TÜBİTAK

Turkish Journal of Agriculture and Forestry

http://journals.tubitak.gov.tr/agriculture/

Research Article

Turk J Agric For
(2014) 38: 224-232

doi:10.3906/tar-1304-86

Antioxidant and radical scavenging activities in fruits of 6 sea buckthorn (*Hippophae rhamnoides* L.) cultivars

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Received: 16.04.2013 • **Accepted:** 12.07.2013 • **Published Online:** 27.01.2014 • **Printed:** 24.02.2014

Abstract: Sea buckthorn (*Hippophae rhamnoides* L.) is a minor fruit crop. The most important breeding activities focused on the production of sea buckthorn fruits as a top-quality food product have been performed in Russia, Germany, and the Czech Republic. Over the past several decades, plant breeders have selected a number of cultivars that show excellent physical and nutritional properties. The aim of this study was to monitor and determine the total contents of phenols and flavonoids as well as antioxidant capacity and scavenging activity of methanolic extracts on reactive oxygen species and lipid peroxidation in some cultivars of sea buckthorn suitable for growing in Central Europe. Altogether, 6 cultivars of this fruit species presented total phenolic contents ranging from 8.62 g of gallic acid kg^{-1} of fresh mass to 14.17 g of gallic acid kg^{-1} of fresh mass. There were high correlations between the contents of phenols and flavonoids on the one hand and the antioxidant properties of sea buckthorn berries on the other. This study indicates that for Central European conditions, sea buckthorn is a promising fruit species that can be used for consumption due to its antioxidant properties.

Key words: Antioxidant capacity, ascorbic acid, flavonoids, *Hippophae rhamnoides*, phenolics

1. Introduction

Reactive oxygen species (ROS) can be generated during normal body function as a human immune system response and can also be acquired from the environment (Jomova and Valko, 2011). Nevertheless, the excessive production of ROS (caused by the influence of current lifestyle, stress, nutrition, etc.) is associated with cellular and metabolic injury, accelerated aging, cancer, cardiovascular diseases, neurodegenerative diseases, and inflammation. Oxidative damage may be prevented or limited by dietary antioxidants (Bonnefoy et al., 2002). Fruits are among the basic foodstuffs with antioxidant properties (Celik et al., 2009; Ochmian et al., 2009; Poledica et al., 2012; Rab and Lisan-Ul-Haq, 2012). These properties are caused by many chemical compounds, e.g., phenolics or flavonoids (Gazdík et al., 2008a, 2008b; Lugasi et al., 2011).

Sea buckthorn (*Hippophae rhamnoides* L.), originating from Europe and Asia, belongs to the oleaster family (Elaeagnaceae), and it is an important fruit crop in the northern region (Dolejsi et al., 1991). Plants are frost-resistant and not very demanding with regard to pedoecological conditions. Moreover, they can be used for antierosion protection and ornamental purposes. A certain disadvantage of these plants is their dioeciousness (Kutina, 1991). The fruit is a berry of an average size of 6–9 mm with a fine, deep orange skin (Hricovsky, 1991). These berries contain high amounts of vitamins (Jurikova et al., 2012b) and show high antioxidant capacity, so it is expected that sea buckthorn berries could therefore be used as a preventive means against neoplastic (Virag et al., 2007) and cardiovascular diseases (Xu et al., 2011). By means of pressing the fruit, it is possible to obtain oil rich in tocopherols, tocotrienols, plant sterols, and carotenoids (Kallio et al., 2002). Sea buckthorn berries are also used for the local production of jams, syrups, and liquors. Dried berries are used for the preparation of various dietetic teas (Beveridge et al., 1999). It has become a cultivated plant due to breeding in Russia. During the past several decades, research in Germany and the Czech Republic has also resulted in cultivars that are valuable for commercial production (Paprstejn, 2009). Some Czech, German, and Russian cultivars (namely Botanicky, Buchlovicky, Hergo,

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Leicora, Ljubitelna, and Trofimovskij) are among the cultivars most utilized in Central Europe (Kutina, 1991; Hricovsky, 1991; Paprstejn, 2009).

The aim of this study was to measure the contents of phenols, ascorbic acid, and flavonoids in the berries of some selected sea buckthorn cultivars originating from Russia, Germany, and the Czech Republic. Another part of this study was the determination of antioxidant capacity and the evaluation of scavenging activity of methanolic extracts from sea buckthorn berries on ROS, namely hydroxyl radical, nitric oxide, superoxide anion, and lipid peroxidation. The main innovation of this work is that for the first time the research was conducted with regard to antioxidant properties in the framework of the particular cultivars listed above, which are suitable for cultivation in the environmental conditions of Central Europe. Moreover, the antioxidant activity of cultivars was determined by 5 methods.

