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ORIGINAL PAPER

Comparative efficiency of different solvents for the anthocyanins extraction from chokeberries and black carrots, to preserve their antioxidant activity

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Abstract
Anthocyanins occur naturally in many blue-, purple- and red-colored fruits and vegetables, and are commonly found in the human diet as natural colorants with proven health benefits. This work aimed to find the most efficient solvents for the anthocyanins extraction from natural matrices (chokeberries and black carrots), able to preserve their antioxidant activity. Four different acidified solvents (methanol, ethanol, acetone, and water) were tested and the extracts were characterized by UV–Vis spectroscopy and High-Performance Liquid Chromatography coupled with Mass Spectrometry. The anthocyanins profile of each extract has been identified. Five monoglycosylated anthocyanins were found in chokeberries and ten anthocyanins (four acylated and four diglycosylated) in black carrots. The antioxidant activities of all extracts (using ABTS, CUPRAC and FRAP assays) were determined concomitantly and ranked. The most efficient extraction was obtained using ethanol and methanol, such extracts showing the highest antioxidant activity for both matrices (black carrots and chokeberry).

Keywords Anthocyanins · Antioxidant activity · Black carrot · Chokeberry · LC–ESI–MS · Solvents

Introduction

Anthocyanins are a subgroup of flavonoids responsible for the blue, purple and red color of many fruits and vegetables that are a common presence in the human diet. Considering the health benefits associated with anthocyanin consumption, these are the proper ingredients for the design of new food products or food supplements (Esatbeyoglu et al. 2016; Li et al. 2017). The changing stability of anthocyanins according to pH, oxygen, light and temperature, the presence of antioxidants as ascorbic acid in their environment, but also their chemical structure impose some limitations on their industrial use (Brenes et al. 2005). Based on this, solvents are needed for the efficient extraction of anthocyanins able to preserve their physical and antioxidant properties. Solvents such as small organic alcohols are preferred for food applications since they can penetrate efficiently the plant tissue, to exert a good affinity and high solubility for anthocyanins (Capello et al. 2007). By now, the acidified organic solvents such as water, acetone, methanol, and ethanol have been considered the most effective extractants for anthocyanins. The acidification improves the stability of anthocyanins, and is usually done with weaker organic acids such as formic or acetic acid, but can also be realized with hydrochloric acid or other mineral acids (Jakobek et al. 2007; Kähkönen et al. 2003). In the presence of moderate to strong acidic conditions and moderate heat, the glycosidic bonds of anthocyanins will be hydrolysed.

In natural sources, the anthocyanidins (aglycones) are found mainly glycosylated with one or more sugar moieties (glucose, xylose, galactose, arabinose), thus forming anthocyanins (Wang and Stoner 2008) or acylated with organic aromatic or aliphatic phenolic acids (cinnamic acid, ferulic,

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synaptic or acetic acids). It was found that in fruits like *Aronia melanocarpa,* the main anthocyanins are derived from cyanidin, glycosylated at the 3-OH position, in contrast to black carrots (*Daucus carota* sp.) where cyanidin-based anthocyanins are mainly acylated (Schwarz et al. 2004).

It is known that the stability and the antioxidant activity of anthocyanins are strongly correlated with their structural characteristics including the type of sugar, number, and position of substituents on the aglycone. No significant difference in the antioxidant activity between cyanidin with glucose or galactose was observed, but cyanidin-3-arabinoside showed less activity than cyanidin-3-glucoside (Kahkonen and Heinonen 2003). Cyanidin aglycon, having more hydroxyl groups, proved to have a higher scavenging activity than pelargonidin; it allows the stabilization of a semiquinone radical and the formation of a stable quinone product (Ali et al. 2016). It was recently reported that the acylated anthocyanins have better stability but a lower antioxidant capacity than the non-acylated ones. The explanation could be that sugar acylation is blocking the hydrogen transfer from hydroxyl groups to the unpaired electrons.

Certainly, the extraction solvent influences the yield of anthocyanins and the overall quality of them, but there is an urgent need to know how efficient was the extraction and what effects on the chemical structure and antioxidant activity of natural compounds solvent have. In this context, researchers are continuously studying the influence of different types of solvents on the anthocyanin's extraction. Silva et al. worked with blueberries as an anthocyanins source and tested for these class of compounds, the extraction procedure of four solvent types: water, ethanol, methanol and acetone, acidified (0.01% HCl) or not (Silva et al. 2017). They observed that out of all solvents tested, acidified ethanol proved to be the best when seeking to obtain a high anthocyanin extract. Moreover, in this study, a comparison between the extraction efficiency of the non-acidified and acidified solvent revealed that methanol and ethanol exhibited the highest capacity to extract anthocyanins, without any difference in yield if the acidification was done. Instead, non-acidified acetone and water demonstrated that both are poor solvents for anthocyanins extraction.

