Short Note

Flavonoids composition of Bulgarian wines and estimation of their intake

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The aim of the study is to assess of flavonoids composition for Bulgarian white and red wines and estimate the intake of flavonoids from wine in the Bulgarian population. This study provides data for myricetin, quercetin, kaempferol, (+)-catechin, (-)-epicatechin and total anthocyanins in three types of white and five types of Bulgarian red wines. The results show that Mavrud, the typical Bulgarian wine type, is the richest source of quercetin (6.9 ± 0.7 mg/L), of (+)-catechin (114.7 ± 5.1 mg/L) and of (-)-epicatechin (28.7 ± 7.0 mg/L). The highest amount of myricetin was detected in Melnik wines (8.9 ± 1.4 mg/L), while the highest total of anthocyanins was in the Cabernet Sauvignon (180.7 ± 83.8 mg/L). Based on Household Budgets Survey, the intake of flavonoids was calculated as between 2.0 to 1.6 mg/day/per capita for 2010-2018 year. The result also shows that, although the white and red wine consumption is about equal, the intake of flavonoids attributed to white wine is about 12% of the total wine intake. Despite the high concentration of flavonoids and their benefits to health, the consumption of wines has decreased in Bulgaria during the last years, reflecting in a low intake of flavonoids.

Keywords: Flavonoids, Bulgarian Wines, Consumption, Intake

INTRODUCTION

Flavonoids are polyphenolic, bioactive compounds, widely distributed in plants, and respectively in human diet as a variety of fruits, vegetables, tea and wine. They possess anti-atherogenic, anti-inflammatory and anti-cancer effects [1 - 4]. The health benefits of flavonoids have encouraged the scientific interest of a wide range of researchers in field of medicine, food science and nutrition, related to food quality assessment and human health. For Bulgaria, this scientific evidence is of particular importance, as our country is among the first places for prevalence of cardiovascular and cancer diseases. For instance, the reasons of deaths in Bulgaria for cardiovascular diseases were 70546 (65%) and 17462 (16%) for cancer in 2018. Bulgaria is an Eastern European country with a high level of cardiovascular morbidity and mortality classified as a "high risk country" in the European Society of Cardiology guidelines for cardiovascular prevention [5]. It is estimated that the increase of saturated fat in the Bulgarian diet is one of the basic risk factors for cardiovascular diseases, cancer, and diabetes. In this aspect, varying the diet, including more flavonoids in food sources could be seen as a step towards the prevention of developing degenerative diseases. One of the relatively dense flavonoids food resources is wine, during the fermentation process the flavonols, flavanols and anthocyanins, located mainly in the skin, seeds and scrapes of grapes, pass into the wine.
Bulgaria has a strong and ancient tradition in wine production. Nevertheless,

the rich flavonoids composition of Bulgarian wines has not been studied well and representative data for flavonols, flavanols and anthocyanins are scattered. Furthermore, data for wine consumption in Bulgaria are limited. The National Survey of nutrition factor for health risk among the population in Bulgaria, 2014, provides only data for average daily intake from alcoholic beverages as mean value of beer and wine consumption (76 g/day) for adults above 19 years of age [6]. The information gap on flavonoids consumption made us set up the task to determine the flavonoids content in Bulgarian wines and estimate their intake in Bulgaria. The aim of the study is to assess the flavonoids composition of Bulgarian white and red wines and estimate the flavonoids intake from wine in the Bulgarian population.

