Impact of phenolic compounds and vitamins C and E on antioxidant activity of sea buckthorn (Hippophaë rhamnoides L.) berries and leaves of diverse ripening times

Impact of phenolic compounds and vitamins C and E on antioxidant activity of sea buckthorn (Hippophaë rhamnoides L.) berries and leaves of diverse ripening times

Irena Sytařová[a], Jana Orsavová[^*], Lukáš Snopek[a], Jiří Mlček[a], tukasz Byczyński[c], Ladislava Mišurcová[a]

[a]*Tomas Bata University in Zlín, Department of Food Analysis and Chemistry, Náměstí T.G. Masaryka 5555, 760 01 Zlín, Czech Republic*

[b]*Tomas Bata University in Zlín, Language Centre, Stefánikova 5670, 760 01 Zlín, Czech Republic*

[c]*University of Agriculture in Krakow, Department of Food Biotechnology, ul Balicka 122, 30-149 Krakow, Poland*

** Corresponding author. E-mail address: orsavova@fhs.utb.cz (J. Orsavová).*

ABSTRACT

Bioactive compounds demonstrating antioxidant activity were analyzed in berries and leaves of nine cultivars of sea buckthorn (Hippophaë rhamnoides L.) of various ripening times. Total polyphenols were ranging between 0.70-3.62 g GAE.kg^{-1} (berries) and 1.88-3.72 g GAE.kg^{-1} (leaves). Leaves were significantly richer source of total flavonoids (14.40-9.44 mg RE.kg^{-1}) in comparison with berries (0.55-4.11 mg RE.kg^{-1}). Phenolic compounds, carotenoids and vitamins were determined using high-performance liquid chromatography with a diode array detection. The content of vitamin C was 0.98-3.65 g.kg^{-1} in berries and 22.81-46.32 g.kg^{-1} in leaves, vitamin E content was 6.98-29.91 g.kg^{-1} in berries and 71.54-153.99 g.kg^{-1} in leaves. Distribution of individual phenolic compounds varied, their total content in berries was considerably lower (76.1-205.2 mg.kg^{-1}) than in leaves (1477.7-8709.0 mg.kg^{-1}). Regarding antioxidant activity, Raisa and Slovan (berries) and Bojan and Maslicnaja (leaves) were evaluated as the best cultivars.

Keywords: Sea buckthorn, antioxidant aktivity, phenolic compounds, carotenoids, vitamins, HPLC-DAD, correlation

1. Introduction

Sea buckthorn (Hippophaë rhamnoides L.) is an ecologically and economically important plant of the Elaeagnaceae family, one of the least demanding frost-resistant trees that can withstand extreme temperatures. This shrub or low tree coming from the Caucasus area, Central Asia and Western Siberia provides sweet and sour orange berries with a high content of various lipophilic and hydrophilic bioactive compounds, such as vitamins (E, K, B, C), polyphenols (flavonoids, phenolic acids), carotenoids (a, p, 8-carotene, lycopene), phytosterols, organic acids, amino acids, essential fatty acids and minerals (**Araya-Farias, Makhlouf, & Ratti, 2011; Arif et al., 2010; Fatima et al., 2015; Stobdan, Korekar, & Srivastava, 2013; Tiitinen, Yang, Haraldsson, Jonsdottir, & Kallio, 2006).** Sea buckthorn berries have been reported as a significant source of four to one-hundred times larger amounts of vitamin C than any other fruits (**Makovics-Zsohár, Hegedus, Rédei, & Papp, 2014**). Additionally, its

other parts, such as leaves, twigs or roots, have been currently investigated to identify the content of important bioactive compounds and have been used for nutraceutical, pharmaceutical and cosmetic purposes (**Jaroszewska & Biel, 2017; Makovics-Zsohár et al., 2014; Morgenstern, Ekholm, Scheewe, & Rumpunen, 2014; Perk, Ceylan, Yanar, Boztas, & Capanoglu, 2016**). Nonetheless, significant differences in chemical composition of berries and leaves depending on the cultivar are known as well as the consequent composition changes caused by the environmental conditions. Particularly contents of carotenoids correlated with the environmental conditions; warm and sunny days with moderate precipitation support their synthesis in sea buckthorn berries (**Andersson, Olsson, Johansson, & Rumpunen, 2009; Kallio, Yang, & Peippo, 2002; Tiitinen, Hakala, & Kallio, 2005; Yang, 2009**). Not only is the sea buckthorn a primary food source of some essential nutrients, but its berries and leaves also contain a wide range of bioactive compounds with antioxidant effects potentially enhancing human health. Extracts of berries and leaves from sea buckthorn are reported to be anti-cancer, anti-aging, antimicrobial, anti-inflammatory and anti-neurodegenerative agents (**Stobdan, Korekar, Srivastava, 2013; Bittová, Krejzová, Roblová, Kubáň, & Kubáň, 2014; Fatima et al., 2015**). Another important influencing factor is high temperature applied during technological processes. It has been published as a factor increasing total phenolics and ellagitannins in the sea buckthorn leaves infusions compared to its infusions in low temperatures; however, thermal processing decreases antioxidant activity (**Ma et al., 2019**). Antioxidant activity (AOA) is a significant factor needed to be pondered to assess a nutritional value of fruit and vegetable. Great health benefits of a diet containing substantial amounts of bioactive substances have been thoroughly investigated and documented (**Cho et al., 2017; Cosmulescu, Trandafir, & Nour, 2017; Gao, Ohlander, Jeppsson, Bjork, & Trajkovski, 2000; Olsson, Gustavsson, Andersson, Nilsson, & Duan, 2004; Wrolstad, 2004**). However, antioxidant properties of plant matter could be influenced by a number of anti-oxidative mechanisms of many various chemical compounds in plant matter as well as by their complex interactions including synergistic or antagonistic effects. Hence, diverse methods employing distinct mechanisms of action are required to determine AOA (**Granato, Shahidi, Wrolstad, Kilmartin, Melton, & Hidalgo, 2018**).

Changes in the amounts and composition of bioactive compounds in various sea buckthorn parts during the annual growth cycle have been studied (**Bittová et al., 2014; Morgenstern et al., 2014**). Diverse ripening times of various fruit cultivars could be an important instrument to prolong the harvest time and moderate financial expenses on the storage. Furthermore, they could also affect occurrence and composition of bioactive compounds in berries and leaves influencing the antioxidant activity of sea buckthorn berries and leaves. Therefore, detailed studies may help employ these facts in practice.

This paper provides considerably valuable information about the impact of determined total contents of polyphenols (TPC), flavonoids (TFC), vitamins C and E, individual carotenoids and phenolic compounds to antioxidant activity (AOA) determined by free radical scavenging (DPPH) and photochemiluminescence (PCL) assays in nine cultivars of sea buckthorn berries and leaves of different maturity times. To assess the contribution of these compounds to AOA, Pearson correlation coefficients (r) were calculated between AOA established by various analytical methods and contents of individual vitamins and phenolic compounds in the extent which has not been published yet.

2. Materials and methods

2.1.Preparation of samples

Samples were prepared from berries and leaves of nine cultivars (Tytii, Hergo, Bojan, Maslicnaja, Dorana, Raisa, Askola, Slovan and Vitaminaja) of sea buckthorn (Hippophae rhamnoides L.) with

diverse ripening times. They originated from the experimental area of Central Control and Testing Institute in Agriculture (UKZUZ) in Bratislava in the cadastral area of Velke Ripnany in the Slovak Republic (188 m a.s.l., GPS coordinates: 48.5085792N, 17.9878006E). The type of soil of this locality is brown soil; topsoil is loamy and subsoil clayey-loamy with the ground water depth of 18 m. In sea buckthorn growing, sawdust mulch was used as the management system fertilized by combined fertilizer Cererit in the dose of 35 g per m^2 applied into the soil in autumn annually. Climatic conditions of this locality are continental with the average yearly temperature of 11.0 °C and precipitation of 621 mm. Monthly temperatures and precipitation in Velke Ripnany in 2016 are shown in **Supplementary Table S1**. Samples represented early ripening cultivars - very early ripening Tytii originated from Finland, early ripening Hergo from Germany and two medium early ripening cultivars Dorana from Germany and Bojan from Slovakia, late ripening cultivars - two medium late ripening cultivars Maslicnaja from Russia and Askola from Germany and three late ripening cultivars - Slovan from Slovakia, Raisa from Finland and Vitaminaja from Russia. Leaves and fully ripe berries were harvested from August to September in 2016. Fresh harvested samples were homogenized and lyophilized by Alpha 1-4 LSC (Christ Gefriertocknungsanlagen GmbH Osterode am Harz, Germany) at -55 °C and 0.120 mbar for 48 h. Further, lyophi-lized samples were subjected to the analyses and results were recalculated to the fresh matter.

2.2. Chemicals and reagents

Acetic acid, ethanol and methanol were obtained from Penta (Prague, Czech Republic) and methanol-HPLC was gained from LabScan (Sowinskiego, Polsko). Phenolic standards for HPLC analyses (flavo-noids - rutin, epigallocatechin, epicatechin, catechin, quercetin, kaempferol; stilbene - resveratrol; phenolic acids - gallic, syringic, protocatechuic, protocatechuic ethylester, 4-hydroxybenzoic, ellagic, hydroxycinnamic, caffeic, ferulic, chlorogenic, neochlorogenic, p-cu-maric and sinapic) were purchased from Sigma Aldrich (St. Louis, MO, USA), all of HPLC-grade. Standards of ascorbic acid and D-alpha-toco-pherol succinate were obtained from AccuStandard (New Haven, CT, USA). Methyl-tert-butyl ether (MTBE) was purchased from Acros Organics (New Jersey, USA) and individual carotenoid standards (P-carotene, lycopene, lutein and zeaxanthin) from Sigma Aldrich (St. Louis, MO, USA). Further chemicals and standards applied in analyses were of analytical grade from Sigma Aldrich (St. Louis, MO, USA).

2.3. Extraction of compounds

The same extraction procedure was used to determine total phenolic content (TPC), total flavonoid content (TFC), HPLC analysis of individual phenolic compounds and antioxidant activity (DPPH). Lyophilized samples of berries and leaves (0.5 g) were extracted by 10 mL of the mixture of water and methanol (70/30, v/v) in shaking water bath (Memmert GmbH + Co.KG, Germany) at 50 °C for 60 min. The mixture was centrifuged at 2 430g for 15 min (Velocity 13µ, Dynamica Scientific Ltd., UK).

Regarding the extraction for vitamin C determination, lyophilized samples of berries and leaves (0.5 g) were extracted by 25 mL of extraction mixture of methanol/H_3PO_4/redistilled water (99/0.5/0.5, v/v/v) in shaker LT 2 (Kavalier, Czech Republic) for 10 min in the dark.

Vitamin E was extracted using lyophilized samples of berries and leaves (1.0 g) in 10 mL methanol in ultrasonic bath PS 04,000 A (Notus-Powersonic,SR) at 40 °C for 60 min.

Individual carotenoids were extracted from lyophilized samples of berries (1.0 g) using 10 mL extraction mixture (hexane/acetone/ ethanol in the ratio of 99/0.5/0.5, v/v/v) in LT 2 shaker for 30 min in the dark. The upper layer of hexane (1 mL) was placed into a round-bottomed flask and was evaporated to dryness. This dry extract was dissolved in the 1 mL of the mixture of tetrahydrofuran/acetonitrile/ methanol in the ratio of 15/30/55, v/v/v.

