Antioxidant Properties and Phenolic Compounds of Vitamin C-Rich Juices

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Abstract: Many studies have shown that bioactive compounds, for example, polyphenols, and so on can play an important role in reducing oxidative stress and protect against various diseases. The sources of these compounds in the human diet include mainly fruit and good quality fruit juices, which may contain polyphenols but also other phytochemicals such as vitamin C. The purpose of the study was to analyze the antioxidant properties of vitamin C-rich juices, which underwent mild processing. The content of total polyphenols (TP, FBBB), total flavonoids (TF), total anthocyanins (TA), and vitamin C as well as the antioxidant capacity (DPPH, ABTS) were evaluated in commercial fruit juices rich in vitamin C (acerola, gojiberry, sea buckthorn, wild rose, cranberry, Japanese quince). Moreover, phenolic acids and selected flavonoids were determined by HPLC methods. Among the examined fruit juices, acerola and wild rose juices contained the highest amounts of vitamin C and total polyphenols, and had the highest antioxidant capacity. Acerola owes its high antioxidant properties mainly to vitamin C, whereas the antioxidant capacity of wild rose is also attributed to its rich content of flavonoids and phenolic acids. Sea buckthorn juice and Japanese quince juice had a lower antioxidant capacity, yet higher than determined for gojiberry and cranberry juices. Total anthocyanins were the highest in cranberry juice. The results showed that the analyzed juices were a valuable source of natural antioxidants. Generally, vitamin C-rich juices are also good source of polyphenols. Vitamin C and polyphenols act synergistically and define the antioxidant properties of juices.

Keywords: antioxidant properties, flavonoids, phenolic acids, polyphenols, vitamin C-rich juices

Practical Application: Bioactive compounds, for example, polyphenols play an important role in reducing oxidative stress and protect against various diseases. Sources of natural antioxidants in human diet include mainly fruit and good quality fruit juices. The study showed that the juices from acerola, gojiberry, sea buckthorn, wild rose, cranberry, Japanese quince were a valuable source of natural polyphenols and vitamin C. These compounds act synergistically and define the antioxidant properties of juices. Among all examined samples, acerola and wild rose juices seem to be the most valuable. Moreover, it's worth noticing that juices underwent mild processing (cold pressed and low pasteurization) retained more bioactive compounds, which affected their higher quality.

Introduction

A diet rich in fruit, vegetables and juices is recommended in latest dietary guidelines. Particularly important is the content of various antioxidants, which play a significant role in reducing oxidative stress. Recently, fruits and products from acerola, goji, sea buckthorn, Japanese quince, wild rose, and cranberry become more popular. They are characterized by the high content of bioactive compounds, especially polyphenols (mainly flavonoids and phenolic acids) and vitamin C.

Malpighia glabra L., popularly called "acerola" in Brazil or "Barbados cherry," is a native species from tropical America. Acerola is considered a functional fruit due to its high vitamin C content and other antioxidants, such as carotenoids, flavonoids, and anthocyanins. Quantities of these compounds, depend on cultivars, environmental conditions and the stage of fruit ripeness (Chaves, de Gouveia, Almeida, Leite, & da Silva, 2004; Horta et al., 2016; Nunes et al., 2011). Acerola extracts may protective oxidative damage through decreasing apoptosis and levels of intracellular reactive oxygen species and improving activity of antioxidant enzymes (Alvarez-Suarez et al., 2017).

Goji fruits (*Lycium barbarum and L. chinense*) are mainly cultivated in northwestern China and are well-known for their beneficial and health-promoting effects (Ruffo et al., 2017). These fruits are traditional food and medicine in East Asia, and they have become increasingly popular in Europe and North America since the early 21st century (Potterat, 2010). Gojiberry has gained popularity in the last few years owing to its public acceptance as "super food" with highly advantageous nutritive and antioxidant properties (Amagase, Sun, & Borek, 2009). Currently, gojiberries are classified as functional food and have many advantages such as: antioxidant properties, immunomodulatory and neuroprotective effects. This wide range of action is the result of bioactive compounds comprised in gojiberries, such as phenolic compounds, vitamin C, carotenoids (Le, Chiu, & Ng, 2007; Ruffo et al., 2017; Zhang, Chen, Zhao, & Xi, 2016) and polysaccharides complex LBB (*Lycium barbarum* polysaccharides) (Li & Zhou, 2007; Wang et al., 2015).

Sea buckthorn (*Hippophae rhamnoides* L.) belongs to the *Elaeagnaceae* family. Sea buckthorn is native to Central Asia and Northwestern Europe. At present, it is also grown in Canada and the USA (Krejcarová, Straková, Suchý, Herzig, & Karásková, 2015).

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The chemical composition of berries depends on a variety, climate conditions, fruit size, ripeness and a processing method. Sea buckthorn is a good source of polyphenols (flavonoids), carotenoids and vitamin C, and other natural antioxidants. Moreover, sea buckthorn is valued for its antioxidant, cardioprotective, antiatherogenic, antidiabetic, hepatoprotective, anticarcinogenic, immunomodulating, antiviral, antibacterial, anti-inflammatory, and vasodilating effects (Krejcarová et al., 2015).

Chaenomeles japonica (Thunb.) Lindl. ex Spach (Japanese quince), which occurs naturally in central and southern Japan, is 1 of the 4 species belonging to the genus *Chaenomeles*, family *Rosaceae*. Japanese quince has long been very popular in East Asia, and since the 19th century has also been grown in northern Europe (Baranowska-Bosiacka et al., 2017). The content of juice in the fruits varied between 41% and 52%, on a fresh weight basis. The juice is very sour and rich in vitamin C (Ros et al., 2004).

The Rosa gender (family *Rosaceae*) contains about 200 species, which are widely distributed in Europe, Asia, North America, and the Middle East (Jiménez et al., 2017). Wild rose (*Rosa canina*) is popular in many European countries such as Portugal, Poland, Germany, Sweden, Romania, and Finland (Barros, Carvalho, Morais, & Ferreira, 2010). Usually, it has been consumed as infusion, jelly or jam, but nowadays it is also used as an ingredient in probiotic yoghurts, juices or beverages (Demir, Yildiz, Alpaslan, & Hayaloglu, 2014; Nadpal et al., 2016). Rosa phytonutrients have different important properties. It has been shown that they have anti-inflammatory, antidiabetic, antimicrobial, and antitumor roles (De la Iglesia, Milagro, Campion, Boque, & Martinez, 2010; Jiménez et al., 2016; Lopes, Daletos, Proksch, Andrade, & Valentão, 2014).