However, by comparing ethanolic (50% v/v aqueous ethanol) and methanolic (90% v/v aqueous ethanol) extracts using the standardized pH differential method, the anthocyanin concentrations of different plum ethanolic extracts were consistently lower. This statement was also supported by HPLC profiling of the anthocyanin content, which showed lower levels of anthocyanins as compared to the methanolic extracts (Johnson et al. 2020).

Anthocyanins, from saffron bio-residues extracted with ethanol (59% v/v) in different solid–solvent ratio (1:10, 1:20, 1:30, 1:50 g/ml), by three advanced extraction technique: conventional solid–liquid extraction (CSLE), ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE). Using a kinetic modeling, it was concluded that the UAE at 1:30 solvent–liquid ratio was an effective method of anthocyanins extraction from floral saffron bio-residues with advantages like lower extraction time and higher extraction yields compared to CSLE and MAE (Da Porto and Natolino 2018).

In this context, our study aimed to examine the effect of different solvents on the composition of anthocyanins extracts and the subsequent antioxidant activity, using chokeberries (*Aronia melanocarpa*) and black carrots (*Daucus carota* sp.) as commonly human-consumed natural anthocyanin-rich sources. Their antioxidant activity was ranked on the resulted data from the three single-electron transfer (SET)-based assays. The best solvents should be chosen according to the type of anthocyanins, as a safe way to preserve the anthocyanins' antioxidant activity.

Materials and methods

Reagents

For extraction and spectrometric or chromatographic analysis, the reagents used were of analytical grade: cyanidin-3-glucoside and cyanidin-3-galactoside were purchased from Extrasynthese (Lyon, France), dimethylsulfoxide (DMSO) from Merck Group (Darmstadt, Germany), hydrochloric acid from VWR International (Radnor, PA, USA), methanol and acetone used for extraction were purchased from Chempur (Karlsruhe, Germany) and ethanol from the Chemical Company (Iasi, Romania).

Preparation of extracts

A mass of 5 g of fresh chokeberries or 10 g of fresh black carrot roots was finely chopped using an Ultraturax (model Miccra D-9 KT; Digitronic GmbH, Bergheim, Germany) and each matrix was subjected to the extraction procedure. Therefore, in triplicate for each matrix, 4 different extracts were obtained, each one with a different solvent: 10-ml methanol (MeOH) 95%, 10-ml ethanol (EtOH) 98%, 10-ml acetone (AC) (70% in water), 10-ml distilled water (WA). All solvents contained 0.01% HCl. Next, the extracts were filtered and the solvents were concentrated in a vacuum rotary evaporator (Rotavapor® model R-124; Buchi, Flawil, Switzerland), at 39 °C and re-dissolved in acidified water.

Determination of total anthocyanins content

The total anthocyanins content (TAC), expressed as cyanidin-3-glucoside equivalents, was determined and calculated using the differential pH method (Giusti and Wrolstad

2001). Each extract was diluted (1:50) using sodium acetate buffer (0.4 M) at pH 1.0 and pH 4.5 and incubated 15 min in the dark. Finally, the extract absorbances were read at 520 nm and 700 nm using the UV–Vis Spectrophotometer (Jasco V-630, International Co. Ltd, Japan). The TAC values were calculated using the following equation: $TAC = A \times MW \times DF \times 1000/\varepsilon x1$.

The results were expressed as mg cyanidin-3-glucoside equivalent per 100-g fresh weight using its molar absorptivity value 34,300 in HCl (ε), and the value of 484.8 for its molecular weight (MW). The dilution factor (DF) was specific to each extract.

Anthocyanins separation and identification using liquid chromatography (HPLC) coupled with photodiode array (PDA) and with electrospray ionization mass spectrometry (ESI^+–MS)

For the HPLC–PDA analysis, all samples were run on an Agilent Technologies 1200 HPLC system (Chelmsford, MA) equipped with G1311A Quaternary Pump, G1322A degasser, G1329A autosampler, and G1315D photo-diode array (PDA) detector. Volumes of 20 µl were injected in the Luna Phenomenex C-18 column (5 µm, 25 cm × 4.6 mm). The mobile phase consisted of 4.5% formic acid in bidistilled water (solvent A) and acetonitrile (solvent B). The flow was maintained at 0.8 ml/min. The PDA full spectra were recorded at 520 nm.

