Bulgarian Chemical Communications, Volume 54, Special Issue B1 (pp. 26-30) 2022 DOI: 10.34049/bcc.54.B1.0359

Assessment of the elemental composition, antioxidant activity, and optical properties of non-traditional Bulgarian fruit wines

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Received: November 17, 2021; Revised: May 19, 2022

Melon wine and white cherry wines purchased from the commercial market were studied. The aim of the present work is to evaluate the elemental composition, antioxidant activity and optical properties of these fruit wines. The concentrations of certain elements in wines are of interest because some of them are regulated, others affect the organoleptic properties of wine, and some elements are essential to the human body. Organic chemicals that contribute to the taste and color of wine are also of healthy interest. For this purpose, the total phenolic content (TPC), the total flavonoid content, the total monomeric and antioxidant activity were determined by four different methods (ABTS, DPPH, FRAP and CUPRAC). A correlation between these parameters and the emission maxima of the fluorescence spectra was obtained for the wavelengths of light excitation 245 nm and 285 nm.

Keywords: fluorescence, antioxidant activity, white wines from melon, white wines with cherry, chemical elements

INTRODUCTION

Fruit wines are made from a variety of fruits, including watermelon and melon. The preparation of these wines has its own peculiarities, but in all other respects they are produced on the same principle as these from grapes, following certain rules. Practically fruit wine can be obtained from almost all fruits, and the color obtained is considered white, red or rosé. In addition to pure fruit wines, wines can be made from various combinations with classic grape wines. Fruit wines or combinations of wines and fruits of apple, blueberry, raspberry, black currant, watermelon, plum, fig, as well as non-traditional for our latitudes tropical fruits such as mango, lemon, pomegranate, lime, passion fruit, kiwi and others can be found on the Bulgarian market. Interest in the production of this type of wine is growing due to the rich aroma and taste qualities that the fruits transmit to the wine [1]. The production of fruit wines from black currant, blueberry, strawberry, raspberry and apricot is already a tradition in many countries of the European Union and the United States [2]. The total phenolic content of wines from raspberries, black currant, blueberries, elderberries, buckwheat, etc. has been studied. Their phenol content is comparable to or higher than that of red grape wines [3 4]. The existence of a positive relationship between the total antioxidant activity of fruit wines and the total phenolic content [5, 6] is confirmed. Last but not least, it is important to determine the mineral content of fruit wines, related to their ecological purity and health safety. Prolonged contact of the product with the facilities in which the various stages of technological processing take place and the use of bentonites may lead to an increase in the content of some elements [7, 8]. Other metals are able to influence the organoleptic properties of fruit wines and are the basis of sensory evaluation of such products.

Although fruit wines are rich in antioxidants and minerals, there are still insufficient data on their chemical composition and physical properties. The aim of the present study is to investigate the optical properties of melon and white cherry wine in combination with Chardonnay. Additionally, the contents of Cu and Pd against their allowable reference values, as well as the concentrations of some elements with potential biological role with health benefits, such as Mg, Zn, Mn and Fe, have also been evaluated.

MATERIALS AND METHODS

Materials

In the present study, two wines were chosen for analysis with the composition:

a) 40% cherry & 60% Chardonnay wine – 3 bottles;

b) 100% melon wine– 3 bottles.

All samples were purchased from the local market and are from the same manufacturer.

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Determination of total phenolic content (TPC)

The total phenolic content in the fruit wines was determined using the Folin–Ciocalteu's reagent [9]. The analysis was performed as 0.2 mL 70 % ethanol extract was mixed with 1 mL of Folin–Ciocalteu reagent diluted five times and then 0.8 mL of 7.5% Na_2CO_3 was added. After 20 min, the absorption was measured at 765 nm against a blank sample. The results were expressed in mg equivalent of gallic acid (GAE) per ml using the calibration curve Y= 12.557x-0.0871 [10].

Determination of total flavonoids content

The total flavonoids content was determined by $Al(NO_3)_3$ reagent. The absorbance was measured at 415 nm. The results were presented as mg equivalents quercetin (QE) per ml according to the calibration curve Y=0.0119x-0.0467 with quercetin as a standard [10].

Total monomeric anthocyanins content (TMA)

Total anthocyanins content was determined using the pH differential method [11] at two wavelengths 520 and 700 nm. The results were presented as cyanidin-3-glycoside per ml.