The North American cranberry (*Vaccinium macrocarpon* Aiton) is widely consumed worldwide, both as fruit and as processed products such as juice, sauces, and extracts. The cranberry fruit is a rich source of phenolic compounds, in particular of flavonoids and proanthocyanidins (PACs) (Sun et al., 2016). Recent studies demonstrated that these phytochemicals of cranberry juice may increase antioxidant capacity in healthy adults (McKay, Chen, Zampariello, & Blumberg, 2015). Moreover, it has antimicrobial, anti-inflammatory, anticancer, neuroprotective, and antihyperglycaemic effects (Casedas, Les, Gomez-Serranillos, Smith, & Lopez, 2017, Sun et al., 2016).

Currently, many studies are conducted on natural bioactive compounds that can play an important role in the fight against oxidative stress. These sources in the human diet include mainly fruit and good quality fruit juices, which may contain polyphenols but also other phytochemicals such as vitamin C. Polyphenols, for example, flavonoids (flavanols, flavones, flavonols, anthocyanins, and proanthocyanidins, and so on) and phenolic acids, have received enormous attention in the recent years. These compounds protect against cardiovascular diseases and exhibit antitumor, antimicrobial, anti-adhesive and anti-inflammatory effects (Du et al., 2013). Vitamin C also plays an important role in preventing the generation of ROS (reactive oxygen species) (De Freitas & Meneghini, 2001; Phaniendra, Jestadi, & Perivasamy, 2015).

Therefore, the purpose of the study was to analyze the antioxidant properties of vitamin C-rich juices, which underwent mild processing. An important aspect of this work was to analyze the structure of polyphenolic compounds found in these juices. Analysis of the antioxidant properties and structure of polyphenolic compounds will provide us with evidence demonstrating whether the analyzed juices are a valuable source of natural antioxidants, which can be help to prevent oxidative stress.

Material and Methods

Samples

Six NFC (not from concentrate) juices, rich in vitamin C, were selected for analyses. The juices were produced by the company Zielona Tłocznia - Piotr Sępkowski (Wola Boglewska, Jasieniec, Poland). Three juices were made from fruit grown in Poland: sea buckthorn (*Hippophae rhamnoides* L., cultivated in northeastern Poland), wild rose (*Rosa canina* L., central Poland) and Japanese quince (*Chaenomeles japonica* (Thunb.) Lindl. ex Spach., eastern Poland). The other 3 juices were pressed from imported fruits: acerola (*Malpighia glabra* L., from Brazil), gojiberry (Lycium chinense Mill., from China), and cranberry (*Vaccinium macrocarpon* Aiton, from Canada). All the juices were cold pressed from whole fruits and submitted to mild continuous flow pasteurization at a temperature not exceeding 85 °C. The juices were naturally turbid, without any additives, which means that water was not added either.

Chemicals

2.6-Dichlorophenyloindophenol, ascorbic acid standard, oxalic acid, DPPH (1,1-diphenyl-2-picrylhydrazyl), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), methanol, acetone, Folin–Ciocalteu reagent, sodium carbonate, gallic acid, sodium hydroxide, Fast Blue BB reagent, sodium nitrite, aluminum chloride, potassium chloride, aqueous sodium acetate, (+)-catechin standard and cyanidin-3-glucoside standard were purchased from Sigma-Aldrich (Steinheim, Germany). Antioxidant Assay Kit (ELISA) was obtained from Cayman Chemical Company. All other solvents used in this study were HPLC (high pressure liquid chromatography) or of analytical grade.

Analytical methods

The analyses were carried out between September and November 2016. The pH of the tested samples of juices was measured with a glass electrode at room temperature.

The determination of vitamin C, antioxidant capacity, total polyphenols, total flavonoids and total anthocyanin content were performed directly in juices or appropriate dilutions. HPLC analyses were conducted in juices (free phenolic acids) or after its alkaline hydrolysis (bound phenolic acids) or acidic hydrolysis (flavonoids) according methods described below.

Analysis of vitamin C

The total vitamin C content was determined according to Polish Norm (1998) as previously described by Hallmann, Lipowski, Marszałek, and Rembiałkowska (2013). A weighed juice sample was extracted in 2% oxalic acid. The solution was filtered. The filtrate was collected and then titrated with the 2.6-dichlorophenyloindophenol (Hallmann et al., 2013; Polish Norm, 1998). The results were expressed as milligrams of ascorbic acid per kilogram of sample (mg AA/kg).

Antioxidant capacity (DPPH assay)

The antioxidant capacity of the samples was determined by a modified Yen and Chen method, using 0.1 mM methanol solution of a 1,1-diphenyl-2-picrylhydrazyl (DPPH, Sigma-Aldrich) (Blois, 1958; Yen & Chen, 1995). This method is widely used to test the antioxidant capacity of fruit, vegetables, and juices. Advantages of a DPPH assay were previously described (Apak et al., 2013; Kapci et al., 2013; Nowak, Gośliński, & Wojtowicz, 2016). A 0.1 mL of sample was added to 2.9 mL DPPH solution and

mixed. The absorbance was measured on a Hitachi U-1900 spectrophotometer at 517 nm after 30 min of incubation at room temperature in the dark. The antioxidant capacity of the samples of juices was expressed as milligrams of Trolox per liter of sample (mg Tx/L).

Antioxidant capacity (ABTS assay)

An Antioxidant Assay Kit (ELISA – Cayman Chemical Company) was used to determine the antioxidant capacity of the samples. The assay relies on the ability of antioxidants in a sample to inhibit the oxidation of ABTS (2,2'-Azino-di-[3ethylbenzthiazoline sulphonate]) to $ABTS^+$ by metmyoglobin. The absorbance was measured on a microplate reader SPEKTROstar Nano (BMG LABTECH) at 750 nm after incubation in a shaker for 5 min at room temperature. The antioxidant capacity was quantified as millimolar Trolox equivalents (mM Trolox).

Total polyphenols content (Folin-Ciocalteu assay)

The content of total polyphenols (TP) of the samples was determined by the Folin–Ciocalteu assay (Singleton, Orthofer, & Lamuela-Raventos, 1999). First, 0.3 mL of sample was added to a 10 mL capacity tube, next 0.05 mL 2N Folin-Ciocalteu reagent and 0.5 mL 20% sodium carbonate solution were added. The mixture was diluted by addition of 4.15 mL distilled water and mixed. The absorbance was measured on a Hitachi U-1900 spectrophotometer at 765 nm after 30 min incubation in the dark at room temperature. The results were expressed as milligrams of gallic acid equivalents per liter of sample (mg GAE/L).