2. Materials and methods

2.1. Description of locality

Fruits were harvested from an experimental germplasm collection of Mendel University in Brno. This orchard is situated in the area of the village Zabcice, approximately 20 km south of Brno, in the Czech Republic. The altitude is 184 m above sea level; the geographical location is 49°01′N, 16°36′E. The average annual temperature and the 50-year average of precipitation are 9 °C (15.6 °C during the growing season) and 553 mm (356 mm during the growing season), respectively. Soils are classified as gleyed alluvial soils developed on Holocene calciferous sediments with a marked accumulation of organic compounds. Regarding the texture, the topsoil is loamy and the subsoil clayey-loamy.

2.2. Collection and the processing of samples for chemical analyses

Within the period of 2011–2012, 100 fully ripe berries were randomly collected from 3 plants of each cultivar during August (Paprstejn, 2009). The fruits of the cultivars were processed immediately after the harvest (within 2 days). Harvested fruits were homogenized in a SJ500 laboratory grinder (MEZOS, Hradec Kralove, Czech Republic), and an average sample was obtained by dividing into quarters. Each parameter was measured in 5 replications. The results were expressed as the average of a 2-year experiment. The age of the experimental plants ranged from 6 to 10 years. Due to the fact that sea buckthorn bears fruit every second year, different plants from the same locality and the same cultivar were used each year.

The following cultivars of sea buckthorn were analyzed: Botanicky and Buchlovicky, which are of Czech origin; Hergo and Leicora, which are German in origin; and Ljubitelna and Trofimovskij, which are Russian cultivars (Rop and Valášek, 2005).

2.3. Sample preparation

The extraction was performed according to the method described by Kim et al. (2003) and modified according to Barros et al. (2007), using the following procedure: 10 g of a fresh sample was homogenized for 10 s in 100 mL of methanol in a SJ500 laboratory grinder (MEZOS). The resulting suspension was placed into Erlenmeyer flasks (120 mL) and left to stand in a water bath with a temperature of 25 °C for a period of 24 h. After the extraction, the contents of the flask were filtrated (13-mm nylon membrane syringe filter, 0.45 μm) and stored at 4 °C for further use. For the measurement of total phenolic content (TPC), antioxidant capacity (TAC), total flavonoid content (TFC), particular ROS, and lipid peroxidation inhibition activity, a LIBRA S6 spectrophotometer (Biochrom Ltd., Cambridge, UK) was used.

2.4. Total phenolic content assay

To measure the total contents of phenolic substances, 0.5 mL of the sample was taken and diluted with water in a 50-mL volumetric flask. Thereafter, 2.5 mL of Folin–Ciocalteu reagent and 7.5 mL of a 20% solution of sodium carbonate were added. The resulting absorbance was measured in the LIBRA S6 spectrophotometer (Biochrom Ltd.) at a wavelength of 765 nm against a blind sample, which was used as a reference. The results were expressed as g of gallic acid equivalent (GAE) kg^{-1} of fresh mass (FM) (Kim et al., 2003).

2.5. Antioxidant capacity by the DPPH assay

The DPPH assay (2,2-diphenyl-1-picrylhydrazyl) was conducted according to the modified method of Brand-Williams et al. (1995) and Thaipong et al. (2006); the stock solution was prepared by dissolving 24 mg of DPPH with 100 mL of methanol, and then stored at –20 °C until needed. The working solution was obtained by mixing 10 mL of the stock solution with 45 mL of methanol to obtain the absorbance of 1.1 ± 0.02 units at 515 nm using the LIBRA S6 spectrophotometer (Biochrom Ltd.). Fruit extracts (150 μL) were allowed to react with 2850 μL of the DPPH solution for 1 h in the dark. The absorbance was then taken at 515 nm.

Antioxidant capacity was calculated as a decrease in the absorbance value using the following formula:

Antioxidant capacity (%) = $(A_0 - A_1/A_0) \times 100\%$,

where A_0 is the absorbance of the control (without the sample) and A_1 is the absorbance of the mixture containing the sample.

The results of the absorbance were converted using a calibration curve of the standard and expressed in ascorbic acid equivalents (AAE) in g kg^{-1} FM (Rupasinghe et al., 2006).

2.6. Total flavonoid content assay

The total flavonoid content was determined following Singleton et al. (1999). In a 10-mL Eppendorf tube, 0.3

mL of the fruit extract, 3.4 mL of 30% ethanol, 0.15 mL of $NaNO_2$ (0.5 mol L^{-1}), and 0.15 mL of $AlCl_3.6H_2O$ (0.3 mol L^{-1}) were added and mixed. After 5 min, 1 mL of NaOH (1 mol L^{-1}) was added, and the mixture was measured at the wavelength of 506 nm. The total flavonoid concentration was calculated from a calibration curve using rutin as the standard. The results were expressed in g kg^{-1} FM.

