Доклади на Българската академия на науките
Comptes rendus de l'Académie bulgare des Sciences

*Tome 77, No 10, 2024*

*CHEMISTRY*

*Analytical chemistry*

# DETERMINATION OF POLYPHENOLS, FLAVONOIDS AND ANTHOCYANINS IN MAVRUD WINE FROM THE HARMANLI REGION. COMPARISON WITH OTHER INVESTIGATED MAVRUD WINES

**Madlena Lazarova**✉, **Dragomir Yankov**

*Received on May 23, 2024*
*Presented by V. Bankova, Member of BAS, on July 30, 2024*

**Abstract**

Native red wine of the Mavrud variety (Harmanli region) was assayed for its phenolic, flavonoid and anthocyanin contents. Total phenolics, flavonoids and anthocyanins of the wine samples were determined by the Folin–Ciocalteu method, the aluminium complexation assay, and the modified method of Ribéreau-Gayon and Stonestreet, respectively. Total phenolic content was determined to be 2990 mg GAE/L, total flavonoid content was 1020 mg CE/L, and the anthocyanin content – 122 mg ME/L. The comparison of the results with those found for other investigated Mavrud wines revealed up to three and two times differences in the phenolic and anthocyanin contents, respectively, while the amount of flavonoids was almost equal.

**Key words:** red wine, Mavrud variety, phenolics, flavonoids, anthocyanins

**Introduction.** Bulgaria has a strong tradition in wine production. Wine is composed of water, ethanol, polysaccharides, acids, glycerol and phenolic compounds. The striving to produce well-coloured, fully flavoured and at the same time harmonious and without unnecessary bitterness wines, directs more studies

---

This work was financially supported by the Bulgarian National Science Fund, Project No KP-06-N47/1/26.11.2020.

`https://doi.org/10.7546/CRABS.2024.10.02`

to investigate the phenolic compounds of grapes and wines. The phenolics extracted from the solid grape parts have an essential role in the quality shaping of the future wine in many aspects: colour and taste characteristics, cloudiness and interaction with proteins during wine clarification [1]. These biomolecules are not only of technological but also of nutraceutical interest, acting as antioxidants through the inhibition of reactive oxygen species. The protective role of polyphenols on human health against neurodegenerative diseases, microbial inflammation, cancer and cardiovascular diseases such as angina, myocardial ischemia and heart failure, has already been well established [2].

Flavonoids are polyphenols bioactive derivatives widely distributed in plants and, respectively, in human diet as various fruits, grains, nuts, vegetables, beverages (tea, coffee, wine) [3]. They account for a significant proportion of the phenolics in red wine and their health benefits have encouraged researchers in the fields of medicine, food and nutrition [4]. Anthocyanins belong to the group of flavonoids and the red colour of grape berries and young wines comes from them. Anthocyanins proportion in red grapes, and, accordingly, in the red wines, greatly depends on the grape varieties and the growing conditions – regional weather characteristics and viticulture practices [5].

Harmanli is a small town in Bulgaria, which spreads between the south slopes of the Sredna Gora and the north slopes of the Rhodope Mountains. The climate is temperate continental and the soils are mainly maroon and woody, which gives its footprint to the characteristics of the wine from this area.

The aim of the present study was to analyze the content of total phenolics (TP), total flavonoids (TF) and total anthocyanins (TA) of the native red wine Mavrud from a Harmanli cellar. A comparison was made between our results and those obtained from other investigated Mavrud wines produced by different wineries/regions in Bulgaria.

**Experimental.** ***Chemicals.*** Folin–Ciocalteau reagent (2N solution), gallic acid, sodium nitrite, (+)-catechin hydrate ($> 96.0\%$) aluminium chloride and sodium bisulfite were obtained from Sigma-Aldrich; sodium carbonate, hydrochloric acid (37%) were purchased from Merck; ethanol (96%) and sodium hydroxide were domestic products (Valerus). All reagents and chemicals were of analytical grade.

***Wine samples.*** For the present study, Mavrud red wine (13.0 vol.%; vintage 2020) was provided by a Bulgarian wine cellar in Harmanli. Data for the following Mavrud wines produced by different wineries and/or regions in Bulgaria was found in the literature and used for comparison (see Table 1).

