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Phenolics content and antioxidant activity of beverages on the Bulgarian market – wines, juices and compotes

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In wine, natural juices and compotes the taste is heavily influenced by the presence of phenolics. They also contribute to the antioxidant activity of fruits and processed foods from them and have health-protecting effect. The phenolic content and antioxidant activity are not included in the standard documentation for food labeling and control. The aim of the present work was to analyze and to compare the content of total phenolics and anthocyanins, as well as the antioxidant potential of alcoholic and non-alcoholic fruit drinks on the Bulgarian market. Commercial natural fruit juices, compotes, red, rosé and white wines on the Bulgarian market were used for testing total phenolics, anthocyanins and antioxidant activity. Among the tested alcoholic drinks the red wines revealed significantly higher polyphenolic (567±33 mg/L) and AC (97.9±40.7 mg/L) content *vs* rosé (323±84 mg/L, $p<0.0001$; 9.9+8.2 mg/L, $p<0.01$) and white (281±42 mg/L, $p<0.0001$; 0.2±0.1 mg/L, $p<0.001$) wines. In the group of the red wines, the highest polyphenolic content (625±13 mg/L) was detected in Merlot wine, and the lowest one in Syrah (534±20 mg/L). The wine Malbec was found to be the richest one in anthocyanins (156.6±1.5 mg/L), while in Mavrud the anthocyanins content was the lowest one (45.2±1.0 mg/L). The tested red wines showed high antioxidant activity, especially strong in Aronia wine (45.55±0.35 mM uric acid equivalent, UAE). In the tested non-alcoholic drinks, the highest polyphenolic content was found in Aronia juice (592±9 mg/L) and compote (556±62 mg/L). The red wines and compotes have been an element of traditional nutrition in Bulgaria and nowadays their input in the healthy diet is reassessed because of their high phenolics content and strong antioxidant potential.

Keywords: Polyphenols, Anthocyanins, Antioxidant capacity, Wine, Compote, Juice

INTRODUCTION

Polyphenolics are the most abundant and widespread compounds in the plant kingdom. They are a product of the secondary plant metabolism and can be found in all plant parts located in the hydrophilic intracellular compartment and in the extracellular fluids. There are currently more than 8,000 phenolic structures, with a common feature - an aromatic ring bound to at least one hydroxyl group (-OH) [1] Phenolics are classified into three important groups: phenolic acids, flavonoids and tannins.

A sub-class of flavonoids are anthocyanins - water-soluble natural pigments responsible for the red, purple and blue colors of the fruits and their products [2]. Depending on acidity of the media, AC exist in various chemical forms with different coloration. They are stable under acidic conditions, and under normal processing and storage they can be transformed to colorless compounds and subsequently to insoluble brown pigments. In the plants AC are present mostly as glycosides, more stable than their non-glycosylated forms (anthocyanidins), which contributes to their resistance to destroying factors such as light, pH and oxidation [3].

Plant polyphenolics are redox-active substances possessing antioxidant activity. Numerous studies have proven causal links between the high polyphenolic content and antioxidant activity. [4, 5]. Due to their antioxidant properties it is believed that they have health -promoting properties including antibacterial, anti-mutagenic, anti-inflammatory and vasodilatory actions [6]. For example, AC, as very efficient antioxidants, play multiple biologic roles such as anti-inflammatory action, inhibition of blood platelet aggregation and antimicrobial activity, treatment of diabetic retinopathy and prevention of cholesterol-induced atherosclerosis [7, 8]. Flavonoids have also been shown to exhibit powerful antioxidant activities and health promoting effects. They act as free radical scavengers and transition metal chelators. Experimental studies have proven that due to their antioxidant effect they can lower oxidative products such as protein carbonyls, DNA base damage and malonaldehyde in blood and in tissues of experimental animals [9].

Wines and fruit juices represent an excellent source of dietary polyphenols. Red wines may contain from 1000 to 4000 mg/L, rosé 500-700 mg/L, and white wines from 200-300 mg/L [10], chokeberry fresh juices from 3002 to 6600 mg/L

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total phenolics [11]. The phenolic compounds present in wines and natural juices strongly contribute not only to their sensory characteristics but also to the antioxidant activities that they possess [10]. Wine phenolics react with free radicals, which makes them potent antioxidants both in wine and *in vivo*. Phenolics in wines are recently attributed to have protective activity against neurodegenerative diseases [12]. The “French Paradox” was also explained with high polyphenolic content and antioxidant activity of red wines.