The extraction of samples aimed for photochemiluminescence assay (PCL) to establish antioxidant activity of water-soluble compounds (ACW) and lipid-soluble compounds (ACL) was proceeded as follows: lyophilized samples of berries and leaves (0.5 g) were extracted using either water (ACW) or methanol (ACL) in shaking water bath at 80 °C for 30 min. Subsequently, extracts were centrifuged at 6749g for 15 min (Velocity 13µ, Dynamica Scientific Ltd., UK). All extracts and supernatants were filtrated using nylon micro filters prior to all the analyses (SYRINGE, Cronus Syringe Filter, Nylon 13 mm x 0.45 |rm, Labicom, Olomouc, Czech Republic).

2.4. Determination of total phenolic (TPC) and flavonoid (TFC) contents

Total phenolic content (TPC) was established using Folin-Ciocalteu method and total flavonoid content (TFC) using $NaNO_2$, AlQ_3-$6H_2O$ and NaOH both according to the protocols reported by **Orsavová, Hlaváčová, Mlček, Snopek, and Mišurcová (2019)** using UV/VIS spectrometer Lambda 25 (PerkinElmer, Waltham, MA, USA). Gallic acid was applied as a standard for TPC and rutin as a standard for TFC. Results were expressed as grams of gallic acid equivalent.kg^{-1} of fresh matter (g GAE. kg^{-1}) and as grams of rutin equivalent.kg^{-1} of fresh matter (g RE. kg^{-1}), respectively.

2.5. Determination of vitamins C and E

Determination of vitamin C and E contents was conducted using methods described by **Orsavová et al. (2019)** using HPLC analysis system UltiMate® 3000 (Dionex, Sunnyvale, CA, USA) with a diode-array detector (DAD). Analyses of both vitamins were conducted uniformly in an isocratic regime, but with different columns, Acclaim column 120 C8 (150 x 2.1 mm; 5 µm) (Dionex, MA, USA) for vitamin C and Kinetex column C-18 (150 x 4.6; 2.6 µm) (Phenomenex, Torrance, CA, USA) for vitamin E. Further analytical conditions for vitamins C and E were as follows: wavelengths - 275 nm and 230 nm, flow rates -0.8 mL.min^{-1} and 1.0 mL.min^{-1}, time of analyses - 10 min and 20 min, respectively. Quantification of vitamin C and E contents was provided applying calibration curves with ascorbic acid and D-alpha-tocopherol succinate as standards, respectively. Data signals were processed by LC ChromeleonTM 7.2 Chromatography Data System (Dionex, Sunnyvale, CA, USA).

2.6. Determination of carotenoids by HPLC

Profile of common carotenoid compounds (carotenes - P-carotene and lycopene, xanthophylls - lutein and zeaxanthin) was analyzed using HPLC device UltiMate® 3000 (Dionex, Sunnyvale, CA, USA) with a diode-array detector (DAD). Carotenoids were separated by YMC Carotenoids - 5 columns (250 x 4.6 mm; 5 µm) (Waters, Milford, MA, USA). The injection volume was 50 µL, flow rate 0.7 mL/min, column temperature 25 °C and analysis time 45 min. Solvent A was methanol and solvent B was the mixture of methanol/methyl-tert-butyl ether (MTBE) in the ratio of 20/80 (v/v). Solvent gradient was set as follows: 0-10 min: 90-75% B, 10-20 min: 75-50% B, 20-25 min: 50-30% B, 25-35 min: 30-10% B, 35-37 min: 10-6% B, 37-39 min: 6-90% B, 39-45 min: 90% B. Chromatograms were registered at 450 nm.

Individual carotenoids were identified comparing their retention times to those of pure standards and quantified by the method of standard addition for individual carotenoids (**Pop, Diaconeasa, Fetea, & Buena, 2015**). Data signals were processed by LC ChromeleonTM 7.2 Chromatography Data System (Dionex, Sunnyvale, CA, USA).

2.7. Determination of phenolic compounds by HPLC

Profile of common individual phenolic compounds was established by HPLC analysis system UltiMate® 3000 (Dionex, Sunnyvale, CA, USA) with a diode-array detector (DAD) and Kinetex column C-18 (150 x 4.6; 2.6 µm) (Phenomenex, Torrance, CA, USA) according to the method by **Sumczynski, Kotásková, Orsavová, and Valášek (2017)**. Individual phenolic compounds were identified using their retention times and quantified by the method of standard addition (**Fig. 1**). Data signals were processed by LC ChromeleonTM 7.2 Chromatography Data System (Dionex, Sunnyvale, CA, USA).

2.8. Antioxidant activity by DPPH and PCL assays

Antioxidant activities were established using DPPH (2,2-diphenyl-1-picrylhydrazyl; Sigma Aldrich, MO, USA) and PCL assays (antioxidant activity of water-soluble compounds - ACW and lipid-soluble compounds - ACL) according to the methods by **Orsavová et al. (2019)**. Briefly, as for DPPH assay - 450 µL of extract was added to 8.55 mL of B-solution of DPPH (10 mL of A-solution DPPH mixed with 45 mL of methanol; A-solution of DPPH was prepared by dissolution of 0.024 g of DPPH in 100 mL of methanol). Absorbance was measured at 515 nm after being maintained in the dark for 60 min using Lambda 25 (Per-kinElmer, Waltham, MA, USA). Trolox (Sigma Aldrich, MO, USA) was used as a standard and the results of antioxidant activity were expressed as grams of Trolox equivalent.kg^{-1} of fresh matter (g TE. kg^{-1}). PCL assays were performed using a volume of 10 mL of berries and leaves extracts using PHOTOCHEM (Analytik Jena AG, Jena, Germany) following ACW and ACL set protocols. Ascorbic acid (ACW) and Trolox (ACL) were used as standards and results were expressed either as grams of ascorbic acid equivalent.kg^{-1} of fresh matter (g AAE.kg^{-1}) or Trolox equivalent.kg^{-1} of fresh matter (g TE. kg^{-1}).

2.9. Statistical analysis

All experiments were repeated three times in two replications for each sample and their results were expressed as means and standard deviations (SD). SPSS 12.0 software (SPSS Inc., Chicago, USA) was used for the statistical evaluation. The level of significance was set to 5% ($p < 0.05$). Furthermore, Pearson correlation coefficients (r) were calculated (Microsoft Office Excel, Redmond, WA, USA). The evaluation of the strength of correlations was provided using the Evans scale (**Evans, 1996**).

3. Results and discussion

Contents of moisture, total polyphenols (TPC) and flavonoids (TFC) in berries and leaves of sea buckthorn cultivars are displayed in Table 1. Moisture in berries reached from 84.2% (Slovan) to 87.4% (Tytii and Maslicnaja) and in leaves from 4.7% (Raisa) to 7.7% (Hergo).

In the investigated specimens, berries of cultivar Bojan (0.70 g GAE.kg^{-1}) and leaves of Hergo (1.88 g GAE.kg^{-1}) presented the lowest amount of total phenolic compounds (TPC). On the other hand, the highest amounts were determined in berries and leaves of Dorana with the values of 3.62 g GAE.kg^{-1} and 3.72 g GAE.kg^{-1}, respectively. TPC contents in the majority of analyzed cultivars were higher than the published value of 1.75 g GAE.kg^{-1} fw in berries of Indian Summer cultivar from Canada (**Araya-Farias et al., 2011**). However, higher content of TPC of 8.58 g.kg^{-1} dw was published in sea buckthorn leaves from Poland (**Jaroszewska & Biel, 2017**). Such a variability of phenolic contents has been recorded due to the influence of number of factors, such as the type of locality and the year of harvest. TPC contents in berries of sea buckthorn cultivars from Iran changed depending on the harvest year between 21.58 g.kg^{-1} in 2014 and 32.16 g.kg^{-1} in 2015 (**Kuhkheil, Badi, Mehrafarin, & Abdossi, 2017**). Similarly, variable TPC contents were recorded in the same cultivars of sea buckthorn berries planted in another Czech locality of Žabčice harvested in different years proving an evident effect of climatic conditions of the specific harvest time and locality. In this study, TPC content of 1.15 g GAE.kg^{-1} was established in Hergo, in contrast to 9.65 g GAE.kg^{-1} recorded in berries of the same cultivar harvested in the years of 2011-2012 (**Rop, Ercişli, Mlcek, Jurikova, & Hoza, 2014**). Interestingly, twice higher TPC content of 83.08 g GAE.kg^{-1} dw was established in sea buckthorn leaves from Ladakh if compared with TPC content of 44.56 g GAE.kg^{-1} dw in berries (**Stobdan et al., 2013**). The impact of the harvest time on TPC content was registered in sea buckthorn leaves from South Korea with TPC content raising from 86.2 to 116.7 g GAE.kg^{-1} dw from June to August (**Cho et al., 2017**).

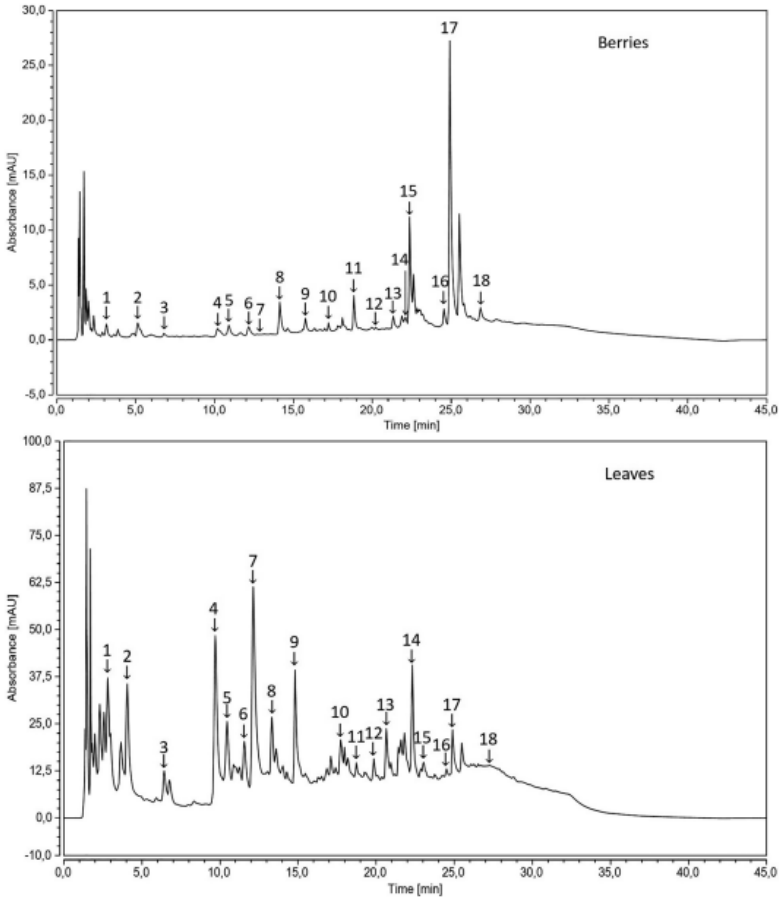

Fig. 1. Characteristic chromatograms of sea buckthorn berries and leaves. Gallic acid (1), protocatechuic acid (2), neochlorogenic acid (3), 4-hydroxybenzoic acid (4), epigallocatechin (5), catechin (6), chlorogenic acid (7), caffeic acid (8), syringic acid (9), epicatechin (10), p-cumaric acid (11), ferulic acid (12), sinapic acid (13), ellagic acid (14), rutin (15), hydroxycynnamic acid (16), protocatechuic ethylester (17), resveratrol (18). Determination of phenolic compounds was

performed in a gradient mode (0-10 min: 10-20% B, 10-16 min: 20-40% B, 16-20 min: 40-50% B, 20-25 min: 50-70% B, 25-30 min: 70% B, 30-40 min: 70-10% B, 40-45 min: 10% B) with using a gradient mobile phase A - redistilled water/acetic acid in the ratio of 99:1 (v/v) and B - redistilled water/acetonitrile/acetic acid in the ratio of 67:32:1 (v/v/v), flow rate - 1 mL.min^{-1}, column temperature - 30 °C, time of analysis - 45 min, wavelength - 275 nm.