Total polyphenols content (Fast Blue BB assay)

Fast Blue BB (FBBB) is a novel method described by Medina to quantify phenolic compounds through direct interaction of polyphenols with the FBBB reagent (Sigma-Aldrich) in an alkaline medium. This method demonstrated higher values of gallic acid equivalents (GAE) than a Folin-Ciocalteu assay (Medina, 2011). 0.2 mL aliquot of 0.1% Fast Blue BB reagent were added to 2 mL of samples, mixed for 1 min and 0.2 mL 5% NaOH was added. The reaction was allowed to complete at room temperature for 90 min. The absorbance was measured on a Hitachi U-1900 spectrophotometer at 420 nm. The results were expressed as gallic acid equivalents (mg GAE/L).

Total flavonoids content

The content of total flavonoids (TF) in juice samples was measured using the colorimetric assay described by Kapci et al. (2013). Briefly, 0.3 mL of 5% $NaNO_2$ was added to 1 mL of juice at zero time. After 5 min, 0.3 mL of 10% $AlCl_3$ was added. At the 6th min, 2 mL of 1M NaOH was added. The mixture was diluted by the addition of 2.4 mL of distilled water and mixed. Absorbance was measured on a Hitachi U-1900 spectrophotometer at 510 nm compared with blank solution. The total flavonoids content was determined by a (+)-catechin (Sigma-Aldrich) standard curve and was expressed as milligrams of catechin equivalents per liter of juice (mg CE/L).

Total anthocyanin content

Total anthocyanin content (TA) was determined by the pH differential method (AOAC Official Method 2005.02) (Lee, Durst, & Wrolstad, 2005). Juices were diluted according to appropriate dilution ratios (one part sample and four parts buffer) by adding both 0.025M KCl (pH 1.0) and 0.4M $CH_3COONa{\cdot}3H_2O$ (pH 4.5) buffer solutions. Samples were mixed and left in the dark for 30 min. Absorbance was measured on a Hitachi U-1900 spectrophotometer at 520 and 700 nm, using $A = [(A_{520} - A_{700})_{pH1.0} - (A_{520} - A_{700})_{pH4.5}]$. Total anthocyanin content was expressed as milligrams of cyanidin-3-glucoside equivalents (molar extinction coefficient = 26,900 L/mol/cm and molecular weight = 449.2 g/mol) per liter of juice (mg cyd-3-glu/L).

HPLC analysis of phenolic acids and flavonoids

Phenolic acids and selected flavonoids were determined by HPLC methods based on the parameters established in our previous studies (Nowak et al., 2016). The analyses were performed using a Dionex LC system equipped with a photodiode array detector (PAD, Dionex) and the absorption spectra were recorded in the range of 200 to 600 nm. The flow rate was 1 mL/min, the column temperature was 30 °C and the injection volume was 20 μL. Qualitative identification was done by comparing the retention times and spectra with the standards. Simultaneous monitoring was performed at 280 nm for phenolic acids and 360 nm for flavonoids.

Phenolic acids were determined according the method described by Krygier, Sosulski, and Hogge (1982). The separation was performed on an Ascentis C18 (Supelco) column (250 mm × 4.6 mm; 5 μm). The binary mobile phase consisted of 0.1% (v/v) formic acid in methanol (eluent A) and methanol–acetonitrile (80:20, v/v; eluent B). The gradient program was as follows: 0 to 5 min (0% B), 7 to 15 min (10% B), 25 min (25% B), 34 min (65% B), 35 to 39 min (100% B), 40 to 45 min (0% B). The examples of chromatograms are presented in Figure 1, 2 and 3.

Flavonoids were determined by a modified Hertog, Hollman, and Enema (1992) method, after its acidic hydrolysis. The separation was performed on an Ascentis (Supelco) C18 column (250 mm x 4.6 mm; 5 μm). The binary mobile phase consisted of 0.1% (v/v) formic acid in water–methanol (75:25, v/v, pH 2.7; eluent A) and 0.1% (v/v) formic acid in methanol (eluent B). The gradient program was as follows: 0 to 2 min (0% B), 10 to 20 min (15% B), 30 min (40% B), 35 to 44 min (100% B), 47 to 51 min (0% B).

Statistical analysis

The results were statistically analyzed by calculating the mean and standard deviation. The interpretation of the results was performed with MS Excel 2010 Analysis ToolPak software, one-way analysis of variance (ANOVA) using the Tukey's *post hoc* test: different letters in the same row or column indicate statistical significance (at least $P < 0.05$).

Results and Discussion

The analyzed fruit juices were sour and had pH between 2.45 and 4.65 (Table 1). The lowest pH values were determined in cranberry juice and in Japanese quince, sea buckthorn and acerola juices (2.45 to 3.19). In other studies, the values of pH were similar, and equaled 2.4 to 2.9 for *Chaenomeles* juice (Ros et al., 2004), 2.91 to 3.0 for sea buckthorn fruits (Green & Low, 2013) and 3.17 to 3.68 for acerola fruits (Souza, Moura, Brito, & Miranda, 2014). Slightly higher pH was identified in the wild rose juice we analyzed (3.93), but this result was approximate to the data given in the literature, that is, 3.50 ± 0.05 (Murathan, Zarifikhosroshaki, Kafkas, & Sevindik, 2016), 4.00 ± 0.07 (Ganhao, Estevez, Kylli, Heinonen, & Morcuende, 2010) and 4.07 (Ercisli, 2007). The highest pH value noted in our study was determined in gojiberry juice (4.65), but even a higher value (6.05 ± 0.15) was reported by