Traditionally in Bulgaria fruits are used as dried food, in the form of juices, syrups, jams, compotes and for wine production. The compotes are traditional Bulgarian fruit drinks. To our knowledge there are no studies on the phenolic content and antioxidant properties of compotes.

Nowadays, with the rise of economy, commercial fruit juices have been proposed as substitutes of fresh ones due to their convenience. However, there are very few data about their phenolic levels and antioxidant properties.

Bulgarian local wines are produced from native and also from the so-called ‘international’ cultivars, including Cabernet Sauvignon, Merlot, Syrah, Grenache, etc. There is a considerable lack of information with regard to polyphenolic composition of Bulgarian red, white and rosé wines and as a result, their antioxidant capacity related to polyphenolic content.

The aim of the present study was to analyze and to compare the content of total phenolics and anthocyanins, as well as the antioxidant potential of alcoholic and non-alcoholic fruit drinks on the Bulgarian market.

EXPERIMENTAL

Selection of alcoholic and non-alcoholic fruit drinks

For this study we selected the most important and representative commercial table wines available on the Bulgarian market. Red, white and rosé wines produced from native cultivars, five different types from each, were randomly selected from the Bulgarian market. Six red, five white and five rosé wines were chosen. The red wines included the cultivars Merlot, Syrah, Mavrud, Zelas, Malbec, and Aronia. From the rosé wines were chosen Pinot Gris, Syrah, Cabernet Sauvignon, Mavrud, and Grenache. The white wines were Sauvignon blanc, Muskat, Traminer, Dimyat, Mavrud, and Chardonnay. Six different types of natural fruit juices were chosen: ”Strawberry”, “Blueberry”, “Forest fruit”, “Sour cherry”, “Black currant”, and “Aronia”. Compotes from five different types of fruits (red currant, strawberry, aronia, blackberry, and sour cherry) were used in the study.

All experiments were carried out in triplicate.

Determination of total phenolics

Total phenolics content was determined by the method of Folin-Ciocalteau with modifications [13, 14]. In brief, 20 μl of sample (wine, fruit juice, compote) was mixed with 1580 μl of deionized water and 100 μl of Folin-Ciocalteu phenol reagent. The solution was incubated for 5 min at room temperature and 300 μl of 7.5% Na_2CO_3 were added. After 2 h incubation in a dark place the optical density was measured at λ=765 nm against blank. The quantitation was done by the method of external calibration using gallic acid as a standard. Working standard solutions (600, 500, 400, 300, 200, 100, 50, 20 μg/ml) were prepared by dilution of the stock gallic acid standard solution. The results were presented in mg/L.

Determination of total monomeric anthocyanin pigments

For the determination of the content of total monomeric anthocyanin pigments in the tested samples the pH-differential method was used. The assay is based on the different coloration of anthocyanins with a change in pH. The colored oxonium form predominates at pH 1.0 and the colorless hemiketal form at pH 4.5. The optical density of each sample was measured at two wavelengths, 510 nm and 700 nm and at two pH values, 1.0 and 4.5 [14]. The appropriate dilution factor was determined until the absorbance of the sample at the λvis-max (510 nm) was within the linear range of the absorbance. Two dilutions of the sample were prepared, one with potassium chloride buffer, pH 1.0, and the other with sodium acetate buffer, pH 4.5, equilibrated at room temperature for 15 min. The optical density was measured at the λ 510 nm and at λ 700 nm. The concentration of monomeric anthocyanin pigment was calculated using the molar absorptivity and molar mass of cyanidine-3-glycoside. The results were expressed in mg/L.

Determination of antioxidant activity

The antioxidant capacity of samples was determined by decolorization of the stable ABTS radical-cation [15, 16]. The method was based on the depletion of the pre-formed $ABTS^{\bullet+}$ radical in the presence of potassium persulfate. The sample (10 μl) was added to 1 ml of $ABTS^{\bullet+}$ solution in PBS (pH 7.4). The optical density was read at 734 nm on zero time and on the 6^{th} minute after adding the sample against PBS as a blank. The quantitation was done by the method of external calibration using uric acid as a standard. The results were expressed in mM UAE. The percentage decrease of the absorbance at 734 nm was calculated by the formula:

$A = [(A_{sample\ t=0min.}\ A_{sample\ t=6min.}) - [(A_{blank\ t=0min.}\ A_{blank\ t=6min.})$

RESULTS AND DISCUSSION

Total polyphenolic content, anthocyanin concentration and antioxidant activity of the tested drinks are presented in Figs. 1 and 2.

