Analysis of bioactive compounds of three sea buckthorn cultivars (*Hippophaë rhamnoides* L. ‘Askola’, ‘Leikora’, and ‘Orangeveja’) with HPLC and spectrophotometric methods

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Summary

Sea buckthorn (SBT) fruit is rich in biologically active compounds. It has considerable health benefits, thus it can serve as functional food ingredient. The aim of this study was to comprehensively characterize the fruit quality traits of three SBT cultivars (‘Leikora’, ‘Askola’, ‘Orangeveja’) grown in Hungary by determining their physicochemical (refraction, titratable acid content, sugar/acid ratio, carbohydrate profile) and antioxidant parameters (by spectrophotometric methods: FRAP, DPPH, and by HPLC method: β -carotene content, phenolic compounds). The statistical analysis was made by SPSS 14.0. Univariate analyses were made to separate the homogenous groups using the Duncan test. Significant differences were shown in the refraction of the cultivars (5.6–7.9 °Brix) and in their titratable acid content (2.35–3.07%), but no statistically significant difference has been found in the sugar/acid ratio (2.41–2.57). Glucose (9.56–12.9 mg L⁻¹) was the main carbohydrate component of the studied cultivars. In addition to that, we detected fructose (1.39–5.4 mg L⁻¹) and mannitol (1.77–9.24 mg L⁻¹). The antioxidant state of the studied cultivars could be well-characterized by their TPC (186–381 mg GAS 100 g⁻¹), DPPH (60.37–79.1 μ g Trolox L⁻¹), FRAP (3335–4887 μ g AA mL⁻¹) and by their β -carotene content (0.37–0.92 mg mL⁻¹); however no significant correlation was found among these antioxidant parameters. We determined the content of quercetin derivatives in the fruits of the SBT genotypes, such as the rutin (22.12–27.52 mg 100 g⁻¹), quercitrin glucoside (50.19–57.72 mg 100 g⁻¹), quercetin dihydrate (5.11–6.45 mg 100 g⁻¹), quercitrin hydrate (1.59–3.34 mg 100 g⁻¹) content, cinnamic acid (78.18–88.93 μ g 100 g⁻¹) and epicatechin (456.73–488.05 mg 100 g⁻¹). We concluded that the antioxidant properties are defined by both the genotype and by the climatic and growing conditions. The fruits of the cultivars ‘Leikora’ and ‘Orangeveja’ can be used as raw material of functional food products, according to their fruit quality traits.

Keywords

Hippophaë rhamnoides, FRAP, DPPH, total polyphenols, carbohydrates, phenolic components, β -carotene

Significance of this study

What is already known on this subject?

- The antioxidative properties of SBT are exceptional; that is why this fruit is a promising candidate as functional food ingredient. It is rich in flavonoid compounds, vitamin C and β -carotene. Several research groups investigated and published about the composition of sea buckthorn. Its role in human nutrition is significant, as it is proved by the research of physicians and biologists.
- Currently, ‘Askola’ is the most significant cultivar of sea buckthorn in Hungary, similarly to other countries of Europe, where there is production. In order to extend the ripening time and for the better utilization of cold chambers during storage, the cultivar ‘Leikora’ with late maturation may be of increasing importance. Furthermore, the thornless ‘Orangeveja’ might be a promising cultivar for easier and faster harvest.
- There are significant differences in the biologically active substances of the varieties; therefore, in addition to the examination of cultivation technology, the comprehensive analytical investigation of different genotypes is also important.

What are the new findings?

- Because of the antioxidant compounds of sea buckthorn, over the past decade it has become a popular ingredient in food products for jams, powders, purée, juices and dietary supplements. However, choosing the right cultivar of berries is very important for the production of food products with functional properties, because the variation of the functional compounds of cultivars is very high. Therefore, the investigation of cultivars is of primary importance for the production of the end-product. The novelty of this article is the analysis of three antioxidant measurement methods on three economically important SBT cultivars (‘Askola’, ‘Leikora’, and ‘Orangeveja’).