***Analytical methods.*** For total phenolic analysis in the investigated wine, the Folin–Ciocalteau method was applied as described by Waterhouse [10]. The measurements were carried out at a wavelength of 765 nm. Gallic acid, considered as equivalent to most phenolics on a mass basis, was used as reference standard. The gallic acid standard solutions used to determine the concentration of TP were

T a b l e 1

Description of the Mavrud wines found in the literature and the one used in this study

| *N* | Wine region and/or producer | Vintage | Reference |
|---|---|---|---|
| 1 | Assenovgrad | 1994 | [6] |
| 2 | Assenovgrad | 1997 | [6] |
| 3 | Peroushtitsa | 1995 | [6] |
| 4 | Peroushtitsa Reserve | 1996 | [6] |
| 5 | Asenovgrad – Assenovgrad Reserve – Peroushtitsa | n/d | [7] |
| 6 | Brestnik | 2004 | [1] |
| 7 | Brestovitza | 2001 | [8] |
| 8 | Agricultural University – Plovdiv | 2000 | [8] |
| 9 | Karabunar | 1996 | [8] |
| 10 | Assenovgrad | 1996 | [8] |
| 11 | Trakiets | 2012 | [9] |
| 12 | Harmanli | 2020 | This article |

within the 100–1000 mg/L range. Before analysis, wine samples were properly diluted. The results were expressed as mg of gallic acid equivalents per liter (mg GAE/L).

The total flavonoid concentration was determined by the aluminium complexation assay [11]. The absorbance of wine samples was measured at 510 nm with catechin used as reference standard. A calibration curve was constructed with catechin standard solutions within the 20–140 mg/L range. Before being analyzed, the wine samples were diluted 10 times. The TF content was expressed as mg of catechin equivalent per liter (mg CE/L).

In red wines, the main anthocyanins usually are the 3-monoglucosides. The total anthocyanin content of the red wine samples was measured spectrophotometrically at 520 nm by the modified method of Ribéreau-Gayon and Stonestreet [12]. An expression with a correction factor for malvidin-3-monoglucoside was used and TA was expressed as mg of malvidin-3-monoglucoside equivalents per liter (mg ME/L).

All colourimetric measurements were carried out on an UV/VIS spectrophotometer (UV-1600PC, VWR, Belgium).

**Results and discussion.** The biological distinction of red grapes and wines comes from their complex of phenolic compounds. The result for total phenolic content determined in our study for the investigated Mavrud wine from Harmanli is shown in Fig. 1, where it is compared with the TP results obtained for Mavrud wines from the other references listed in Table 1. As it can be seen, the content of total phenolics varies considerably among the wine samples examined, with an average value of 2085 mg GAE/L. The most significant difference in TP is between the wine samples from Mavrud-Brestnik and Mavrud-Harmanli, where the TP content of the first one is more than three times lower than the second

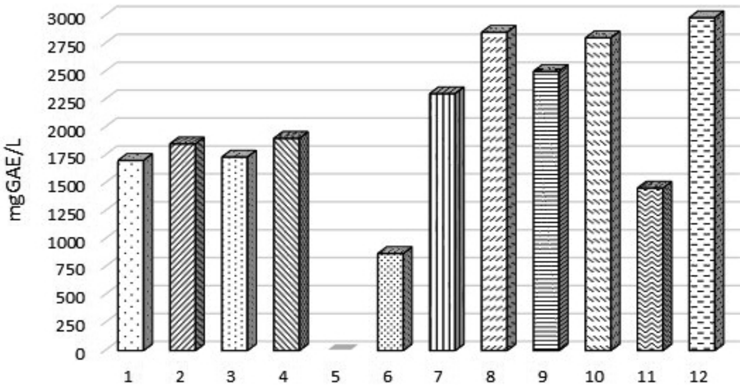

Fig. 1. Total phenolic content of Mavrud wines:
(see Table 1 for specifying numbering); 5 – n/d

one. Our result is in accordance with the TP content of the four Mavrud wines reported by Gugulias et al. [8].

The divergence between results could be justified by different factors affecting the phenolic extraction from grapes, such as climate change, soil variety, ripening stage, place of growing and vine cultivation. For example, Pérez-Magariño and González-San José [13] have investigated two varieties of red grape and have acknowledged that the wines of each variety after 18 months of ageing had different phenolic contents probably due to the different maturity state of the grapes used. Another research has proven the effect of vintage [14]. The authors stated that in the year 2001 lower TP levels were estimated in comparison with the year 2002 which was an extraordinarily warm year. The different winemaking techniques are also recognized as influencing the content of TP in wines. Ivanova et al. [15] have shown that sulphur dioxide, applied in winemaking as effective antioxidant and antimicrobial agent, affects the content of TP. In this research, two doses of $SO_2$ were used, 30 and 70 mg/L, in order to check its influence on phenolics extraction during maceration. Higher concentrations of TP were measured in the wines with higher $SO_2$-dose, since it prevented the phenolic oxidation and in this way resulted in higher extraction of polyphenols. Studies have confirmed the significance of fermentation temperature on polyphenols extraction, although not in an unambiguous way. The study done by Girard et al. [16] demonstrated an increase in the TP content with temperature rise from 15 to 30 °C. Other researchers [17] stated that the highest content of TP was obtained after 6 days of cold maceration (15 °C).

