported a correlation between the antioxidant capacity and anthocyanin content in grape juices of the Isabella variety cultivated under Brazilian tropical semi-arid climate conditions. Baiano (2020) conducted a study on the phenolic composition and antioxidant activity of experimental and industrially obtained grape juices from table grapes and found a significant correlation between the pronounced antioxidant activity and the content of total phenols.

The present study aimed to define the phenolic composition and antioxidant *in vitro* potential of grape juices obtained by different treatments and combinations of white grapevine varieties and apple juice grown in the region of the town of Pleven, Central Northern Bulgaria.

## MATERIALS AND METHODS

### *Grapevine varieties, applied treatments and formed experimental juice variants*

The study was conducted in the autumn of 2022 and the spring of 2023. Grapes from the interspecies white hybrid grapevine varieties Druzhba and Naslada, which have increased resistance to stress factors, were used as raw material. These grapes were grown at the Experimental Base of the Institute of Viticulture and Enology (IVE) in Pleven. Druzhba is a white grapevine variety created by complex interspecies hybridization (Misket hamburgski × Save Vilar 12 375) x (Zarya Severa × Misket hamburgski) and approved in 1983. The variety was included in the Official Varietal List of Bulgaria in 2012. It 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 content of sugars is 19-21%, with titratable acids 6.5-7.5 g/dm$^3$ (Radulov et al., 1992; Roychev, 2012). The other used variety, Naslada, is also a white hybrid grapevine variety obtained from the crossing of Misket Hamburgski

× Villar Blanc and approved in 1976. The grapes of the variety reach technological maturity in the first half of September. The variety is characterized by resistance to downy mildew and increased resistance to grey rot, powdery mildew, and low winter temperatures. At technological maturity, grapes accumulate 18-20% sugars at 7-8 g/dm$^3$ titratable acids (Radulov et al., 1992; Roychev, 2012).

The grapes used for the experiment were healthy and had an optimal ratio (for the grape juice production) of sugars and titratable acids. The obtaining of grape juice follows the following technological operations, with two conservation treatments applied – thermally and with a preservative. The technological operations with heat treatment included the following processes: Crushing the grapes ► Draining ► Pressing ► Sulphitation (20 mg/dm$^3$ $SO_2$) ► Clarification of the must and decanting ► Pasteurization of the clear part in a plate heat exchanger at mode: temperature: 75 – 80 °C, time of delay: 10 sec. ► Cooling ► Bottling.

The technological operations with added preservative included the processes: Crushing of the grapes ► Draining ► Pressing ► Sulphitation (20 mg/dm$^3$ $SO_2$) ► Clarification of the must and decanting ► Addition of preservative potassium sorbate in an amount - 200 mg/dm$^3$ ► Bottling

Four experimental variants were formed and analyzed: V1 – Pasteurized grape juice /Druzhba variety/ with added ascorbic acid (10 g/100 dm$^3$); V2 – Pasteurized grape juice /Naslada variety/; V3 – Pasteurized grape juice / Naslada variety/ + apple juice (ratio 50:50); V4 – Grape juice /Naslada variety/ + potassium sorbate.

***Chemical parameters of grape juices***

The main components of the chemical content of the studied grape juice variants were researched according to the methods generally accepted in wine practice (Ivanov et al., 1979). The sugar content (g/dm$^3$) was determined hydrometrically, by using a Dujardin hydrometer. The content of titratable acids (TA, g/dm$^3$) was analyzed by titration with 0.1 N NaOH. The actual acidity (pH) of the experimental variants was established potentiometrically, using a pH meter.

***Determination of the grape juice's phenolic content***

The phenolic content of the experimental juices was established by determination of total phenolic compounds (TPC, mg/dm$^3$ gallic acid equivalents) using Singleton et Rossi method with Folin–Ciocâlteu reagent (Ivanov et al., 1979), determination of the content of flavonoid phenolic compounds (FPC, mg/dm$^3$ catechin equivalent) and non-flavonoid phenolic compounds (NPC, mg/dm$^3$ of caffeic acid equivalents) by Sommers' method (Chobanova, 2007).

***Determination of the antioxidant (DPPH) activity of grape juices***

The antioxidant activity was determined according to the method of Wang et al. (1996), as antiradical activity against the stable DPPH (2,2-diphenyl-1-picrylhydrazyl) radical (Sigma Aldrich, Germany). The grape juice samples were diluted to a total extract of 600.00 mg/dm$^3$ and 400.00 mg/dm$^3$, and the analysis was carried out on the samples thus diluted. The control sample used was prepared with distilled water instead of juice. Freshly prepared solution (100 µm) of DPPH in ethanol was used as a free radical. In a test tube, 0.5 cm$^3$ of the sample and 2.5 cm$^3$ of the freshly prepared solution of DPPH were mixed. The values of the molecular absorption of light (spectrophotometrically at a wavelength of 515 nm) of the control and the experimental samples were measured at reaction times of 5 and 15 min, counted from the moment of reagents mixing. The antiradical activity was expressed in %.