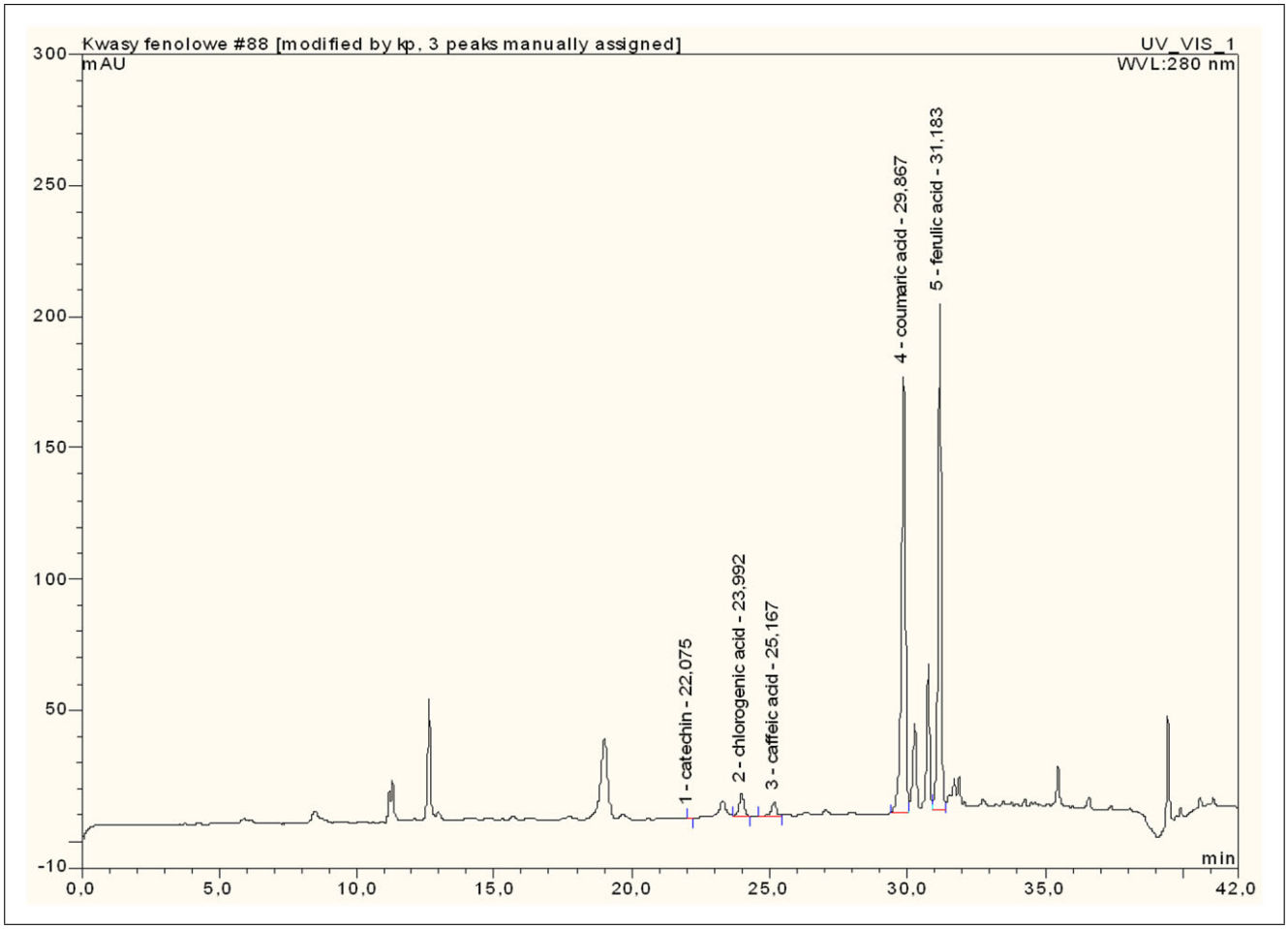

Figure 1–Free phenolic acids of gojiberry juice.

Istrati, Vizireanu, Iordachescu, Dima, and Garnai (2013) in dried gojiberries.

All the analyzed juices had large quantities of vitamin C. The highest vitamin C content was determined in acerola juice: 9283 mg/kg. Less vitamin C was found in wild rose and gojiberry juices. Mezadri, Villaño, Fernández-Pachón, García-Parrilla, and Troncoso (2008) determined a slightly higher level of vitamin C in acerola juices than identified in our study, namely 9440 to 17970 mg/kg. The literature reports different levels of vitamin C, but determined in fruit and depending on the ripeness of acerola: 9570 mg/kg for ripe fruit and 24240 mg/kg for the green stage (De Assis, Lima, & de Faria Oliveira, 2001). In other studies as well, unripe acerola had a higher content of vitamin C than ripe fruit (81040 mg/kg compared with 44476 mg/kg) (Horta et al., 2016; Nunes et al., 2011). The content of vitamin C in different extracts from wild rose (*Rosa canina*) ranged from 560 to 3730 mg/kg (Nadpal et al., 2016) and these values were lower than determined in our study. The differences resulted from various extraction methods, that is, water and methanol extracts of fresh and air-dried wild rose fruits (Nadpal et al., 2016). Higher concentrations of vitamin C in the fruit of *Rosa canina* were reported in some other investigations: 7544.8 ± 1002 for ripe fruit (Murathan et al., 2016) and 4110 to 22000 mg/kg for fruit or flesh (Kazaz, Baydar, & Erbas, 2009). In turn, the goji juice in our study had a lower vitamin C content than acerola and wild rose juices (Table 1), but the result was much higher than reported by Ionica, Nour, and Trandafir (2012) for fresh and dried goji fruits (299 to 458 mg/kg) or showed by Potterat (2010) for goji fruits (420 mg/kg). In our research, the vitamin C content in sea buckthorn juice was 3255 mg/kg, thus being higher than in the study by Christaki (2012) 1540 mg/kg in juice and comparable to the result obtained by Sabir, Maqsood, Ahmed, Shah, and Khan (2005) 2500 to 3330 mg/kg but in berries. Other reports provide higher levels of vitamin C in sea buckthorn fruits, for example, 3940 to 5730 mg/ kg (Rop, Ercişli, Mlcek, Jurikova, & Hoza, 2014) and 3600 to 25000 mg/kg (Bal, Meda, Naik, & Satya, 2011). The difference of vitamin C content of sea buckthorn depends on species/varieties and analyzed material (berries or juices). The content of vitamin C in Japanese quince juice was 1978 mg/kg, thus being higher than elsewhere, for example, 200 to 1120 mg/kg of fresh C*haenomeles* juice (Ros et al., 2004) and 1000 to 1720 mg/kg of Japanese quince fresh fruit (Baranowska-Bosiacka et al., 2017). It has been demonstrated that consumption of vitamin C in the form of juice causes a substantial increase in the content of this vitamin in plasma (Duthie et al., 2006), which can aid an organism in reducing oxidative stress. Vitamin C present in acerola juice is an example of an antioxidant compound that can chelate metals, thus preventing the generation of ROS (De Freitas & Meneghini, 2001; Phaniendra et al., 2015), apart from playing a role in the regulation of DNA repair enzymes (Jomova & Valko, 2011).

