Anthocyanin content and antioxidant activity of various red fruit juices

Lidija Jakobek#, Marijan Šeruga, Martina Medvidović-Kosanović and Ivana Novak
Department of Applied Chemistry and Ecology, Faculty of Food Technology, J. J. Strossmayer University of Osijek, Kuhačeva 18, HR-31000 Osijek/Croatia

Summary
Anthocyanin content and antioxidant activity of various red fruit juices (black currant, red raspberry, blackberry, sour cherry, sweet cherry, strawberry, chokeberry, and elderberry juice) has been evaluated in this study by using HPLC, pH-differential, and DPPH method. Anthocyanins were the predominant phenolic components (66 % in elderberry juice, 56 % in black currant juice) or represented considerable portion in total polyphenol content of some juices (40 % in blackberry juice, 33 % in chokeberry juice). Amount of anthocyanins determined by HPLC method ranged from 202 to 6287 mg l^{-1} in strawberry and elderberry juice, respectively. Anthocyanins present in investigated red fruit juices were derivatives of cyanidin, delphinidin, pelargonidin and peonidin. Chokeberry, elderberry, blackberry and sour cherry juice were characterized by cyanidin derivatives, black currant juice by delphinidin and cyanidin derivatives and strawberry juice by pelargonidin derivatives. The major anthocyanins in red raspberry and sweet cherry juice were derivatives of cyanidin although peonidin (in sweet cherry juice) and pelargonidin derivatives (in red raspberry juice) were found in low amount. Antioxidant activity varied from 4 to 72 µmol TE/ml in sweet cherry and chokeberry juice, respectively. High correlation was observed between antioxidant activity and total anthocyanin content of investigated red fruit juices. Overall results showed that red fruit juices can serve as a good source of bioactive phytochemicals in human diet. Chokeberry, elderberry and black currant juice were the richest in anthocyanin content and showed the strongest antioxidant activity, as well. Therefore, these three juices can be regarded as good candidates for raw materials in production of functional foods.

Zusammenfassung
In dieser Studie wurden Anthocyane-Konzentration sowie antioxidative Aktivität von Fruchtsäfte aus verschiedenen Beerenfrüchte-Sorten und Kirschen (schwarze Johannisbeere, Himbeere, Brombeere, Sauer- und Süßkirschen, Erdbeere, Aronia und Holunderbeere) mittels HPLC, pH-Differenzial und DPPH-Methode bestimmt. In einigen Säften bilden Anthocyane den größten Anteil der Gesamtpolyphenole (z. B. 66 % im Holunderbeersaft, 56 % im schwarzen Johannisbeersaft), oder stellen einen bedeutenden Anteil am Gesamtpolyphengehalt mancher Säfte (40 % im Brombeersaft, 33 % im Aroniasaft) dar. Die Anthocyane-Konzentration, mittels HPLC bestimmt, variierte von 202 mg/l im Erdbeersaft bis 6287 mg/l im Holunderbeersaft. Bei den aus Beerenfrüchten und Kirschen hergestellten Fruchsäften vorhandenen Anthocyanen handelt es sich um Cyanidin-, Delphinidin-, Pelargonidin-, und Peonidinderivate. So wurden im Aronia-, Holunderbeere-, Brombeere- und Sauerkirschensaft Cyanidinderivate gefunden. Im schwarzen Johannisbeersaft wurden Delphinidin- und Cyanidin-, im Erdbeersaft Pelargonidinderivate gefunden. Die im Himbeer- und Süßkirschensaft enthaltenen Anthocyane sind größtenteils Cyanidinderivate, obwohl auch darin, aber nur in kleinen Mengen, Peonidinderivate (im Süßkirschensaft) und Pelargonidinderivate (im Himbeersaft) vorhanden sind. Die antioxidative Aktivität variierte im Bereich von 4 µmol Troloxäquivalent/ml im Süßkirschensaft bis zu 72 µmol Troloxäquivalent/ml im Aroniasaft. Es wurde eine starke Korrelation zwischen der antioxidativen Aktivität und der Anthocyan-Konzentration in den geprüften Säften aus Beerenfrühten und Kirschen festgestellt. Alle Ergebnisse weisen darauf hin, dass Säfte aus Beerenfrüchten und Kirschen eine geeignete Quelle an bioaktiven Phytochemikalien für die menschliche Ernährung darstellen. Aronia-, Holunderbeer- und schwarzer Johannisbeersaft enthalten die größte Anthocyane-Konzentration und zeigen die stärkste antioxidative Aktivität. Diese Säfte sind daher zur Herstellung funktioneller Lebensmittel geeignet.

Keywords: Anthocyanins, red fruit juices, berry juices, antioxidant activity / Anthocyane, Beerenfrüchtesäfte, antioxidative Aktivität

Introduction

Considerable interest has been developed over the years in fruits and vegetables due to their potential biological and health-promoting effects. Numerous epidemiological studies indicate that an increase in the consumption of fruits and vegetables is associated with a decrease in the incidence of various diseases like cardiovascular disease, stroke, and cancer[1–5]. The protective effect of fruits and vegetables has been attributed to their bioactive antioxidant constituents, including vitamins, carotenoids, polyphenols[1,6]. Among various antioxidants present in fruits and vegetables, polyphenols (including anthocyanins) have received much attention since being reported to have a positive influence on human health[1].

Anthocyanins belong to the class of phenolic compounds. They are water-soluble glycosides and acylglycosides of anthocyanidins. The most common naturally occurring anthocyanins are the 3-O-glycosides or 3,5-di-O-glycosides of cyanidin, delphinidin, peonidin, petunidin, pelargonidin and malvidin[7]. Anthocyanins are important polyphenolic components of fruits, especially berries[8–13]. They are potent antioxidants *in vitro*[14] and may be protective against many degenerative diseases[7]. Numerous studies have shown that anthocyanins are absorbed in their original glycosylated forms in humans[15–18]. They appear in urine after supplementation with berries or berry extracts but in very low

Corresponding author, E-mail: ljakobek@ptfos.hr,
Tel.: +385-31-224-300; Fax: +385-31-207-115

concentrations[15,16,18]. Anthocyanins have also been found in human plasma in very low concentrations[15–18]. Recent research emphasized the importance of anthocyanins in development of cancer therapy, because it was found that the anthocyanins could decrease the *in vitro* invasiveness of cancer cells[19].

