HPLC CHARACTERIZATION OF COMMERCIAL AVAILABLE JUICES FROM ROMANIA

CARACTERIZAREA CROMATOGRAFICĂ A UNOR SUCURI DE PE PIAȚA ROMANEASCĂ

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Abstract. *Commercial available juices of beetroot (Beta vulgaris), elderberry (Sambucus nigra) and chokeberry (Aronia melanocarpa), were purified using C18 SPE cartridges in order to obtain anthocyanin-rich fraction. However obtained extracts were analyzed by high-performance liquid chromatography coupled with photodiode array detection and also electrospray ionization-mass spectrometry (LC/PDA/ESI-MS). Chokeberry and elderberry purified extract contained 7 kinds of glycosilated anthocyanins. The major ones were cyanidin-3-O-sambubioside and cyanidin-3-O-galactoside in elderberry, chokeberry respectively. Beetroot juice was found to contain another type of plant pigments, betalains which were recently studied for their antioxidant potential. Total polyphenol (TP) content was analyzed by using Folin-Ciocalteu method.*
Keywords*: Berry juices, anthocyanins, betacyanins, HPLC*

Rezumat. *Sucurile comerciale de sfeclă roșie (Beta vulgaris), soc (Sambucus nigra) și aronia (Aronia melanocarpa), au fost purificate prin tehnica SPE folosind coloanele C18 Sep Pack, în scopul de a obține fracții bogate de antociani. Prin urmare extractele obținute au fost analizate prin Cromatografia de lichide de înaltă performanță cuplată cu dioda şi de asemenea, spectrometrie de masă (LC/PDA/ESI-MS). Extractele purificate de aronia şi soc conțin 7 tipuri de antociani glicozilați. Antocianii majoritari au fost cianidin-3-O-sambubiozid și cianidin-3-O-galactozid în soc, aronia respectiv. Sucul de sfecla rosie conține un alt tip de pigmenți naturali, betalanine, studiate recent pentru potențialul lor antioxidant. Evaluarea polifenolilor totali a fost realizată prin utilizarea metodei Folin-Ciocalteu.*
Cuvinte cheie: *sucuri, fructe, antociani, batalanine, HPLC*

INTRODUCTION

A number of different fruits and vegetables beverage shows to have antioxidant potential due to their high content of polyphenols especially anthocyanins. Blueberry, elderberry and chokeberry are the most common *berries* consume in Romania, have strong antioxidant activity and related health benefits.

Anthocyanins are water soluble pigments belonging to the class of plant secondary metabolites named flavonoids. They are present in different

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plant organs, such as fruits, flowers, stems, leaves and roots (Brouillard, 1982). The main sources of anthocyanin are red fruits, mainly berries and red grapes, cereals, principally purple maize, vegetables, red wine among drinks, also anthocyanins can be found in roots, bulbs, grasses and many other sources (Giusti and Wrolstad, 2003, Markham et al., 2000). The large variety of anthocyanins sources found in nature makes them the most complex and interesting group of plant pigments. When this class of compounds is missing from the plant sometimes they are replaced with betalanins. Anthocyanins and betalains have never been reported in the same plant till now (Henriette M.C.Azeredo, 2006). Betalains are water-soluble nitrogen-containing pigments, synthesized from the amino acid tyrosine into two structural groups: betacyanins, having red-violet color and betaxanthins which is yellow-orange (Henriette M.C.Azeredo, 2006). The main sources of betalains are red and yellow beetroot (*Beta vulgaris* L. ssp. *vulgaris*), seeds or amaranth leaf (*Amaranthus* sp.) (Cai et al., 1998). In order to their isolation and purification different kinds of chromatographic methods have been developed such as paper chromatography, thin-layer chromatography, column chromatography high speed countercurrent chromatography and high-performance liquid chromatography. The most convenient method is solid-phase extraction (SPE) because is the simplest, most effective and do not imply any sophisticate equipment low cost (He and Giusti 2011). The objectives of the current study were (1) isolation of anthocyanin rich fraction from commercial juices using C18 SPE cartridges (2) HPLC characterization of obtained fraction, (3) determination of total polyphenol.

MATERIAL AND METHOD

Anthocyanin Sources. Commercially available bio chokeberry, beetroot and elderberry juices were purchased from available local market.

Chemicals All HPLC reagents, anthocyanin standards were purchased from Sigma Aldrich (Germany). Gallic acid (GAE) (purity 97.5%), 2, 2-diphenyl-1-picrylhydrazyl (DPPH) 95% purity, were obtained also from Sigma-Aldrich (Darmstadt, Germany).

Anthocyanins semipurification was done according to the protocol described by Giusti and Wrolstad, 2008.