The LC–ESI^+–MS data were recorded by directing the LC flow to a Quadrupole 6110 mass spectrometer (Agilent Technologies, Chelmsford, MA) equipped with an ESI probe. The spray voltage was set at 3000 V. Nitrogen was used as nebulizer gas and nebulizer pressure was set to 40 psi with a source temperature of 100 °C. Mass spectra were acquired in positive ion and full scan mode in a range of 260–1000 m/z. Molecular ions and fragment ions were determined by setting the fragmentation voltage at 70 and 130 eV. The identification of anthocyanins was carried out based on the elution order, molecular mass (m/z) and occurrence of fragments, as well compared with the literature data. The quantification of anthocyanins was done using cyanidin-3-galactoside as standard for the calibration curve (data not shown).

Antioxidant activity assays

Scavenging effect on $ABTS^+$radical

The scavenging ability of each extract against the radical anion $ABTS^+$ was determined in 96-well plates according to the procedure described previously (Arnao et al. 2001). Absorbance was measured at 734 nm, after 6 min of incubation in the dark at room temperature, with the microplate reader (BioTek Instruments, Winooski, VT). Results were expressed as mM Trolox/g fresh weight (FW).

Cupric reducing antioxidant capacity (CUPRAC) assay

The cupric ion-reducing antioxidant capacity of each extract was determined according to a previously described method (Apak et al. 2007). Each absorbance was measured with the spectrophotometer (JASCO V-630 series, International Co., Ltd., Japan) at 450 nm against the blank reagent. The standard curve was prepared with different Trolox concentrations and the results were expressed as mM Trolox/ g FW.

Ferric reducing/antioxidant power (FRAP) assay

This determination is based on the reduction of ferric 2,4,6-tris(2-pyridyl)-1,3,5-triazine [Fe (III)-TPTZ] to the ferrous complex at a low pH, followed by spectrophotometric recording (Benzie and Strain 1999). The reagent was prepared by mixing 10-mmol 2,4,6-Tris(2-pyridyl)-triazine (TPTZ)/L reagent with 40-mmol/L ferric chloride in acetate buffer (pH 3.6). The results were calculated and expressed as mM Trolox/ g FW.

Global antioxidant capacity

The weighted mean which reflects the global antioxidant capacity of each extract, using ABTS, CUPRAC, and FRAP, was calculated, being expressed in mM Trolox/g FW, according to the model previously published (Tabart et al. 2009). This global antioxidant capacity was applied for the ranking of extracts depending on the solvent type.

Results and discussion

Total anthocyanin content

The total anthocyanin content (TAC) *t* for each extract obtained from the selected fresh matrices, chokeberry fruits and black carrot roots, was determined by the pH differential method (Table 1). For chokeberry extracts, TAC values ranged between 23.42 ± 1.50 mg Cy-3-glu/100 g FW (WA) and 240.40 ± 17.50 mg/100 g FW (EtOH). Literature data suport our TAC values, calculated by the pH differential method, and expresses values in the same interval scale, ranging from 176.18 ± 19.4 to 366.16 ± 1.4 mg/100 g FW for methanolic extracts of *Aronia* cultivars samples (Rugina et al. 2015). Recently, for methanolic extracts from *Aronia melanocarpa*, the TAC values were reported to range from 141 ± 9 to 147 ± 17 mg/100 g dry weight (DW) (Tolić et al. 2015).

Table 1 The total anthocyanins content (TAC) expressed in mg Cy-3-glu/100 g FW from chokeberries and black carrots using MeOH, EtOH, AC and WA extracts

Sample (mg Cy-3-glu/ 100 g FW)	MeOH	EtOH	AC	WA
Chokeberry	240.5 ± 17.50	225.4 ± 20.55	116.7 ± 11.66	23.4 ± 1.50
Black carrots	275.9 ± 20.22^{ns}	383.1 ± 30.20**	221.7 ± 20.18**	18.3 ± 1.10**

The results are expressed as means ± SD. Statistical analysis of all the data between groups were performed by Student's *t*-test. The *p*-value ≤ 0.05 was considered statistically significant

*($p < 0.05$)

**($p < 0.01$)

^{ns}($p < 0.05$)

Data obtained here for TAC of black carrots extracts varied between 18.27 ± 1.10 (WA) and 383.05 ± 30.20 (EtOH), being in the same interval scale as values found in literature for ethanolic black carrot extracts 270.3 ± 27.12 mg/100 g (Saleema et al. 2018), or 93.8 ± 3 mg/100 g FW and 126.4 ± 6 mg/100 g FW (Ćujić et al. 2016). Instead, TAC values for an anthocyanins extract obtained with a mixture of MEOH:WA:EtOH (70/29.5/0.5, v/v/v) from black carrots, by an UV–VIS spectrophotometric method, were 33.81 mg/100 g FW (Smeriglio et al. 2018).