What is the expected impact on horticulture?

- Choosing the appropriate cultivar is essential for the manufacture of finished products with functional properties. The results of our experiments supply applicable information for growers about choosing and planting the right cultivar.

Introduction

The European sea buckthorn (SBT, *Hippophaë rhamnoides* L.) is a deciduous shrub, which belongs to the *Eleagnaceae* family (*Rosales*). The berries of SBT are rarely consumed in fresh form, but the fruit juice, pulp, peel, and the seed oil are widely used in several countries. Due to its bioactive compounds, such as high vitamin C, flavonoid, carotenoid, and tocopherol content, SBT is considered to be an excellent ingredient of functional food products (Suryakumar and Gupta, 2011; Németh et al., 2011; Christaki, 2012; Dóka et al., 2015; Krejcarová et al., 2015). The medicinal value of SBT was first mentioned by Gurevich (1956), and since then many articles have been published about the various beneficial effects of SBT on human health. SBT proved to be efficient against several common diseases such as diabetes (Wang et al., 2008; Kim, 2013), gastric ulcer (Xu et al., 2007; Xing et al., 2002; Huff et al., 2012), and cardiovascular diseases (Sayegh et al., 2014). It has wound healing (Upadhyay et al., 2009; Upadhyay et al., 2011; Edraki et al., 2014), and had antitumor (Li and Liu, 2014; Ali and Ahmad, 2015; Chakraborty et al., 2015) effects as well.

SBT can be grown under the most extreme environmental conditions, thus it is widely used in landscape protection (Lu, 2001). In addition, there is an increasing interest towards functional food products, made of fruits that are rich in bioactive compounds such as SBT (Abuajah et al., 2015). For this reason, SBT growing is an intensively improving field; also the breeding of new cultivars is an important issue. Fruit size, colour, texture, and other traits that allow easier harvest have all been considered during the breeding, but special attention has to be paid to the chemical composition of the cultivars, because SBT is primarily grown for its bioactive compounds (Shalkevich et al., 2009). SBT has a very large genetic diversity, and accordingly, the variability of the biologically active compounds is very high, depending on the specific cultivar. We have very few data about the fruit quality of different cultivars, which should be evaluated in order to get a better image of their value and to clarify the possibilities of their utilization. The aim of this study is to explore the bioactive components of three well known SBT cultivars ('Askola', 'Leikora', and 'Orangeveja') which are of great importance in the growing and fruit processing.

Materials and methods

Plant material

The berries of the German sea buckthorn (*Hippophaë rhamnoides* L.) cultivars 'Leikora' and 'Askola', and the Siberian cultivar 'Orangeveja' (Table 1) were collected from a commercial orchard (latitude 46°25'05"N, longitude 18°55'08"E) near Fajsz, located in South Hungary. The orchard was established in 1994 on alluvial meadow soil, which formed on the calcareous floodplain of the Danube. Berries of the SBT cultivars were harvested manually from early August to October (Table 1) in the middle of September, when the berries began to soften and had orange colouring that is characteristic for the cultivar. Following the fruit harvest, samples (3 kg culti-

var⁻¹) were homogenised in a blender and stored in freezer at -28°C until analysis. After thawing the physicochemical parameters and antioxidant properties were measured. Three parallel measurements were conducted for each sample.

Chemicals used during the measurements

For the identification and quantification analytical HPLC grade standards of different phenolic compounds such as rutin [153-18-4], epicatechin [490-46-0], quercitrin [522-12-3], quercetin dihydrate [6151-25-3] and quercetin hydrate [849061-97-8], also hydrochloric acid, butylated hydroxytoluene (BHT) and methanol (MeOH), as solvents were purchased from Sigma-Aldrich Chemical Co. (3050 Spruce Street, St. Louis, MO 63103, USA). The standards (0.5 g mL⁻¹) were dissolved in methanol containing 1% HCl and 1% BHT and a 100× dilution was used as the working standard for HPLC.