***Statistical analysis***

Descriptive statistics were calculated to ascertain the mean and standard deviation (± SD) of the observed variables. The statistical analysis was conducted using IBM® SPSS software (Landau and Everitt, 2004). One-way Analysis of Variance (ANOVA) was employed to validate significant differences among the examined values, with significance set at $P<0.05$. Tukey's post-hoc tests were subsequently performed to identify significant differences between group means, where applicable. Pearson correlation coefficients (R) were computed to assess the re-

lationships between variables, with statistical significance determined via a two-tailed test at the alpha level of 0.05.

## RESULTS AND DISCUSSION

There was a slight disparity in sugar content among the variants, with Variant 4 boasting the highest mean sugar content at 9.63 ± 0.05 g/dm³, closely followed by Variant 1 at 9.49 ± 0.05 g/dm³. Conversely, Variants 2 and 3 exhibited comparatively lower mean sugar levels at 9.35 ± 0.05 g/dm³ and 9.25 ± 0.02 g/dm³, respectively (Table 1).

In terms of total acidity, significant discrepancies emerge across the variants. Variant 3 notably distinguished itself with the highest mean acidity, registered at 7.53 ± 0.05 g/dm³, followed by Variant 2 at 5.25 ± 0.00 g/dm³. In contrast, Variants 1 and 4 displayed relatively lower mean acidity levels (Table 1).

Regarding pH levels, Variant 3 exhibited the lowest mean pH of 4.21 ± 0.01, indicating higher acidity, whereas Variant 1 displayed the highest mean pH level of 4.40 ± 0.01. Variants 2 and 4 lay in intermediate positions, with Variant 4 slightly edged out Variant 2 with a mean pH of 4.29 ± 0.00 compared to 4.31 ± 0.01, respectively (Table 1).

The results found in our study regarding the content of titratable acids (TA) showed differences compared to the data of Pavloušek and Kumšta (2011), who investigated primary metabolites in 11 newly selected grapevine varieties. They found a slightly higher content of titratable acids in unheated must, ranging from 7.96 g/dm³ to 9.55 g/dm³, and the pH levels found were lower than those obtained in the present study and ranged from 3.13 to 3.56. It was established that regimes of fruit juices thermal treatment at temperatures equal to or higher than 80 °C, with a short delay of 30 sec, led to a decrease in the content of citric and ascorbic acids in combined grape-fruit juices (Igual et al., 2010; Petruzzi et al., 2017). In this way, the total titratable acids were reduced, which also explained their lower levels in our study, in contrast to the studies that examined non-thermally processed juices. Korzenski and Molnar (2014) investigated the influence of different types of thermal treatments (by water bath, microwaves and electric heater) on grape must at a temperature of 70 °C. They found that heat treatment resulted in an increase in pH levels from 2.76 in the untreated control to 3.05 in the electric heater-treated sample. The obtained result correlated with the higher pH levels found in our study, which, given the findings of the above team, were due to their heat treatment.

Table 2 displays the content of total phenolic compounds (TPC), flavonoid phenolic compounds (FPC), and non-flavonoid phenolic compounds (NPC) in grape juices across the four variants. Collectively, the findings consistently position Variant 3 as the top performer across all phenolic compound categories, underscoring its potential nutritional significance, followed by Variant 2, while Variant 4 preceded Variant 1 with the lowest TPC, FPC and NPC levels.

In our study, the content of total phenolic compounds (TPC) across the four variants ranged from 453.3 ± 5.8 mg GAE/dm³ to 646.70 ± 5.8 mg GAE/dm³. These findings

**Table 1.** Main chemical indicators of grape juices

| Chemical indicators | Variant 1 | | Variant 2 | | Variant 3 | | Variant 4 | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Sugars, g/dm³ | 9.49 | 0.05 | 9.35 | 0.05 | 9.25 | 0.02 | 9.63 | 0.05 |
| TA, g/dm³ | 4.55 | 0.05 | 5.25 | 0.00 | 7.53 | 0.05 | 4.28 | 0.00 |
| pH | 4.40 | 0.01 | 4.31 | 0.01 | 4.21 | 0.01 | 4.29 | 0.00 |

**Table 2.** Content of total phenolic compounds (TPC), flavonoid phenolic compounds (FPC) and non-flavonoid phenolic compounds (NPC) in the grape juice of the studied variants

| Phenolic compounds | Variant 1 | | Variant 2 | | Variant 3 | | Variant 4 | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| TPC, mg GAE/dm$^3$ | 453.3 | 5.8 | 593.3 | 5.8 | 646.7 | 5.8 | 480.0 | 0.0 |
| FPC, mg CE/dm$^3$ | 240.3 | 3.5 | 575.3 | 1.4 | 911.4 | 10.1 | 362.2 | 2.2 |
| NPC, mg CA/dm$^3$ | 84.2 | 0.1 | 141.5 | 0.3 | 208.7 | 0.1 | 116.5 | 8.8 |

align with the research of Moreno-Montero et al. (2015), who observed a variation in total phenols in commercial Spanish white and red grape juices ranging from 126.0 mg GAE/dm$^3$ to 1728.0 mg GAE/dm$^3$. This comparison suggests that our results fall within the range of phenolic content reported in commercial grape juices. In comparison to previous studies, the present study observed higher total phenolic content in grape juices.