MATERIALS AND METHODS

WINE SAMPLES

Flavonoids content in white wine was determined in 3 different wine types – Traminer, Chardonnay and Muscat. Flavonoids content of red wines was determined from data on 15 Bulgarian red wines, including three Bulgarian wine types Gamza, Mavrud, and Melnik and two international wine types – Cabernet Sauvignon and Merlot, produced in Bulgaria. For each wine type 3 different wines, from different wine regions and/or producers were analysed, as follows: Gamza (Lyaskovetz, Novo Selo, Lovico Suhindol); Mavrud (Perouchtisa, Asenovgrad, Asenovgrad - Reserva); Melnik (Harsovo, Damyanitza, Damyanitza - Reserva); Merlot (Stambolovo - Reserva, Lovico Suhindol - Reserva, Targoviste - Reserva); Cabernet Sauvignon (Suhindol - Reserva, Svistov - Reserva, Targoviste - Reserva); Chardonet (Loviko Suhindol, Domain Boyar, Vinex Preslav); Muskat (Loviko Suhindol, Ruse, Targoviste) and Traminer (Han Krum - Domain Boyar, Targoviste, Han Krum - Vinex Preslav).

DETERMINATION OF FLAVONOLS

The content of flavonols – myricetin, quercetin and kaempferol was determined, with HPLC analyses after acid hydrolysis of 15 ml wine samples with 1.2 M HCl in 50% methanol, by refluxing for 2 hours at 90°C, according Hertog et al. [7]. An antioxidant tertiary butylhydroquinone (TBHQ 2 mg/ml in methanol) was added during refluxing for stability of flavonoid aglycones. A HPLC analysis was performed with UV detection at 360 nm wavelength. A method of internal standard (IS) was applied for the quantitative determination and morin was used as IS. The limit of detection was 0.7 mg/L.

DETERMINATION OF FLAVANOLS

Flavanols content – (+)-catechin and (-)-epicatechin was determined after dilution of wine samples with 80% methanol in 1:1 ratio (v/v) for white wines and 1:10 ration (v/v) for red wines. After centrifuge of the samples on 220 × g, a HPLC determination was performed with fluorescent detection at λ_{EX} = 280 nm and λ_{EM} = 315 nm. The limit of detection of the method was 0.03 mg/L.

DETERMINATION OF TOTAL ANTHOCYANINS

Total Anthocyanin content of red wine samples was measured by spectrophotometric method of Somers and Evans [8]. In brief 10 ml of 1M HCl was added to 0.1 ml of filtered red wine. The absorbance at 520 nm was measured after 3.5 h in cuvette of 1 cm optical pathway. The absorbance value obtained is multiplied by 101 (A_{520}^{HCl}). At the same time, 5µl of 15% $NaHSO_3$ are added to 330 µl filtered red wine and the sample was homogenised. After 1 minute the absorbance is measured at 520 nm in 0.1 cm cuvette. The values obtained were multiplied by 10 ($A_{520}^{SO_2}$).
The anthocyanin content was calculated in mg/L by the equation:

$$\text{Total Antocyanins} = 20 \times (A_{520}^{HCl} - A_{520}^{SO_2})$$

STATISTICAL ANALYSIS

In this work the results are reported as a mean value in mg/L of the 3 wine samples analysed per wine type. To assess the variation of the results, the standard deviation of the mean (±SD) was also calculated by using Microsoft Office Excel 2007.

ESTIMATION OF WINE FLAVONOIDS INTAKE

Wine consumption was taken from the official data of National Statistical Institute, on the basis of Household budget survey for period 2010 – 2018 year (Republic of Bulgaria, NSI, Household consumption, *https://www.nsi.bg/en/content/5712/annual-data*). They were determined using the balanced method and are presented as an average value per year, per capita. Combining the data from national statistic and our results for flavonoids content of wines, we have estimated the wine flavonoids intake per day, per capita in Bulgaria.

RESULTS AND DISCUSSION

In Table I, consolidated data for flavonoids content of Bulgarian white and red wines are presented. The results show that only quercetin was detected in white wines among flavonols representatives. Flavanols are the main flavonoid representatives in white wines, reaching maximum value of 30.9 ± 1.4 mg/L for (+)-catechin in Muskat wine. According to literature data, anthocyanins are not presented in white wines and correspondingly they have been not determined.