Juices rich in vitamin C were characterized by valuable antioxidant characteristics, originating from the content of polyphenols. The highest antioxidant capacity determined by the DPPH method was demonstrated by acerola and wild rose juices

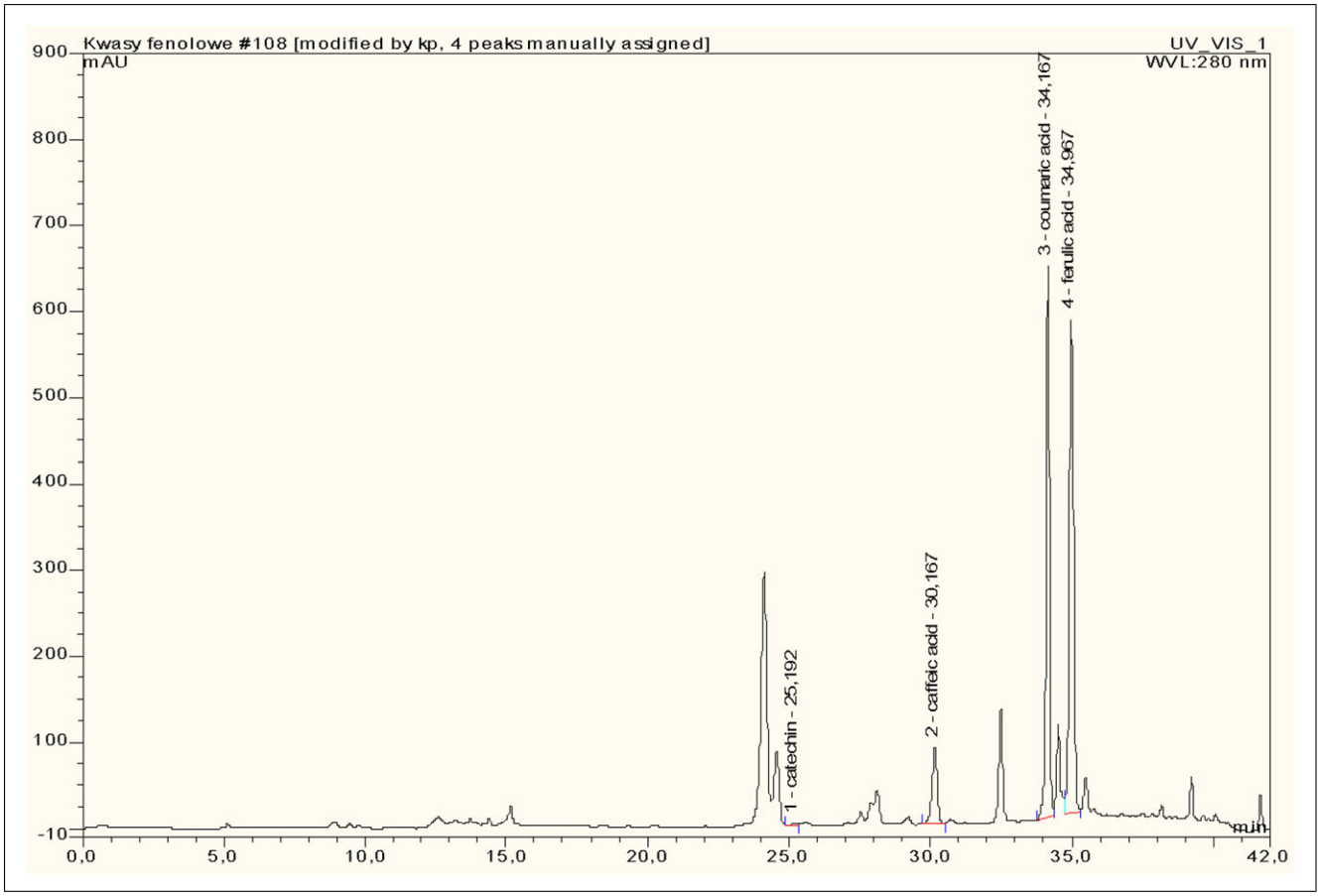

Figure 2–Bound phenolic acids of gojiberry juice.

(5149 ± 446 and 4895 ± 53 mg Tx/L, respectively), that is the juices with the highest vitamin C content. Japanese quince juice had about half lower antioxidant capacity: 2580 ± 101 mg Tx/L. The lowest antioxidant capacity was determined in cranberry and gojiberry juices: 884 ± 3.0 and 877 ± 9.0 mg Tx/L, respectively (Table 1). Analogous results on the antoxidant capacity of the analyzed juices were achieved using a rapid Antioxidant Assay Kit test, based on the ELISA method. Here, too, acerola and wild rose juices had the highest antioxidant capacity (21.4 mM Tx each), followed by Japanese quince juice (17.9 mM Tx). The lowest antioxidant capacity was determined for cranberry and sea buckthorn juices: 7.9 and 9.8 mM Tx, respectively.

In other studies, the total antioxidant activity (TAA) of acerola fruit was 32.93 to 140.04 μmol TEAC/g FW, being the highest in unripe fruit (Souza et al., 2014). Nunes et al. (2011) observed that the amount of DPPH needed to capture free radicals is lower in unripe than in ripe acerola, and the antioxidant capacity of unripe fruits in IC50 was twice as high as in a sample of ripe fruits (Horta et al., 2016; Nunes et al., 2011). Lower values of antioxidant capacity have been determined in various *Rosa canina* L. biotypes 6.3 to 12,78 mM Trolox/kg (Roman, Stanila, & Stanila, 2013).

Acerola juice and wild rose juice, that is the juices with the highest antioxidant capacity, were also distinguished by the highest total content of polyphenols (TP), that is, 13707 ± 9.0 and 13188 ± 87 mg GAE/L (Table 1). Other researchers report the total content of polyphenols to be slightly lower, that is, 10560 mg (Alves, Brito, Rufino, & Sampaio, 2008) and 10630 ± 531 mg GAE/kg (Rufino et al., 2010) or higher, that is, 15616.7 (ripe fruit) up to 43388.9 mg GAE/kg (unripe fruit) (Souza et al., 2014) than in the acerola juice analyzed in our study. Also, the total content of polyphenols in wild rose fruit was higher than revealed in our study, and ranged from 11750 ± 2220 to 46040 ± 8770 mg GAE/kg, depending on the type of extract (ethanol/methanol/water). The highest TP content was detected in the water extract and the lowest in ethanolic extract (Ganhao et al., 2010). Various researchers have identified the content of total polyphenols in *Rosa canina* from 1760 to 96000 mg of GAE/kg (Egea, Sánchez-Be, Romojaro, & Pretel, 2010; Ercisli, 2007; Fattahi, Jamei, & Sarghein, 2012; Nadpal et al., 2016; Roman et al., 2013; Su, Yin, Charles, Zhou, & Moore, 2007). These studies concerned different species/biotypes, samples type (fresh or lyophilized fruit), and various extracts (acetone or methanol), which affected the content of total polyphenols. The content of total polyphenols of sea buckthorn juice determined in our study was 4784 ± 35 mg GAE/L, thus being lower than in other studies, but analyzed in fruits, where it varied from 8620 to 14170 mg GAE/kg, depending on a variety of sea buckthorn (Rop et al., 2014). The content of total polyphenols in Japanese quince juice was determined at 6852 ± 44 mg GAE/L and approximated the results reported by Fronc and Oszmiański (1994), that is 6450 mg GAE/kg. Lower amounts of TP in Japanese quince juice were reported by Ros et al. (2004), 1850 to 4760 mg GAE/L, and 2840 mg/L on average. The lowest total content of polyphenols in our study was found in cranberry and gojiberry juices: 3504 ± 182 and 4028 ± 176 mg GAE/L, respectively (Table 1). Similar results were observed in other studies, for example, TP in cranberry was 2241 to 4982 mg/kg (Cesoniene, Jasutiene, & Sarkinas, 2009) and