Frankel et al. (1998) examined commercial white grape juices from Westfield, New York, reporting total phenolic content ranging from 254.0 mg GAE/dm$^3$ to 389.0 mg GAE/dm$^3$, which was lower than the levels found in our study. Similarly, Rodriguez et al. (2019) investigated grape juices from Rio De Janeiro, Brazil, and reported total phenolic content in white grape juices ranging from 193.0 mg GAE/dm$^3$ to 343.0 mg GAE/dm$^3$, again lower than our findings. Additionally, Lee et al. (2008) found lower levels of total phenols (ranging from 57.95 mg GAE/dm$^3$ to 205.64 mg GAE/dm$^3$) in Korean commercial grape juice compared to our study results. The differences in total phenolic content are due to the specifics of the terroir where the vines are grown. These soil and climatic characteristics, unique to each region, have a fundamental influence on the ultimate accumulation of phenols in the grapes and are a factor in their biological potential. The disparities in total phenolic content could be attributed to the unique terroir of each vineyard where the grapes are grown. The terroir encompasses the soil composition, climate conditions, topography, and other environmental factors specific to each region, all of which play a crucial role in shaping the accumulation of phenolic compounds in the grapes. These environmental factors directly influence the grapevine's biological processes, including secondary metabolite production, ultimately impacting the phenolic composition of the grapes.

The content of flavonoid phenolic compounds (FPC) ranged from 240.3 ± 3.5 mg CE/dm$^3$ to 911.4 ± 10.1 mg CE/dm$^3$. This group consists of monomeric phenols and includes flavones, flavonols, flavonones, catechins, proanthocyanidins and anthocyanins (the latter being present only in red grapes) (Harbone, 1980).

The non-flavonoid phenolic compounds (NPC) in the studied variants ranged from 84.2 ± 0.1 mg CA/dm$^3$ to 208.7 ± 0.1 mg CA/dm$^3$. This group of phenols consists of subgroups with representatives that exhibit confirmed biological effects: bactericidal activity, anticholesterolemic effect (phenolic acids); antioxidant activity, preventive effect against cardiovascular diseases, anticarcinogenic effect, immunomodulation (resveratrol) (Wang et al., 1996; Jang et al., 1997; Lu and Serrero, 1999; Chobanova, 2012; Craveiro et al., 2017).

The data on the established antioxidant activity of the investigated grape juices at different extracts (600.0 and 400.0 mg/dm$^3$) and reaction times (5 and 15 min) are presented in Figure 1.