Regarding total flavonoid contents (TFC), the lowest values were determined in berries of Tytii in the amount of 0.55 mg RE.kg^{-1} and in leaves in two cultivars Dorana and Slovan - 14.40 mg RE.kg^{-1}. The highest TFC content of 4.11 mg RE.kg^{-1} was established in berries of Dorana, whereas TFC contents in leaves were substantially higher varying from 14.40 mg RE.kg^{-1} (Dorana and Slovan) to 49.58 mg RE.kg^{-1} (Bojan). Analogously to TPC contents, higher TFC amount of 4.98 g RE.kg^{-1} was published in berries of cultivar Hergo from another Czech locality of Žabčice (**Rop et al., 2014**) contrasting with the content of 1.35 g RE.kg^{-1} in the same cultivar analyzed in this study. Similarly, established TFC contents in leaves were much lower than published amounts in leaves from South Korea varying from 16.0 to 21.8 g catechin (C).kg^{-1} depending on the harvest time (**Cho et al., 2017**). Surprisingly high TFC content of 2546.2 mg quercetin.kg^{-1} dw was registered in sea buckthorn leaves from Poland (**Jaroszewska & Biel, 2017**).

3.2. Content of vitamins C and E by HPLC

Sea buckthorn berries are an abundant source of vitamin C. Its content in analyzed fruits was generally affected by the ripening time. As can be seen in **Table 1**, lower amounts of vitamin C from 0.98 g.kg^{-1} (Bojan) to 3.18 g.kg^{-1} (Dorana) were determined in early ripening cultivars while in late ripening cultivars vitamin C contents were higher in the range from 3.46 g.kg^{-1} (Ascola and Raisa) to 3.65 g.kg^{-1} (Slovan). Vitamin C value of 1.49 g.kg^{-1} established in berries of Hergo exceeded the published amount of 0.87 g.kg^{-1} in berries of the same cultivar from another Czech locality of Žabčice harvested in 2006 (**Řezníček & Plšek, 2008**).

Table 1 Moisture, contents of total polyphenols (TPC) and flavonoids (TFC), vitamins C and E and carotenoids in sea uckthorn fruits and leaves.

Fruit cultivars	Moisture [%]		Polyphenols (TPC) [g GAE.kg^{-1}]		Flavonoids (TFC) [mg RE.kg^{-1}]		Vitamin C [g.kg^{-1}]		Vitamin E [mg.kg^{-1}]	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Berries										
Tytii	87.4 ±	0.6^{a,b}	1.26 ±	0.01^{a}	0.55 ±	0.03^{a}	2.01 ±	0.01^{a}	19.82 ±	
Hergo	86.4 ±	1.0^{b,c,d}	1.15 ±	0.01^{b}	1.35 ±	0.07^{b}	1.49 ±	0.01^{b}	16.10 ±	
Bojar	86.5 ±	0.4^{c,d}	0.70 ±	0.01^{c}	1.52 ±	0.05^{c}	0.98 ±	0.00^{e}	6.98 ±	
Dorana	84.9 ±	0.2^{e}	3.62 ±	0.05^{e}	4.11 ±	0.25^{e}	3.18 ±	0.00^{g}	11.83 ±	
Maslenaja	87.4 ±	0.2^{a,b}	2.55 ±	0.02^{d}	3.47 ±	0.17^{d}	2.25 ±	0.00^{d}	29.91 ±	
Askola	85.6 ±	0.2^{e}	2.66 ±	0.02^{g}	1.84 ±	0.14^{g}	3.46 ±	0.02^{f}	14.49 ±	
Raisa	85.9 ±	0.4^{c,d}	2.90 ±	0.01^{f}	1.04 ±	0.04^{f}	3.46 ±	0.00^{f}	7.60 ±	
Slovan	84.2 ±	0.6^{d,e}	3.20 ±	0.03^{h}	1.26 ±	0.03^{h}	3.65 ±	0.00^{g}	7.43 ±	
Vitaminaja	84.9 ±	0.2^{e}	2.55 ±	0.01^{i}	1.07 ±	0.04^{f}	3.53 ±	0.00^{h}	10.90 ±	
Leaves										
Tytii	4.8 ±	0.2^{a,b}	2.77 ±	0.06^{a}	35.84 ±	1.41^{a,b}	32.59 ±	0.11^{a}	153.99 ±	
Hergo	7.7 ±	0.3^{a}	1.88 ±	0.15^{b}	28.47 ±	1.19^{c}	22.81 ±	0.14^{b}	128.21 ±	
Bojar	4.9 ±	0.2^{a,b}	3.46 ±	0.27^{c,d}	49.58 ±	3.26^{d}	46.32 ±	0.06^{c}	111.59 ±	
Dorana	5.9 ±	0.2^{a}	3.72 ±	0.25^{c}	14.40 ±	0.47^{f}	45.30 ±	0.15^{e}	101.66 ±	
Maslenaja	6.8 ±	0.1^{d}	3.68 ±	0.32^{c}	42.44 ±	3.16^{e}	41.70 ±	0.08^{d}	108.31 ±	
Askola	5.7 ±	0.2^{e,f}	2.50 ±	0.12^{g}	39.79 ±	1.45^{e}	29.85 ±	0.04^{g}	71.54 ±	
Raisa	4.7 ±	0.1^{a}	2.96 ±	0.25^{c,d}	38.24 ±	2.68^{b,c}	40.00 ±	0.05^{f}	123.02 ±	
Slovan	5.9 ±	0.3^{e,f}	2.45 ±	0.11^{e}	14.40 ±	0.47^{f}	33.21 ±	0.10^{b}	74.38 ±	
Vitaminaja	5.2 ±	0.2^{a,g}	2.85 ±	0.05^{a}	33.00 ±	1.48^{a}	38.79 ±	0.13^{i}	83.65 ±	

Fruit cultivars	Vitamin E [mg.kg^{-1}]	β–carotene [mg.kg^{-1}]		Lycopene [mg.kg^{-1}]		Lutein [mg.kg^{-1}]		Zeaxanthin [mg.kg^{-1}]	
	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Berries									
Tytii	0.11^{a}	5.79 ±	0.01^{a}	< 0.01		0.48 ±	0.00^{a}	1.68 ±	0.02^{a}
Hergo	0.73^{b}	6.55 ±	0.02^{b}	< 0.01		0.33 ±	0.02^{b}	2.06 ±	0.01^{b}
Bojar	0.03^{c}	2.33 ±	0.03^{c}	< 0.01		1.14 ±	0.01^{c}	1.48 ±	0.01^{c}
Dorana	0.05^{c}	2.33 ±	0.05^{e}	< 0.01		0.24 ±	0.01^{e}	0.87 ±	0.00^{e}
Maslenaja	0.23^{d}	5.22 ±	0.07^{d}	0.71 ±	0.03^{a}	0.09 ±	0.00^{d}	0.72 ±	0.01^{d}
Askola	0.21^{g}	9.09 ±	0.08^{g}	< 0.01		0.07 ±	0.00^{g}	1.17 ±	0.01^{g}
Raisa	0.06^{f}	61.81 ±	0.08^{f}	6.04 ±	0.06^{b}	0.20 ±	0.02^{f}	1.97 ±	0.01^{f}
Slovan	0.09^{h}	44.86 ±	0.04^{h}	4.29 ±	0.04^{c}	0.21 ±	0.02^{f}	1.62 ±	0.00^{h}
Vitaminaja	0.28^{i}	95.74 ±	0.07^{i}	13.26 ±	0.11^{d}	0.26 ±	0.01^{e}	2.96 ±	0.01^{i}
Leaves									
Tytii	2.68^{a}								
Hergo	4.76^{b}								
Bojar	0.42^{c}								
Dorana	1.73^{e}								
Maslenaja	0.92^{d}								
Askola	0.52^{g}								
Raisa	0.26^{f}								
Slovan	0.38^{h}								
Vitaminaja	1.93^{i}								

Results are expressed in fresh matter as means ± SD, n = 5. Data in the same column followed by the same letters do not significantly differ by Tukey's test ($p < 0.05$).

A great variability of vitamin C content in sea buckthorn berries has been published; lower vitamin C content of 1.85 $g.kg^{-1}$ fw in berries of cultivar Indian Summer from Canada (**Araya-Farias et al., 2011**) was contrasting with higher amounts of 4.36 $g.kg^{-1}$ and 4.14 $g.kg^{-1}$ in berries from Germany and Romania, respectively (**Gutzeit, Baleanu, Winterhalter, & Jerz, 2008**). What is more, the type of locality seems to be an important factor influencing vitamin C content as well; in juice from wildly growing Hippophae rhamnoides subsp. sinensis from China a higher content of vitamin C (4-13 $g.L^{-1}$) was determined in comparison with the values of 0.02-2 $g.L^{-1}$ in juice from berries of Hippophae rhamnoides subsp. mongolica from Russia (**Kallio et al., 2002**). A significant variability of vitamin C content was reported also in berries from different areas in Iran in the range from 1.47 $g.kg^{-1}$ to 8.96 $g.kg^{-1}$ (**Kuhkheil et al., 2017**). Concerning the influence of the ripening time, a decreasing trend of vitamin C content from September to November was registered in juice from both wild and cultivated berries Hippophae rhamnoides subsp. mongolica from Finland (**Kallio et al., 2002**). Similarly, variable vitamin C contents were published in connection with the maturity of sea buckthorn berries from Pakistan. The highest vitamin C content was recorded in medium ripening stages while its content was lower at the beginning of berries growth and after the fruit color had been developed (Arif et al., 2010). Similarly, inconsistent vitamin C contents were reported in dependence on different temperature during the ripening period; the highest vitamin C amounts were determined in 2002, in the season with significantly sunnier days than in 2003 and 2004 (**Tiitinen et al., 2005, 2006**). Ascorbic acid is a crucial signaling molecule whose concentration must be strictly regulated. The way of its synthesis depends on the particular cell specialization. Therefore, vitamin C content in plant parts differs due to the specific plant characteristics, such as the age, type of plant tissue or cell compartment, and also due to the environmental conditions including the light intensity and time of the day (**Ohkawa, Kanayama, Chiba, Tiitinen, & Kanahama, 2009; Orsavová et al., 2019; Valpuesta & Botella, 2004**). Considerably higher vitamin C amounts were determined in sea buckthorn leaves if compared with berries. Diverse ripening times did not influence vitamin C content in leaves; however, statistically significant differences among various cultivars were established. The lowest value of 22.81 $g.kg^{-1}$ was recorded in Hergo. The richest cultivars were Bojan and Dorana with vitamin C contents of 46.32 $g.kg^{-1}$ and 45.30 g.kg-1, respectively. Analyzed vitamin C contents exceeded the published value of 2.22 $g.kg^{-1}$ dw in sea buckthorn leaves from Poland (**Jaroszewska & Biel, 2017**).