Certainly, differences between TAC values in literature are likely to appear due to different steps in extraction procedure included by other authors, or other solvents mixture used in extraction, or even from the different anthocyanins content in different varieties of chokeberries/black carrots used in experiments. However, from both matrices selected here, the black carrot roots prove to have larger content of anthocyanins than chokeberries.

Data reported here are in agreement with other literature reports, for red fruits, and sustains that ethanol is the best solvent when compared to water, acetone, hexane, ethyl acetate and methanol (Galvan d'Alessandro et al. 2012; Lao and Giusti 2018), and methanol as well, as a second choice (Canuto et al. 2016; Ştefănuţ et al. 2011; Wang et al. 2016). Both solvents are economically affordable and could be classified as food-friendly solvents (Alfonsi et al. 2008), which makes anthocyanins extracts available for food industry utilization.

Separation, identification, and quantification of anthocyanins by HPLC–PDA and LC–ESI$^+$–MS analysis

Data resulting from the ESI$^+$–MS identification, including retention times, molecular ions and m/z values of the aglycons can be found in Table 2.

In chokeberries, five anthocyanins were identified: cyanidin-3-galactoside (1), cyanidin-3-glucoside (2) cyanidin-3-arabinoside (3), cyanidin-3-xyloside (4), and a minor cyanidin-pentoside derivative (5) (Fig. 1), data similar with previously published ones (Kulling and Rawel 2008; Määttä-Riihinen et al. 2004; Vlachojannis et al. 2015). Cyanidin-3-galactoside was identified as the major compound of the extract, with m/z = 449 and aglycon fragment m/z = 287 (cyanidin).

In the black carrots, 10 anthocyanin derivatives (peaks 6–15) were separated and identified in the black carrots, four of them being glycosylated and six as acylated derivatives (Table 1). Cyanidin-3-xylosyl-(feruloyl-glucosyl)-galactoside, with m/z = 919 and aglycon fragment m/z = 287 (cyanidin), was the main acylated anthocyanin found in black carrots. Other studies confirm it as major anthocyanins in black carrots (Gras et al. 2015, 2016).

LC–ESI$^+$–MS quantification analysis data sustained the statement resulting from pH differential analysis that EtOH and MeOH extraction capacity for anthocyanins was higher than AC and WA.

The major compound found in chokeberries, cyanidin-3-galactoside, accounted for 64.4% (147.1 ± 4.2 mg Cy-3-gal/100 g FW) of total anthocyanins in the methanol extracts; respectively, 65% (142.3 ± 2.14 mg Cy-3-gal/100 g FW) when the ethanol extraction was done. As can be observed, insignificant differences were obtained between methanolic and ethanolic extraction, in the anthocyanin content determined by LC–ESI$^+$–MS analysis (Tables 2, 3). The compounds identified here are consistent with those reported by other authors (Kulling and Rawel 2008; Määttä-Riihinen et al. 2004; Vlachojannis et al. 2015; Wu et al. 2004).

Total individual anthocyanins content in black carrots sample was higher when alcohols were used for their extraction. Cyanidin-3-xylosyl-(feruloyl-glucosyl)-galactoside accounted for 64.7% (193.4 ± 4.6 mg Cy-3-gal/100 g FW) when extraction was done with MeOH; respectively, 82.7% (303.0 ± 5.9 mg Cy-3-gal/100 g FW) when extraction procedure was realized with EtOH. Individual anthocyanins identified and quantified in black carrots (Table 2)

Table 2 Anthocyanins and their characteristic spectral data resulted from LC–ESI$^+$–MS analysis: chokeberries (peaks 1–5) and black carrots (peaks 6–17)

Peak	R_t(min)	m/z		UV–Vis λ_{max} (nm)	Compound
		M^+	Aglycon		
1	19.990	449	287	277, 524	Cy-3-galactoside
2	22.874	449	287	276, 516	Cy-3-glucoside
3	25.395	419	287	275, 526	Cy-3-arabinoside
4	31.209	419	287	278, 517	Cy-3-xyloside
5	33.814	419	287	276, 528	Cy-pentoside
6	17.31	743	287	295, 372, 517	Cy-3-xylosyl-glucosyl-galactoside
7	20.58	581	287	295, 328, 527	Cy-3-xylosyl-galactoside
8	25.82	949	287	289, 332, 533	Cy-3-xylosyl(sinapoylglucosyl) galactoside
9	26.43	863	287	243, 327, 534	Cy-3-xylosyl(p-hydroxybenzoyl glucosyl) galactoside
10	27.55	919	287	283, 331, 529	Cy-3-xylosyl(feruloylglucosyl) galactoside
11	28.72	889	287	292, 318, 527	Cy-3-xylosyl(cumaroylglucosyl) galactoside
12	29.31	595	301	294, 328, 530	Peo-3-xylosylgalactoside
13	30.21	933	301	295, 328, 533	Peo-3-xylosylglucosylgalactoside
14	30.74	963	301	290, 330, 530	Peo-3-xylosyl(sinapoylglucosyl) galactoside
15	31.12	903	301	287, 330, 530	Peo-3-xylosyl(feruloylglucosyl) galactoside