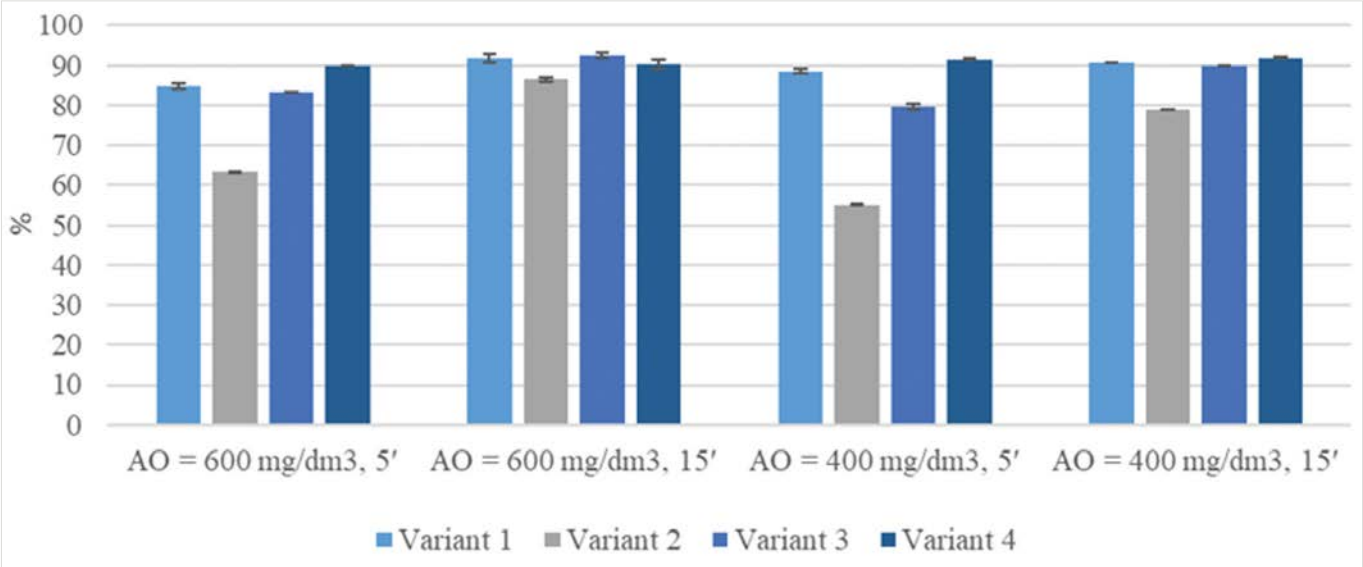


**Figure 1.** Antioxidant activity of grape juices at total extracts (600.0 and 400.0 mg/dm$^3$) and reaction times (5 and 15 min)

For a total extract concentration of 600.0 mg/dm$^3$, at the 5-minute mark, Variant 4 demonstrated the highest mean antioxidant activity at approximately 89.9%, followed by Variant 1 with approximately 84.75%, and Variant 3 with 83.23%. Variant 2 trailed behind with a mean activity of approximately 63.34%. By the 15-minute mark, Variant 3 exhibited the highest mean antioxidant activity at approximately 92.39%, followed closely by Variant 1 at approximately 91.71%. Variant 4 displays a mean activity of approximately 90.19%, while Variant 2 remained lower at approximately 86.42%.

For a total extract concentration of 400.0 mg/dm$^3$ at the 5$^{th}$ minute mark, Variant 4 exhibited the highest mean antioxidant activity at approximately 91.5%, followed by Variant 1 with approximately 88.57% and Variant 3 at 79.46%. Variant 2 displayed a lower mean activity of approximately 55.17%.

Based on the data from Figure 1, it is evident that Variant 4 exhibited the highest antioxidant activity across most measurements. However, it's noteworthy that at 600.0 mg/dm$^3$ and a reaction time of 15 minutes, Variant 4 ranked third, while Variant 1 consistently secured the second position across all measurements. Variant 3 generally ranked third in most instances, except for the second measurement at the 15$^{th}$ minute with 600.0 mg/dm$^3$, where it took the first position. Overall, the data highlighted the varying antioxidant activities of grape juices under different conditions, with Variant 4 being the most potent in most scenarios.

At a total extract of 600.0 mg/dm$^3$ after 5 minutes of the reaction with the DPPH radical, the antioxidant activity between the four investigated variants varied from 63.34 ± 0.93% (Variant 2) to 89.93 ± 0.03% (Variant 4). An increase in the radical-elimination capacity with antioxidant activity ranged from 86.42 ± 0.56% (Variant 2) to 91.71 ± 0.16 (Variant 1) was found at the 15$^{th}$ minute of the reaction with the same extract. The higher antioxidant activities of Variants 1 and 4 were due to the specific treatments applied. The influence of ascorbic acid in Variant 1, which acts as a strong antioxidant, increased the activity accordingly. The potassium sorbate added as a preservative also has an antioxidant effect and the ability to increase the radical-elimination capacity of the sample.

The reported trends stayed the same, even with the established antioxidant activity at a total extract of 400.0 mg/dm$^3$. At the 5$^{th}$ minute of the reaction with the radical in this total extract, a variation in the radical scavenging activity was found from 55.17 ± 0.63% in Variant 2 to 91.51 ± 0.03% in Variant 4. At the 15$^{th}$ minute of the reaction with the same extract, a similar dynamics of vari-

ation was recorded – from the lowest activity in Variant 2 (78.76 ± 1.08%) to the highest in Variant 4 (91.78 ± 0.14%). The differences between Variants 1 and 4, which showed the highest antioxidant activity, were not significant, but their high activity was probably due to the addition of ascorbic acid and potassium sorbate and their influence. Landbo and Meyer (2008) found that the addition of ascorbic acid to European grape juices improved their antioxidant potential and the ability of the juices to inhibit LDL peroxidation *in vitro*.

A complex statistical data processing was carried out, which revealed important dependencies between all the studied indicators. The ANOVA analysis (Table 3) was conducted for various factor levels and conditions, including different concentrations of total extract (AO) at 600.0 mg/dm$^3$ and 400.0 mg/dm$^3$, and at two different reaction times of 5 minutes and 15 minutes. For all components (TPC, FPC, and NPC), the F-values were large and associated *P*-values were extremely small ($P<0.0001$), indicating highly significant differences between the groups.