Besides fruits and vegetables, a relevant part of intake of polyphenolic phytochemicals (including anthocyanins) is supplied by fruit juices. Juices are suitable food products in terms of ingestion of health protective phytochemicals. Bioactive components may even be better absorbed from juices than from plant tissues, as it was demonstrated for ascorbic acid[20]. Some studies have shown that the intake of antioxidant berry juice (containing white grape, black currant, elderberry, sour cherry, blackberry and aronia juice) can increase plasma antioxidant capacity, which suggests an improvement in antioxidant status and indicates that antioxidant constituents of the juice might decrease lipid oxidation within plasma compartment[20]. Consumption of polyphenol rich juice (containing apple, orange and mango extracts) enhanced antioxidant status, reduced oxidative DNA damage and stimulated immune cell functions[21].

There is still not enough knowledge about health effects of fruits, vegetables, juices or antioxidant concentrates. Much more research is therefore needed on the composition of antioxidants in natural antioxidant mixtures, juices or fruits and on antioxidant effects of these products[22]. Many reports have been written about the antioxidant activity and anthocyanin profiles of various fruits or fruit extracts[8–14,23–27], but relatively few have been based on red fruit juices[28,29]. A need for such data still exists because of increasing popularity of red fruit juice consumption during the last time, and because of increasing consumer awareness concerning the nutritional value of all foods (including these juices).

Therefore, the objective of this work was to evaluate various natural red fruit juices (black currant, red raspberry, blackberry, sour cherry, sweet cherry, strawberry, chokeberry, and elderberry juice) regarding the amount of anthocyanins and antioxidant activity and to determine individual anthocyanic compounds present in these juices. Furthermore, a concentration of total polyphenolics was measured in order to determine the portion of anthocyanins in total polyphenol content of red fruit juices.

Materials and methods

Chemicals

For this work, anthocyanin standards cyanidin-3-O-glucoside chloride (kuromanin chloride), cyanidin-3-O-rutinoside chloride (keracyanin chloride), delphinidin-3-O-glucoside chloride (myrtillin chloride), pelargonidin-3-O-glucoside chloride (callistephin chloride) and peonidine-3-O-glucoside chloride) were purchased from *Extrasynthèse* (Genay/France). Methanol (HPLC grade) was obtained from *Merck* (Darmstadt/Germany) and ortho-phosphoric acid (85 %, HPLC grade) was purchased from *Fluka* (Buchs/Switzerland). Hydrochloric acid (36.5 %), potassium chloride, sodium acetate trihydrate, Folin-Ciocalteau reagent were obtained from *Kemika* (Zagreb/Croatia). Trolox ((±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH$^{\cdot}$) were purchased from *Sigma-Aldrich* (St. Louis, MO/USA).

Samples

Fruits [black currant (*Ribes nigrum)*, red raspberry (*Rubus idaeus*), blackberry (*Rubus fruticosus*), sour cherry (*Prunus cerasus*), sweet cherry (*Prunus avium*), strawberry (*Fragaria anannassa*), chokeberry (*Aronia melanocarpa*), and elderberry (*Sambucus nigra*)] were harvested in Slavonia region (Croatia) at the commercial maturity stage. Immediately after harvesting, fruits were frozen and stored at –20° C until analysis.

Sample preparation

For total anthocyanin, total polyphenols and antioxidant activity determination, fruits (~500 g) were thawed at room temperature for 30 min and then processed in juice extractor (Juicer, *Philips*) in order to obtain natural fruit juice. Three replicates of juice (20 ml each) were centrifuged at 4000 rpm for a 1 h. All juices were analysed the same day when they were prepared.

Determination of total polyphenols

Total polyphenols were determined by Folin-Ciocalteau micro method[30]. An aliquot (20 µl) of appropriately diluted fruit juice (red raspberry, blackberry, sour cherry, sweet cherry, strawberry, 1:1 (v/v); black currant 1:2 (v/v); elderberry, chokeberry 1:6 (v/v) with H_2O) was mixed with 1580 µl of distilled water and 100 µl of Folin-Ciocalteau reagent. 300 µl of sodium carbonate solution (200 g/l) was added to the mixture which was then shaken. After incubation at 40 °C for 30 min in water bath, absorbance of the mixture was read against the prepared blank at 765 nm. Total polyphenolics were expressed as mg of gallic acid equivalents (GAE) per l of fruit juice. Data presented are mean ± standard deviation (SD).

Determination of total anthocyanins

Total anthocyanins were estimated by a pH-differential method[31]. Two dilutions of natural fruit juices were prepared, one with potassium chloride buffer (pH 1.0) (1.86 g KCl in 1 l of distilled water, pH value adjusted to 1.0 with concentrated HCl), and the other with sodium acetate buffer (pH 4.5) (54.43 g $CH_3CO_2Na \cdot 3H_2O$ in 1 l of distilled water, pH value adjusted to 4.5 with concentrated HCl), diluting each by the previously determined dilution factor (sweet cherry 1:5 (v/v); strawberry 1:10 (v/v); red raspberry 1:20 (v/v), sour cherry and blackberry 1:30 (v/v); black currant 1:25 (v/v); elderberry and chokeberry 1:200 (v/v)). Absorbance was measured simultaneously at 510 and 700 nm after 15 min of incubation at room temperature. The content of total anthocyanins was expressed in mg of cyanidin-3-O-glucoside equivalents (CGE) per l of fruit juice using a molar extinction coefficient (ε) of cyanidin-3-O-glucoside of 26 900 l mol^{-1} cm^{-1} and molar weight (MW) (449.2 g mol^{-1}). Data presented are mean ± standard deviation (SD).