2.7. ROS scavenging activity assay

For the measurement of ROS activity, a 10% fruit extract was prepared in phosphate buffer (50 mmol L^{-1}, pH 7.0). The hydroxyl radical scavenging activity was assayed according to Ghiselli et al. (1998): 1 mL of the extract was mixed with 0.8 mL of a reaction buffer (phosphate buffer, 20 mmol L^{-1}, pH 7.4; deoxyribose, 1.75 µmol/L; iron ammonium sulfate, 0.1 µmol L^{-1}; and ethylenediaminetetraacetic acid, 0.1 µmol L^{-1}). Next, 0.1 mL of H_2O_2 (0.01 mol L^{-1}) was added to the reaction solution. The solution was incubated for 10 min at 37 °C prior to the addition of 0.5 mL of 1% thiobarbituric acid and 1 mL of 2.8% trichloroacetic acid. The mixture was boiled for 10 min and cooled rapidly. The absorbance of the mixture was measured at 532 nm with the LIBRA S6 apparatus (Biochrom Ltd.).

The assay of nitric oxide scavenging activity was performed according to the method of Green et al. (1982); 1 mL of the extract was mixed with 1 mL of the reaction solution containing sodium nitroprusside (10 mmol L^{-1}) in phosphate buffer (20 mmol L^{-1}, pH 7.4). Incubation at 37 °C for 1 h followed, and 0.5 mL of the aliquot was then mixed with 0.5 mL of Griess reagent. The absorbance was measured at 540 nm.

The superoxide anion scavenging activity was conducted according to the method based on the reduction of cytochrome *c* (Beissenhirtz et al., 2004); 1 mL of the extract was mixed with 1 mL of solution containing xanthine oxidase (0.07 U mL^{-1}), xanthine (100 µmol L^{-1}), and cytochrome *c* (50 µmol L^{-1}). After incubation at 20 °C for 3 min, the absorbance at 550 nm was determined.

All tests were performed in triplicate. The scavenging activities of hydroxyl radical, nitric oxide, and superoxide anion were calculated as follows:

(%) inhibition = $(A_0 - A_1/A_0) \times 100\%$,

where A_0 is the absorbance of the control (without the sample) and A_1 is the absorbance of the mixture containing the sample.

2.8. Lipid peroxidation inhibition activity

The inhibition of lipid peroxidation was assayed by using 5 µg of rat liver homogenized in 20 mL of Tris-HCl buffer (50 mmol L^{-1}, pH 7.6). Next, 0.1 mL of the liver homogenate was incubated with the sample (0.2 mL of a 25% extract), 0.1 mL of KCl (30 mmol L^{-1}), 0.1 mL of $FeSO_4$ (0.16 mmol L^{-1}), and 0.1 mL of ascorbic acid (0.06 mmol L^{-1}) at 37 °C for 1 h. Thereafter, 1 mL of 1% thiobarbituric acid (TBA) and 1 mL of 15% trichloroacetic acid were added. The final solution was heated at 100 °C in a boiling water bath for 15 min, cooled with ice for 10 min, and then centrifuged at 5000 rpm for 10 min using an MPW-54 apparatus (Unimed, Prague, Czech Republic). The absorbance of the supernatant was measured at 532 nm using the LIBRA S6 spectrophotometer (Biochrom Ltd.). The blank was performed by substituting Tris-HCl buffer (50 mmol/L, pH 7.6) for the sample. The percentage of inhibition of the formation of TBA-reactive substances was calculated as:

(%) inhibition = $(A_0 - A_1/A_0) \times 100\%$,

where A_0 is the absorbance of the control (without the sample) and A_1 is the absorbance of the mixture containing the sample (Anup et al., 2006).

2.9. Determination of ascorbic acid

The determination of ascorbic acid content (AAC) was ascertained according to the modified method of Miki (1981), using 5 g of the homogenized fruit weighed in an Erlenmeyer flask by adding 25 mL of extractant methanol, H_2O, and H_3PO_4 at a ratio of 99:0.5:0.5. The flask with the samples was placed into a water bath with a temperature of 25 °C, and the samples were extracted for 15 min. To keep the samples from being exposed to sunlight, the flask was covered with aluminum foil during the preparation. After the extraction, the contents of the flask were filtrated through a No. 390 paper Filtrapak (Petr Lukes, Uhersky Brod, Czech Republic). Before injection, the filtrate prepared in this way was diluted in a ration of extractant and filtrated again through a nylon 0.45-µm membrane filter (Petr Lukes). The instruments used for ascorbic acid analysis consisted of a solvent delivery pump (Model 582, ESA Inc., Chelmsford, MA, USA), a Model 5010A guard cell with a working electrode potential K1 = 600 mV, K2 = 650 mV (ESA Inc.), a Model Supelcosil LC-8 (150.0 × 4.6 mm) 5-µm particle size chromatographic column (Sigma-Aldrich, St. Louis, MO, USA), and a Coulochem III electrochemical detector (ESA Inc.). The chromatographic conditions were constant: at 30 °C, a mobile phase comprising methanol, H_2O, and H_3PO_4 at 99:0.5:0.5 was used (filtrated through a nylon 0.2-µm filter); the type of elution was isocratic; and the flow rate of the mobile phase was 1.1 mL/min. The content of ascorbic acid was calculated as g kg^{-1} FM.