Antioxidant activity

DPPH radical-scavenging ability. Wine sample (0.15 ml) was added to 2.85 ml of freshly prepared 0.1mM methanol solution of DPPH. The reduction of absorbance at 517 nm was measured by spectrophotometer against blank containing methanol. The percent inhibition was also calculated. The results were expressed in mM Trolox® equivalents (TE)/ml [10].

ABTS+ radical scavenging ability. The ABTS+ solution (2.85 ml) was mixed with 0.15 ml of fruit wine sample. After 15 min at 37°C in darkness, the absorbance was measured at 734 nm against ethanol. The percent inhibition was also calculated. The results were expressed in mM Trolox® equivalents (TE)/g dw [10].

FRAP assay. The FRAP reagent was prepared before analysis by mixing 10 parts of 0.3M acetate buffer (pH 3.6), 1 part of 10 mM 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) in 40 mM HCl and 1 part of 20 mM $FeCl_3 \times 6H_2O$ in distilled water. FRAP reagent (3.0 ml) was mixed with 0.1 ml of wine extract. After 10 min at 37°C in darkness, the absorbance of the sample was measured at 593 nm [12].

CUPRAC assay. Wine extract (0.1 ml) was mixed with 1 ml of $CuCl_2 \times 2H_2O$, 1 ml of methanol solution of neocuproine, 1 ml of 0.1M ammonium acetate buffer and 1 ml of distilled H_2O. After 20 min at 50°C in darkness, the samples were cooled to room temperature and the absorbance was measured at 450 nm. The results were expressed in mM Trolox® equivalents (TE)/g dw [10].

Fluorescence spectra

Fluorescence spectra were measured with an optical spectrometer (AvaSpec-2048, Avantes) with an operating range from 200 nm to 1100 nm. The used sources are LEDs operating at wavelengths of 245 nm, 265 nm, 275 nm and 295 nm. The resolution of the spectrometer is 8 nm for an input slit of 200 μm. An optical fiber with a diameter of 200 μm is used to bring the light to the probe and to measure the scattered and fluorescent light. A collimator with a lens with an aperture of D=5 mm is used to collect more light and send it to the receiver.

For each wine, three fluorescence spectra were recorded for three different bottles, purchased from the market. During the investigation the average spectra are presented for each excitation wavelength of the sample.

Determination of elements

A sample of about 5 g was weighed on an analytical balance and treated with 3-4 mL of HNO_3 (65%, Suprapur®, Merck) on a hot plate to remove the organic part. The residue was transferred into a 25-ml flask and diluted with distilled water [13]. Multielement standard solution 5 for ICP (TraceCERT®, Merck) was used for the preparation of working standard solutions for calibration for ICP-OES iCAP 7000 SERIES (Thermo Fisher Scientific, USA).

Statistical analysis:

The ANOVA single factor analysis and descriptive statistic methods are used. Each parameter was measured in triplicate and the average results and standard deviation are presented in the experimental results.

RESULTS AND DISCUSSION

The fluorescence spectra were obtained by excitation of melon and cherry&Chardonnay wines by light with wavelength 245 nm, 285 nm, 370 nm, 380 nm, 390 nm, 400 nm and 420 nm. The best ratio between the intensity of excitation and intensity of emission was found for excitation light from the UV region. For that reason only these spectra are given in figures 1a and 1b.

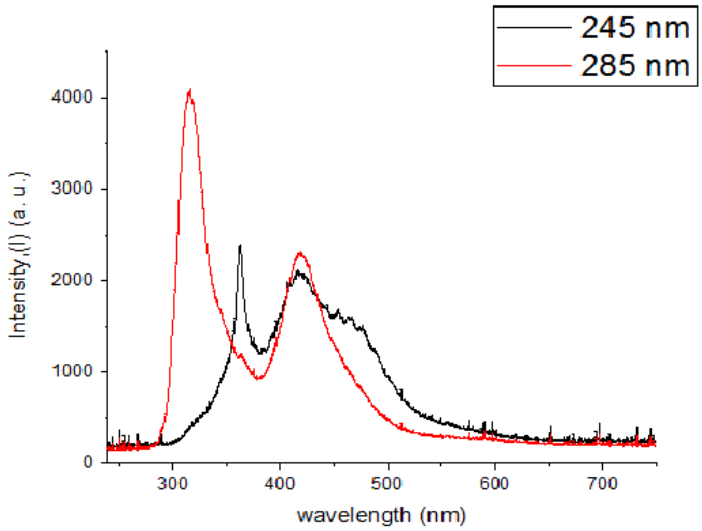

Figure 1a. Fluorescence spectra of melon wine, obtained by using excitation light in the UV-VIS region