Determination of antioxidant activity

Antioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. In the DPPH method[9], five dilutions of each fruit juice with two replicates were analyzed. Reaction solution was prepared by mixing 50 µl of diluted fruit juice with 300 µl of methanolic DPPH· solution (1mM) and brought to 3 ml with methanol. The solution was kept in dark at room temperature for 15 minutes. The absorbance (A_{juice}) was read against the prepared blank (50 µl diluted fruit juice, 2950 µl methanol) at 517 nm. A DPPH· blank solution was prepared (300 µl of 1mM DPPH· solution, 2.7 ml of methanol) and measured daily. Percent inhibition of DPPH radical was calculated for each dilution of juice according to formula:

$$\% \text{ inhibition} = [(A_{DPPH} - A_{juice})/A_{DPPH})] \times 100$$

where A_{DPPH} is the absorbance value of the DPPH· blank solution, A_{juice} is absorbance value of the sample solution.

Calibration curve of Trolox was constructed by linear regression of the absorbance value versus concentration (0–2500 µmol l^{-1}). For each dilution of fruit juice, antioxidant activity was calculated on the basis of the Trolox calibration curve and expressed in µmol of Trolox equivalent (TE) per ml of fruit juice. Percent of inhibition of DPPH radical of each dilution of fruit juice was plotted against antioxidant activity (µmol TE/ml). Using the curve obtained, antioxidant activity was calculated for 50 % of DPPH· inhibition. Final results are expressed as µmol of TE per ml of juice needed to reduce DPPH radical by 50 %.

HPLC analysis of anthocyanins

For HPLC analysis, fruits (~500 g) (random selection) were thawed at room temperature for 30 min and then processed in juice extractor (Juicer, *Philips*) in order to obtain natural fruit juice. Three replicates of juice (20 ml each) were centrifuged at 4000 rpm for a 1 hour and diluted 1:3 (v/v) with 0.1 % solution of HCl prepared in methanol. The samples were filtered through a 0.45 µm syringe filter (VariSep PTFE, 0.45 µm, 25 mm-Varian) before they were injected into HPLC apparatus. All juices were analysed on HPLC system immediately, the same day when they were prepared.

The analytical HPLC system employed consisted of a Varian LC system (USA) equipped with a ProStar 230 solvent delivery module, ProStar 310 UV-Vis Detector, and ProStar 330 PDA Detector. Anthocyanin compounds separation was done in an OmniSpher C18 column (250 x 4.6 mm inner diameter, 5 µm, *Varian*, USA) protected with guard column (ChromSep 1 cm x 3 mm, *Varian*, USA). The data were collected and analysed on *IBM* computing system equipped with Star Chromatography Workstation software (version 5.52).

Elution was employed with a mobile phase A consisting of 0.5 % phosphoric acid in water and mobile phase B consisting of 100 % HPLC grade methanol as follows: 0–38 min from 3 % B to 65 % B; from 38–45 min, 65 % B; with flow rate = 1 ml min^{-1}. Operating conditions were as follows: column temperature 20 °C; injection volumes, 10 µl of the standards and samples. A 10-minute re-equilibration period was used between individual runs. UV-Vis spectra were recorded in wavelength range from 190–600 nm (detection wavelength was 520 nm).

Identification and peak assignment of anthocyanins in all fruit juices was based on comparison of their retention times and spectral data (190–600 nm) with those of authentic standards. Additional identification of anthocyanins present in fruit juices was carried out by spiking the juices with anthocyanin standards. Anthocyanin profiles of fruit juices were compared with those found in literature[8,10–12,14,23–29] which gave additional information on anthocyanin identification. Calibration curves of the standards were made by diluting stock standards in 0.1 % HCl solution prepared in methanol to yield 1–100 mg l^{-1} (cyanidin-3-glucoside); 0.5–200 mg l^{-1} (cyanidin-3-rutinoside); 5–100 mg l^{-1} (peonidin-3-glucoside); 1–100 mg l^{-1} (delphinidin-3-glucoside) and 1–100 mg l^{-1} (pelargonidin-3-glucoside). Identified anthocyanins were quantified using calibration curve of cyanidin-3-glucoside. Data presented are mean ± standard deviation (SD).

Analytical quality control

All anthocyanic compounds showed a linear response within range studied (r^2 = 0.9958–0.9997). Precision of method was evaluated by determining within-day variation of the HPLC analysis (within-day precision-repeatability). To gain the data for studying repeatability, each juice sample was analysed three times within one day. Coefficients of variation (CV) of peak areas varied between 0.04 and 5.95 %. Recoveries were measured by adding known amounts of standards (10–50 mg l^{-1}) to fruit juices prior to HPLC analysis. The recoveries ranged from 80 to 92 % for cyanidin-3-glucoside, from 74 to 84 % for cyanidin-3-rutinoside, from 90 to 95 % for peonidin-3-glucoside, from 89 to 93 % for delphinidin-3-glucoside and from 95 to 101 % for pelargonidin-3-glucoside. In the calculation of final results, no correction for recovery was applied to data. The following limits of detection were estimated using a signal-to-noise ratio of 3:0.64 mg l^{-1} by cyanidin-3-glucoside, 1.18 mg l^{-1} by cyanidin-3-rutinoside, 0.41 mg l^{-1} by peonidin-3-glucoside, 0.35 mg l^{-1} by delphinidin-3-glucoside and 0.92 mg l^{-1} by pelargondin-3-glucoside.

Statistical analysis

Correlation and regression analyses were performed using Statistica 7.1 (*Statsoft*, Tulsa/USA). Differences at $p \leq 0.05$ were considered significant.