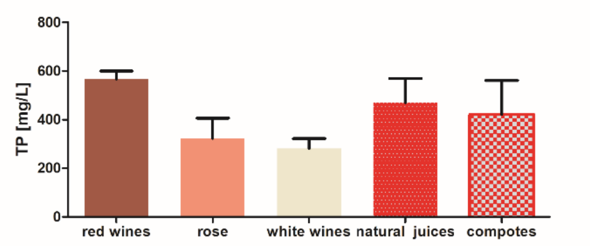

Fig. 1. Total phenolic content of wines, natural fruit juices and compotes from the Bulgarian market. TP – total phenolics

Fig. 2. Total monomeric anthocyanin content of wines, natural fruit juices and compotes from the Bulgarian market. AC – total monomeric anthocyanins

The highest polyphenolic content was established for the red wines, followed by natural fruit juices, compotes, rosé wines, and white wines. Among the tested alcoholic drinks the red wines revealed significantly higher average polyphenolic content (567±32 mg/L) *vs* rosé (323±84 mg/L, $p<0.0001$) and white wines (281±42 mg/L, $p<0.0001$). The natural fruit juices and compotes revealed similar polyphenolic content 470±99 mg/L *vs* 421±139 mg/L, respectively (Fig. 1).

The highest total monomeric anthocyanin content was established in the red wines (97.9±40.7 mg/L), followed by compotes (47.4±42.2 mg/L), natural fruit juices (36.1±16.4 mg/L), rosé wines (9.9±8.2 mg/L). The white wines were with the lowest content of anthocyanins 0.2±0.1 mg/L (Fig. 2).

Regarding the antioxidant capacity of the tested drinks, the red wines represent the highest average antioxidant capacity (24.50±11.30 mM) followed by the compotes (6.30±1.40 mM UAE), natural juices (5.30±1.90 mM), white wines (4.00±0.40 mM), and rosé wines (3.50±0.80 mM) (Fig. 3).

Fig. 3. Antioxidant capacity of wines, natural fruit juices and compotes from the Bulgarian market. TAC – total antioxidant capacity

The polyphenolic content of the different wine cultivars, natural fruit juices and compotes is presented in Table 1.

In the group of the red wines, the highest polyphenolic content (625±13 mg/L) was detected for Merlot wine, and the lowest for Syrah (534±20 mg/L). The wine Malbec was found to be the richest one in anthocyanins (156.6±1.5 mg/L), while in Mavrud their concentration was the lowest one (45.2±1.0 mg/L). The tested red wines showed high antioxidant capacity, especially strong for aronia wine (45.54±0.35 mM UAE). Among the non-alcoholic drinks, the highest polyphenolic content was found for aronia natural juice (592±9 mg/L) and compote (556±62 mg/L)

The established high content of total polyphenols and anthocyanins found in aronia drinks is consistent with the results of Nvenuti *et al.* [17] who detected high polyphenolic and anthocyanin content in non-alcoholic aronia drinks.

Surprisingly, a weak correlation between the anthocyanin content and the antioxidant capacity was found in the red wines Mavrud and Malbec. This is probably due to the fact that the pH differential method used in our study is selective for monomeric anthocyanins only. According to Arnous *et al.* [18] polymeric anthocyanin pigments are likely to contribute to the overall antioxidant capacity of the drink.

Aronia compote, aronia juice and strawberry juice revealed low antioxidant capacity, while their polyphenolic content was high. At the same time all cultivars red wines showed the opposite relationships. Moreover, we found a strong positive correlation between anthocyanin content and antioxidant capacity for rosé wines ($r=0.965$, $p<0.01$). Possible explanation is that the technologies for producing wines, juices, and compotes are quite different. The wine does not undergo heat treatment while the juices and the compotes are pasteurized or sterilized at high temperature. Changes that occur during their thermal treatment and storage may lead to significant losses of anthocyanins and low antioxidant capacity [19-21]. In support of this assumption are also the results for aronia wine, juice and compote. We found high total polyphenolic and anthocyanin content for these three drinks, but only the aronia wine showed high antioxidant capacity - 45.54 mM UAE *vs* 8.28 mM UAE for the juice and 6.00 mM UAE for the compote.