β-carotene determination was carried out with β-carotene standard [7235-40-7] and analytical HPLC grade solvents: methanol (MeOH), acetonitrile (ACN), tetrahydrofuran (THF) that were purchased from Sigma-Aldrich Chemical Co. (3050 Spruce Street, St. Louis, MO 63103, USA). The stock solution was prepared from β-carotene standard; 0.0916 mg β-carotene was dissolved in 1 mL THF and then diluted (ten-fold) in a mixture of ACN:MeOH:THF (50:45:5, V/V/V) before injecting on the HPLC column. For the identification and quantification of sugars analytical grade D-glucose [50-99-7], D-fructose [57-48-7] and D-mannitol [69-65-8] were purchased also from Sigma-Aldrich Chemical Co. (3050 Spruce Street, St. Louis, MO 63103, USA), and were used as HPLC standards along with Fe³⁺-2,4,6-tripyridyl-S-triazine [3682-35-7] and 2,2-diphenyl-1-picrylhydrazyl [1898-66-4] for the FRAP and DPPH assays.

Physicochemical analyses

Determination of soluble solid content (SSC) was fulfilled based on Codex Alimentarius 3-1-558/93 formula using ATAGO PR-101 refractometer. Total titratable acid (TA) content was determined according to the Nr. 12147:1998 Hungarian Standard. The results were expressed as the percent of malic acid. The maturity index (MI) was calculated using the equation SSC/TA=MI.

Spectrophotometric methods

1. Sample preparation for the spectrophotometric measurements. After thawing, the frozen fruit pulp was centrifuged with a Hettich EBA 21 (Hettich Instruments, Massachusetts, USA) laboratory centrifuge at 15,000 rpm for 5 minutes and polyphenol content, FRAP and DPPH were determined from the diluted supernatant as needed on a Hitachi U2800A UV-VIS spectrophotometer (Chiyoda, Tokyo, Japan).

2. Total phenolic content. The total polyphenol content (TPC) was measured photometrically with Folin-Ciocalteu reagents at λ = 765 nm. The calibration curve was made from gallic acid using the method of Singleton and Rossi (1965). Three parallel measurements were conducted for each sample.

TABLE 1. Characteristics of the three sea buckthorn cultivars.

Cultivar	Origin	Weight (g berry ⁻¹)	Berry color	Ripening time
'Askola'	Germany	0.23–0.30	Deep orange	Late August
'Leikora'	Germany	0.23–0.30	Bright orange	September to October
'Orangeveja'	Siberia	0.45–0.60	Reddish-orange	Early August

3. FRAP assay. The ferric reducing antioxidant power (FRAP) assay was carried out according to Benzie and Strain (1996). The FRAP assay is based on the reduction of the Fe^{3+} -2,4,6-tripyridyl-S-triazine [3682-35-7] complex to the ferrous form (Fe^{2+}) and the intensity of the reaction is monitored by measuring the change of absorption at 593 nm. The final results were expressed as micrograms of Ascorbic Acid equivalents (AA) per liter of pulp ($\mu\text{g AA L}^{-1}$). Three parallel measurements were conducted for each sample.

4. DPPH assay. The hydrogen donating or radical scavenging ability of the centrifuged sea buckthorn pulps were measured spectrophotometrically using the stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl) [1898-66-4], on 517 nm (Blois, 1958). All measurements were performed in triplicate. The calibration curve of Trolox was plotted as a function of the percentage of DPPH radical scavenging activity. The final results were expressed as milligrams of Trolox equivalents (TE) per liter of pulp (mg TE L^{-1}). Higher absorbance of the reaction mixture indicates lower free radical scavenging activity. Three parallel measurements were conducted for each sample.