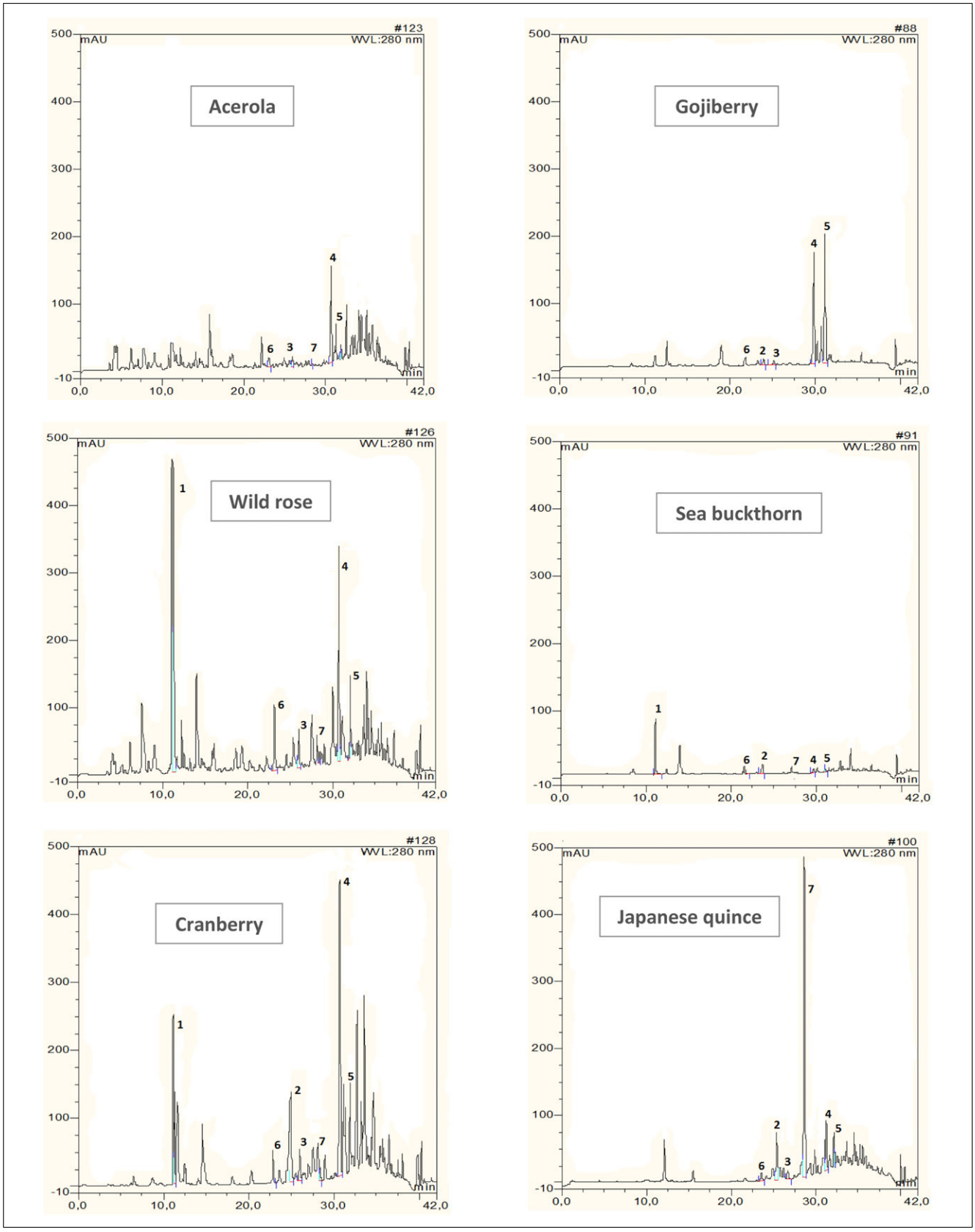

Figure 3–Free phenolic acids and flavanols of analyzed juices.
1 – gallic acid; 2 – chlorogenic acid; 3 – caffeic acid; 4 – coumaric acid; 5 – ferulic acid; 6 – catechin; 7 – epicatechin.

Table 1–Antioxidant capacity and polyphenols of juices.

	Acerola	Gojiberry	Sea buckthorn	Wild rose	Cranberry	Japanese quince
pH	3.19 ± 0.2^{c}	4.65 ± 0.2^{a}	3.05 ± 0.1^{c}	3.93 ± 0.2^{b}	2.45 ± 0.2^{d}	2.95 ± 0.1^{c}
Vitamin C (mg AA kg^{-1})	9283 ± 426^{a}	4237 ± 217^{b}	3255 ± 147^{c}	4548 ± 163^{b}	250 ± 15^{e}	1978 ± 106^{d}
DPPH (mg Tx L^{-1})	5149 ± 446^{a}	877 ± 9.0^{d}	1909 ± 61^{c}	4895 ± 53^{a}	884 ± 3.0^{d}	2580 ± 101^{b}
ABTS (mM Tx)	21.4^{a}	12.9^{c}	9.8^{d}	21.4^{a}	7.9^{d}	17.9^{b}
Total Polyphenols (mg GAE L^{-1})	13707 ± 9.0^{a}	4028 ± 176^{d}	4784 ± 35^{c}	13188 ± 87^{a}	3504 ± 182^{d}	6852 ± 44^{b}
Total Flavonoids (mg CE L^{-1})	1574 ± 45^{c}	1391 ± 28^{c}	2513 ± 43^{b}	5864 ± 27^{a}	527 ± 5.0^{d}	2641 ± 20^{b}
Total Anthocyanins (mg cyd-3-glu L^{-1})	55.7 ± 3.2^{b}	44.5 ± 2.6^{b}	22.3 ± 0.9^{c}	27.8 ± 1.3^{c}	200.4 ± 12.1^{a}	77.9. ± 2.9^{b}

Data are mean ± SD ($n = 5$). Statistical analysis was performed by one-way ANOVA using the Tukey's *post hoc* test: different letters in the same row indicate statistical significance (at least $p < 0.05$).
AA = ascorbic acid; Tx = Trolox equivalents; GAE = gallic acid equivalents; CE = catechin equivalents.

in gojiberry 1742.7 to 3510 mg GAE/kg, depending on whether it was fresh or dehydrated fruit (Ionica et al., 2012; Istrati et al., 2013).