Results for total anthocyanin content of the Mavrud (Harmanli) samples examined is given in Fig. 2 in comparison with the TA content obtained for the other investigated Mavrud wines in Table 1. In this case, the values obtained are in a considerable range as well, averaging 149 mg ME/L, and the difference between the highest and lowest values amounts to 2.2 times. These dissimilarities

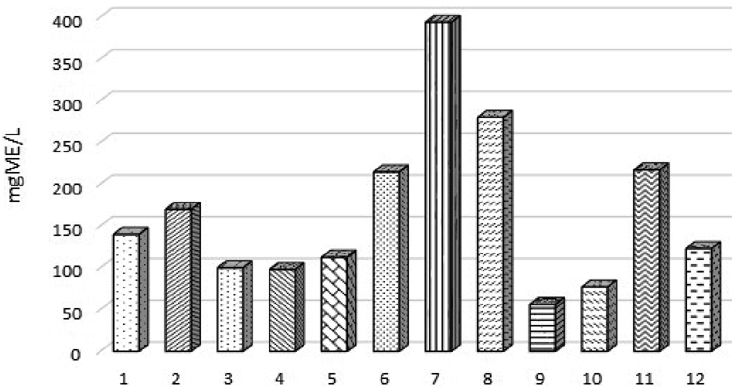

Fig. 2. Total anthocyanins content of Mavrud wines
(see Table 1 for specifying numbering)

are explained by variance in the winemaking technologies which exert a different impact on the anthocyanin fraction. Various researchers have reported that wine maceration time is vital for the concentration of anthocyanins in the must. YOKOTSUKA et al. [18] reported that TA content increased rapidly until day 4, then slowly decreased over time due to reactions of degradation and condensation with tannin. A similar trend has been observed by KELEBEK et al. [19]. The TA content increased from days 3 to 6. In another study anthocyanin levels were found to increase gradually during the initial two to three days of maceration and later decrease during prolonged contact [20]. Wine ageing in bottles also has influence on the anthocyanins content. Intensive decrease of total anthocyanins during the storage period was observed for the wines produced with three days of maceration, while those macerated for ten days were more stabilized and experienced lower decrease in TA content [21]. It was shown that the harvesting date is also relevant for the anthocyanins content. Grape harvest of Mavrud variety on September 1, 2014 and October 10, 2014 has led to 135 and 294.3 mg ME/L anthocyanins, respectively, in the resulting wines [1].

Scarce information has been found in the literature concerning data on Mavrud wine flavonoids. Our result is presented in Fig. 3 together with the one obtained by TOCHEV et al. [9] who have also analyzed the TF content in Mavrud wine. Both values are quite similar – 1020 and 1099 mg CE/L, respectively. The results obtained indicate that there is no specific correlation between the amounts of the individual groups of compounds measured. For example, the investigated wine Mavrud-Harmanli has twice as much TP than Mavrud-Trakiets, 43% less anthocyanins, and the amount of TF of both wines is almost equal.

**Conclusions.** The contents of TP, TF and TA were determined by colourimetric methods to analyze the native red wine Mavrud from a Harmanli cellar. The results from this study were compared with those obtained from other investigated Mavrud wines produced by various wineries/regions in Bulgaria. The

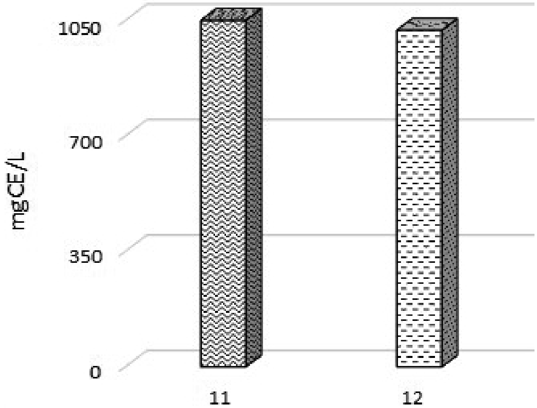

Fig. 3. Total flavonoids content of Mavrud wines
(see Table 1 for specifying numbering)

comparison revealed divergence between results in the TP content, as well as in the TA content, with differences in the values up to three and two times, respectively. These differences are determined by factors like climate change, soil variety, diverse vinicultural practices, all of which are shown to have an impact on the phenolic extraction from grapes, alongside with variance in the winemaking techniques.

## REFERENCES

[1] Stoyanov N. (2007) Research on the Phenolic Compounds of Grapes and Wines from Cabernet Sauvignon and Mavrud Varieties, Dissertation, University of Food Technologies – Plovdiv, 18–106, (in Bulgarian).