HPLC analyses

1. Identification and quantification of carbohydrates.

Sample preparation. 1000 g fruit per cultivar was collected. The fruits were stoned, and then crushed with a blender. Then the 5-g fruit samples were placed into 50-mL Falcon tubes and 20 mL distilled water was added. Thereafter, the samples were shaken on 200 rpm frequency for four hours with a Büchler SM-30 control shaker (Edmund Büchler GmbH, Hechingen, Germany). One mL of supernatant was placed into a 1.5-mL Eppendorf tube and centrifuged (Hettich 23R, Andreas Hettich GmbH and Co. KG, Tuttlingen, Germany) for 5 min on 15,000 rpm. One mL supernatant was then pipetted and filtered through a Millex®-HN Syringe Driven Filter Unit (SLHN 013 NL, 0.45 μm , Millipore Ltd., 290 Concord Road, Billerica, MA 01921, USA).

The standards were first dissolved in double distilled water at a concentration of 0.01 g per 50 mL, and a 1:50 dilution of these solutions was used for the HPLC analysis. Each measurement was repeated three times.

HPLC conditions. The HPLC equipment (Waters Corporation, 34 Maple Street, Milford, MA 01757, USA) consisted of the following hardware: 2414 Refractive Index Detector, 1525 Binary HPLC Pump, Column heater and 717plus automatic injector. The equipment was controlled by the EMPOWER™ 2 software program.

The sugars were separated on a Sugar-Pak™ column (Sugar-Pak I column 10 μm , 6.5 mm $\times$ 300 mm) placed in a thermostat at 90°C. The mobile phase was a 0.0001 M aqueous solution of Ca-EDTA [304695-78-1]. The flow rate was 0.5 mL min^{-1} , leading to a pressure of 450 ± 10 psi on the column. The running time of chromatogram has been set to 30 min. The injected sample quantity was 20 μL . The retention time was 10.292 min for glucose, 11.300 min for fructose and 12.191 min for mannitol.

2. Identification and quantification of phenolic components.

Sample preparation for phenolic components. One-g-samples were extracted in 10 mL methanol (containing 1% HCL and 1% BHT) for 12 h in the dark at 4°C, using an Edmund Bühler SM 30 control shaker (200 rpm min^{-1}). The supernatant was decanted and centrifuged in Eppendorf tubes in a Hettich Mikro 22R centrifuge (15,000 rpm min^{-1} for 5 min). The supernatant was filtered on a 0.45 μm Millex®

HV Syringe Driven Filter Unit (SLHV 013 NL, PVDF Durapore), purchased from Millipore Co. (Bedford, MA, USA), and injected into the HPLC system. The quantities of the individual phenolic compounds are given in $\mu\text{g g}^{-1}$.

Analytical conditions. The Waters High-Performance Liquid Chromatograph (purchased from Waters Co., 34 Maple Street, Milford, MA, USA) was equipped with 2487 Dual λ Absorbance Detector, a 1525 binary HPLC pump, and in-line degasser, a column thermostat (set at 40°C) and an 717plus autosampler (set at 5°C), and was controlled using Empower TM2 software. A Kinetex C18 2.6 μm 150 $\times$ 4.6 mm column (Phenomenex, 411 Madrid Avenue Torrance, CA 90501-1430, USA) was installed. The gradient mobile phase was A: $\text{H}_2\text{O}:\text{MeOH}:\text{H}_3\text{PO}_4=940:50:1$, B: MeOH (0–30 min: A 100%–10%, 30–30.1 min: 10%–100%, 30.1–31: A 100%) with a flow rate 1 mL min^{-1} , the pressure in the column was 4200 ± 10 psi at a column temperature of 30°C. The running time was 25 min. Each injected volume was 20 μL . The sampling rate was 10 pt sec^{-1} , and the phenolic components were monitored at a wavelength of 280 nm. The retention times of the standards were rutin (28.8 min), quercitrin (24.4 min), quercetin dihydrate (22.26 min), quercetin hydrate (22.83 min), epicatechin (15.76 min).

3. Identification and quantification of β -carotene.

Extraction and determination of β -carotene by HPLC. The extraction of carotenoids from 5 g sea buckthorn fruits was carried out under subdued light with 15 mL THF for 12 h at +4°C, using Edmund Büchler SM 30-control shaker (Hechingen, Germany) at 150 rpm. The supernatant of extraction was decanted to 1.5-mL Eppendorf-tubes and centrifuged at 15,000 rpm (8163.1 g) (Hettich Mikro 22R centrifuge, Tuttlingen, Germany) for 5 min at -5°C.