Table I - Flavonoids content of white and red Bulgarian wines

		Flavonols						Flavanols				Anthocyanins	
		Myricetin		Quercetin		Kaempferol		(+)-Catechin		(-)-Epicatechin		Total Anthocyanins	
		mg/L											
Wine	**n**	**Mean value**	**SD**	**Mean value**	**SD**	**Mean value**	**SD**	**Mean value**	**SD**	**Mean value**	**SD**	**Mean value**	**SD**
White Wine													
Traminer	3	nd		1.1	0.1	nd		27.0	2.9	7.1	1.9	na	
Chardonnay	3	nd		1.0	0.6	nd		28.5	1.5	4.9	3.5	na	
Muskat	3	nd		1.3	0.8	nd		30.9	1.4	6.4	6.8	na	
Red Wine													
Gamza	3	1.7	1.3	1.9	1.5	nd		79.5	3.6	17.4	3.5	35.9	5.4
Mavrud	3	5.5	1.1	6.9	0.7	1.2	0.4	114.7	5.1	28.7	7.0	112.7	23.7
Melnik	3	8.9	1.4	5.2	0.6	1.4	0.6	121.3	2.1	19.4	2.3	62.0	35.2
Merlot	3	4.1	1.1	4.9	2.7	1.4	0.4	76.0	2.0	16.0	1.6	152.7	22.1
Cabernet Sauvignon	3	4.5	0.7	5.5	1.5	1.1	0.7	76.8	2.8	19.1	1.4	180.7	83.8

n – number of samples, nd – not detected, na – not analyzed

Table II - Wine consumption in Bulgaria and estimation of flavonoids intake

	Year								
Consumption L/year/per capita	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**
Wine	5.5	5.2	5.5	5.5	5.5	4.6	4.4	4.3	4.6
White wine	2.42	2.29	2.42	2.42	2.42	2.02	1.94	1.89	2.02
Red wine	2.81	2.65	2.81	2.81	2.81	2.35	2.24	2.19	2.35
Intake mg/day/per capita	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**
Total flavonoids by White Wine	0.24	0.23	0.24	0.24	0.24	0.20	0.19	0.19	0.20
Total flavonoids by Red Wine	1.79	1.69	1.79	1.79	1.79	1.50	1.43	1.39	0.15
Total flavonoids by Wine	2.03	1.91	2.03	2.03	2.03	1.70	1.62	1.58	1.70

Not only quercetin, but also myricetin and kaempferol were detected in red wines. We could point out that red wines are one of the few foods and beverage products containing all three representatives of flavonols. The flavanols content in red wines is much higher than those of white wines whith a maximum value of 121.3 ± 2.1 mg/L for (+)-catechin in Melnik wine (Tab. I).
Combined data for flavonoids in wines can be found in two international databases: USDA Database for Flavonoids content in selected foods, 2015 [9] and Phenol-Expoler [10]. Our results for (-)-epicatechin quantity in white wines (4.9-7.1 mg/L) are comparable with those in USDA (mean value 5.5 mg/L) [12]. However, the (+)-catechin content (27.0-30.9 mg/l) in Bulgarian white wines is about 4 times higher than the mean value reported in USDA Database (7.7 mg/L) [9]. This result emphasises the fact that wines from different geographical origin can have major differences in their polyphenolic load. For instance, in the publication of Frenkel et al. [11 14] the flavanols content of two Californian Chardonnay samples is 46-43 mg/L and 60-17 mg/L for (+)-catechin and (-)-epicatechin, correspondingly. In our study the data for (+)-catechin and (-)-epicatechin for Chardonnay wine, produced in Bulgaria, are significantly lower – 28.5 ± 1.5 mg/L and 4.9 ± 3.5mg/L respectively. In opposite, Soleas at al. [12] have presented data for 11 Chardonnay samples, produced in Ontario region, Canada, where (+)-catechin and (-)-epicatechin had a mean value of 3.8 mg/L and 1.7 mg/L, which is considerably a lower amount compared to our results.
Our data for quercetin content in white wine vary in close boundaries (1.0 ± 0.6 - 1.3 ± 0.8 mg/L) and are more than 2 times higher than the average value for quercetin, reported in USDA Database (0.4 mg/L) [9]. We could not compare our results for Muskat wine with wines from different origin since this wine type is typical for Bulgaria and no other HPLC analysis data have been found. Our results for quercetin and (+)-catechin in Cabernet Sauvignon wines are very close to the literature data in the USDA Database – 5.8 mg/L and 77.0 mg/L respectively, however the amount of (-)-epicatechin in Bulgarian Cabernet-Sauvignon wine (19.1 ± 1.4 mg/L) is about 5 times lower than data reported in USDA (106.6 mg/L) [9]. Our results show that the (+)-catechin content is higher than that of (-)-epicatechin in all red wine samples. These results are also confirmed by Phenol-Explorer data [10].
Commonly used methods for estimating food consumption and nutrient intake include observed-weighed food record data, 24-hour recall, Food and Agriculture Organisation Food Balance Sheets, Food Fre-