3.3. Content of individual carotenoids by HPLC

Profiles of individual carotenes (β-carotene and lycopene) and xanthophylls (lutein and zeaxanthin) in sea buckthorn berries are displayed in **Table 1** showing differences between various cultivars. In accordance with published data, β-carotene was determined in the highest amount and its content was significantly affected by the ripening time. Considerably high contents were established in the range from 44.86 $mg.kg^{-1}$ (Slovan) to 95.74 $mg.kg^{-1}$ (Vitaminaja) in late ripening cultivars contrasting with the content of 2.33 $mg.kg^{-1}$ in early ripening cultivars (Bojan and Dorana). Similarly, β-carotene content of 54.4 $mg.kg^{-1}$ was published in sea buckthorn berries in Ljublitelskaja cultivar from Sweden (**Andersson et al., 2009**); however, low average β-carotene contents of 0.25 $g.kg^{-1}$ dw in 2014 and 0.22 $g.kg^{-1}$ dw in 2015 were documented in berries of various cultivars from Iran (**Kuhkheil et al., 2017**). A great variability of β-carotene contents from 19 $mg.kg^{-1}$ to 74 $mg.kg^{-1}$ dw was recorded in different sea buckthorn cultivars from Romania (**Pop et al., 2014**). Interestingly, considerably high amounts of β-carotene were published in berries of wild growing sea buckthorn berries from Iran with contents varying in connection with the harvest season; in 2014-250 $mg.kg^{-1}$ and in 2015-220 $mg.kg^{-1}$ dw (**Kuhkheil et al., 2017**). In this study, lycopene was determined only in four cultivars. The lowest

amount of 0.71 mg.kg^{-1} was established in cultivar Maslicnaja while the highest content of 13.26 mg.kg^{-1} in Vitaminaja which is in accordance with lycopene content of 11.3 mg.kg^{-1} published in cultivar Ljublitelskaja from Sweden (**Andersson et al., 2009**). Generally higher lycopene amounts have been documented; in five cultivars from Romania ranging from 14 mg.kg^{-1} to 23 mg.kg^{-1} dw (**Pop et al., 2014**) and high contents in wild growing berries from Iran - 180 mg.kg-1 harvested in 2014 and 160 mg.kg^{-1} in 2015 (**Kuhkheil et al., 2017**).

Concerning xanthophylls lutein and zeaxanthin, their contents were not affected by the ripening time. Lutein was determined in very small amounts between 0.07 mg.kg^{-1} (Askola) and 1.14 mg.kg^{-1} (Bojan). Published studies have mainly documented higher lutein amounts -4.0 mg.kg^{-1} in cultivar Ljublitelskaja from Sweden (**Andersson et al., 2009**) and 21 mg.kg^{-1} and 14 mg.kg^{-1} dw in two cultivars from Romania; however, it was not detected in further four cultivars from the same locality at all (**Pop et al., 2014**). Zeaxanthin was determined in higher amounts than lutein varying from 0.72 mg.kg^{-1} (Maslicnaja) to 2.96 mg.kg^{-1} (Vitaminaja). Nonetheless, these findings are much lower than the published value of 42.3 mg.kg^{-1} in cultivar Ljublitelskaja from Sweden (**Andersson et al., 2009**) and the contents in six cultivars from Romania reaching from 18 mg.kg^{-1} to 25 mg.kg^{-1} dw (**Pop et al., 2014**).

3.4. Determination of individual phenolic compounds by HPLC

The list of individual phenolic compounds contained in sea buckthorn berries and leaves are illustrated in **Tables 2 and 3**. Total contents of phenolic compounds (total-PP) were notably higher in leaves than in berries and were not affected by the ripening time. Total-PP content significantly differed in cultivars; it rose in berries from 76.7 mg.kg^{-1} (Bojan) to 206.5 mg.kg^{-1} (Dorana) and in leaves from 1483.2 mg.kg^{-1} (Hergo) to 8691.5 mg.kg^{-1} (Maslicnaja). If berries and leaves are compared, distribution of phenolic compounds varied as well. Flavo-noids were presented in berries in substantially higher amounts than phenolic acids, except for early ripening cultivars Tytii and Hergo with balanced contents of flavonoids and phenolic acids. In leaves, phenolic acid contents were mostly predominant without the effect of the ripening time, except for two cultivars Dorana and Vitaminaja with a slightly higher content of flavonoids. Even though ripening time does not seem to be the most significant influencing factor, total flavonoids contents (total-FL) differed depending on the specific cultivar in the range from 58.9 mg.kg^{-1} (Tytii) to 181.0 mg.kg^{-1} (Dorana) in berries and from 610.6 mg.kg^{-1} (Askola) to 3798.5 mg.kg^{-1} (Maslicnaja) in leaves. Concerning flavanols, only rutin (RU) was determined in the amounts from 1.00 mg.kg^{-1} (Bojan and Askola) to 25.5 mg.kg^{-1} (Maslicnaja) in berries and from 7.3 mg.kg^{-1} (Bojan) to 23.7 mg.kg^{-1} (Askola) in leaves. Very high RU contents of 242-1352 mg.kg^{-1} fw were published in sea buckthorn leaves of two cultivars from Canada (**Fatima et al., 2015**) and 313.8 mg.kg-**1** dw from India (**Stobdan et al., 2013**).

Table 2 Content of phenolic compounds in sea buckthorn berries by reversed-phase chromatography.

Phenolics [mg.kg^{-1}]	Berries																							
	Tytii			Hergo			Bojan			Dorana			Maslienaja			Askola			Raisa			Slovan			Vitaminaja		
	Mean		SD	Mean		SD	Mean		SD	Mean		SD	Mean		SD	Mean		SD	Mean		SD	Mean		SD	Mean		SD
RU	8.7	±	0.0^{a}	6.7	±	0.0^{b}	1.0	±	0.0^{c}	8.4	±	0.1^{e}	25.5	±	0.2^{d}	1.0	±	0.0^{e}	10.7	±	0.0^{f}	12.7	±	0.0^{g}	14.9	±	0.1^{h}
EGC	43.5	±	0.4^{a}	33.8	±	1.0^{b,c}	36.2	±	0.5^{d}	62.3	±	0.4^{f}	45.8	±	0.6^{e}	34.5	±	0.8^{b,c}	40.6	±	0.5^{g}	32.9	±	0.7^{b}	35.2	±	0.7^{c}
EC	2.3	±	0.0^{a}	0.5	±	0.0^{b}	1.1	±	0.1^{c,d}	< 0.01			2.4	±	0.1^{a}	2.5	±	0.1^{a}	1.4	±	0.0^{f}	1.1	±	0.0^{c}	1.2	±	0.0^{d}
C	5.4	±	0.0^{a}	20.6	±	0.3^{b}	21.0	±	0.5^{b}	110.3	±	0.2^{d}	36.4	±	0.8^{c}	42.9	±	0.5^{f}	54.2	±	1.3^{g}	42.4	±	0.9^{f}	47.4	±	0.5^{h}
Total - FL	58.9	±	0.4^{a}	61.6	±	1.3^{a}	59.3	±	1.1^{a}	181.0	±	0.7^{c}	110.1	±	1.7^{b}	80.9	±	1.4^{d}	106.9	±	1.8^{c}	89.1	±	1.6^{e}	98.7	±	1.3^{f}
Stilbene RES	1.0	±	0.0^{a}	0.1	±	0.0^{b}	0.5	±	0.0^{c}	0.5	±	0.0^{c}	1.7	±	0.0^{d}	1.5	±	0.0^{f}	3.7	±	0.0^{e}	3.5	±	0.0^{g}	5.7	±	0.0^{h}
GA	0.3	±	0.0^{a}	0.2	±	0.0^{b}	0.4	±	0.0^{c}	7.0	±	0.0^{e}	0.5	±	0.0^{d}	1.2	±	0.0^{g}	0.5	±	0.0^{g}	0.4	±	0.0^{b}	0.5	±	0.0^{f}
SI	1.3	±	0.0^{a}	14.0	±	0.3^{b}	1.6	±	0.0^{c}	0.1	±	0.0^{e}	0.6	±	0.1^{d}	0.7	±	0.0^{g}	4.2	±	0.1^{f}	4.3	±	0.2^{f}	5.5	±	0.2^{h}
PK	1.9	±	0.0^{a}	3.5	±	0.1^{b}	1.6	±	0.0^{c}	0.1	±	0.0^{e}	0.7	±	0.1^{d}	0.1	±	0.0^{g}	0.9	±	0.0^{f}	1.7	±	0.0^{c}	1.2	±	0.0^{h}
PKBE	2.9	±	0.1^{a}	9.7	±	0.0^{b}	1.3	±	0.0^{c}	0.8	±	0.0^{e}	7.0	±	0.2^{d}	0.5	±	0.0^{g}	3.8	±	0.0^{f}	4.1	±	0.0^{b}	5.8	±	0.0^{f}
HB	0.1	±	0.0^{a}	0.2	±	0.0^{b}	0.1	±	0.0^{a}	0.5	±	0.0^{d}	0.2	±	0.0^{c}	0.1	±	0.0^{f}	0.5	±	0.0^{f}	0.6	±	0.0^{g}	0.9	±	0.1^{h}
EL	12.1	±	0.1^{a}	6.3	±	0.0^{b}	4.3	±	0.0^{c}	0.6	±	0.0^{e}	2.3	±	0.1^{d}	0.4	±	0.0^{g}	8.6	±	0.0^{f}	8.7	±	0.0^{b}	9.7	±	0.2^{f}
Total - DBA	18.6	±	0.2^{a}	33.9	±	0.4^{b}	9.3	±	0.0^{c}	9.1	±	0.0^{d}	11.3	±	0.5^{d}	3.0	±	0.0^{e}	18.5	±	0.1^{a}	19.8	±	0.2^{a}	23.6	±	0.5^{f}
HCA	3.3	±	0.1^{a}	0.1	±	0.0^{b}	0.1	±	0.0^{c}	0.4	±	0.0^{e}	0.3	±	0.0^{d}	0.2	±	0.0^{h}	1.3	±	0.0^{f}	1.7	±	0.0^{g}	1.1	±	0.1^{h}
CA	< 0.01			0.1	±	0.0^{a}	< 0.01			0.3	±	0.0^{c,d}	0.1	±	0.0^{b}	0.1	±	0.0^{b}	0.3	±	0.0^{c}	0.4	±	0.0^{g}	0.2	±	0.0^{f}
FIR	2.0	±	0.0^{a}	5.6	±	0.0^{b}	0.8	±	0.0^{c}	1.3	±	0.0^{e}	2.3	±	0.0^{d}	0.8	±	0.0^{g}	1.0	±	0.0^{f}	1.1	±	0.1^{h}	1.2	±	0.0^{h}
CHL	23.1	±	0.0^{a}	14.2	±	0.4^{b}	0.6	±	0.1^{c}	1.9	±	0.2^{d}	< 0.01						1.5	±	0.0^{g}	1.7	±	0.2^{d}	1.4	±	0.1^{e}
NCHL	0.4	±	0.0^{a}	3.7	±	0.1^{b}	2.3	±	0.0^{c}	0.9	±	0.0^{e}	1.4	±	0.0^{d}	0.8	±	0.0^{g}	2.4	±	0.0^{f}	2.7	±	0.2^{f}	3.1	±	0.1^{h}
CU	0.5	±	0.0^{a}	1.9	±	0.0^{b}	3.2	±	0.1^{c}	9.8	±	0.1^{e}	4.3	±	0.0^{d}	6.5	±	0.1^{g}	7.5	±	0.0^{f}	7.0	±	0.0^{h}	8.6	±	0.1^{i}
SP	0.5	±	0.0^{a}	0.3	±	0.0^{b}	0.6	±	0.0^{c}	1.3	±	0.1^{d}	0.5	±	0.0^{e}	0.9	±	0.0^{f}	0.9	±	0.0^{g}	1.0	±	0.0^{h}	1.2	±	0.0^{f}
Total - DCA	29.8	±	0.1^{a}	25.9	±	0.3^{b}	7.6	±	0.2^{c}	15.9	±	0.3^{e}	8.9	±	0.0^{d}	9.3	±	0.1^{g}	14.9	±	0.1^{f}	15.6	±	0.5^{d}	16.8	±	0.4^{h}
Total - PP	108.3	±	0.7^{a}	121.5	±	2.2^{b}	76.7	±	1.3^{c}	206.5	±	1.0^{e}	132.0	±	2.2^{d}	94.7	±	1.5^{g}	144.0	±	1.9^{f}	128.0	±	2.3^{b}	144.8	±	2.2^{f}