Cy cyanidin, *Peo* peonidine, *gal* galactoside, *glc* glucoside; *ara* arabinoside, *xyl* xyloside, *sin* sinapic acid, *p* hydroxybenzoic acid, *fer* ferulic acid, *coum* coumaric acid

are similar to the data found in literature (Algarra et al. 2014; Montilla et al. 2011; Sadilova et al. 2009).

Antioxidant capacity

In the current study, three single-electron transfer (SET)-based assays [2,20-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), cupric ion-reducing antioxidant capacity (CUPRAC) and ferri- reducing antioxidant power (FRAP)] were chosen, to measure the antioxidant capacity of chokeberries and black carrots extracts.

According to ABTS, CUPRAC, and FRAP assays data (Figs. 2, 3, 4), the highest antioxidant activity values for chokeberry extract were seen when methanolextraction was used. In contrast, in the case of the black carrot extract, ABTS, CUPRAC, FRAP data revealed the highest antioxidant activity when the ethanolic extraction was done. There are some possible explanations to be given regarding the different values obtained of antioxidant activities between assays for samples. For instance, a variation could come from the different reaction mechanism that each assay used. Moreover, other classes of antioxidant compounds found in the extract could influence the final antioxidant activity of the sample, even though the anthocyanins represent the major class of compounds.

Also, the different chemical structure of anthocyanins could contribute to the antioxidant activity of the sample. For instance, it is known that the increasing number of hydroxyl groups may enhance the antioxidant activity; meanwhile, glycosylation may diminish it when compared to that of the corresponding aglycons. But glycosylated non-acylated anthocyanins exert a higher antoxidant activity than the acylated ones (Zhao et al. 2017). The explanation for this could be that the acylation of the sugar improves the chemical stability of their molecular structure, thus blocking the transfer of a hydrogen atom from the hydroxyl groups to the unpaired electrons (Blando et al. 2018). This statement can be sustained by our data obtained by ABTS and FRAP assays, where chokeberry extract, containing monoglycosylated anthocyanins, exerted a higher antioxidant activity than that recorded for black carrots, having most acylated anthocyanins.

In chokeberries, the glycosylation site on anthocyanins is the 3 position, the preferred one, with the highest antioxidant activity. The order of antioxidant potency reported in the literature for sugars is