The supernatant was filtered through 0.45 μm Millex HN syringe filter unit (SLHV 013 NL, PVDF Durapore, Millipore Co., Billerica, MA, USA), and finally injected onto the HPLC. Each extraction was performed in triplicate.

HPLC instrument from Waters Co. (USA) includes the 2487 dual absorbance detector (analytical wavelength 450 nm), 1525 binary HPLC pump (temperature of the sample compartment set to +5°C), column thermostat (set to 30°C), in-line degasser AF and 717 plus autosampler (temperature of the sample compartment set to 5°C). Empower TM2 software was used to control the analysis. The column type was Symmetry C18, 5 μm , 4.6 $\times$ 150 mm. The pressure on the column was 11.55 ± 0.07 MPa and the volume injected into the column was 20 μL . The conditions regarding mobile phase were modified after Bushway (Bushway, 1986). The mixture ACN:MeOH:THF (50:45:5, V/V/V) flowing at 1 mL min^{-1} was used as eluent.

Statistical analysis

The statistical analysis was made by SPSS 14.0. Univariate analyses were made to separate the homogenous groups using the Duncan test. The RSD value of the determination was 5% ($n=5$).

Results and discussion

Physicochemical properties of sea buckthorn cultivars

Former research studies proved that there are huge differences in the soluble solid content of sea buckthorn cultivars (2.9–22.7°Brix) (Shyrko and Radzyuk, 1989; Zhang et al., 1989). In the present research, we investigated the fruit quality of two German ('Askola', 'Leikora') and one Siberian ('Orangeveja') SBT cultivar. Their soluble solid content varied

TABLE 2. Physicochemical properties of sea buckthorn cultivars (*Hippophaë rhamnoides* L. 'Askola', 'Leikora', and 'Orangeveja').

	SSC (Brix°)	TA (%)	MI
'Askola'	7.9±0.1 c	3.07±0.02 b	2.57±0.02 a
'Leikora'	5.6±0.15 a	2.35±0.02 a	2.41±0.08 a
Orangeveja'	6.2±0.06 b	2.39± 0.06 a	2.58±0.09 a

SSC: soluble solid content.

TA: total titratable acid.

MI: maturity index.

between 5.6 and 7.9°Brix. The highest value was measured in the fruits of 'Askola', while the lowest in the fruits of 'Leikora' (Table 2). Statistically significant differences were observed among the examined cultivars. We got slightly lower values than Raffo et al. (2004), who studied both 'Askola' (8.8°Brix) and 'Leikora' (7.5°Brix), or Tiitinen et al. (2005) (7.4–12.6°Brix). Considerably higher values were measured in Turkish (10.1–14.8°Brix) (Ercisli et al., 2007), and Chinese (10.2–22.7°Brix) genotypes (Zhang et al., 1989; Tong et al., 1989).

The fruit of 'Askola' has the highest titratable acid content (3.07 g 100 g⁻¹), similarly to the highest water-soluble solid content. The lower values of 'Leikora' and 'Orangeveja' were nearly equal to each other. The SSC and TA values of the investigated cultivars were different, their sugar/acid ratio did not differ in a statistically significant amount (2.41–2.58). The titratable acid content measured in Turkish wild genotypes (2.64–4.54 g 100 g⁻¹) (Ercisli et al., 2007), and in hybrid populations (3.25–4.46 g 100 g⁻¹) (Tang, 2001) is in line with our results.

Sugars and acids play an important role in determining the sensory attributes of the berry. It is known (Kallio et al., 1999; Ma et al., 1989; Zhang et al., 1989) that Chinese genotypes have similar sugar/acid ratio (MI: maturity index) as the cultivars investigated in this study. Nevertheless, this ratio of the Chinese varieties was given as a result of the high titratable acid content (3.5–9.1 g 100 g⁻¹) along with the high water-soluble solid content. According to our findings, low titratable acid content (2.35–3.07 g 100 g⁻¹) was along with the low water-soluble solid content. The sugar- and the acid content of the fruits, as well as their relative proportions, fundamentally determine the taste of the fruit, also the processing possibilities.