Results are expressed in fresh matter as means ± SD, n = 5. Data in the same column followed by the same letters do not significantly differ by Tukey's test ($p < 0.05$). Quercetin and kaempferol were not detected, t-cinnamic acid < 0.01. Flavonoids:

RU (rutin), EGC (epigallocatechin), EC (epicatechin), C (catechin), FL (flavonoids), RES (resveratrol). Phenolic acids: GA (gallic), SI (syringic), PK (protocatechuic), PKEE (protocatechuic ethylester), EL (ellagic), HB (4-hydroxybenzoic), DBA (total of benzoic acid derivates), HCA (hydroxycinnamic), CA (caffeic), FER (ferulic), CHL (chlorogenic), NCHL (neochlorogenic), CU (*p*-cumaric), SP (sinapic), DCA (total of cinnamic acid derivates), PP (phenolics).

Table 3 Content of phenolic compounds in sea buckthorn leaves by by reversed-phase chromatography.

Phenolics [mg.kg^{-1}]	Leaves																													
	Tytii			Hergo			Bojan			Dorana			Muslenaja			Askola			Raisa			Slovan			Vitaminaja		
	Mean		SD	Mean		SD	Mean		SD	Mean		SD	Mean		SD	Mean		SD	Mean		SD	Mean		SD	Mean		SD	Mean		SD
RU	17.5	±	0.2^{a}	17.8	±	0.3^{a}	7.3	±	0.4^{b}	20.4	±	0.0^{d}	10.2	±	0.1^{c}	23.7	±	0.2^{f}	19.4	±	0.1^{e}	21.7	±	0.0^{g}	16.2	±	0.2^{h}
EGC	1067.3	±	15.9^{b}	548.5	±	8.0^{b}	66.9	±	29.7^{c}	1435.5	±	3.0^{e}	1740.0	±	9.3^{d}	479.6	±	2.9^{g}	231.4	±	14.3^{f}	1479.8	±	26.5^{h}	2189.5	±	121^{i}
EC	21.7	±	0.1^{a}	10.8	±	0.6^{b}	27.0	±	0.2^{c}	6.2	±	0.1^{e}	18.3	±	0.2^{d}	7.2	±	0.1^{g}	29.1	±	0.3^{f}	28.7	±	0.1^{f}	23.5	±	0.1^{h}
C	260.0	±	3.1^{a}	115.7	±	0.1^{b}	589.8	±	0.3^{c}	296.4	±	0.9^{e}	2030.0	±	18.6^{d}	100.1	±	0.4^{g}	1385.2	±	25.9^{f}	1170.1	±	5.5^{h}	399.8	±	7.5^{i}
Total - FL	1366.5	±	19.3^{a}	692.8	±	9.0^{b}	1288.0	±	30.6^{c}	1758.5	±	4.0^{e}	3798.5	±	28.2^{d}	610.6	±	3.6^{g}	1665.1	±	40.6^{f}	2700.3	±	32.1^{h}	2629.0	±	199^{i}
Stilbene RES	2.5	±	0.1^{a}	3.7	±	0.1^{b}	7.9	±	0.1^{c}	3.6	±	0.0^{e}	4.5	±	0.0^{d}	4.3	±	0.1^{g}	2.9	±	0.0^{f}	3.4	±	0.0^{h}	2.2	±	0.0^{g}
GA	34.0	±	0.3^{a}	38.1	±	0.1^{b}	65.2	±	0.0^{f}	36.4	±	0.0^{e}	68.8	±	1.2^{d}	28.4	±	1.4^{g}	102.4	±	0.1^{c}	98.7	±	1.0^{h}	56.1	±	0.0^{g}
SI	14.3	±	0.7^{a}	14.2	±	0.9^{a}	26.4	±	0.3^{b}	11.6	±	0.2^{d}	23.5	±	0.4^{c}	25.2	±	0.5^{f}	20.8	±	0.5^{e}	27.6	±	0.4^{g}	10.7	±	0.4^{h}
PK	23.9	±	0.6^{a}	19.9	±	0.1^{b}	8.5	±	0.1^{c}	10.6	±	0.0^{e}	9.3	±	0.2^{d}	11.1	±	0.1^{g}	7.5	±	0.3^{f}	10.3	±	0.1^{h}	2.6	±	0.0^{g}
PKBE	14.8	±	0.2^{a}	92.3	±	0.0^{b}	41.6	±	0.1^{c}	4.1	±	0.2^{e}	79.9	±	0.1^{d}	1.4	±	0.0^{g}	39.3	±	0.0^{f}	65.2	±	0.1^{h}	52.4	±	0.6^{i}
HB	6.3	±	0.1^{a}	4.1	±	0.2^{b}	8.5	±	0.1^{c}	5.3	±	0.1^{d}	3.9	±	0.1^{b}	8.2	±	0.1^{f}	3.3	±	0.0^{e}	4.5	±	0.0^{g}	9.1	±	0.3^{h}
EL	124.0	±	0.3^{a}	81.9	±	0.1^{b}	133.8	±	0.0^{f}	2.3	±	0.0^{e}	114.8	±	0.6^{d}	5.7	±	0.0^{g}	165.8	±	0.3^{f}	143.8	±	0.1^{h}	163.2	±	0.9^{i}
Total - DBA	217.3	±	1.6^{a}	250.5	±	1.4^{b}	284.0	±	0.6^{c}	70.3	±	0.5^{e}	300.2	±	2.6^{d}	80.0	±	2.1^{f}	339.1	±	1.2^{d}	350.1	±	1.7^{g}	294.1	±	2.2^{g}
HCA	3.0	±	0.1^{a}	1.0	±	0.0^{b}	2.5	±	0.0^{f}	1.9	±	0.0^{e}	8.5	±	0.1^{d}	1.5	±	0.1^{g}	0.9	±	0.0^{f}	2.4	±	0.0^{h}	3.7	±	0.1^{i}
CA	9.3	±	0.4^{a}	3.1	±	0.1^{b}	28.8	±	0.4^{c}	56.5		0.8^{e}	6.4	±	0.2^{d}	7.0	±	0.1^{g}	38.8	±	0.9^{f}	41.8	±	0.6^{h}	6.2	±	0.0^{g}
FBR	10.9	±	0.1^{a}	24.7	±	0.1^{b}	6.2	±	0.0^{f}	3.2	±	0.0^{e}	6.6	±	0.0^{d}	4.7	±	0.1^{g}	4.7	±	0.0^{f}	5.1	±	0.0^{g}	5.1	±	0.0^{g}
CHL	969.5	±	2.0^{a}	198.9	±	2.2^{b}	2242.6	±	1.9^{c}	911.0	±	6.4^{e}	3906.3	±	32.5^{d}	545.1	±	15.7^{g}	2529.6	±	3.5^{f}	2321.2	±	4.4^{h}	1463.5	±	122^{i}
NCHL	229.5	±	2.9^{a}	227.4	±	1.9^{e}	299.7	±	6.9^{b}	215.8	±	1.4^{d}	553.4	±	3.9^{c}	264.0	±	5.2^{f}	181.5	±	3.4^{e}	108.8	±	1.7^{g}	198.6	±	0.4^{h}
CU	32.2	±	0.3^{a}	75.6	±	0.1^{b}	22.5	±	0.1^{c}	105.9	±	0.5^{e}	82.3	±	0.4^{d}	64.3	±	0.3^{g}	25.9	±	0.1^{f}	42.6	±	0.0^{h}	31.2	±	0.7^{i}
SP	26.0	±	0.2^{a}	5.5	±	0.0^{e}	27.8	±	0.3^{b}	8.9	±	0.4^{d}	24.4	±	0.4^{c}	42.8	±	0.4^{g}	107.2	±	0.4^{f}	103.3	±	0.3^{h}	79.4	±	2.3^{i}
Total - DCA	1280.4	±	6.0^{a}	536.2	±	4.4^{b}	2630.1	±	9.6^{c}	1303.2	±	9.5^{e}	4588.3	±	37.9^{d}	929.4	±	21.9^{g}	2888.6	±	7.9^{f}	2625.2	±	7.0^{h}	1787.7	±	157^{i}
Total - PP	2866.7	±	27.0^{a}	1483.2	±	14.9^{b}	4210.9	±	40.9^{c}	3135.6	±	14.0^{e}	8691.5	±	68.7^{d}	1624.3	±	27.7^{g}	4895.7	±	49.7^{f}	5679.0	±	40.9^{h}	4713.0	±	378^{i}

Results are expressed in fresh matter as means ± SD, n = 5. Data in the same column followed by the same letters do not significantly differ by Tukey's test ($p < 0.05$). Quercetin and kaempferol were not detected, t-cinnamic acid < 0.01. Flavonoids:

RU (rutin), EGC (epigallocatechin), EC (epicatechin), C (catechin), FL (flavonoids), RES (resveratrol). Phenolic acids: GA (gallic), SI (syringic), PK (protocatechuic), PKEE (protocatechuic ethylester), EL (ellagic), HB (4-hydroxybenzoic), DBA (total of benzoic acid derivates), HCA (hydroxycinnamic), CA (caffeic), FER (ferulic), CHL (chlorogenic), NCHL (neochlorogenic), CU (p-cumaric), SP (sinapic), DCA (total of cinnamic acid derivates), PP (phenolics).