**Table 3.** Analysis of variance (ANOVA) for antioxidant activity and phenolic compounds

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Total phenolic compounds | Between Groups | 151,733.33 | 3 | 50,577.78 | 2,528.89 | 0.00 |
| | Within Groups | 400.00 | 20 | 20.00 | | |
| | Total | 152,133.33 | 23 | | | |
| Flavonoid phenolic compound | Between Groups | 1,556,172.11 | 3 | 518,724.04 | 21,606.12 | 0.00 |
| | Within Groups | 480.16 | 20 | 24.01 | | |
| | Total | 1,556,652.27 | 23 | | | |
| Non-flavonoid phenolic compounds | Between Groups | 50,270.61 | 3 | 16,756.87 | 1,092.22 | 0.00 |
| | Within Groups | 306.84 | 20 | 15.34 | | |
| | Total | 50,577.45 | 23 | | | |
| AO = 600.0 mg/dm$^3$, 5′ | Between Groups | 2,451.82 | 3 | 817.27 | 2,216.48 | 0.00 |
| | Within Groups | 7.38 | 20 | 0.37 | | |
| | Total | 2,459.20 | 23 | | | |
| AO = 400.00 mg/dm$^3$, 5′ | Between Groups | 4,894.04 | 3 | 1,631.35 | 8,436.83 | 0.00 |
| | Within Groups | 3.87 | 20 | 0.19 | | |
| | Total | 4,897.91 | 23 | | | |
| AO = 600.0 mg/dm$^3$, 15′ | Between Groups | 128.18 | 3 | 42.73 | 621.67 | 0.00 |
| | Within Groups | 1.38 | 20 | 0.07 | | |
| | Total | 129.55 | 23 | | | |
| AO = 400.0 mg/dm$^3$, 15′ | Between Groups | 659.65 | 3 | 219.88 | 886.96 | 0.00 |
| | Within Groups | 4.96 | 20 | 0.25 | | |
| | Total | Total | 664.60 | 23 | | |

Therefore, there was strong evidence to reject the null hypothesis and conclude that the concentrations of total phenolic compounds, flavonoid phenolic compounds, and non-flavonoid phenolic compounds in grapes significantly affected the levels of antioxidant activity. The high F-values suggested that the variability between groups was much larger than the variability within groups, further supporting the significance of the observed differences.

The analysis of variance (ANOVA) (Table 3) revealed significant differences in the levels of total phenolic compounds, flavonoid phenolic compounds, and non-flavonoid phenolic compounds among different groups. Specifically, the antioxidant treatments at varying concentrations and reaction times also exhibited significant variations in antioxidant activity. These findings underscored the importance of both the type of phenolic compounds present and the conditions under which antioxidant treatments were applied in influencing the overall antioxidant activity of grape juices. Additionally, the relatively low within-group variability indicated consistent results within each treatment group, further supporting the reliability of the observed differences. The results from the Tukey HSD multiple comparisons test showed significant differences in the mean values of phenolic compounds and antioxidant activities across the grape juice variants. Each comparison indicated significant differences ($P<0.05$) between specific grape variants, providing insights into the unique biochemical profiles of different grape juices.

Table 4 shows the results for correlations among the variables studied. Sugars exhibited strong negative associations with titratable acids (r = -0.860, $P<0.001$), total

**Table 4.** Pearson correlations among analyzed variable pairs

| Variables | Variables | Pearson Correlation | Sig. (2-tailed) |
|---|---|---|---|
| Sugars | Titratable acids | -0.860** | 0.000 |
| Sugars | Total phenolic compounds | -0.856** | 0.000 |
| Sugars | Flavonoid phenolic compound | -0.820** | 0.000 |
| Sugars | Non-flavonoid phenolic compounds | -0.749** | 0.000 |
| Sugars | AO = 600.0 mg/dm$^3$, 5’ | 0.514* | 0.010 |
| Sugars | AO = 400.0 mg/dm$^3$, 5’ | 0.583** | 0.003 |
| AO = 600.0 mg/dm$^3$, 5’ | Total phenolic compounds | -0.481* | 0.017 |
| AO = 600.0 mg/dm$^3$, 5’ | Flavonoid phenolic compound | -0.248 | 0.243 |
| AO = 600.0 mg/dm$^3$, 5’ | Non-flavonoid phenolic compounds | -0.161 | 0.452 |
| AO = 400.0 mg/dm$^3$, 5’ | Total phenolic compounds | -0.615** | 0.001 |
| AO = 400.0 mg/dm$^3$, 5’ | Flavonoid phenolic compound | -0.398 | 0.054 |
| AO = 400.0 mg/dm$^3$, 5’ | Non-flavonoid phenolic compounds | -0.319 | 0.129 |
| AO = 600.0 mg/dm$^3$, 15’ | Total phenolic compounds | -0.130 | 0.546 |
| AO = 600.0 mg/dm$^3$, 15’ | Flavonoid phenolic compound | 0.096 | 0.656 |
| AO = 600.0 mg/dm$^3$, 15’ | Non-flavonoid phenolic compounds | 0.142 | 0.508 |
| AO = 400.0 mg/dm$^3$, 15’ | Total phenolic compounds | -0.432)* | 0.035 |
| AO = 400.0 mg/dm$^3$, 15’ | Flavonoid phenolic compound | -0.193 | 0.367 |
| AO = 400.0 mg/dm$^3$, 15’ | Non-flavonoid phenolic compounds | -0.114 | 0.597 |