Results and discussion

Natural red fruit juices were analyzed using a pH-differential and Folin-Ciocalteau method in order to examine their total anthocyanin (TA) and total polyphenol (TP) content. The portion of anthocyanins in total polyphenol concentration was evaluated by calculating TA/TP ratio. The concentrations of total anthocyanins, total polyphenols and TA/TP ratio are shown in Table 1. Anthocyanins were found in the highest concentrations in elderberry, chokeberry and black currant juices (4189 mg l^{-1}, 3042 mg l^{-1}, 1544 mg l^{-1}, respectively) whereas the concentrations of anthocyanins in blackberry, sour cherry, sweet cherry, red raspberry and strawberry juice were significantly lower (740 mg l^{-1}, 369 mg l^{-1}, 257 mg l^{-1}, 217 mg l^{-1}, 206 mg l^{-1}, respectively). Data presented by other authors also confirmed that the amount of total anthocyanins were higher in chokeberry and black currant than in other red fruits like blackberry, raspberry or red currant[9].

Polyphenols were found in the highest concentrations in chokeberry, elderberry and black currant juices (9154 mg l^{-1}, 6362 mg l^{-1}, 2771 mg l^{-1}, respectively). High concentrations of polyphenols were found in sour cherry and blackberry juice as well (2054 mg l^{-1}, 1831 mg l^{-1}, respectively), while sweet cherry, strawberry and red raspberry juice had relatively lower concentrations of polyphenols (1567 mg l^{-1}, 1272 mg l^{-1}, 1234 mg l^{-1}, respectively). According to data presented by other authors, the concentrations of polyphenols were higher in chokeberry and black currant than in red currant, blackberry and raspberry[9].

Anthocyanins were the predominant polyphenolic components in elderberry (66 %) and black currant juice (56 %) or represented significant portion in total polyphenol concentration of chokeberry (33 %) or blackberry juice (40 %). The portion of anthocyanins in red raspberry

Tab. 1 Concentrations of total anthocyanins (TA) (mg CGE/l)[a], total polyphenols (TP) (mg GAE/l)[a], antioxidant activity of red fruit juices (µmol TE/ml)[b], and TA/TP ratio

	Total anthocyanins [mg/l]	Total polyphenols [mg/l]	Antioxidant activity [µmol TE/ml]	TA/TP
Black currant	1543.89 ± 5.5	2770.94 ± 63.9	30.15	0.56
Red raspberry	217.39 ± 5.2	1234.27 ± 54.8	8.20	0.18
Blackberry	739.93 ± 37.5	1831.21 ± 111.6	8.75	0.40
Sour cherry	369.36 ± 2.4	2054.43 ± 140.2	12.52	0.18
Sweet cherry	256.60 ± 2.5	1566.84 ± 130.2	4.07	0.16
Strawberry	205.98 ± 2.2	1271.85 ± 106.9	4.39	0.16
Chokeberry	3042.20 ± 196.3	9154.47 ± 595.4	72.44	0.33
Elderberry	4188.63 ± 257.0	6361.89 ± 298.9	62.14	0.66

[a] values are means ± SD (n=3); [b] antioxidant activity determined after 15 min