The highest content of total flavonoids was determined in wild rose juice 5864 ± 27 mg CE/L (Table 1). This result was twice as high as in Japanese quince juice and in sea buckthorn juice, and 4-fold higher than in acerola and gojiberry juice. The lowest content of total flavonoids was detected in cranberry juice. Other researchers showed that the content of flavonoids is higher in ripe acerola fruit than in unripe one. Eccleston et al. (2002) proved that sea buckthorn was a good source of flavonoids and contained 1182 mg/L, which is half the content determined in our study. The lowest amounts were reported by Hallmann, Orpel, and Rembiałkowska (2011), where total flavonoids equaled 171 mg/kg of fresh quince juice. In other studies, gojiberry also contained flavonoids, but the variety *L. chinense* had a lower content of flavonoids than *L. barbarum* did (Mocan et al., 2014). The total content of flavonoids determined in wild rose juice was much higher in our study than reported by Roman et al. (2013) 1013 ± 33.3 to 1632 ± 54.5 mg QE/kg frozen pulp. However, Yoo, Lee, Lee, Moon, and Lee (2008) reported a higher content of flavonoids (4000 mg QE/kg fresh fruit).

In our study, total anthocyanins were the highest in cranberry juice, where their content reached 200 mg cyd-3-glu/L. A much lower content of total anthocyanins was found in Japanese quince juice as well as in acerola and gojiberry juices (Table 1). In other studies, the content of anthocyanins in cranberry was much higher and equaled 407 to 2073 mg/kg (Cesoniene et al., 2009) but these results originated from analyses of cranberry fruit rather than cranberry juice. The high content of anthocyanins in cranberry juice has been reported in our previous studies (Nowak, Gośliński, & Szwengiel, 2017). On the other hand, Casedas et al. (2017) showed that total anthocyanin content in cranberry juice was much lower than in blueberry juice. Rufino et al. (2010) reported a higher content of total anthocyanins in acerola fruit than determined in our research, namely 189 ± 9.0 mg/100 g of fresh matter. Unripe acerola fruits had a higher level of these compounds (Souza et al., 2014). Anthocyanins were also detected in Japanese quince fruits (Du et al., 2013). In turn, the content of anthocyanins in wild rose fruits (*R. canina*) was similar to the one determined in our study and equaled 27.5 mg/kg (Murathan et al., 2016) or 20.4 mg/kg (Ercisli, 2007). A relatively low content of anthocyanins in gojiberries suggests that the orange color of the fruit may be due to a large content of carotenoids, mostly zeaxanthin (Ruffo et al., 2017). Anthocyanins were not detected in sea buckthorn berries (Chen, Xin, Yuan, Su, & Wei, 2014; Green & Low, 2013). In our study, sea buckthorn juice was found to contain small amounts of total anthocyanins, the lowest ones among all the analyzed juices.

Table 2–The correlation coefficients (R^2).

	DPPH	ABTS	TP	TF	TA	Vit. C
DPPH		0.900	0.991	0.606	0.446	0.886
ABTS	0.900		0.915	0.594	0.497	0.713
TP	0.991	0.915		0.585	0.417	0.867
TF	0.606	0.594	0.585		0.579	0.245
TA	0.446	0.497	0.417	0.579		0.423
Vit. C	0.886	0.713	0.867	0.245	0.423	

Furthermore, we determined the correlation of analytical methods used. The correlation coefficients (R^2) for antioxidant properties of analyzed juices ranged from 0.245 to 0.991 (Table 2). DPPH and ABTS assays showed a high correlation coefficient ($R^2 = 0.900$). In addition, both methods demonstrated a high linear correlation with TP (0.991 and 0.915, respectively), which confirms that polyphenols decided about antioxidant capacity more than vitamin C content. On the other hand, the lowest correlation coefficients were determined for TA (from 0.417 to 0.579), which demonstrated that anthocyanins were minor contributors of the antioxidant properties of analyzed juices.

Our analysis of polyphenolic compounds performed by the HPLC method showed that Japanese quince juice had the highest content of flavanols, especially epicatechins (148.4 ± 3.6 mg/kg), among all the analyzed juices (Table 3). Sea buckthorn and cranberry juices contained most flavonols, mostly quercetin (cranberry) and kaempferol (sea buckthorn). Cranberry juice also contained most flavones, mainly apigenin. Considering the quantities of phenolic acids found in the analyzed juices, it was concluded that the highest amounts of gallic and chlorogenic acids were detected in wild rose and Japanese quince juices, respectively. Cranberry and gojiberry juices were a good source of coumaric acid. The latter was also quite rich in ferulic acid. Gojiberry juice contained most resveratrol, which belongs to stilbenes.

Other studies confirm that Japanese quince juice is a rich source of flavanols, mostly epicatechin and catechin (Wojdyło, Oszmiański, & Bober, 2008). Large quantities of epicatechin and procyanidin B2 were also found in *Chaenomeles japonica* by Du et al. (2013). Similarly to our study, other authors have concluded that sea buckthorn is a good source of flavonoids, mainly quercetin and kaempferol (Chen et al., 2013; Fatima et al., 2012). Bittová, Krejzová, Roblová, Kubáň, and Kubáň (2014), who carried out analyses of phenolic compounds in sea buckthorn, concluded that the content of gallic acid was from 7.9 to 15.9 mg/kg, depending on the time of harvest of sea buckthorn fruits. The concentrations of coumaric acid and ferulic acids in sea buckthorn were

Table 3–HPLC analysis of selected phenolic compounds of juices (mg kg^{-1} ± SD).