* Correlation is significant at the 0.05 level (2-tailed); ** correlation is significant at the 0.01 level (2-tailed)

phenolic compounds (r = -0.856, $P<0.001$), flavonoid phenolic compounds (r = -0.820, $P<0.001$), and non-flavonoid phenolic compounds (r = -0.749, $P<0.05$). Additionally, moderate positive correlations were found between sugars and antioxidant treatments at 600.0 mg/dm³ for 5 minutes (r = 0.514, $P<0.05$) and 400.0 mg/dm³ for 5 minutes (r = 0.583, $P<0.01$). Antioxidant treatments at different concentrations and durations showed varying relationships with phenolic compounds. Notably, there were negative correlations with total phenolic compounds at 600.0 mg/dm³ for 5 minutes (r = -0.481, $P<0.05$) and 400.0 mg/dm³ for 5 minutes (r = -0.615, $P<0.001$). Additionally, a moderate negative correlation was observed at 400.0 mg/dm³ for 15 minutes (r = -0.432, $P<0.05$). The correlation between AO at 400.0 mg/dm³ for 5 minutes and flavonoid phenolic compounds was nearly significant (r = -0.398, P = 0.054). Other correlations with total phenolic compounds, flavonoid, and non-flavonoid phenolic compounds were not statistically significant.

## CONCLUSIONS

The following conclusions could be drawn from the conducted research:

- The investigated grape juices (thermally treated, with the addition of ascorbic acid and potassium sorbate) showed a normal state in terms of the three main chemical indicators - content of sugars, titratable acids, and pH. A strong correlation dependence ($P<0.001$) was proved between the concentration of sugars and titratable acids. Sugar content did not affect NPC ($P<0.05$), but had a slight effect on antioxidant activity in both extracts (600.0 and 400.0 mg/dm³).
- It was statistically proven that the concentration of the three groups of phenolic compounds (TPC, FPC, and NPC) significantly influenced ($P<0.0001$) and affected the levels of antioxidant activity exhibited by the juices.
- The statistical analysis revealed significant differences when comparing the groups of phenolic compounds within each variant, as well as the antioxidant activities at different total extracts and reaction times in each tested variant. These findings indicated the distinct influence of each phenolic group and the impact of varying conditions on the antioxidant potential of each investigated variant.

The present study enriched the nutritional science with valuable data proven by statistical confirmation of the dependencies and concentration presence of phenolic compounds from different groups, as well as their influence on the *in vitro* antioxidant potential of grape juices from the region of Pleven, Central Northern Bulgaria. The study proved substantial availability of biologically active substances in the composition of the studied grape juices and the manifestation of a high antioxidant effect, which could serve as an important factor in increasing the antioxidant capacity in the blood, helping to protect against cellular damage caused by oxidative stress in the human body.

## REFERENCES

Akter, R., Rahman, H., Behl, T., Chowdhury, M., Manirujjaman, M., Bubul, I., Elshenaw, S., Tit, D., Bungan, S. (2021) Prospective role of polyphenolic compounds in the treatment of neurodegenerative diseases. CNC and Neurological Disorders – Drug Targets, 20 (5), 430-450.
DOI: https://doi.org/10.2174/1871527320666210218084444

Almulaiky, Y., Alshawafi, W., Al-Talhi, H., Zeyadi, M., Anwar, F., Al-Abbasi, F., Khan, R., Kumar, V. (2017) Evaluation of the antioxidant potential and antioxidant enzymes of some Yemeni grape cultivars. Free Radicals and Antioxidants, 7 (1), 74-79.
DOI: https://doi.org/10.5530/fra.2017.1.11

Baiano, A. (2020) Phenolic compounds and antioxidant activity of experimental and industrial table grape juices. Journal of Food Processing and Preservation, 44 (9), e14655.
DOI: https://doi.org/10.1111/jfpp.14655

Beara, I., Torović, L., Pintać, D., Majkić, T., Orčić, D., Mimica-Dukić, N., Lesjak, M. (2017) Polyphenolic profile, antioxidant and neuroprotective potency of grape juices and wines from Fruška Gora region (Serbia). International Journal of Food Properties, 20 (S3), S2552-S2568.
DOI: https://doi.org/10.1080/10942912.2017.1375512

Burin, V., Falcão, L., Gonzaga, L., Fett, R., Rosier, J., Bordignon-Luiz, M. (2010) Colour, phenolic content and antioxidant activity of grape juice. Food Science and Technology, 30 (4), 1027-1032. DOI: https://doi.org/10.1590/s0101-20612010000400030

Cazarin, C., Correa, L., da Silva, J., Batista, A., Furlan, P., Biasoto, A., Pereira, G., Rybka, A., Junior, M. (2013) Tropical Isabella grape juices: bioactive compounds and antioxidant power depends on harvest season. Journal of Food Science and Engineering, 3, 64-70.