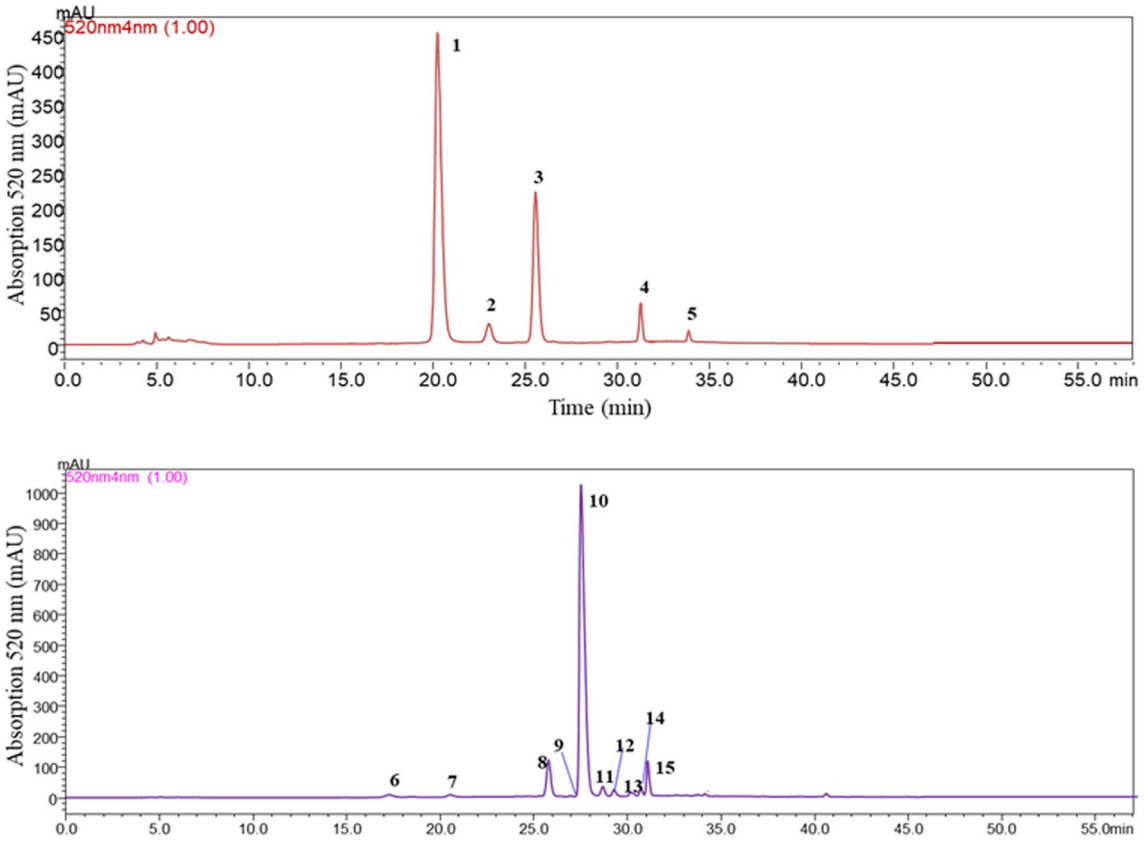

Fig.1 Chromatograms of chokeberries (1–5) and black carrots (6–15) from the MeOH extract, recorded at 520 nm. For peak identification see Table 1

3-glucoside > 3-rhamnoside > 3-arabinoside ≈ 3-galactoside (Zheng and Wang 2003).

Correlations between total anthocyanins content and antioxidant capacity

To be able to compare the different data resulted from the antioxidant assays, the antioxidant values for each assay were expressed as mM Trolox equivalents/g FW (Table 4) and the weighted average was calculated according to Tabart et al. (2009): the antioxidant capacity value of each sample (chokeberry and black carrot), as determined for each solvent (methanol, ethanol, acetone, water) by the specified method, was divided by the average capacity determined for the whole set of compounds by the same assay, summing the three (ABTS, CUPRAC, FRAP) results of this calculation, and dividing the sum by three. Thus, after the weighted mean calculation, a ranking order for extraction solvents was established (chokeberries methanol > ethanol > acetone > water and for black carrots ethanol > acetone > methanol > water) (Tabart et al. 2009).

To estalish the correlation between the antioxidant activity and the anthocyanins content, determined both by the colorimetric method (TAC) and the chromatographic one (TIA, total individual anthocyanins), the Pearson's correlation coefficient (r) was calculated for each sample. In the pH differential method, cyanidin-3-glucoside was used as standard for anthocyanins quantification, being the structural isomer of cyanidin-3-galactoside used in HPLC analysis. Thus, overall strong correlation between the antioxidant activity and TAC ($r = 0.97$ [ABTS], $r = 0.88$ [CUPRAC], $r = 0.94$ [FRAP]) could be observed for chokeberries extract, as well as for TIA ($r = 0.94$ [ABTS], $r = 0.83$ [CUPRAC], $r = 0.92$ [FRAP]). For the black carrots extract, Pearson's correlation showed a high interrelationship between antioxidant methods and TAC ($r = 0.92$ [ABTS], 0.91 [CUPRAC] and 0.76 [FRAP]); respectively, TIA ($r = 0.89$ [ABTS], 0.88 [CUPRAC] and 0.72 [FRAP]). These positive correlations between antioxidant assays and anthocyanin content suggest that the antioxidant capacity of both extracts would derive more from the presence of the anthocyanins in the extract, rather than from the contribution of other phenolic compounds.

Table 3 Individual anthocyanin concentration of chokeberries (peaks 1–5) and black carrots (peaks 6–15) samples extracted in MeOH, EtOH, AC and WA