Such a considerable variability of RU content in sea buckthorn leaves has been published due to the application of different extraction procedures; higher RU content of 8377 mg.kg^{-1} dw was detected inethanol extract if compared with water extract of 6939 mg.kg^{-1} dw in sea buckthorn leaves from Turkey (**Perk et al., 2016**). The impact of the time of harvest on RU content in leaves from Sweden was observed; the highest content of 1310 mg.kg^{-1} dw was detected at the end of April while in the middle of July its content decreased to 387 mg.kg^{-1} dw and finally, it rose to 471 mg.kg^{-1} dw at the end of July (**Morgenstern et al., 2014**). Similarly, changeable RU values reflecting different harvest times were published in leaves from the Czech Republic: the highest contents of 11.8 mg.kg^{-1} dw and 13.3 mg.kg^{-1} dw were detected in April and May, respectively, then it decreased to 6.7 mg.kg^{-1} dw during July with a subsequent rise to 10.8 mg.kg^{-1} dw during August and lastly, during September a dramatic decline to 4.2 mg.kg^{-1} dw was recorded (**Bittová et al., 2014**). Flavonols - quercetin (QUE) and kempferol (KEM) - were not detected either in berries or leaves which is in accordance with published data concerning the absence of QUE in sea buckthorn leaves from India (**Stobdan et al., 2013**). On the other hand, in contrast to these analyzed cultivars, in berries from Canada QUE was reported varying from 6.7 mg.100 g^{-1} fw to 17.5 and KEM from 3.2 mg.100 g^{-1} fw to 7.2 and RU was not identified (**Fatima et al., 2015**). Similarly, in leaves from Canada QUE was determined in the range of 43.9-105 mg.100 g^{-1} fw and KEM of 51.0-147.7 mg.100 g^{-1} fw (**Fatima et al., 2015**). What is more, substantially changeable contents of QUE and KEM were monitored in sea buckthorn leaves depending on variable harvest times in South Korea (**Cho et al., 2017**) and in Sweden (**Morgenstern et al., 2014**). The main part of flavonoids was comprised by flavanols with predominant epigallocatechin (EGC) and catechin (C). In berries, the contents of C ranged from 5.4 mg.kg^{-1} (Tytii) to 110.3 mg.kg^{-1} (Dorana) and EGC from 32.9 mg.kg^{-1} (Slovan) to 62.3 mg.kg^{-1} (Dorana). Epicatechin (EC) was detected only in small amounts from 0.5 mg.kg^{-1} (Hergo) to 2.4 mg.kg^{-1} (Maslic-naja) and in cultivar Dorana it was not detected at all. In leaves, EGC was recorded in abundant amounts reaching from 231.4 mg.kg^{-1} (Raisa) to 2189.5 mg.kg^{-1} (Vitaminaja), C was determined in the range from 100.1 mg.kg^{-1} (Ascola) to 2030.0 mg.kg^{-1} (Maslicnaja) and EC was established in the smallest amounts varying from 6.2 mg.kg^{-1} (Dorana) to 29.1 mg.kg^{-1} (Raisa). Comparably to flavonols, such a great variability of flavanol (EGC and C) contents in sea buckthorn leaves was published in connection with differing harvest times in Sweden (**Morgenstern et al., 2014**) as well as in the Czech Republic (**Bittová et al., 2014**). Stilbene resveratrol (RES) was determined in low amounts from 0.1 mg.kg^{-1} (Hergo) to 5.7 mg.kg^{-1} (Vitaminaja) in berries and from 2.2 mg.kg^{-1} (Vitaminaja) to 7.9 mg.kg^{-1} (Bojan) in leaves.

Distribution of phenolic acids from groups of benzoic acid derivatives (total-DBA) and hydroxycinnamic acid derivatives (total-DCA) varied in different cultivars. In berries, their contents were not influenced by the ripening time and total-DBA contents were higher than total-DCA contents except for three late ripening cultivars. However, in leaves DCA formed a major proportion of phenolic acids in all cultivars. Total-DBA contents ranged from 3.0 mg.kg^{-1} (Askola) to 33.9 mg.kg^{-1} (Hergo) in berries and from 70.3 mg.kg^{-1} (Dorana) to 350.1 mg.kg^{-1} (Slovan) in leaves. Total-DCA contents varied from 7.6 mg.kg^{-1} (Bojan) to 29.8 mg.kg^{-1} (Tytii) in berries and from 536.2 mg.kg^{-1} (Hergo) to 4588.3 mg.kg^{-1} (Maslicnaja) in leaves. Ellagic acid (EL) was predominant in berries of five cultivars and in leaves of seven cultivars. Its content in berries increased from 0.4 mg.kg-1 (Askola) to 12.1 mg.kg^{-1} (Tytii) and from 2.3 mg.kg^{-1} (Dorana) to 165.8 g.kg^{-1} (Raisa) in leaves. Very high EL content was established in leaves from South Korea and it rose from 4941 to 6722 mg.kg^{-1} dw during June and August (**Cho et al., 2017**). On the

other hand, 4-hydroxybenzoic acid (HB) was determined in the lowest amounts in berries in the range from 0.1 mg.kg^{-1} (Tytii, Bojan and Askola) to 0.9 mg.kg-1 (Vitami-naja), its content in leaves was slightly higher from 3.3 mg.kg^{-1} (Raisa) to 9.1 mg.kg^{-1} (Vitaminaja). Gallic acid (GA) was recorded in berries in the lowest amount of 0.2 mg.kg^{-1} in cultivar Hergo and the highest content of 7.0 mg.kg^{-1} in Dorana whilst in leaves its larger amounts varied from 28.4 mg.kg^{-1} (Askola) to 102.4 mg.kg^{-1} (Raisa). Changeable GA contents in leaves depending on different harvest times were recorded in Sweden (**Morgenstern et al., 2014**) and also in South Korea (**Cho et al., 2017**). Regarding syringic acid (SI), the smallest amounts were determined in berries of Dorana cultivar in the amount of 0.1 mg.kg^{-1} and of 10.7 mg.kg^{-1} in leaves of Vitaminaja cultivar while the highest contents were detected in berries of Hergo - 14.0 mg.kg-1 and in leaves of Slovan cultivar - 27.6 mg.kg^{-1}. Remaining acids of DBA group were established in relatively small amounts; proto-catechuic acid (PK) from 0.1 mg.kg^{-1} (Dorana and Askola) to 3.5 mg.kg^{-1} (Hergo) in berries and from 2.6 mg.kg^{-1} (Vitaminaja) to 23.9 mg.kg^{-1} (Tytii) in leaves. Finally, the lowest amounts of ethyl ester protocatechuic acid (PKEE) were recorded in the same cultivar Askola in berries - 0.5 mg.kg^{-1} and leaves - 1.4 mg.kg^{-1} and its highest contents in Hergo cultivar - 9.7 mg.kg^{-1} in berries and 92.3 mg.kg^{-1} in leaves. Concerning DCA group, β-cumaric acid (CU) was established in prevailing amounts from 0.5 mg.kg^{-1} (Tytii) to 9.8 mg.kg^{-1} (Dorana) in berries while in leaves it constituted a smaller proportion of DCA reaching from 22.5 mg.kg^{-1} (Bojan) to 105.9 mg.kg^{-1} (Dorana). Smaller and variable CU contents in leaves from the Czech Republic were documented depending on the growth cycle (**Bittová et al., 2014**). Chlorogenic acid (CHL) was abundant DCA in berries of only two cultivars: Tytii - 23.1 mg.kg^{-1} and Hergo -14.2 mg.kg^{-1}. Otherwise, it was determined only in small amounts ranging from 0.6 mg.kg^{-1} in Bojan to 1.9 mg.kg^{-1} in Dorana; and in Maslicnaja and Ascola cultivars it was not detected at all. In contrast to berries, CHL occurred predominantly in leaves of majority cultivars. Its content varied from 198.9 mg.kg^{-1} in Hergo to 3906.3 mg.kg^{-1} in Maslicnaja, except for the leaves of Hergo cultivar, in which neo-chlorogenic acid (NCHL) was the most abundant DCA in the amount of 227.4 mg.kg^{-1}. In other cultivars its content fluctuated from 0.4 mg.kg^{-1} (Tytii) to 3.7 mg.kg^{-1} (Hergo) in berries. In leaves, the lowest NCHL content of 108.8 mg.kg^{-1} was determined in Slovan and the highest of 553.38 mg.kg^{-1} in Maslicnaja cultivar. Ferulic acid (FER) was established in the range from 0.8 mg.kg^{-1} (Bojan and Ascola) to 5.6 mg.kg^{-1} (Hergo) in berries while in leaves its content was higher reaching from 3.2 mg.kg^{-1} in Dorana to 24.7 mg.kg^{-1} in Hergo. Si-napic acid (SP) was determined from 0.3 mg.kg^{-1} in Hergo to 1.3 mg.kg^{-1} in Dorana in berries and in leaves in the amounts from 5.5 mg.kg^{-1} in Hergo to 107.2 mg.kg^{-1} in Raisa. Finally, caffeic acid (CA) was presented in berries at the least amounts from 0.1 mg.kg^{-1} (Hergo, Maslicnaja and Ascola) to 0.4 mg.kg^{-1} (Slovan); in Tytii and Bojan cultivars CA was not detected at all. In leaves CA contents reached from 3.1 mg.kg^{-1} in Hergo to 56.5 mg.kg^{-1} in Dorana. Inconsistent contents of FER and CA in sea buckthorn leaves from the Czech Republic were documented reflecting variable growth periods (**Bittová et al., 2014**).

3.5. Antioxidant activity (AOA) by free radical scavenging (DPPH) and water-soluble (ACW) or lipid-soluble (ACL) capacities

Antioxidant activity (AOA) was assessed by employing DPPH, ACW and ACL to include a broader scope of bioactive compounds with various antioxidant mechanisms counting with their mutual impact. AOA values recorded in berries and leaves of various sea buckthorn cultivars are shown in Table 4. Substantial differences in AOA in berries and leaves have been observed. In berries, values of antioxidant activity established by ACW and ACL exceeded the values obtained by DPPH. Regarding AOA recorded in berries, DPPH ranged from 1.08 g TE.kg^{-1} in Hergo to 4.67 g TE.kg^{-1} in Slovan. The highest values of AOA in berries were established by ACW reaching from 5.72 g AAE.kg^{-1} in Bojan to

49.85 g AAE.kg^{-1} in Dorana. AOA determined by ACL was lower from 3.19 g TE.kg^{-1} in Bojan to 25.16 g TE.kg^{-1} in Raisa.

Table 4 Antioxidant activities of sea buckthorn berries and leaves.

Fruit cultivars	PPH [g TE.kg^{-1}]			ACW [g AAE.kg^{-1}]			ACL [g TE.kg^{-1}]		
	mean		SD	mean		SD	mean		SD
Berries									
Tytii	1.27	±	0.10^{a}	14.91	±	0.10^{a}	8.81	±	0.19^{a}
Hergo	1.08	±	0.10^{a}	12.78	±	0.48^{b}	3.73	±	0.04^{b}
Bojan	1.15	±	0.10^{a}	5.72	±	0.31^{c}	3.19	±	0.02^{c}
Dorana	3.44	±	0.56^{c,e}	49.85	±	1.25^{e}	17.75	±	0.15^{e}
Maslicnaja	2.51	±	0.09^{b}	21.94	±	0.16^{d}	14.72	±	0.63^{d}
Askola	3.31	±	0.10^{c}	47.31	±	1.05^{g}	20.62	±	0.70^{g}
Raisa	4.55	±	0.16^{d}	42.04	±	1.27^{f}	25.16	±	0.52^{f}
Slovan	4.67	±	0.21^{d}	37.65	±	0.27^{h}	24.29	±	0.70^{f}
Vitaminaja	3.77	±	0.06^{e}	35.85	±	0.14^{i}	19.79	±	0.31^{g}
Leaves									
Tytii	43.57	±	0.17^{a}	43.92	±	1.06^{a}	51.24	±	0.26^{a}
Hergo	19.60	±	0.26^{b}	33.15	±	0.06^{b}	22.31	±	0.21^{b}
Bojan	47.12	±	0.38^{c}	55.56	±	1.57^{c}	79.84	±	1.51^{c}
Dorana	54.17	±	0.32^{e}	37.49	±	0.11^{e}	42.50	±	0.98^{e}
Maslicnaja	45.85	±	0.53^{d}	48.91	±	0.39^{d}	66.97	±	1.32^{d}
Askola	34.00	±	0.21^{g}	34.31	±	1.68^{b}	41.68	±	1.18^{e}
Raisa	39.71	±	1.21^{f,i}	42.59	±	0.18^{a}	54.85	±	0.87^{f}
Slovan	21.43	±	0.51^{h}	40.55	±	0.18^{f}	64.24	±	1.03^{g}
Vitaminaja	41.70	±	0.91^{f}	33.73	±	1.35^{b}	36.29	±	0.56^{h}

Results are presented in fresh matter as means ± SD, n = 5. Data in the same column followed by the same letters do not significantly differ by Tukey's test (p < 0.05).