Chacar, S., Tarighi, M., Fares, N., Faivre, J.F., Louka, N., Maroun, R. (2018) Identification of phenolic compounds-rich grape pomace extracts urine metabolites and correlation with gut microbiota modulation. Antioxidants, 7 (6), 75. DOI: https://doi.org/10.3390/antiox7060075

Chiavaroli, A., Balaha, M., Acquaviva, A., Ferrante, C., Cataldi, A., Menghini, L., Rapino, M., Orlando, G., Brunetti, L., Leone, S., Recinella, L., di Giacomo, V. (2021) Phenolic characterization and neuroprotective properties of grape pomace extracts. Molecules, 26 (20), 6216. DOI: https://doi.org/10.3390/molecules26206216

Chobanova, D. (2007) Guide to Exercises in Oenology. Plovdiv, Bulgaria: Academic Press of University of Food Technologies. [in Bulgarian]

Chobanova, D. (2012) Enology. Part I: Composition of wine. Plovdiv, Bulgaria: Academic Press of University of Food Technologies. [in Bulgarian]

Craveiro, M., Cretenet, G., Mongellaz, C., Matias, M., Caron, O., Pedroso de Lima, M., Zimmermann, V., Solary, E., Dardalhon, V., Dulić, V., Taylor, N. (2017) Resveratrol stimulates the metabolic reprogramming of human CD4+ T cells to enchance effector function. Science Signaling, 10 (501), 3024. DOI: https://doi.org/10.1126/scisignal.aal3024

da Silva, J., Cazarin, C., Correa, L., Batista, A., Furlan, C., Biasoto, A., Pereira, G., Camargo, A., Junior, M. (2015) Bioactive compounds of juices from two Brazilian grape cultivars. Journal of the Science of Food and Agriculture, 96, 1990-1996. DOI: https://doi.org/10.1002/jsfa.7309

Day, A., Kemp, H., Bolton, C., Hartog, M., Stansbie, D. (1997) Effect of concentrated red grape juice consumption on serum antioxidant capacity and low-density lipoprotein oxidation. Annals of Nutrition and Metabolism, 41 (6), 353-357. DOI: https://doi.org/10.1159/000178006

Fang, F., Jing-Ming, L., Ping, Z., Ke, T., Wei, W., Qui-Hong, P., Wei-Dong, H. (2008) Effect of grape variety, harvest date, fermentation vessel and wine aging on flavonoid concentration in red wines. Food Research International, 41 (1), 53-60. DOI: https://doi.org/10.1016/j.foodres.2007.09.004

Frankel, E., Bosanek, C., Meyer, A., Silliman, K., Kirk, L. (1998) Commercial grape juices inhibit the *in vitro* oxidation of human low density lipoproteins. Journal of Agricultural and Food Chemistry, 46, 834-838. DOI: https://doi.org/10.1021/jf9707952

Ghanem, C., Bouajila, J., Rizk, Z., El Beyrouthy, M., Sadaka, C., Gürer, E., Souchard, J.P., Taillandier, P., Sharifi-Rad, J., Nehme, N., El Rayess, Y. (2023) Comparative analysis of pre-fermentation treatments on phenolic compounds and bioactivity in *Vitis vinifera* var. Syrah and var. Cabernet Sauvignon grapes. Nutrire, 48, 25. DOI: https://doi.org/10.1186/s41110-023-00207-w

Harborne, J.B. (1980) Plant phenolics. In: Bell, E. A., Charlwood, B. V., eds. Encyclopedia of Plant Physiology, volume 8 Secondary Plant Products. Berlin Heidelberg New York: Springer-Verlag, pp. 329–395.

Igual, M., Garcia-Martinez, E., Camacho, M., Martinez-Navarrete, N. (2010) Effect of thermal treatment and storage on the stability of organic acids and the functional value of grapefruit juice. Food Chemistry, 118, 291-299. DOI: https://doi.org/10.1016/j.foodchem.2009.04.118

Ivanović, Lj., Milašević, I., Đurović, D., Topalović, A., Knežević, M., Mugoša, B., Vrvić, M. (2016) Application of plant biotechnology techniques in antioxidant production. Agriculture and Forestry, 62 (1), 325-342.