HPLC-PDA identification and quantification of anthocyanin. Analyses were performed on a Shimadzu HPLC system equipped with a binary pump delivery system LC-20 AT (Prominence), a degasser DGU-20 A3 (Prominence), diode-array SPDM20 A UV-VIS detector (DAD) and a Luna Phenomenex C-18 column (5µm, 25 cm x 4.6 mm). The mobile phase consisted in: solvent A - formic acid (4.5%) in bidistilled water and solvent B - acetonitrile. The gradient elution system was: 10% B, 0-9 min; 12% B, 9-17 min; 25% B 17-30 min; 90% B, 30-50 min; 10% B, 50-55 min. The flow rate was 0.8 ml/min and the analyses were performed at 35°C. The chromatograms were monitored at 520 nm. The anthocyanin quantification was performed using cyanidin-3-*O*-sambubioside.

HPLC–MS Analysis were performed with a Quadrupole 6110 mass spectrometer (Agilent Technologies, Chelmsford, MA) equipped with an ESI probe equipped with an electrospray ion source. Mass spectra were recorded between 260 and 1000 m/z.

Total phenolic content. The total concentration of polyphenol in the purified juices was determined using Folin-Ciocalteu colorimetric method with minor modifications

(Singleton et al., 1999. The results were expressed as milligram of gallic acid per 100 grams.

RESULTS AND DISCUSSION

Prior to chromatographic analysis the purified anthocyanin rich-fraction was dissolved in acidified water and filtered through 0.45μm.The quantification was done using cyanidin 3-*O*-sambubioside standard curve. Anthocyanin identification of purified juices was done using were identified based on their retention time, UV-VIS spectra compared with pure standards and also were compared with anthocyanin profiles of fruit juice found in literature. (Heinonen et al., 2002). As a confirmation LC/PDA/ESI-MS analysis was done. Peaks identification is summarized din Table 1. Both chokeberry and elderberry juices content only cyanidin based pigment.

Chokeberry juice contained a mixture of four different cyanidin-glycosides. Cyanidin-3-galactoside and cyanidin-3-arabinoside were found to be the majors compounds, these anthocyanins represented together about 60% from total anthocyanin content. Cyanidin-3-xyloside (57.3 mg Cy-3-sam /100ml FJ) and cyanidin-3-glucoside (68.5 mg Cy-3-sam /ml FJ) were found in low concentration. Published data are in accordance with the results obtained in this study for chokeberry juice (Nakajima J., et al., 2004, Lidija Jakobek et al., 2007). Total anthocyanin content obtained for elderberry juice was 416.9 mg cy-3-same/100 ml FJ. The majors anthocyanins identified were cyanidin-3-*O*-sambubioside-5-glucosid and cyanidin-3-*O*-sambubioside representing 40% and 21% respectively from total anthocyanin content. The obtained results are in good correlation with data reported previously (Lidija Jakobek et al., 2007).

Beetroot juice was characterized by the presence of betalanins, another class of water-soluble plant pigments. In this case the major compound was Betanin, representing about 42%. The obtained data was similar whit those found in literature (Czapski, 1990, , Florian et al., 2006, Peter et al., 2011). Concentrations of anthocyanins in analyzed juices (mg/100ml), calculated using HPLC method and percentage distribution of anthocyanin are presented in Table 2. The recorded PDA chromatograms of purified juices (520nm absorption) are shows in Figure 1.

Table 1

Compounds, UV-Vis and mass spectral data of analyzed juices.

Sample name	Peak no	Identified compound	λ_{max}	[M + H]+ ion, fragments)
Beetroot	1	Betanin	245, 536	551/549/389
	2	Isobetanin	290, 536	551/549/389
	3	Betanidin	244, 534	551/398
	4	Neobetd 5-glc	245, 469	549.1/387
	1	Cyanidin-3-*O*-sam-5-glucoside	279, 528	743, 611, 287
	2	Cyanidin-3,5 -diglucoside	278, 518	611, 449, 287

Elderberry	3	ND	280, 518	
	4	Cyanidin-3-*O*-sambubioside	279, 518	581/287
	5	Cyanidin-3-*O*-glucoside	279, 517	449 /287
Chokeberry	1	Cyanidin-3-*O*-galactoside	279, 517	449 /287
	2	Cyanidin-3-*O*-glucoside	280, 517	449/ 287
	3	Cyanidin-3-O-arabinoside	279, 517	419 /287
	4	Cyanidin-3-O-xiloside	279, 517	454/ 287

Total polyphenol (TP) values in purified analyzed juices as gallic acid equivalents were in the range of 82.9-110 mg GAE/100 ml. Among all the samples analyzed, beetroot juice shows to have the highest total phenolics content (110mg GAE/100ml) follow by elderberry (54.3 mg GAE/100ml) and chokeberry (28.9 mg GAE/100ml). In a previous study, Lidija Jakobek et al., 2007, reported values comparable with those obtained in this study (91.5 mg GAE/100ml for chokeberry juice and 63.6 mg GAE/100ml for elderberry juice. The results are presented in Table 2.