Peak	Anthocyanins (mg Cy-3-gal/100 g FW)	MeOH	EtOH	AC	WA
1	Cy-3-galactoside	147.1 ± 4.2^{a}	142.3 ± 2.14^{a}	68.2 ± 2.81^{b}	21.4 ± 2.85^{c}
2	Cy-3-glucoside	8.91 ± 1.24^{a}	7.62 ± 1.20^{a}	3.66 ± 1.52^{bc}	1.91 ± 0.12^{c}
3	Cy-3-arabinoside	58.6 ± 2.14^{a}	52.6 ± 1.89^{b}	21.0 ± 2.49^{c}	9.03 ± 2.18^{d}
4	Cy-3-xyloside	9.45 ± 1.47^{a}	7.53 ± 2.54^{a}	3.11 ± 1.45^{bc}	1.94 ± 0.14^{c}
5	Cy-pentoside	4.07 ± 1.24^{ab}	6.73 ± 2.47^{a}	1.23 ± 0.21^{b}	/
	Total	228.1 ± 5.1	216.8 ± 2.0	97.3 ± 8.5	34.3 ± 1.1
6	Cy-3-xylosyl-glucosyl-galactoside	7.66 ± 1.45^{a}	5.95 ± 1.42^{ab}	4.56 ± 1.22^{b}	0.32 ± 0.01^{c}
7	Cy-3-xylosyl-galactoside	8.74 ± 0.89^{a}	4.87 ± 1.23^{b}	1.10 ± 0.54^{cd}	0.33 ± 0.02^{d}
8	Cy-3-xylosyl(sinapoylglucosyl)galactoside	12.6 ± 2.16^{a}	12.4 ± 2.81^{a}	4.39 ± 1.15^{bc}	1.03 ± 0.78^{c}
9	Cy-3-xylosyl(p-hydroxybenzoylglucosyl)galactoside	7.31 ± 1.48^{a}	1.27 ± 0.16^{cd}	1.56 ± 0.24^{bc}	0.29 ± 0.01^{cd}
10	Cy-3-xylosyl(feruloylglucosyl)galactoside	193.4 ± 4.6^{b}	303.0 ± 5.9^{a}	161.0 ± 4.23^{c}	10.4 ± 1.58^{d}
11	Cy-3-xylosyl(cumaroylglucosyl)galactoside	14.0 ± 1.87^{a}	11.4 ± 1.73^{ab}	9.67 ± 2.15^{b}	0.49 ± 0.01^{c}
12	Peo-3-xylosylgalactoside	8.11 ± 1.25^{a}	3.84 ± 0.78^{bc}	2.29 ± 0.58^{cd}	0.41 ± 0.01^{d}
13	Peo-3-xylosylglucosylgalactoside	/	/	17.2 ± 1.28^{a}	0.42 ± 0.01^{b}
14	Peo-3-xylosyl(sinapoylglucosyl)galactoside	/	/	2.05 ± 0.87^{a}	0.27 ± 0.01^{b}
15	Peo-3-xylosyl(feruloylglucosyl)galactoside	29.3 ± 2.58^{a}	18.7 ± 2.48^{b}	4.20 ± 1.25^{cd}	3.41 ± 0.06^{d}
	Total	281.2 ± 2.0	361.5 ± 2.0	208.0 ± 1.4	17.4 ± 0.3

All anthocyanins are quantified as equivalents of cyanidin-3-galactoside. All samples were analyzed in triplicate. One sample resulted from one matrix type, by extracting anthocyanins with one of the four solvents taken into study (MeOH, EtOH, AC and WA)

[a,b,c,d]Different letters indicate a significant difference, determined using the multiple comparison Tukey's test ($p < 0.05$)

Same letter indicates a non-significant difference

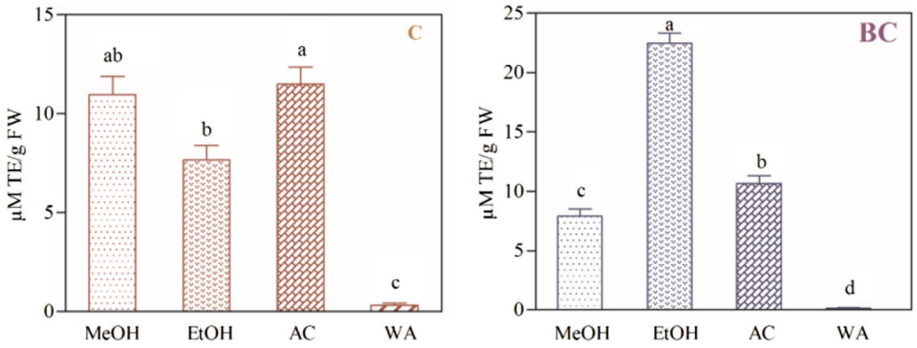

Fig.2 Scavenging effect on $ABTS^{\cdot+}$radical in chokeberries (C) and black carrots (BC) extracts. a,b,c,d—different letters indicate a significant difference, determined using the multiple comparison Tukey's test ($p < 0.05$). Same letter indicates a non-significant difference