Ivanov, T., Gerov, S., Yankov, A., Bambalov, G., Tonchev, T., Nachkov, D., Marinov, M. (1979) Guide to wine technology. Plovdiv, Bulgaria: Hr. Publishing House G. Danov. [in Bulgarian]

Jang, M., Cai, L., Udeani, G., Slowing, K., Thomas, C., Beecher, C., Fong, H., Farnsworth, N., Kinghorn, A., Metha, R., Moon, R., Pezzuto, J. (1997) Cancer chemopreventive activity of resveratrol, a natural product from grapes. Science, 275 (5297), 218 – 220. DOI: https://doi.org/10.1126/science.275.5297.218

Korzenszky, P., Molnár, E. (2014) Examination of heat treatments at preservation of grape must. Potravinarstvo, 8, 38-42. DOI: https://doi.org/10.5219/328

Krivokapić, S., Otović, M., Perović, S., Damjanović Vratnica, B. (2022) Total phenols, flavonoids, anthocyanins and antioxidant activity of wild pomegranate (*Punica granatum* L.) biowaste from Montenegro. Agriculture and Forestry, 68 (2), 157-165. DOI: https://doi.org/10.17707/agricultforest.68.2.11

Landau S., Everitt B. (2004) A handbook of statistical analyses using SPSS. Florida, USA: Chapman and Hall/CRC Press LLC. DOI: https://doi.org/10.1201/9780203009765

Landbo, A.K., Meyer, A. (2008) Ascorbic acid improves the antioxidant activity of European grape juices' ability to inhibit lipid peroxidation of human LDL *in vitro*. International Journal of Food Science and Technology, 36 (7), 727-735. DOI: https://doi.org/10.1046/j.1365-2621.2001.00515.x

Lee, H., Jung, B., Park, J., Hwang, I., Kim, S., Choi, J., Lee, S., Chung, S. (2008) Antioxidant activity and total phenolic contents of grape juice products in the Korean market. Korean Journal of Food Preservation, 15 (3), 445-449.

Lu, R., Serrero, G. (1999) Resveratrol, a natural product derived from grape, exhibits antiestrogenic activity and inhibits the growth of human breast cancer cells. Journal of Cellular Physiology, 179 (3), 297-304.

Moreno-Montero, M., Olalla-Herrera, M., Gimenez-Martinez, R., Navarro-Alarcon, M., Rutian-Henares, J. (2015) Phenolic compounds and antioxidant activity of Spanish commercial grape juices. Journal of Food Composition and Analysis, 38, 19-26. DOI: https://doi.org/10.1016/j.jfca.2014.10.001

Pastrana-Bonilla, E., Akoh, C., Sellappan, S., Krewer, G. (2003) Phenolic content and antioxidant capacity of *Muscadine grapes*. Journal of Agricultural and Food Chemistry, 51 (18), 5497-5503. DOI: https://doi.org/10.1021/jf030113c

Pavloušek, P., Kumšta, M. (2011) Profiling of primary metabolites in grape of interspecific grapevine varieties: sugars and organic acids. Czech Journal of Food Science, 29, 361-372. DOI: https://doi.org/10.17221/257/2010-cjfs

Petruzzi, L., Campaniello, P., Speranza, B., Corbo, M., Sinigaglia, M., Bevilacqua, A. (2017) Thermal treatments for fruit and vegetable juices and beverages: A literature overview. Comprehensive Reviews in Food Science and Food Safety, 2017, 668-691. DOI: https://doi.org/10.1111/1541-4337.12270

Radulov, L., Babrikov, D., Georgiev, S. (1992) Ampelography with basics of winemaking. Sofia: Zemizdat. [in Bulgarian]

Rasines-Perea, Z., Teissedre, P.L. (2017) Grape polyphenols effects in human cardiovascular diseases and diabetes. Molecules, 22 (1), 68. DOI: https://doi.org/10.3390/molecules22010068

Rodriguez, R.F.C., Lima, A., Melo, A.C.F.L., Trindade, R.A. (2019) Physicochemical characterization, bioactive compounds and *in vitro* antioxidant activities of commercial integral grape juices. International Food Research Journal, 26 (2), 469-479.

Roychev, V. (2012) Ampelography. Plovdiv: Academic Publishing House of the University of Plovdiv. [in Bulgarian]

Trifunović, S., Topalović, A., Knežević, M., Vajs, V. (2015) Free radicals and antioxidants: antioxidative and other properties of swiss chard (*Beta vulgaris* L. subsp. cicla). Agriculture and Forestry, 61 (2), 73-92. DOI: https://doi.org/10.17707/agricultforest.61.2.06

Wang, H., Cao, G., Prior, R.L. (1996) Total antioxidant capacity of fruits. Journal of Agriculture and Food Chemistry, 44 (3), 701-705. DOI: https://doi.org/10.1021/jf950579y

Yi, W., Fishar, J., Akoh, K. (2005) Study of anticancer activities of Muscadine grape phenolics *in vitro*. Journal of Agricultural and Food Chemistry, 53 (22), 8804-8812. DOI: https://doi.org/10.1021/jf0515328