Compounds	Acerola	Gojiberry	Sea buckthorn	Wild rose	Cranberry	Japanese quince
PHENOLIC ACIDS						
Free phenolic acids						
- gallic acid	Nd^{c}	Nd^{c}	7.3 ± 0.1^{b}	38.9 ± 0.1^{a}	3.5 ± 0.1^{b}	Nd^{c}
- chlorogenic acid	Nd^{d}	2.9 ± 0.1^{c}	5.4 ± 0.3^{c}	Nd^{d}	8.8 ± 0.1^{b}	26.9 ± 0.1^{a}
- caffeic acid	0.5 ± 0.1^{c}	0.9 ± 0.1^{c}	Nd^{d}	1.4 ± 0.1^{b}	1.6 ± 0.1^{b}	2.7 ± 0.1^{a}
- coumaric acid	3.3 ± 0.1^{c}	18.4 ± 0.4^{a}	0.9 ± 0.1^{d}	6.5 ± 0.1^{b}	20.7 ± 0.1^{a}	7.2 ± 0.1^{b}
- ferulic acid	0.4 ± 0.1^{c}	22.3 ± 1.9^{a}	Tr^{d}	0.2 ± 0.1^{c}	Nd^{d}	1.9 ± 0.1^{b}
Bound phenolic acids						
- caffeic acid	1.0 ± 0.1^{c}	6.4 ± 0.5^{b}	Tr^{d}	0.8 ± 0.1^{c}	8.9 ± 0.1^{b}	21.5 ± 0.2^{a}
- coumaric acid	8.4 ± 0.1^{c}	18.5 ± .1.6^{a}	0.9 ± 0.1^{d}	8.2 ± 0.1^{c}	20.4 ± 0.1^{a}	15.6 ± 0.2^{b}
- ferulic acid	4.5 ± 0.1^{b}	15.5 ± 0.1^{a}	0.4 ± 0.1^{c}	0.8 ± 0.1^{c}	5.7 ± 0.1^{b}	0.4 ± 0.1
- galic acid	0.7 ± 0.1^{c}	Nd^{d}	6.7 ± 0.1^{b}	49.0 ± 0.1^{a}	1.1 ± 0.1^{c}	Nd^{d}
FLAVONOIDS						
Flavanols						
- catechin	1.5 ± 0.1^{b}	0.7 ± 0.1^{b}	0.4 ± 0.1^{b}	51.0 ± 0.1^{a}	1.0 ± 0.1^{b}	55.5 ± 0.1^{a}
- epicatechin	Tr^{d}	Nd^{d}	0.3 ± 0.1^{c}	1.9 ± 0.1^{b}	1.6 ± 0.1^{b}	148.4 ± 3.6^{a}
Flavonols						
- quercetin	13.6 ± 1.1^{c}	4.4 ± 0.4^{d}	27.5 ± 3.1^{b}	5.4 ± 0.1^{d}	52.6 ± 1.1^{a}	12.3 ± 0.8^{c}
- kaempferol	3.7 ± 0.1^{b}	1.1 ± 0.4^{c}	52.2 ± 5.0^{a}	0.4 ± 0.1^{c}	0.7 ± 0.1^{c}	Nd^{d}
- myricetin	0.9 ± 0.2^{c}	5.4 ± 0.1^{b}	Nd^{d}	3.0 ± 0.1^{b}	12.4 ± 0.4^{a}	Nd^{d}
Flavones						
- apigenin	Nd^{c}	Nd^{c}	Nd^{c}	0.8 ± 0.1^{b}	12.5 ± 0.1^{a}	Nd^{c}
- luteolin	0.4 ± 0.1^{a}	Nd^{b}	Nd^{b}	0.4 ± 0.1^{a}	Nd^{b}	Nd^{b}
STILBENES						
- resveratrol	3.4 ± 0.1^{c}	12.0 ± 0.4^{a}	4.0 ± 0.3^{c}	7.3 ± 0.1^{b}	1.8 ± 0.1^{d}	2.0 ± 0.1^{d}

Data are mean ± SD (n = 3). Statistical analysis was performed by one-way ANOVA using the Tukey's *post hoc* test: different letters in the same row indicate statistical significance (at least $p < 0.05$).
Nd = nondetected (detection limit 0.1); Tr= trace amount.

2.3 to 22.3 and 2.4 to 10.5 mg/kg, respectively (Bittová et al., 2014). The levels of these compounds were therefore higher than in our study, but the quoted analyses were made on fruits. Caffeic acid as well as epicatechin were not detected in sea buckthorn. Also, the content of catechins was much higher than in our study, and reached 4.1 to 22.2 mg/kg (Bittova et al., 2014). Quercetin and myricetin derivatives were detected in various cranberry genotypes (Abeywickrama, Debnath, Ambigaipalan, & Shahidi, 2016). Same as in our study, Mocan et al. (2014) detected higher quantities of coumaric and ferulic acids in goji fruits. Moreover, apart from *p*-coumaric acid Forino, Tartaglione, Dell'Aversano, and Ciminiello (2016) demonstrated the presence of caffeic acid, rutin, and scopoletin in dried goji fruits. Similarly to our results, myricetin, quercetin, and kaempferol were identified (Le et al., 2007). In our experiment, wild rose juice had the highest quantities of catechin and galic acid. In another study, this fruit contained large amounts of procyanidins and catechins as well as smaller quantities of flavonols (Ganhao et al., 2010). Jimenez et al. (2017) demonstrated the highest content of rutin, followed by catechin and myricetin. The same researchers also demonstrated the presence of gallic acid and caffeic acid in *Rosa canina*, but did not detect coumaric acid. All these compounds were present in the wild rose juice we analyzed. Furthermore, Mezadri et al. (2008) identified the presence of 5 different polyphenolic compounds in acerola fruit, using the HPLC approach. They were rutin, chlorogenic acid, epigallocatechin gallate, epicatechin, and procyanidin B1. These compounds were not detected in our study. Acerola also contains numerous anthocyanins, mainly cyanidin 3-rhamnoside and pelargonidin 3-rhamnoside (Brito, de Araújo, Alves, & Carkeet, 2007; De Rosso et al., 2008). Same as in our study, acerola also comprised small amounts of quercetin, but rutin was the dominant compound (Correia, Borges, Medeiros, & Genovese, 2011; Horta et al., 2016; Souza et al., 2014).

Conclusions

The highest antioxidant capacity was determined in acerola and wild rose juices. Acerola owed these mainly to vitamin C content, whereas the high antioxidant capacity of wild rose was associated with vitamin C and polyphenols (especially flavonoids and phenolic acids). Japanese quince juice presented relatively high antioxidant capacity, which it owed mostly to flavanols (epicatechin and catechin) and phenolic acids. Among the analyzed juices, the lowest antioxidant capacity, total polyphenols and total flavonoids demonstrated gojiberry and cranberry juices. HPLC analysis showed the lowest level of flavonols, but relatively high amount of phenolic acids in this juices. The highest content of total anthocyanins had cranberry juice, however it did not affect on antioxidant properties of this juice. The antioxidant capacity of the analyzed juices depended not only on their high content of vitamin C but also on the presence of polyphenols. These compounds may show synergistic action. However, correlation coefficients showed that polyphenols decided about antioxidant capacity more than vitamin C content. The results justify the conclusion that the analyzed juices are a valuable source of natural antioxidants, which could play an important role in the fight against oxidative stress.

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Author Contributions

D. Nowak: contributed to the study design, collected test data, analysis and interpretation of results, helped to statistical analysis, and drafted the manuscript. M. Gośliński: helped to the studies and statistical analysis, editing of the manuscript. E. Wojtowicz and

K. Przygoński partly helped with analysis. All authors approved the final version.

Conflict of Interest

The authors declare that they have no conflict of interest.

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