quency Questionnaires, and Household Consumption and Expenditures Surveys [13]. A representative household survey that assesses food consumption over a whole calendar year might be regarded as a “gold standard” for the measurement of food availability [14]. The data from the Bulgarian Household Budget survey (National Statistical Institute) show that the wine consumption in Bulgaria decreased from 5.5 to 4.3 L/per capita for the last 8 years. These data do not make a difference between red and white wines. The data for estimated white and red wine consumption in Bulgaria has been provided by Bulgarian Vintage and Wine Camera (www.bulgarianwines.com) and show that red wines constitute 51% and white wine 45% of the wine consumption in the country. On the basis of these data and average values of total flavonoids content (sum of flavonols, flavanols and anthocyanins) which are 36.1 mg/L for white vines and 232.4 mg/L for red wines, the flavonoids intake was calculated to be in the range of 2.03 to 1.58 mg/day/per capita for 2010-2018 year (Tab. II).

The result also shows that, although the white wine consumption is about equal to that of red wine, the flavonoids intake attributed to white wine is about 12% of the total intake from wines. This is due to the absence of anthocyanins and of the considerably lower amount of (+)-catechin. We could compare our data with the study of Chun et al. [15] for the dietary flavonoid intake and major food source of U.S. Adults, where total flavonoids intake from wine was estimated to be 4 mg/day, based on the food frequency questionnaire (FFQ) and data from USDA Flavonoids Food Composition Database. In a French study of dietary intake of polyphenols, a total polyphenol intake was estimated to be 1245 mg/day for red wine consumers with 11.1% of the intake coming from red wines. The study was carried out by FFQ and using a Phenol-Explored database [16]. However, the data do not provide information on the flavonoid intake from wines, since the flavonoids intake was estimated as 73 mg/day per capita for food class of Alcoholic beverages, comprising wines and blond beer [19]. In this respect, our data, based on original analytical results for Bulgarian wines, show a first attempt to estimate a wine’s flavonoids’ consumption in Bulgaria and are an informative snapshot for dietary habits and flavonoids’ antioxidant intake. We can notice that wine consumption and, respectively, the intake of flavonoids is lower in Bulgaria compared to the US and France.

The traditional Bulgarian diet used to be characterised with wide wine consumption. However, during the last decades there is an obvious trend of beer and high spirits intake to rise and of wine consumption to decline in Bulgarian population. The scientific community should not promote alcohol intake in general, but taking into consideration the high prevalence of non-communicable diseases in Bulgaria, we could support the mild consumption of traditional flavonoid-rich Bulgarian red wines to replace the wide spread consumption of high spirits. This is part of our effort, along with the strict control of other risk factors, to reverse the negative “Bulgarian cardiovascular paradox”, consisting of a contradiction that, although Bulgarian diet provides a vast diversity of healthy food choices, the prevalence of cardiovascular diseases in the population is very high.

CONCLUSIONS

An original data for flavonoids composition of Bulgarian white and red wines is presented in this study, along with the first attempt to estimate of their intake. Our results have shown that, despite the relatively high content of flavonoids and their good quality, the consumption of wines has decreased in Bulgaria during the last years, reflecting in a low intake of flavonoids.

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