Table 1. Phenolic composition and antioxidant capacity of alcoholic and non-alcoholic fruit drinks from Bulgarian market

Drinks	Type	Total phenolics [mg/L]	Total monomeric anthocyanins [mg/L]	TAC [mM UAE]
Red wine	Merlot	625±13	103.4±7.9	24.37±0.14
	Syrah	534±20	130.5±1.4	20.33±0.22
	Mavrud	544±42	45.2±1.	24.77±0.10
	Zelas	551±16	72.8±2.8	12.01±0.12
	Malbec	571±2	156.6±1.5	20.23±0.13
	Aronia	576±9	79.2±1.3	45.54±0.35
Rosé wine	Pinot Gris	283±14	0.6±0.1	3.04±0.06
	Syrah	250±7	19.6±0.7	4.52±0.12
	Cabernet Sauvignon	391±29	17.3±0.3	4.18±0.12
	Mavrud	433±4	5.4±0.1	3.08±0.01
	Grenache	257±15	6.4±0.3	2.79±0.02
White wine	Sauvignon blanc	255±10	0.1±0.01	4.08±0.01
	Muskat	304±21	0.5±0.3	3.52±0.10
	Traminer	223±14	0.1±0.05	4.20±0.13
	Dimyat and Mavrud	330±5	0.3±0.2	4.42±0.02
	Chardonnay	291±13	0.2±0.1	3.72±0.01
Natural fruit juices	Strawberry	508±4	27.5±3.6	5.82±0.06
	Blueberry	326±11	15.5±1.3	2.76±0.04
	Forest fruit	553±18	35.6±2.2	6.04±0.07
	Sour cherry	414±15	29.6±1.1	3.64±0.06
	Black currant	428±3	62.8±2.6	5.48±0.68
	Aronia	592±9	45.7±3/3	8.28±0.03
Compotes	Red currant	239±21	2.8±0.2	6.60±0.06
	Strawberry	308±8	23.0±1.2	8.52±0.17
	Aronia	556±62	113.0±2.5	6.00±0.03
	Blackberry	519±6	39.0±0.9	4.80±0.07
	Sour cherry	485±15	62.0±1.0	5.64±0.08

CONCLUSION

The red wines and compotes have been an element of traditional Bulgarian cuisine and nowadays their input in the healthy diet is reassessed, because of their high phenolics content and strong antioxidant potential.

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ФЕНОЛНО СЪДЪРЖАНИЕ И АНТИОКСИДАНТНА АКТИВНОСТ НА НАПИТКИ ОТ БЪЛГАРСКИЯ ПАЗАР – ВИНА, СОКОВЕ И КОМПОТИ

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(Резюме)

Вкусът на вината, натуралните сокове и компотите се влияе силно от присъствието на феноли. Последните допринасят и за антиоксидантната активност на плодовете и продуктите от тяхната преработка и имат здравословно действие. Фенолното съдържание и антиоксидантният ификт не са включени в стандартната документация за етикетиране и контрол на храните. Целта на настоящата работа е да определи и сравни съдържанието на общи феноли и антоцианини (AC), както и антиоксидантния ефект на алкохолни и безалкохолни плодови напитки от българския пазар. Анализирани са търговски натурални плодови сокове, компоти, червени, розе и бели вина от българския пазар. Сред изследваните алкохолни напитки червените вина имат по-високо съдържание на полифеноли (567±33 mg/L) и AC (97.9±40.7mg/L) в сравнение с розе (323±84 mg/L, $p<0.0001$; 9.9+8.2 mg/L, $p<0.01$) и белите вина (281±42 mg/L, $p<0.0001$; 0.2±0.1 mg/L, $p<0.001$). В групата на червените вина най-високо полифенолно съдържание има мерло (625±13 mg/L), а най-ниско –сира (534±20 mg/L). Виното малбек е най-богато на антоцианини (156.6±1.5 mg/L), докато в мавруд съдържанието на антоцианини е най-ниско (45.2±1.0 mg/L). Изследваните червени вина проявяват висока антиоксидантна активност, особено виното от арония (45.55±0.35 mM UAE). В изследваните безалкохолни напитки най-високо полифенолно съдържание е установено в сок и компот от арония (съответно 592±9 mg/L и 556±62 mg/L). Червените вина и компотите са били елемент от традиционната храна в България и понастоящем техният принос към здравословната диета се преоценява поради високото им съдържание на общи феноли и силния антиоксидантен потенциал.