[2] Fraga C. G., K. D. Croft, D. O. Kennedy, F. A. Tomás-Barberán (2019) The effect of polyphenols and other bioactives on human health, Food Funct., **10**(2), 514–528.

[3] Zeinali M., S. A. Rezaee, H. Hosseinzadeh (2017) An overview on immunoregulatory and anti-inflammatory properties of chrysin and flavonoids substances, Biomed. Pharmacother., **92**, 998–1009.

[4] Choy K. W., D. Murugan, X. F. Leong et al. (2019) Flavonoids as natural anti-inflammatory agents targeting nuclear factor-kappa B (NF$\kappa$B) signaling in cardiovascular diseases: A mini review, Front. Pharmacol., **10**, 1295.

[5] González-Neves G., J. Franco, L. Barreiro et al. (2007) Varietal differentiation of Tannat, Cabernet-Sauvignon and Merlot grapes and wines according to their anthocyanic composition, Eur. Food Res. Technol., **225**(1), 111–117.

[6] Tsanova-Savova S., S. Dimov, F. Ribarova (2002) Anthocyanins and color variables of Bulgarian aged red wines, J. Food Compos. Anal., **15**, 647–654.

[7] Tsanova-Savova S., I. Gruev, R. Ribarov (2021) Flavonoids composition of Bulgarian wines and estimation of their intake, Riv. Ital. Sostanze Grasse, **XCVIII**, 125–129.

[8] Gugulias N., L. Masheva, N. Mashev (2004) Studies of total phenols contents, anthocyans and antioxidant activity of some Bulgarian and Greek red wines, J. Environ. Prot. Ecol., **5**, 555–562.

[9] Tochev D., M. Karsheva, D. Pilev et al. (2020) Phenolic compounds in peels, seeds, marcs and wines from Mavrud grapes, J. Chem. Technol. Metall., **55**(4), 772–777.

[10] Waterhouse A. L. (2002) Determination of Total Phenolics. In: Current Protocols in Food Analytical Chemistry, (Ed. R. E. Wrolstad), I1.1.1-I1.1.8, John Wiley & Sons, Inc.

[11] Zhishen J., T. Mengcheng, W. Jianming (1999) The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals, Food Chem., **64**, 555–559.

[12] Rivas-Gonzalo J. C., Y. Gutierrez, E. Hebrero, C. Santos-Buelga (1992) Comparisons of methods for the determination of anthocyanins in red wines, Am. J. Enol. Vitic, **43**, 210–214.

[13] Pérez-Magariño S., M. L. González-San José (2006) Polyphenols and colour variability of red wines made from grapes harvested at different ripeness grade, Food Chem., **96**, 197–208.

[14] Lachman J., M. Šulc, K. Faitová, V. Pivec (2009) Major factors influencing antioxidant contents and antioxidant activity in grapes and wines, Int. J. Wine Res., **1**, 101–121.

[15] Ivanova-Petropulos V., Á. Dörnyei, L. Márk et al. (2011) Polyphenolic content of Vranec wines produced by different vinification conditions, Food Chem., **124**, 316–325.

[16] Girard B., D. Yuksel, M. A. Cliff et al. (2001) Vinification effects on the sensory, color and GC profiles of Pinot Noir wines from British Colombia, Food Res. Int., **34**, 483–499.

[17] Şener H., H. K. Yildirim (2013) Influence of different maceration time and temperatures on total phenols, colour and sensory properties of Cabernet Sauvignon wines, Food Sci. Technol. Int., **19**, 523–533.

[18] Yokotsuka K., S. Michikatsu, N. Ueno, V. L. Singleton (2000) Colour and sensory characteristics of Merlot red wines caused by prolonged pomace contact, J. Wine Res., **11**, 7–18.

[19] Kelebek H., A. Canbas, S. Selli (2009) Effects of different maceration times and pectolytic enzyme addition on the anthocyanin composition of Vitis vinifera cv. Kalecik Karasi wines, J. Food Process. Preserv., **33**, 296–311.

[20] Sacchi K. L., L. F. Bisson, D. O. Adams (2005) A review of the effect of winemaking techniques on phenolic extraction in red wines, Am. J. Enol. Vitic., **56**, 197–206.

[21] Ivanova V., B. Vojnoski, M. Stefova (2012) Effect of winemaking treatment and wine aging on phenolic content in Vranec wines, J. Food Sci. Technol., **49**(2), 161–172.

*Institute of Chemical Engineering, Bulgarian Academy of Sciences,*
*Akad. G. Bonchev St, Bl. 103, 1113 Sofia, Bulgaria*
*e-mails*: m.lazarova@iche.bas.bg, yanpe@bas.bg