Published AOA of 1.87 pM TE.g^{-1} fw assessed in extracts from sea buckthorn berries from Romania were significantly higher than AOA values of 0.59 pM TE.g^{-1} dw recorded in extracts from apricot (**Pop et al., 2015**). Elevated AOA value of 5.70 pM TE.g^{-1} fw in sea buckthorn berries from Romania was also documented (**Cosmulescu et al., 2017**). Concerning AOA of sea buckthorn leaves, the impact of ripening time on AOA values has not been observed. And similarly, differences in AOA values in leaves determined by different methods have not been as great as in berries. The lowest AOA values quantified by all methods were determined in Hergo cultivar - 19.60 g TE.kg^{-1} (DPPH), 33.15 g AAE.kg^{-1} (ACW) and 22.31 g TE.kg^{-1} (ACL). On the other hand, the highest AOA values by DPPH method were detected in Dorana - 54.17 g TE.kg^{-1} and Bojan cultivar - 47.12 g TE.kg^{-1} aligned with the highest AOA values quantified by further methods: 55.56 g AAE.kg^{-1} (ACW) and 79.84 g TE.kg^{-1} (ACL), both in Bojan. Notably higher values of DPPH were published in leaves from Korea which varied with the ripening time rising from 155.5 g AAE.kg^{-1} dw in June to 206.0 g AAE.kg^{-1} dw in August. Similarly, higher values of 227.0 g AAE.kg^{-1} dw in June and 323.0 g AAE.kg^{-1} dw in August were established by ABTS (**Cho et al., 2017**). The extraction method influenced AOA values by DPPH in leaves from Turkey - 132.4 g TE.kg^{-1} dw was documented in water extract while lower value of 89.6 g TE.kg^{-1} dw was recorded in ethanol extract (**Perk et al., 2016**).

3.6. Correlations between antioxidant activities and phenolic compounds and vitamins C and E contents

Generally, AOA could be influenced by many factors - by presence of diverse chemical compounds which could be further affected by the type of cultivar, plant part, ripening and harvest time and environmental conditions of the specific locality. Last but not least, synergistic or antagonistic effects of these compounds play a crucial role in the resulting AOA. Possible impacts of analyzed vitamins C and E and phenolic compounds on the resulting AOA established by DPPH, ACW and ACL were assessed by regression analysis using Pearson correlation coefficients summarized in **Table 5**. Variability in

correlations between monitored factors in berries and leaves might reflect diverse ways of metabolism of bioactive compounds present in different plant parts. Very strong correlations were established between methods

determining AOA, especially between DPPH and ACW (r = 0.9158) and ACW and ACL (r = 0.8606) in berries, and between ACW and ACL (r = 0.9249) in leaves. Polyphenolics seem to be the most potent antioxidants as could be concluded from very strong correlations in berries between TPC and DPPH (r = 0.9044), ACL (r = 0.8133) and ACW (r = 0.8963) which is in accordance with the published correlation between DPPH and TPC (r = 0.8904) in sea buckthorn berries (**Rop et al., 2014**). Similarly, a very strong correlation between method of fluorescence recovery after photobleaching (FRAP) and TPC (r = 0.937) in berries of bird cherry (Prunuspadus L.) was documented proving it as a significant source of bioactive compounds with health benefits traditionally used in folk medical and food applications (**Donno, Mellano, De Biaggi, Riondato, Rakotoniaina, & Beccaro, 2018**). However, in the analyzed sea buckthorn leaves a very strong correlation was found only between TPC and DPPH (r = 0.9150). Regarding total carotenoids (TCC), a strong positive correlation was found only with ACW (r = 0.6702) and moderate correlation with ACL (r = 0.5870) in leaves. A correlation between TCC and DPPH (r = 0.3642) in berries was rather weak which is in agreement with the similar published correlation between TCC and ABTS (r = 0.36) in berries of Trofi-movskaja cultivar from Sweden. Nevertheless, between TCC and ABTS were established strong (r = 0.78) and very strong (r = 0.87) correlations in Botanitjeskaja and Aromatnaja cultivar, respectively (**Gao et al., 2000**). Additionally, weak correlations between TFC and all methods applied in AOA determination were found in contrast to the published significant very strong correlation (r = 0.8345) in berries (**Rop et al., 2014**). Concerning vitamins, vitamin C strongly affected AOA quantified by DPPH both in berries (r = 0.8247) and leaves (r = 0.7968) which is in accordance with the published very strong correlation between vitamin C and DPPH (r = 0.9312) in berries according to Rop et al. (2014). Even though vitamin C showed significant very strong correlations with photoluminescence methods only in berries - with ACL (r = 0.9292) and with ACW (r = 0.9126), vitamin E correlated weakly with all the methods both in berries and leaves. Regarding individual flavonoids, rutin performed a moderate positive correlation with ACL (r = 0.5374) in berries and ACW (r = 0.5396) in leaves. However, a strong positive correlation was established between rutin and ACL (r = 0.6065) in leaves. Similarly, catechin correlated moderately with DPPH (r = 0.5022) in berries and with ACL (r = 0.5910) in leaves and strong positive correlations were found between catechin and ACL (r = 0.6383) in berries and with ACW (r = 0.6626) in leaves. Finally, epicatechin correlated moderately only with ACW (r = 0.5641) in leaves.

Table 5 Correlations between antioxidant activity (by DPPH, ACL, ACW) and phenolics, carotenoids and vitamins.

	Pearson correlation coefficients at $p < 0.05$					
	DPPH		ACL		ACW	
	Berries	Leaves	Berries	Leaves	Berries	Leaves
ACL	**0.6958**	0.4182	–	–	–	–
ACW	**0.9158**	0.3256	**0.8606**	**0.9249**	–	–
TPC	**0.9044**	**0.9150**	**0.8133**	0.3858	**0.8963**	0.2748
TFC	0.1371	0.1571	0.2148	0.2653	0.0574	0.1728
TCC	0.3642	0.2361	0.3969	0.5870	0.4705	**0.6702**
Vitamin C	**0.8247**	**0.7968**	**0.9292**	0.2868	**0.9126**	0.3627
Vitamin E	−0.3530	0.1695	−0.4014	0.1770	−0.3617	−0.1180
Flavonoids						
Flavonols						
RU	0.3982	0.1921	0.5374	**0.6065**	0.3170	0.5396
Flavanols						
EGC	−0.0061	0.2500	0.0688	−0.0418	−0.1085	0.0595
EC	−0.1842	−0.0619	0.3729	0.4901	0.1715	0.5641
C	0.5022	0.0980	**0.6383**	0.5910	0.4248	**0.6626**
Total flavanols	0.3982	0.1921	0.5374	**0.6065**	0.3170	0.5396
Stilbene						
RES	−0.3344	−0.1754	−0.3613	0.4836	−0.4275	0.4836
Phenolic acids						
DBA						
GA	0.1429	−0.1360	0.4148	0.4864	0.0886	**0.6252**
SI	−0,1684	−0.1985	−0.2662	0.5617	−0.2501	**0.7228**
PC	−0.3569	−0.2591	**−0.6422**	−0.0692	−0.4844	−0.2504
PCEE	−0.1677	−0.5176	−0.3842	0.0800	−0.2308	0.0121
HB	0.5899	0.2092	0.4743	−0.1987	0.5308	−0.1626
EL	0.0445	−0.1014	−0.2550	0.4623	0.0256	0.4611
Total DBA	−0.1216	0.2658	−0.3302	**0.7049**	−0.2085	**0.7638**
DCA						
HCA	−0.0780	0.3235	−0.0818	0.2760	0.0292	0.2662
CA	**0.8475**	0.2056	0.4146	0.3791	**0.6736**	0.4701
FER	−0.4767	−0.5622	−0.5514	−0.2688	−0.5930	−0.5112
CHL	−0.5701	0.2475	−0.5681	**0.7146**	−0.5548	**0.7820**
CU	**0.7903**	0.0884	**0.7499**	−0.3888	**0.7057**	−0.4203
NCHL	0.1831	0.3467	−0.0604	0.2535	0.0945	0.1348
SP	**0.7207**	−0,1813	**0.7738**	0,0866	**0.6841**	0,2459
Total DCA	−0.2708	−0.3395	−0.3261	−0.4974	−0.3317	−0.3497

ACL (lipid-soluble antioxidant capacity), ACW (water-soluble antioxidant capacity), TPC (total polyphenols), TFC (total flavonoids), TCC (total carotenoids); Flavonoids: RU (rutin), EGC (epigallocatechin), EC (epicatechin), C (catechin), RES (resveratrol); Phenolic acids: GA (gallic), SI (syringic), PC (protocatechuic), PCEE (protocatechuic ethylester), HB (4-hydroxybenzoic), EL (ellagic), DBA (total of benzoic acid derivatives), HCA (hydroxycinnamic), CA (caffeic), FER (ferulic), CHL (chlorogenic), CU (p-cumaric), NCHL (neochlorogenic), SP (sinapic), DCA (total of cinnamic acid derivatives); significant correlations are in bold.

Contributions of individual phenolic acids to AOA varied greatly. Regarding DBA group, significant strong positive correlation was observed in leaves between syringic acid and ACW (r = 0.7228) and moderate positive correlations were found between syringic acid and ACL (r = 0.5617) and between 4-hydroxybenzoic acid with DPPH (r = 0.5899) and ACW (r = 0.5308) in berries. Furthermore, significant strong negative correlation was established between proto-catechuic acid and ACL (r = -0.6422) in berries and moderate correlation was found between protocatechuic ethylester and DPPH (r = -0.5176) in leaves. As far as DCA group is concerned, significant positive correlations were established in berries between caffeic acid and either DPPH (r = 0.8475) or ACW (r = 0.6736). Similarly, p-cu-maric and sinapic acids correlated substantially in berries with all the methods. The values were for p-cumaric acid: DPPH (r = 0.7903), ACL (r = 0.7499) and ACW (r = 0.7057) and for sinapic acid: DPPH (r = 0.7207), ACL (r = 0.7738) and ACW (r = 0.6841). In leaves, strong positive correlations were found only for chlorogenic acid with photochemiluminiscence methods - ACL (r = 0.7146) and ACW (r = 0.7820) which is in contrast to its moderate negative correlations with all the methods in berries - DPPH (r = -0.5701), ACL (r = -0.5681) and ACW (r = -0.5548). Correspondingly, moderate negative correlations were found for ferulic acid with DPPH (r = -0.5622) in leaves, with ACL (r = -0.5514) in berries and finally with ACW both in berries (r = -0.5930) and leaves (r = -0.5112).