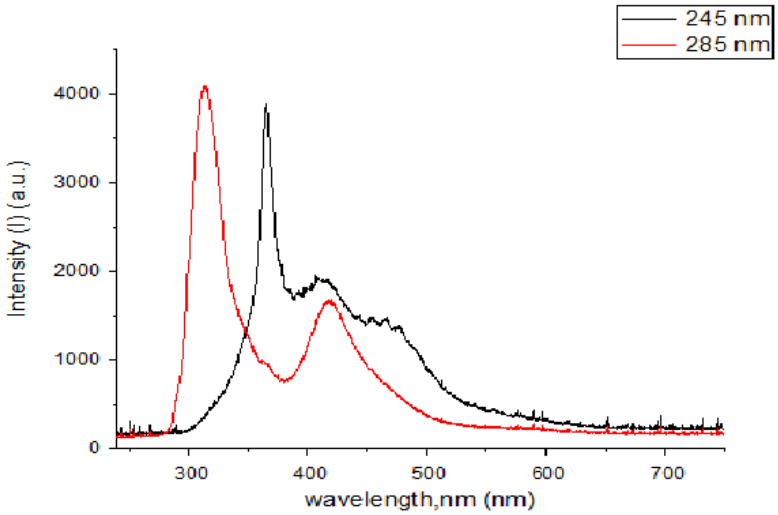

Figure 1b. Fluorescence spectra of cherry&Chardonnay wine, obtained by using excitation light in the UV-VIS region.

The spectra are characterized with two maxima:

- The first one is between 320 and 365 nm;
- The second one is between 420 and 427 nm.

The similar wavelength for fluorescence maximum of wine are reported by Dufour *et al.* of French and German grape wines [14]. According to Rodríguez-Delgado the fluorescence maximum in the region (320 - 426) nm for excitation wavelength (262 - 285) nm is connected with the presence of caffeic acid in the wine [15]. The fluorescence maximum in the region (320-366) nm for excitation wavelength (278 – 285) nm is connected with gallic acid [16].

The difference between the fluorescence maxima for cherry&Chardonnay and melon wine was in the shape and in the maximum values of the fluorescence intensity. This can be explained with the different content of phenolic components in these fruits and the different technology of production of the wine.

Total phenolic content and total flavonoids content were evaluated (Table 1). The cherry&Chardonnay wine and melon wine possessed similar total phenolic contents. However, flavonoids content of white cherry wine is slightly higher than that of melon wine.

Various authors [17-20] reported that the highest total phenolic content (TPC) is demonstrated by the fruit wines from red cherry (1.081-2.711) mg GAE/ml, black currant (0.941-3.086) mg GAE/ml and blackberry (1.055-2.705) mg GAE/ml. Apple wine possesses the lowest TPC (0.244-0.644) mg GAE/ml [20]. In our case the results for TPC white cherry wine are lower than those reported by Brat *et al.* [21] – 0.94 mg GAE/g, but they are close to those reported for apple wines.

Table 1. Phenolic content and antioxidant activity in the fruit wine samples

	TPC, mg GAE/ml	Total flavonoids, mg QE/ml	TMA, mg cyanidin-3-glycoside/ml	Antioxidant activity, mM TE/ml			
				DPPH	ABTS	FRAP	CUPRAC
Cherry& Chardonnay	0.34±0.02	6.61±0.01	N.D.	1.25±0.01	1.98±0.03	1.11±0.01	3.38±0.05
Melon	0.35±0.01	5.86±0.03	N.D.	1.35±0.02	2.02±0.02	1.22±0.01	3.61±0.06

Table 2. Content of Mg, Fe, Cu, Zn, and Mn in fruit wine samples (RSD varied between 2–5%)

	Mg, mg kg^{-1}	Fe, mg kg^{-1}	Cu, mg kg^{-1}	Zn, mg kg^{-1}	Mn, mg kg^{-1}
Cherruy& Chardonnay*	46.8	0.68	0.11	0.18	0.24
Melon*	50.6	0.62	0.09	0.13	0.25

* Pb<0.17 mg kg^{-1} and Cd<0.01 mg kg^{-1}

The total phenolic content in melon wine is similar to that for grapefruit (0.39 mg GAE/g and 0.47 mg GAE/g) [21]. Total phenolic content in both wines under study is similar to that in white wine. TPC in cherry wine is equal to those in white wine from Dimyat and Mavrud (0.33 mg GAE/ml) [22] and from Chardonnay (0.29 mg GAE/ml) [19]. TPC in melon wine is higher than that of some rose wine. It is reported that Grenache and Souvingnon blanc types of rosé wine have TPC 0.257 mg GAE/ml and 0.255 mg GAE/ml, repectively [22].