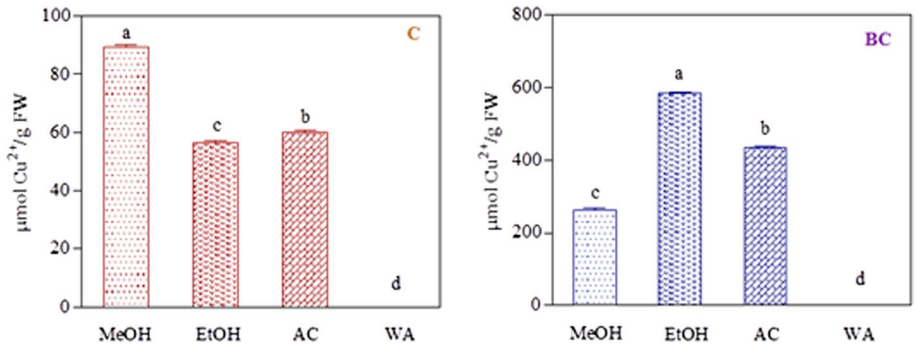

Fig.3 Cupric reducing antioxidant capacity (CUPRAC) assay in chokeberries (C) and black carrots (BC) extracts. a,b,c,d—different letters indicate a significant difference, determined using the multiple comparison Tukey's test ($p < 0.05$). Same letter indicates a non-significant difference

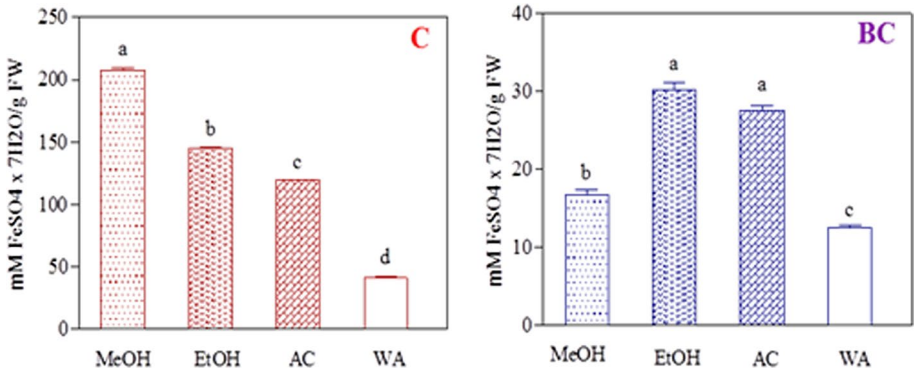

Fig.4 Ferric Reducing/Antioxidant Power (FRAP) assay in chokeberries (C) and black carrots (BC) extracts. a,b,c,d—different letters indicate a significant difference, determined using the multiple comparison Tukey's test ($p < 0.05$). Same letter indicates a non-significant difference

Table 4 Statistical ranking of the solvents' extracting capacity as based on the weighted mean for chokeberries (C) and black carrot (BC)

Ranking	C		Ranking	BC	
	Solvent	Weighted mean		Solvent	Weighted mean
1	MeOH	1.60	1	EtOH	1.80
2	EtOH	1.24	2	AC	1.22
3	AC	1.03	3	MeOH	0.79
4	WA	0.12	4	WA	0.20

The antioxidant activity was found to be positively correlated with their anthocyanin content also in blackberries, red raspberries, black raspberries and strawberries (Castañeda-Ovando et al. 2009; Dai et al. 2009).

Conclusion

In conclusion, data reported here led us to conclude that selecting the proper solvent for anthocyanins extraction could preserve the physical and antioxidant properties of these natural compounds. The selection of the proper solvent for anthocyanins extraction can be done according to thematrix of anthocyanins. The sources of anthocyanins used here: chokeberry fruits (*Aronia melanocarpa*) and black carrot roots (*Daucus carota* sp.), one conaining monoglycosylated anthocyanins and the other one both acylated and diglycosylated anthocyanins, were two different matrices for which the extraction solvent was particular to them. For instance,the extraction could be more efficient, for fruits as chokeberries, using methanol that has a high capacity to penetrate efficiently the fruit tissue, being a solvent with a great affinity and a high solubility for monoglycosylated cyanidin-based anthocyanins. In contrast, for vegetables as black carrots, the highest capacity to penetrate efficiently the root tissue is fulfilled by ethanol, which proves to be the solvent with high affinity for acylated and diglycosylated anthocyanins extraction, able to preserve their antioxidant activity too. This statement is also sustained by data resulted from antioxidant activity studies of three single-electron transfer (SET)-based assays, all done to establish by calculating weighted average the rank of solvents.. Either ethanol or methanol are both economically affordable solvents, that qualifies them for natural compound extraction required in food industry, mostly in procedures in which, finally, thealcohol removal is fullfiled.. Our research could provide new knowledge in the extraction field of anthocyanins and sustain their further exploration and application in the food industry.

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Data availability The data used to support the findings of this study are available from the corresponding author upon request.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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