Carbohydrate profile of sea buckthorn cultivars

Major part of the soluble solid content of SBT consists of sugar compounds. Nowadays, the number of people suffering from diabetes is very high, so it is essential to be aware of the quantity and quality of the sugar content of the food ingredients. Consuming foods containing fructose is advantageous, because fructose has a relatively low glycemic index, thus useful in diabetic diets (Bantle, 2009).

Three carbohydrate compounds were identified by the HPLC measurement in the fruits: glucose, fructose, and mannitol (Figure 1). Statistically significant differences were observed between the carbohydrate profiles; however, glucose was the main sugar component. Similarly to our results, the glucose content was reported to be dominant in the fruits of Chinese and Russian cultivars (Kallio et al., 1999; Ma et al., 1989) and in the fruits of German cultivars (Raffo et al., 2004). According to our results the amounts of glucose, fructose and mannitol were 11.95–15.26 mg mL⁻¹, 1.75–6.75 mg mL⁻¹, 1.32–6.21 mg mL⁻¹ respectively (Table 3).

These results were in accordance with earlier works (Kallio et al., 1999; Ma et al., 1989; Makinen et al., 1980). In the fruits of the cultivar 'Askola' the glucose content was significantly lower than in the other two cultivars. In contrast, this cultivar contained significantly higher amount of fructose and mannitol. Similarly, Raffo et al. (2004) measured lower glucose content in the fruits of 'Askola' (1.62 mg mL⁻¹) than in the fruits of 'Leikora' (6.4 mg mL⁻¹); however, the values of both cultivars were slightly lower than our results. No statistically significant difference was found between the glucose content of 'Leikora' and 'Orangeveja', but the fruits of 'Leikora' had significantly higher fructose and mannitol content than the other cultivars. In previous studies (Tang, 2002; Ma et al., 2016), as well as in our study, the fructose content differed significantly in all genotypes.

Antioxidant properties of sea buckthorn cultivars

Sea buckthorn berry is a rich source of antioxidants and bioactive components beneficial for human health. Previous studies had also shown, that the fruits of SBT cultivars have a strong antioxidant effect, but there is considerable variance among the cultivars (Gao et al., 2000; Korekar et al., 2014). In this study, the antioxidant state of the investigated sea buckthorn cultivars was characterized by the following four antioxidant parameters: total polyphenol content, water-soluble antioxidant capacity (FRAP), hydrogen donor activity (DPPH), and the β -carotene content (Table 4; Figure

FIGURE 1. Typical chromatogram of the sugar determination of STB berries. Retention times: Glucose: 10.292; Fructose: 11.300 and Mannitol: 12.191. Column: Sugar-Pak I 10 μ m, 6.5 mm $\times$ 300 mm. Mobile phase: 0.0001 M Ca-EDTA [304695-78-1] aqueous solution. Flow rate: 0.5 mL min⁻¹, pressure: 450 $\pm$ 10 psi on the column. The running time: 30 min. Injected sample quantity: 20 μ L.

TABLE 3. Carbohydrate profile (glucose, fructose, mannitol and the glucose/fructose ratio) of sea buckthorn cultivars (*Hippophaë rhamnoides* L. 'Askola', 'Leikora', and 'Orangeveja').

	Glucose	Fructose	Mannitol	Glucose/Fructose
	(mg mL ⁻¹)			
'Askola'	9.56±0.28 a	5.40±0.1 c	4.96±0.14 c	1.77±0.02 a
'Leikora'	12.90±0.83 b	2.77±0.11 b	2.66±0.15 b	4.66±0.37 b
'Orangeveja'	12.21±0.44 b	1.39±0.43 a	1.06±0.05 a	9.24±0.05 c

TABLE 4. Polyphenol content of sea buckthorn cultivars (*Hippophaë rhamnoides* L. 'Askola', 'Leikora', and 'Orangeveja').