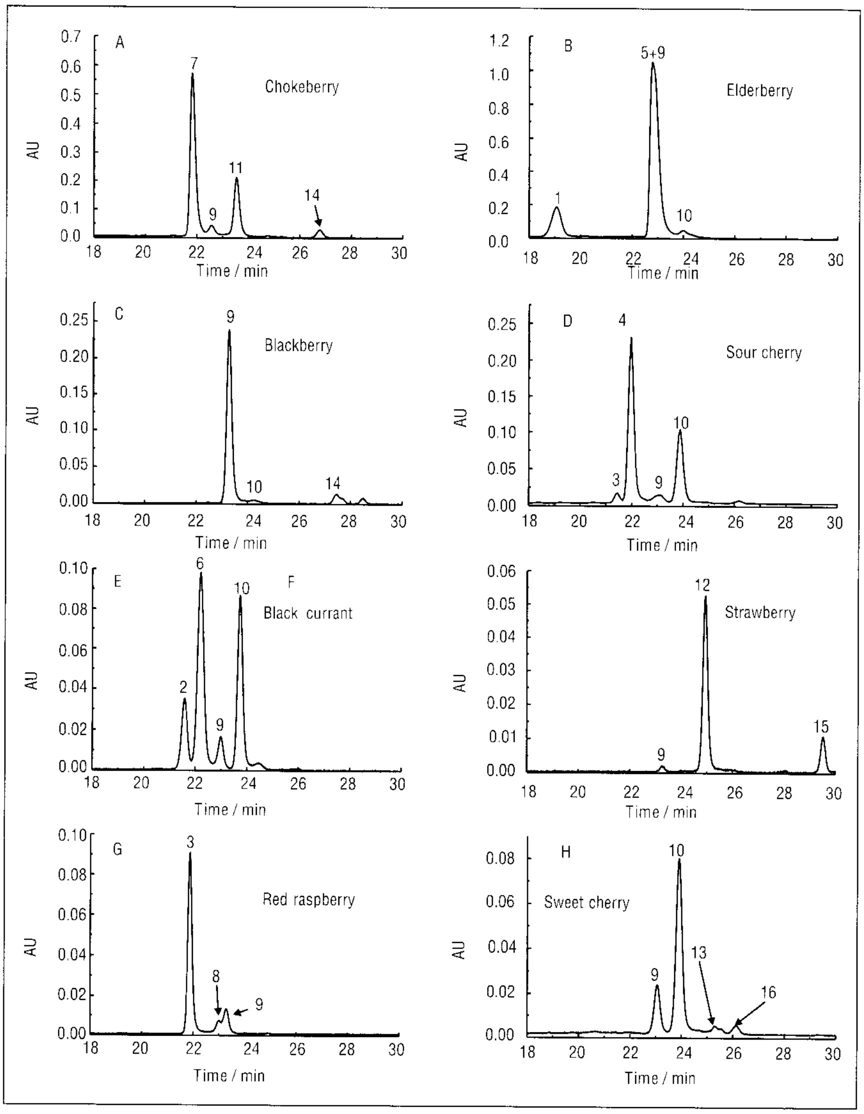

Fig. 1 HPLC chromatograms of the anthocyanin compounds of red fruit juices. Peak identification: **(1)** cyanidin-3-sambubioside-5-glucoside; **(2)** delphinidin-3-glucoside; **(3)** cyanidin-3-sophoroside; **(4)** cyanidin-3-glucosyl-rutinoside, **(5)** cyanidin-3-sambubioside; **(6)** delphinidin-3-rutinoside; **(7)** cyanidin-3-galactoside; **(8)** pelargonidin-3-sophoroside; **(9)** cyanidin-3-glucoside; **(10)** cyanidin-3-rutinoside; **(11)** cyanidin-3-arabinoside, **(12)** pelargonidin-3-glucoside; **(13)** peonidin-3-glucoside; **(14)** cyanidin-3-xyloside; **(15)** pelargonidin-3-rutinoside; **(16)** peonidin-3-rutinoside

Tab. 2 Concentrations of anthocyanins in natural red fruit juices (mg/l)[a] determined by HPLC method and percentage distribution of anthocyanins

Juice	Concentration as Cy-3-glu equivalents [mg/l]	Total anthocyanins [%]
	Chokeberry	
cy-3-gal	1816.6 ± 4.9	68.9
cy-3-glu	74.3 ± 0.1	2.8
cy-3-ara	647.1 ± 1.0	24.5
cy-3-xyl	99.8 ± 0.1	3.8
Total	2637.8 ± 6.1	100.0
	Elderberry	
cy-3-sam-5-glu	949.1 ± 9.9	15.1
cy-3-sam + cy-3-glu	5226.6 ± 18.9	83.1
cy-3-rut	111.7 ± 5.9	1.8
Total	6287.4 ± 34.7	100.0
	Blackberry	
cy-3-glu	743.3 ± 2.7	90.4
cy-3-rut	10.8 ± 0.0	1.3
cy-3-xyl	68.1 ± 1.2	8.3
Total	822.2 ± 3.9	100.0
	Sour Cherry	
cy-3-sopho	11.7 ± 0.8	3.9
cy-3-glu-rut	183.3 ± 0.7	61.4
cy-3-glu	10.6 ± 0.3	3.5
cy-3-rut	93.0 ± 1.6	31.2
Total	298.6 ± 3.4	100.0
	Black Currant	
dp-3-glu	222.3 ± 10.9	13.8
dp-3-rut	706.4 ± 38.9	43.9
cy-3-glu	99.9 ± 1.7	6.2
cy-3-rut	579.5 ± 12.3	36.0
Total	1608.1 ± 63.8	99.9
	Strawberry	
cy-3-glu	7.3 ± 0.7	3.6
pg-3-glu	159.2 ± 2.0	78.7
pg-3-rut	35.9 ± 0.0	17.7
Total	202.4 ± 2.7	100.0
	Red Raspberry	
cy-3-sopho	239.4 ± 2.8	79.8
pg-3-sopho	18.0 ± 0.5	6.0
cy-3-glu	42.5 ± 0.7	14.2
Total	299.9 ± 4.0	100.0
	Sweet Cherry	
cy-3-glu	71.6 ± 1.5	20.2
cy-3-rut	252.9 ± 8.0	71.2
pn-3-glu	16.0 ± 1.1	4.5
pn-3-rut	14.7 ± 0.2	4.1
Total	355.2 ± 10.8	100.0

(18 %), sour cherry (18 %), sweet cherry (16 %) and strawberry juice (16 %) was considerably lower (Tab. 1).

In order to separate and determine individual anthocyanic compounds present in red fruit juices, HPLC method was applied. The HPLC chromatograms of red fruit juices recorded at 520 nm are presented in Figure 1. The amounts of anthocyanins in red fruit juices are shown in Table 2.

Chokeberry, elderberry, blackberry and sour cherry juices contained only cyanidin based pigments. Chokeberry juice contained a mixture of four different cyanidin-glycosides: 3-galactoside, 3-arabinoside, 3-glucoside and 3-xyloside of cyanidin. Cyanidin-3-galactoside and cyanidin-3-arabinoside were found in high concentrations (1817 mg l^{-1}, 647 mg l^{-1}, respectively, >93 % of total anthocyanin content) whereas the concentrations of cyanidin-3-xyloside (100 mg l^{-1}) and cyanidin-3-glucoside (74 mg l^{-1}) were relatively low. The data presented by other authors are showing that the main anthocyanins in chokeberry are cyanidin-3-galactoside and cyanidin-3-arabinoside; the following are cyanidin-3-xyloside and cyanidin-3-glucoside[8,12,28], which is consistent with our results.

The major anthocyanins in elderberry juice were cyanidin based [cyanidin-3-sambubioside and cyanidin-3-glucoside eluted together (5227 mg l^{-1}), cyanidin-3-sambubioside-5-glucoside (949 mg l^{-1}) and cyanidin-3-rutinoside (112 mg l^{-1})], with cyanidin-3-sambubioside and cyanidin-3-glucoside predominating (83 % of total anthocyanin amount). Data presented by other authors also confirmed that the major anthocyanins in elderberry are cyanidin-based, with cyanidin-3-sambubioside and cyanidin-3-glucoside predominating[8,12,28,29] like in our study.