4. Conclusion

Sea buckthorn (Hippophae rhamnoides L.) has been generally known for a rich content of bioactive substances, particularly of those with beneficial antioxidative properties. Present study has only proved that both berries and leaves of sea buckthorn are an excellent source of bioactive compounds, such as vitamin C, carotenoids and phenolic compounds with a significant power of antioxidant activity. Great differences have been observed between the analyzed factors in various cultivars, in berries and leaves. AOA values seem not to be fundamentally influenced by ripening time in sea buckthorn leaves as both the highest and lowest AOA values were established in early ripening cultivars Bojan and Hergo, respectively. However, in berries, the lowest AOA values were determined in early ripening cultivars Bojan, Hergo and Tytii, while higher AOA values were established in late and middle late ripening Raisa, Slovan, Maslicnaja, Ascola and surprisingly, also early ripening Dorana cultivar. That is due to high contents of vitamin C corresponding with the highest values of Pearson correlation coefficients between vitamin C and all used methods of AOA determination. What is more, Pearson correlation coefficients indicate that vitamin C has a stronger impact on AOA than carotenoids do. Considering vitamin E, the influence of ripening time to its content has not been confirmed and low values of correlation coefficients with values of AOA may show its weak effect on AOA in berries and leaves. To assess the extent of contribution of phenolic compounds to antioxidant activity of sea buckthorn berries and leaves it is essential to consider their potential synergistic or antagonistic effects reflected by different Pearson correlation coefficients between individual phenolic compounds and AOA.

In this study, the time of ripening has been confirmed as a significant factor causing high contents of vitamin C and P-carotene and consequently, initiating higher values of AOA in berries of late ripening cultivars; however, it has not shown the same effect in leaves. Concerning all examined factors, Vitaminaja has been evaluated as the best cultivar regarding the results of analyses in berries and Maslicnaja in leaves.

References

Andersson, S. C., Olsson, M. E., Johansson, E., & Rumpunen, K. (2009). Carotenoids in sea buckthorn (Hippophae rhamnoides L.) berries during ripening and use of pheophytin a as a maturity marker. Journal of Agricultural and Food Chemistry, 57(1), 250-258.

Araya-Farias, M., Makhlouf, J., & Ratti, C. (2011). Drying of sea buckthorn (Hippophae rhamnoides L.) berry: Impact of dehydration methods on kinetics and quality. Drying Technology, 29(3), 351-359.

Arif, S., Ahmed, S. D., Shah, A. H., Hassan, L., Awan, S. I., Hamid, A., & Batool, F. (2010). Determination of optimum harvesting time for Vitamin C, oil and mineral elements in berries sea buckthorn (Hippophae rhamnoides). Pakistan Journal of Botany, 42(5), 3561-3568.

Bittová, M., Krejzová, E., Roblová, V., Kubáň, P., & Kubáň, V. (2014). Monitoring ofHPLC profiles of selected polyphenolic compounds in sea buckthorn (Hippophae rhamnoides L.) plant parts during annual growth cycle and estimation of their antioxidant potential. Central European Journal of Chemistry, 12(11), 1152-1161.

Cosmulescu, S., Trandafir, I., & Nour, V. (2017). Phenolic acids and flavonoids profiles of extracts from edible wild fruits and their antioxidant properties. International Journal of Food Properties, 20(12), 3124-3134.

Donno, D., Mellano, M. G., De Biaggi, M., Riondato, I., Rakotoniaina, E. N., & Beccaro, G. L. (2018). New findings in Prunus padus L. fruits as a source of natural compounds: Characterization of metabolite profiles and preliminary evaluation of antioxidant activity. Molecules, 23, 725.

Evans, J. D. (1996). Straightforward statistics for the behavioral sciences. Pacific Grove, CA: Brooks/Cole Publishing.

Fatima, T., Kesari, V., Watt, I., Wishart, D., Todd, J. F., Schroeder, W. R., & Krishna, P. (2015). Metabolite profiling and expression analysis of flavonoid, vitamin C and tocopherol biosynthesis genes in the antioxidant-rich sea buckthorn (Hippophae rhamnoides L.). Phytochemistry, 118, 181-191.

Gao, X., Ohlander, M., Jeppsson, N., Bjork, L., & Trajkovski, V. (2000). Changes in antioxidant effects and their relationship to phytonutrients in fruits of sea buckthorn (Hippophae rhamnoides L.) during maturation. Journal of Agricultural and Food Chemistry, 48(5), 1485-1490.

Granato, D., Shahidi, F., Wrolstad, R., Kilmartin, P., Melton, L. D., Hidalgo, F. J.... Finglas, P. (2018). Antioxidant activity, total phenolics and flavonoids contents: Should we ban in vitro screening methods? Food Chemistry, 264, 471-475.

Gutzeit, D., Baleanu, G., Winterhalter, P., & Jerz, G. (2008). Vitamin C content in sea buckthorn berries (Hippophae rhamnoides L. ssp. rhamnoides) and related products: A kinetic study on storage stability and the determination of processing effects. Journal of Food Science, 73(9), C615-C620.

Cho, C. H., Jang, H., Lee, M., Kang, H., Heo, H. J., & Kim, D. O. (2017). Sea buckthorn (Hippophae rhamnoides L.) leaf extracts protect neuronal PC-12 cells from oxidative stress. Journal of Microbiology and Biotechnology, 27(7), 1257-1265.

Jaroszewska, A., & Biel, W. (2017). Chemical composition and antioxidant activity of leaves of mycorrhized sea-buckthorn (Hippophae rhamnoides L.). Chilean. Journal of Agricultural Research, 77(2), 155-162.

Kallio, H., Yang, B., & Peippo, P. (2002). Effects of different origins and harvesting time on vitamin C, tocopherols, and tocotrienols in sea buckthorn (Hippophae rhamnoides) berries. Journal of Agricultural and Food Chemistry, 50(21), 6136-6142.

Kuhkheil, A., Badi, H. N., Mehrafarin, A., & Abdossi, V. (2017). Chemical constituents of sea buckthorn (Hippophae rhamnoides L.) fruit in populations of central Alborz Mountains in Iran. Research Journal of Pharmacognosy, 4(3), 1-12.

Ma, X., Moilanen, J., Laaksonen, O., Yang, W., Tenhu, E., & Yang, B. (2019). Phenolic compounds and antioxidant activities of tea-type infusions processed from sea buckthorn (Hippophae rhamnoides) leaves. Food Chemistry, 272, 1-11.

Makovics-Zsohár, N., Hegedus, A., Rédei, R., & Papp, N. (2014). The antioxidant capacity of sea buckthorn (Hippophae rhamnoides L.) berries depends on the genotype and harvest time. International Journal of Horticultural Science, 3, 27-30.

Morgenstern, A., Ekholm, A., Scheewe, P., & Rumpunen, K. (2014). Changes in content of major phenolic compounds during leaf development of sea buckthorn (Hippophae rhamnoides L.). Agricultural and Food Science, 23(3), 207-219.

Ohkawa, W., Kanayama, Y., Chiba, E., Tiitinen, K., & Kanahama, K. (2009). Changes in sugar, titratable acidity, and ascorbic acid content during fruit development in sea buckthorn (Hippophae rhamnoides L.). Journal of the Japanese Science, 78(3), 288-293.

Olsson, M. E., Gustavsson, K. E., Andersson, S., Nilsson, Á., & Duan, R. D. (2004). Inhibition of cancer cell proliferation in vitro by fruit and berry extracts and correlations with antioxidant levels. Journal of Agricultural and Food Chemistry, 52(24), 7264-7271.

Orsavová, J., Hlaváčová, I., Mlček, J., Snopek, L., & Mišurcová, L. (2019). Contribution of phenolic compounds, ascorbic acid and vitamin E to antioxidant activity of currant (Ribes L.) and gooseberry (Ribes uva-crispa L.) fruits. Food chemistry, 284, 323-333.

Perk, A. A., Ceylan, F. D., Yanar, O., Boztas, K., & Capanoglu, E. (2016). Investigating the antioxidant properties and rutin content of Sea buckthorn (Hippophae rhamnoides L.) leaves and branches. African Journal of Biotechnology, 15(5), 118-124.

Pop, E. A., Diaconeasa, Z. B., Fetea, F., & Buena, A. (2015). Carotenoids, tocopherols and antioxidant activity of lipophilic extracts from sea buckthorn berries (Hippophae rhamnoides), apricot pulp and apricot kernel (Prunus Armeniaca). Bulletin UASVM Food Science and Technology, 72(2), 14-21.

Pop, R. M., Weesepoel, Y., Socaciu, C., Pintea, A., Vincken, J. P., & Gruppen, H. (2014). Carotenoid composition of berries and leaves from six Romanian sea buckthorn (Hippophae rhamnoides L.) varieties. Food Chemistry, 147, 1-9.

Rop, O., Erci§li, S., Mlcek, J., Jurikova, T., & Hoza, I. (2014). Antioxidant and radical scavenging activities in fruits of 6 sea buckthorn (Hippophae rhamnoides L.) cultivars. Turkish Journal of Agriculture and Forestry, 38(2), 224-232.

Řezníček, V. & Plšek, J. (2008). Sea buckthorn (Hippophae rhamnoides L.)-the effective source of vitamin C. In Proceedings of the Fifth Conference on Medicinal and Aromatic Plants of Southeast European Countries, (5th CMAPSEEC), Brno, Czech Republic, 2-5 September, 2008. Mendel University of Agriculture and Forestry in Brno.

Stobdan, T., Korekar, G., & Srivastava, R. B. (2013). Nutritional attributes and health application of seabuckthorn (Hippophae rhamnoides L.) - A review. Current Nutrition & Food Science, 9(2), 151-165.

Stobdan, T., Chaurasia, O. P., Korekar, G., Yadav, A., & Singh, S. B. (2010). Attributes of Seabuckthorn (Hippophae rhamnoides L.) to meet nutritional requirements in high altitude. Defence Science Journal, 60(2), 226-230.

Sumczynski, D., Kotásková, E., Orsavová, J., & Valášek, P. (2017). Contribution of individual phenolics to antioxidant activity and in vitro digestibility of wild rices (Zizania aquatica L.). Food Chemistry, 218, 107-115.

Tiitinen, K. M., Yang, B., Haraldsson, G. G., Jonsdottir, S., & Kallio, H. P. (2006). Fast analysis of sugars, fruit acids, and vitamin C in sea buckthorn (Hippophae rhamnoides L.) varieties. Journal of Agricultural and Food Chemistry, 54(7), 2508-2513.

Tiitinen, K. M., Hakala, M. A., & Kallio, H. P. (2005). Quality components of sea buckthorn (Hippophae rhamnoides) varieties. Journal of Agricultural and Food Chemistry, 53(5), 1692-1699.

Valpuesta, V., & Botella, M. A. (2004). Biosynthesis of L-ascorbic acid in plants: New pathways for an old antioxidant. Trends in Plant Science, 9(12), 573-577.

Wrolstad, R. E. (2004). Anthocyanin pigments-bioactivity and coloring properties. Journal ofFood Science, 69(5), C419-C425.

Yang, B. (2009). Sugars, acids, ethyl P-D-glucopyranose and a methyl inositol in sea buckthorn (Hippophae rhamnoides) berries. Food Chemistry, 112(1), 89-97.