	Rutin	Quercetin glucoside	Quercetin dihydrate	Quercetin hydrate	Cinnamic acid	Epicatechin
	(mg 100 g ⁻¹)				(μg 100 g ⁻¹)	
'Askola'	27.4 ±0.34 a	57.72±0.36 a	5.11±0.05 a	1.59±0.02 a	88.93±0.71 b	488.05±2.37 b
'Leikora'	22.12±0.68 a	50.19±0.13 b	6.07±0.19 a	2.68±0.07 b	83.91±0.85 ab	480.45±1.67 b
'Orangeveja'	27.52±0.48 a	50.58±0.27 b	6.45±0.10 a	3.34±0.08 c	78.18±0.31 a	456.73±1.89 a

TABLE 5. Antioxidant characteristics of sea buckthorn cultivars (*Hippophaë rhamnoides* L. 'Askola', 'Leikora', and 'Orangeveja').

	TPC (mg GAE 100 g ⁻¹)	DPPH (μg Trolox L ⁻¹)	FRAP (μg AA mL ⁻¹)	β-carotene (mg mL ⁻¹)
'Askola'	186±36 a	68.37±8.05 ab	3335±192 a	0.37±0.06 a
'Leikora'	295±28 b	60.37±6.41 a	4663±234 b	0.84±0.11 b
'Orangeveja'	381±14 c	79.1±3.92 b	4887±251 b	0.92±0.38 b

2). Furthermore, six flavonoid components were detected by HPLC (Table 5).

1. Analysis of the polyphenol content. Polyphenols have one of the most strong antioxidant effects among the different kinds of components. Based on previous research studies, SBT fruits have high polyphenol content, but the total polyphenol content greatly varies depending on the cultivar. According to Ercisli et al. (2007), who studied Turkish genotypes, their TPC value varies between 213.1 and 553.8 mg GAE 100 g⁻¹. Even 11-fold difference (from 964 to 10,704 mg GAE 100 g⁻¹) was measured by Korekar et al. (2014) in the TPC content of different Indian populations (trans-Himalayan region). The polyphenol content of SBT cultivars is affected by both their genetic background and by the growing and climatic conditions (Sabir et al., 2005; Tiitinen et al., 2005; Ercisli et al., 2007). The fruits of the studied SBT cultivars also had significantly different ($p < 0.005$) TPC values (1.86–3.81 g GAE kg⁻¹). The fruit of 'Askola' had the lowest TPC value, while the fruit of 'Orangeveja' the highest. The statistically significant difference in the TPC values of the cultivated varieties under the same climatic conditions can clearly be explained by the different genetic material. According to Rop et al. (2014), the 'Leikora' fruits collected near Brno, in the Czech Republic, had more than three times higher TPC value (974 mg GAE 100 g⁻¹) than the 'Leikora' fruits grown under Hungarian growing and climatic conditions.

Mostly the high polyphenol content of the SBT berries are responsible for their health benefits, most of all their flavonoid glucoside content. According to Ma et al. (2016), SBT genotypes are rich source of flavonol glycosides with contents ranging from 23 mg to 250 mg 100 g⁻¹ of fresh berries. The most important flavonol components of the SBT fruits are the isorhamnetin glycosides, such as isorhamnetin-3-

FIGURE 2. The typical chromatogram of STB berries, the peak at 25.892 belongs to β-carotene. The other peaks were not determined. Analytical wavelength: 450 nm, 1525 column thermostat: 30°C. Column type: Symmetry C18, 5 μm, 4.6 × 150 mm, column pressure: 11.55±0.07 MPa, the injected volume was 20 μL. The conditions regarding mobile phase were modified after Bushway (Bushway, 1986). The mixture ACN:MeOH:THF (50:45:5, V/V/V) flowing at 1 mL min⁻¹ was used as eluent.