Cyanidin-3-glucoside was the major anthocyanin in blackberry juice (743 mg l^{-1}, 90 % of total anthocyanin content) whereas the concentrations of cyanidin-3-xyloside (68 mg l^{-1}) and cyanidin-3-rutinoside (11 mg l^{-1}) were considerably lower. Previous study confirmed that the main anthocyanin in blackberry is cyanidin-3-glucoside (from 44 to 95 % of total peak area in various blackberry samples)[23]. Cyanidin-3-rutinoside ranged from trace amount to 53 %, whereas cyanidin-3-xyloside, cyanidin-3-malonylglucoside and cyanidin-3-dioxalylglucoside were detected but at much lower concentrations[23].

Sour cherry juice contained -3-glucosylrutinoside (183 mg l^{-1}, 61.4 %), -3-rutinoside (93 mg l^{-1}, 31.2 %), -3-glucoside (11 mg l^{-1}, 3.5 %) and -3-sophoroside (12 mg l^{-1}, 3.9 %) of cyanidin. These data are in accordance with those found in literature[24,25,29].

Black currant juice was characterized by the presence of rutinosides and glucosides of delphinidin and cyanidin with the rutinosides being the most abundant (delphinidin-3-rutinoside 706 mg l^{-1}, cyanidin-3-rutinoside 580 mg l^{-1}; 79.9 % of total anthocyanin content). The data reported in the literature are showing that the main anthocyanins in black currant are rutinosides and glucosides of delphinidin and cyanidin[8,12,14,28,29] which agrees with our results.

Pelargonidin derivatives predominated in strawberry juice (pelargonidin-3-glucoside, 159 mg l^{-1}, pelargonidin-3-rutinoside, 36 mg l^{-1}). These anthocyanins represented together

> 96 % of total anthocyanin amount. The concentration of cyanidin-3-glucoside was low (7 mg l^{-1}, 4 %). Previous studies confirmed that strawberry is characterized by the same pelargonidin and cyanidin glycosides as in our study and that pelargonidin glycosides predominate in strawberry[11,26].

The main anthocyanin found in red raspberry juice, which represented 79.8 % of total anthocyanin content, was cyanidin-3-sophoroside (239 mg l^{-1}). Cyanidin-3-glucoside and pelargonidin-3-sophoroside were found in relatively lower amount (43 mg l^{-1}, 18 mg l^{-1}, respectively). Previous study confirmed that anthocyanins present in red raspberry (early cultivar Heritage) are cyanidin-3-sophoroside, cyanidin-3-glucoside and pelargonidin-3-sophoroside which is in accordance with our results[27]. However, anthocyanin profiles of various raspberry cultivars are very heterogenous; the main red raspberry anthocyanins are cyanidin and pelargonidin derivatives [11,27,29] but in some late cultivars malvidin and delphinidin derivatives were found as well[27].

The dominant anthocyanin in sweet cherry juice was cyanidin-3-rutinoside (253 mg l^{-1}), with cyanidin-3-glucoside being second (72 mg l^{-1}). These two anthocyanins represented together 91.4 % of total anthocyanin amount, while the concentrations of peonidin-3-rutinoside and peonidin-3-glucoside were low (15 mg l^{-1}, 16 mg l^{-1}, respectively). The data reported in the literature are showing that sweet cherry contains cyanidin, peonidin and pelargonidin derivatives[10,11]. The major anthocyanins are cyanidin-3-rutinoside and cyanidin-3-glucoside like in our study, while 3-rutinoside and 3-glucoside of pelargonidin and peonidin are present but at much lower concentrations[10,11].

In order to evaluate antioxidant activity of chosen red fruit juices, DPPH· assay was applied and the results are presented in Table 1. All investigated juices exhibited potent radical scavenging activity. The strongest radical scavenging activity showed chokeberry, elderberry, and black currant juice (72 µmol TE/ml, 62 µmol TE/ml µmol, 30 µmol TE/ml respectively) while activities of other juices were considerably lower (sour cherry 13 µmol TE/ml; blackberry 9 µmol TE/ml; red raspberry 8 µmol TE/ml; strawberry 4 µmol TE/ml; sweet cherry 4 µmol TE/ml). There are already a number of reports on the antioxidant activity of berry extracts by several methods such as oxygen radical absorbance capacity[12] or DPPH radical scavenging capacity[8,9] indicating that chokeberry, elderberry and black currant possess strong antiradical activities. Antioxidant activity of chokeberry juice concentrate against DPPH radical was stronger than antioxidant activity of black currant, elderberry, red currant, strawberry, red raspberry and cherry concentrate[28].

The correlation between antioxidant activity measured by DPPH methods and total polyphenols and total anthocyanins are presented in Figure 2. The concentration of total polyphenols were found to correlate with the antioxidant activities of juices (r = 0.97***). The concentration of total anthocyanins correlate with antioxidant activity as well