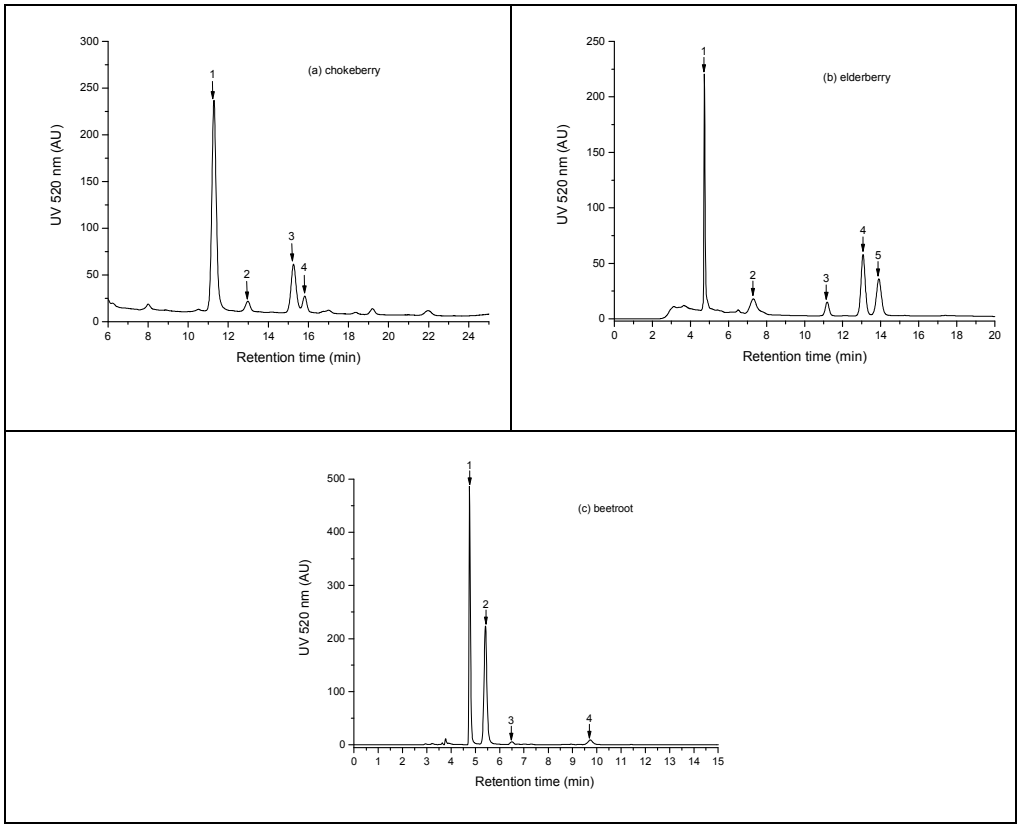

Fig. 1 - HPLC-PDA chromatograms of purified juices (a) Chokeberry; (b) Elderberry; beetroot (c). Peaks identification is shown in Table 1.

Table 2

Concentration of anthocyanins and polyphenol in commercial juices analyzed

Juice/Compound	Concentration Cy-3-sam mg/100ml	Total anthocyanins %	Total polyphenol content mgGAE/100ml
Beetroot			
Betanin	381.4	42.5	110
Isobetanin	374,4	41.7	
Betanidin	63.4	7.08	
Neobetd 5-glc	77.23	8.6	
Total	**895.5**	100	
Elderberry			
Cyanidin-3-*O*-sambubioside-5-glucosid	184.7	40	54.3
Cyanidin-3,5 -diglucoside	57.1	12.4	
unknown	42.3	9.2	
Cyanidin-3-*O*-sambubioside	98.6	21.4	
Cyanidin-3-*O*-glucoside	79	17.1	
Total	**416.9**	100	
Chokeberry			
Cyanidin-3-*O*-galactoside	79.8	37.4	82.9
Cyanidin-3-*O*-glucoside	68.5	19.3	
Cyanidin-3-O-arabinoside	152.9	23.4	
Cyanidin-3-O-xiloside	57.3	19.8	
Total	**358.69**	100	

CONCLUSIONS

1. Cyanidin glycosides are the predominant anthocyanins in chokeberry and elderberry purified extract

2. Beetroot purified juice was found to be rich in another class of plant pigment, betalains.

3. Moreover this study demonstrates that phytochemical composition of commercial available juices is complex and complete and it makes these juices to be recommended as daily consumption.

4. This is the first paper to report anthocyanin, betalanin and polyphenol composition of commercial available juices in Romania.

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