O-rutinoside, isorhamnetin-3-*O*-glucoside-7-*O*-rhamnoside and quercetin derivatives such as quercetin-3-*O*-glucoside and quercetin-3-*O*-rutinoside (Rösch et al., 2004; Yang et al., 2009). We determined the quercetin derivatives of the SBT berries, such as the rutin, quercetin glucoside, quercetin dihydrate, quercitrin hydrate, and the cinnamic acid and epicatechin content (Table 4).

The berries of the examined cultivars contain high amounts of rutin (22.12–27.52 mg 100 g⁻¹), but there was no statistical difference in the rutin content of the cultivars. The fruits of 'Askola' contain significantly higher amount of quercitrin glucoside than the other two cultivars, which have similar content to each other. Also, similar amounts of quercetin were shown in the cultivars, though 'Askola' had slightly lower content than the other two. The quercitrin hydrate content was the lowest in 'Askola', and the highest in 'Orangeveja', while the cinnamic acid content was the highest in 'Askola' and the lowest in 'Orangeveja'. The fruits of 'Askola' and 'Leikora' contained nearly the same amount of epicatechin, but the epicatechin content of 'Orangeveja' was much lower.

2. Antioxidant capacity of the STB cultivars. The fruit of 'Leikora' had the lowest hydrogen donor activity, while the highest value was measured in the fruit of 'Orangeveja' this time as well (Table 5). The lowest water-soluble solid content was shown in the 'Askola' fruits; nearly equal values were shown in 'Leikora' and 'Orangeveja'. Korekar et al. (2014) found considerable differences between the FRAP values of different Indian SBT populations (from 180 to 1355 µg mL⁻¹); however, these values were substantially lower than that we have measured.

The correlation between the parameters of antioxidant capacity (TPC-FRAP) had been investigated in several research works. Some of these studies showed strong correlation between the antioxidant parameters (Velioglu et al., 1998), while others could not find strong correlation (Kahkonen et al., 1999; Ercisli et al., 2007). Our results do not suggest strong correlation between the results of different antioxidant methods (TPC, FRAP, DPPH).

3. Analysis of the β-carotene content of the STB cultivars. The main carotenoids of SBT genotypes are zeaxanthin, β-carotene and β-kryptoxanthin (Raffo et al., 2004). The colour of the berry is defined primarily by these components, since it was confirmed in several earlier studies that carotenoids are responsible for evolving the yellowish and/or orangish fruit colour, the intensity of which depends on the number of double bonds and types of functional groups of the fruit (Khoo et al., 2011; Lancaster et al., 1997; Bartley and Scolnik, 1995).

Based on the literature, among the antioxidant properties of the SBT genotypes, β-carotene content has the highest variance (Korekar et al., 2014). We determined the β-carotene content of the examined SBT cultivars by HPLC method (Figure 2). We came to the similar conclusion as Raffo et al. (2004), that 'Askola' has considerably lower β-carotene content than 'Leikora'. The difference between 'Leikora' and 'Orangeveja' was not statistically significant (Table 5).

According to the results of this experiment the cultivar 'Orangeveja' has outstanding antioxidant properties compared to other fruits (Fu, 2011). It has considerable β-carotene content, and among the three examined cultivars it had the highest values when any of the other antioxidant parameters were measured.

Conclusions

We can conclude that the investigated SBT cultivars have strong overall antioxidant properties. Based on the measured antioxidant parameters, 'Askola' has the lowest values, excluding the DPPH, and 'Orangeveja' the highest. Our results and the literature data led us to the conclusion that the antioxidant properties might be defined by both the genotype and the growing and climatic conditions. Under the Hungarian growing and climatic conditions, the 'Askola' sea buckthorn cultivar might serve as raw material for products with reduced sugar content for diabetic consumers. While the fruit quality of 'Leikora' and 'Orangeveja' makes them suitable as functional food ingredients that could also be beneficial to human health. In order to confirm these assumptions, further research is needed and the changes of these ingredients during the food processing technologies should be monitored along with the less frequently utilized parts of the berry (seed and skin). It should be clarified, to what extent they contain these valuable components, and what is the most efficient method to extract them and utilise them for food industrial purposes.

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