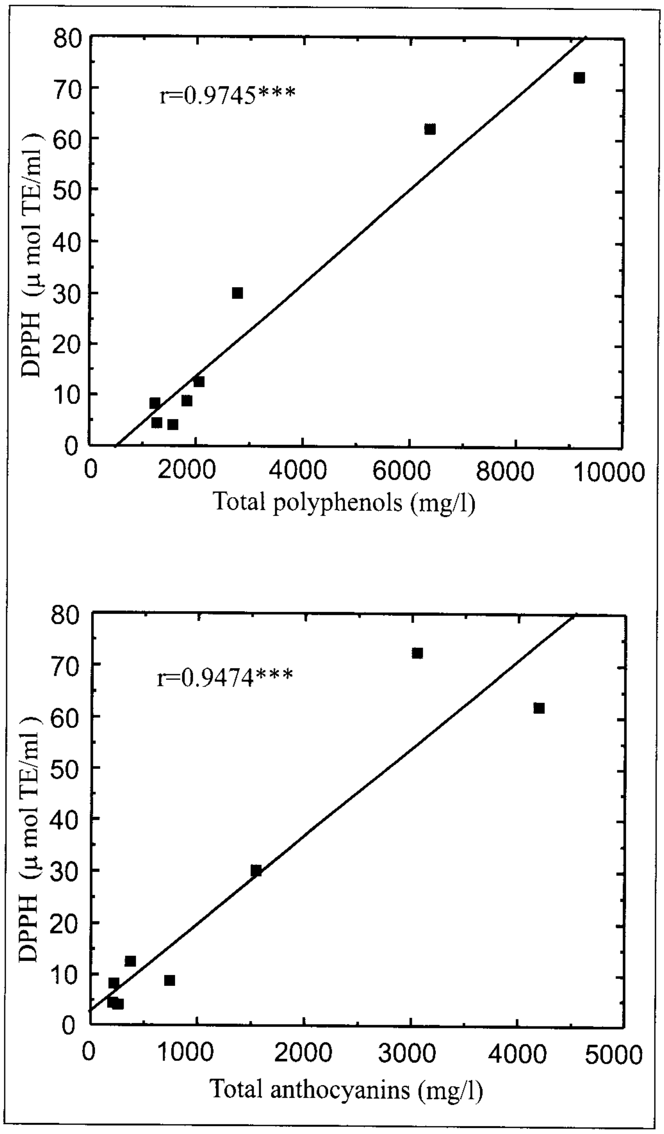

Fig. 2 Correlation plots of DPPH values versus total polyphenol and total anthocyanin contents of red fruit juices. The correlation coefficients (r) are marked in each plot; significant correlations are marked with asterisks (*** = significance at P ≤ 0.001)

(r = 0.95***) but the correlation coefficient was lower for total anthocyanins versus antioxidant activity than for the total polyphenols versus antioxidant activity. According to the data presented by others, the best linear relationship was observed between antioxidant capacity and total polyphenols of various red fruits (r^2 = 0.96) than between antioxidant capacity and total anthocyanins (r^2 = 0.95)[12] which agrees with our results.

Red fruit juices evaluated in this study are showing significant variations in anthocyanin content and profile. Some juices have low amount of anthocyanins, like strawberry and red raspberry juice, whereas the amount of these phytochemicals is very high in chokeberry, elderberry and black currant juice. Anthocyanins are the predominant phenolic components in elderberry and black currant juice or represent significant part in total polyphenol concentration of chokeberry and blackberry juice. Every juice evaluated possess unique anthocyanin pattern. Chokeberry, elderberry, blackberry and sour cherry juice contain only cyanidin based pigments, black currant juice is characterized by delphinidin and cyanidin, and strawberry juice by pelargonidin derivatives. The major anthocyanins in red raspberry and sweet cherry juice are derivatives of cyanidin although peonidin (in sweet cherry juice) and pelargonidin derivatives (in red raspberry juice) are present in low amount. Although all juices can serve as a good source of bioactive phytochemicals in the human diet, chokeberry, elderberry and black currant juice stand out in high anthocyanin content and high antioxidant activity. From the view of the anthocyanin content and antioxidant activity these juices can be regarded as good candidates for raw materials in production of health beneficial functional foods. Moreover, delphinidin-3-glucoside, delphinidin-3-rutinoside and cyanidin-3-glucoside were reported to have strong antiradical activity among various anthocyanins[32]. Therefore, black currant (which is abundant in delphinidin derivatives) and blackberry (abundant in cyanidin-3-glucoside) juice can serve as good source of these individual anthocyanins.

Acknowledgements
This work was supported by the Croatian Ministry of Science, Education, and Sports (Project number 0113006).