As expected, anthocyanins were not detected in the investigated wines. Similar results are reported for apple wine [20].

The antioxidant and pharmacological effects of fruit wines are due to the phenolic compounds - anthocyanins, flavanols and other flavonoids. These compounds also improve the sensory characteristics of wines such as color and astringency [16]. The most studied phenolic compounds in fruit wines are flavonoids, because they are widely present in the plants and they possess antioxidant properties. The mportant class of flavonoids is flavanols such as myricetin and quercetin, which are spread in red berry wines [23]. Czyzowska reported that flavanols' content of cherry wines is 10 times higher than that of grape wine [24]. In our case cherry wine (6.61 mg QE/ml) is superior to melon wine (5.86 mg QE/ml) in total flavonoids.

The main compounds with antioxidant activity are flavonoids and phenolic acids, carotenoids and vitamins. The order of antioxidant activity (AOA) in the fruit wines is reported in [19] – AOA decreases from bilberry, blackberry, black mulberry, sour cherry, strawberry, raspberry, apricot, quince, apple and melon. Kalkan Yildirim [19] reported AOA of fruit wines in the following decreasing rank: bilberry > blackberry > black mulberry > sour cherry > strawberry > raspberry > apricot > quince, apple > melon. AOA is determined by us using 4 different methods: ABTS, DPPH, FRAP and CUPRAC. All methods revealed a higher AOA of melon wine compared with the white cherry wine. The highest antioxidant activity in our case was found by CUPRAC assay, where the antioxidant potential was twice higher in comparison with the radical scavenging capacity determined by DPPH method. Therefore, the investigated wines demonstrated better antioxidant potential by using the principle of metal-reducing activity.

The concentrations of Cu, Cd, Mg, Mn, Fe, Zn and Pb in the fruit wine samples were determined (Table 2). The analyzed elements belong to three groups in terms of human health: main elements such as Mg (essential in amount> 50 mg/day), trace elements Fe, Cu, Zn and Mn (essential in concentrations< 50 mg/day) and toxic elements Pb and Cd [25]. The content of metals in grape wines is regulated by the legislation of the European Union – 0.20 mg kg^{-1} for Pb and 1 mg L^{-1} for Cu [26]; additionally the OIV recommends up to 5 mg L^{-1} Zn content [27]. The concentrations of all three elements in the studied fruit wines remain below the normative/ recommended values. Cd concentrations are extremely low and below detection limits.

The mineral composition of wines is determined by many factors: type of fruit, climatic conditions, soil characteristics, cultivation methods, technological procedures and equipment, fermentation, bottling, etc. According to Pohl [28], who summarizes the research of a number of authors on the mineral composition of different types of wine from different regions of the world, the content of Mg, Fe and Mn varies widely (7.8–718 mg L^{-1} for Mg, 0.06–23.7 mg L^{-1} for Fe, 0.1–5.5 mg L^{-1} for Mn). As can be seen from Table 2, both wines fit perfectly within the specified limits.

A comparison with blackberry wines from Croatia [29] and sour cherry wine from Serbia [30] shows that the wines we studied are more than 3 times poorer in iron and manganese, while in magnesium there is no significant difference.

As can be seen, the content of the elements can vary widely due to the great variety of fruits and grapes, which leads to their difficulty in comparison, and it can be concluded that Mg> Fe> Mn> Zn> Cu> Cd.

CONCLUSIONS

- There are no significant differences in the elemental composition of the two studied fruit wines.
- The difference between the fluorescence maxima for cherry&Chardonnay and melon wine is in the form and in the maximum values of the fluorescence intensity. This can be explained by the different content of phenolic components in these fruits and the different technology of wine production.
- Melon wine demonstrates slightly higher AOA than cherry&Chardonnay wine for each method used. A positive correlation can be established between the antioxidant activity of AOA and the total phenolic content, but not between AOA and flavonoids content. The highest antioxidant activity was found by CUPRAC analysis, where the antioxidant potential was twice as high as DPPH radical scavenging methods. Therefore, the studied wines demonstrate better antioxidant potential by using the principle of metal-reducing activity.

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