References
1) *Kris-Etherton, P. M., K. D. Hecker, A. Bonanome, S. M. Coval, A. E. Binkoski, K. F. Hilpert, A. E. Griel,* and *T. D. Etherton*: Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. Am. J. Med. **113,** 71–88 (2002).
2) *Joshipura, K. J., F. B. Hu, J. E. Manson, M. J. Stampfer, E. B. Rimm, F. E. Speizer, G. Colditz, A. Ascheiro, B. Rosner, D. Spiegelman,* and *W. C. Willett*: The effect of fruit and vegetable intake on risk for coronary heart disease. Ann. Intern. Med. **134,** 1106–1114 (2001).
3) *Knekt, P., J. Kumpulainen, R. Järvinen., H. Rissanen, M. Heliövaara, A. Reunanen,* and *T. Hakulinen*: Flavonoid intake and risk of chronic diseases. Am. J. Clin. Nut. **76,** 560–568 (2002).
4) *Le Marchand, L., S. P. Murphy, J. H. Hankin, L. R. Wilkens,* and *L. N. Kolonel*: Intake of flavonoids and lung cancer. J. Natl. Cancer Inst. **92,** 154–160 (2000).
5) *Garcia-Closas, R., C. A. Gonzalez, A. Agudo,* and *E. Riboli*: Intake of specific carotenoids and flavonoids and the risk of gastric cancer in Spain. Cancer Causes Control **10,** 71–75 (1999).
6) *Shi, H., N. Noguchi,* and *E. Niki*: Introducing natural antioxidants. In *Pokorny, J., N. Yanishlieva,* and *M. Gordon* (eds.): Antioxidants in food, pp. 147–158, Woodhead Publishing Limited, Cambridge (2001).
7) *Clifford, M. N.*: Anthocyanins-nature occurence and dietary burden. J. Sci. Food Agr. **80,** 1063–1072 (2000).
8) *Nakajima, J., I. Tanaka, S. Seo, M. Yamazaki,* and *K. Saito*: LC/PDA/ESI-MS profiling and radical scavenging activity of anthocyanins in various berries. J. Biomed. Biotechnol. **5,** 241–247 (2004).
9) *Benvenuti, S., F. Pellati, M. Melegari,* and *D. Bertelli:* Polyphenols, anthocyanins, ascorbic acid, and radical scavenging activity of *Rubus, Ribes,* and *Aronia.* J. Food Sci. **69,** 164–169 (2004).
10) *Mozetič, B.,* and *P. Trebše*: Identification of sweet cherry anthocyanins and hydroxycinnamic acids using HPLC coupled with DAD and MS detector. Acta Chim. Slov. **51,** 151–158 (2004).
11) *Wu, X.,* and *R. L. Prior*: Systematic identification and characterization of anthocyanins by HPLC-ESI-MS/MS in common foods in the United States: fruits and berries. J. Agr. Food Chem. **53,** 2589–2599 (2005).
12) *Wu, X., L. Gu, R. L. Prior,* and *S. McKay*: Characterization of anthocyanins and proanthocyanidins in some cultivars of *Ribes, Aronia,* and *Sambucus* and their antioxidant capacity. J. Agr. Food Chem. **52,** 7846–7856 (2004).
13) *Kim, D. O., H. J. Heo, Y. J. Kim, H. S. Yang,* and *C. Y. Lee*: Sweet and sour cherry phenolics and their protective effects on neuronal cells. J. Agr. Food Chem. **53,** 9921–9927 (2005).
14) *Kähkönen, M. P., J. Heinämäki, V. Ollilainen,* and *M. Heinonen*: Berry anthocyanins: isolation, identification and antioxidant activities. J. Sci. Food Agr. **83,** 1403–1411 (2003).
15) *Netzel, M., G. Strass, M. Herbst, H. Dietrich, R. Bitsch,* and *T. Frank*: The excretion and biological antioxidant activity of elderberry antioxidants in healthy humans. Food Res. Int. **38,** 905–910 (2005).
16) *Mülleder, U., M. Murkovic,* and *W. Pfannhauser*: Urinary excretion of cyanidin glycosides. J. Biochem. Biophys. Methods **53,** 61–66 (2002).
17) *Miyazawa, T., K. Nakagawa, M. Kudo, K. Muraishi,* and *K. Someya*: Direct intestinal absorption of red fruit anthocyanins, cyanidin-3-glucoside and cyanidin-3,5-diglucoside, into rats and humans. J. Agr. Food Chem. **47,** 1093–1091 (1999).
18) *Milbury, P. E., G. Cao, R. L. Prior,* and *J. Blumberg*: Bioavailability of elderberry anthocyanins. Mech. Ageing Dev. **123,** 997–1006, (2002).
19) *Chen,P. N., S. C. Chu, H. L. Chiou, W. H. Kuo, C. L. Chiang,* and *Y. S. Hsieh*: Mulberry anthocyanins, cyanidin 3-rutinoside and cyanidin 3-glucoside, exhibited an inhibitory effect on the migration and invasion of a human lung cancer cell line. Cancer Lett. **235,** 248–259 (2006).
20) *Netzel, M., G. Strass, C. Kaul. I. Bitsch, H. Dietrich,* and *R. Bitsch*: In vivo antioxidative capacity of a composite berry juice. Food Res. Int. **35,** 213–216 (2002).
21) *Bub, A., B. Watzl, M. Blockhaus, K. Briviba, U. Liegibel, H. Müller, B. L. Pool-Zobel,* and *G. Rechkemmer*: Fruit juice consumption modulates antioxidant status, immune status and DNA damage. J. Nutr. Biochem. **14,** 90–98. (2003).
22) *Heinonen, I. M.,* and *A. S. Meyer*: Antioxidants in fruits, berries and vegetables. In *Jongen, W.* (ed.): Fruit and vegetable processing, pp.23–51, Woodhead Publishing Limited, Cambridge (2002).
23) *Fan-Chiang, H. J.,* and *R. E. Wrolstad*: Anthocyanin pigment composition of blackberries. J. Food Sci. **70,** C198–C202 (2005).
24) *Blando, F., C. Gerardi,* and *I. Nicoletti*: Sour cherry (*Prunus cerasus* L.) anthocyanins as ingredients for functional foods. J. Biomed. Biotechnol. **5,** 253–258 (2004).
25) *Blando, F., A. P. Scardino, L. De Bellis, I. Nicoletti,* and *G. Giovinazzo*: Characterization of in vitro anthocyanin-producing sour chery (*Prunus cerasus* L.) callus cultures. Food Res. Int. **38,** 937–942 (2005).
26) *Gill, M. I., D. M. Holcroft,* and *A. A. Kader*: Changes in strawberry anthocyanins and other polyphenols in response to carbon dioxide treatments. J. Agr. Food Chem. **45,** 1662–1667 (1997).
27) *De Ancos, B., E. Gonzalez,* and *M. P. Cano*: Differentiation of raspberry varieties according to anthocyanin composition. Z. Lebensm. Unters. Forsch. A **208,** 33–38 (1999).
28) *Bermúdez-Soto, M.,* and *F. A. Tomás-Barberán*: Evaluation of commercial red fruit juice concentrates as ingredients for antioxidant functional juices. Eur. Food Res. Technol. **219,** 133–141 (2004).
29) *Goiffon, J., P. P. Mouly,* and *E. M. Gaydou*: Anthocyanic pigment determination in red fruit juices, concentrated juices and syrups using liquid chromatography. Anal. Chim. Acta **382,** 39–50 (1999).
30) *Waterhouse, A.*: (May, 10th 2006). http://waterhouse.ucdavis.edu/phenol/folinmicro.htm
31) *Giusti, M. M.,* and *R. E. Wrolstad*: Anthocyanins. Characterization and measurement with UV-visible spectroscopy. In *Wrolstad, R. E.* (ed.): Current protocols in food analytical chemistry, pp. F1.2.1.–F1.2.13. Wiley, New York (2001).
32) *Kähkönen, M. P.,* and *M. Heinonen*: Antioxidant activity of anthocyanins and their aglycons. J. Agr. Food Chem. **51,** 628–633 (2003).