2.10. Statistical analysis

The data obtained were analyzed statistically by analysis of variance (ANOVA) and Tukey's multiple range tests for comparison of means (Snedecor and Cochran, 1968). Correlation functions were calculated using the statistical package Unistat, v. 5.1, and Microsoft Office Excel, v. 2010.

3. Results

The results of chemical analyses are given in Tables 1 and 2. All results are expressed as a 2-year average, and it should be mentioned that there were no statistically significant differences between the individual years.

Concerning phenolic contents, they ranged from 8.62 g GAE kg^{-1} FM (the Buchlovicky cultivar) to 14.17 g GAE kg^{-1} (the Trofimovskij cultivar).

Antioxidant capacity was measured by means of the DPPH test. The highest antioxidant capacity was found in the Ljubitelna cultivar (18.11 g of AAE kg^{-1} FM), which is of Russian origin. In our work, the correlation coefficient between TPC and TAC was $r^2 = 0.8904$. In addition to the highest content of antioxidant capacity, the highest contents of flavonoids and ascorbic acid (Table 1) were also determined in the Russian sea buckthorn cultivars.

When comparing all cultivars used in our work, the flavonoids content ranged from 4.18 g of rutin kg^{-1} FM to 7.97 g of rutin kg^{-1} FM. High flavonoid content is typical in sea buckthorn fruits.

The ascorbic acid content ranged from 3.94 g kg^{-1} FM to 5.73 g kg^{-1} FM. In the species studied, ascorbic acid was—similarly to flavonoids and total phenolics—highly correlated with the antioxidant effects of these berries (Table 3).

The highest values of scavenging activity of ROS and lipid peroxidation were also recorded in the Russian cultivars. The Czech and German cultivars showed statistically significantly lower contents of substances influencing their antioxidant capacity; this was manifested in a reduced inhibitory efficiency against ROS (see Table 2). Methanolic extract of the Trofimovskij cultivar reduced the percentages of inhibition of nitric oxide, superoxide anion, hydroxyl radical, and lipid peroxidation by 48.52%, 49.65%, 34.03%, and 24.70%, respectively.

The coefficients of correlations existing between the total contents of phenolics, ascorbic acid, flavonoids, and antioxidant capacity and scavenging effect on ROS and lipid peroxidation are given in Tables 3 and 4.

Table 1. Total phenolic contents (g GAE kg^{-1} FM), antioxidant capacity (g AAE kg^{-1} FM), total flavonoid content (mg kg^{-1} FM), and ascorbic acid content (g kg^{-1} FM) of fruits of particular sea buckthorn cultivars; n = 10.

Cultivar	Total phenolic content	Antioxidant capacity	Total flavonoid content	Ascorbic acid
Botanicky	9.31 ± 1.02 a	11.26 ± 1.29 a	4.79 ± 0.54 a	4.10 ± 0.81 a
Buchlovicky	8.62 ± 1.51 a	12.85 ± 1.95 a	4.18 ± 0.86 a	3.94 ± 0.93 a
Hergo	9.65 ± 1.34 a	11.58 ± 1.11 a	4.98 ± 0.35 a	4.18 ± 0.66 a
Leicora	9.74 ± 1.20 a	11.50 ± 1.26 a	5.04 ± 0.68 a	4.09 ± 0.80 a
Ljubitelna	14.01 ± 1.65 b	18.11 ± 1.74 b	7.35 ± 0.80 b	5.73 ± 0.70 b
Trofimovskij	14.17 ± 1.43 b	17.38 ± 1.41 b	7.97 ± 0.75 b	5.51 ± 0.45 b

Different letters in each column indicate significant differences in means at $P < 0.05$ according to Tukey's test.

Table 2. Scavenging effect of sea buckthorn methanolic extract (10%) on nitric oxide (percentage of inhibition), superoxide anion (percentage of inhibition), hydroxyl radical (percentage of inhibition), and lipid peroxidation (percentage of inhibition); n = 10.

Cultivar	Hydroxyl radical (%)	Nitric oxide (%)	Superoxide anion (%)	Lipid peroxidation (%)
Botanicky	27.45 ± 1.82 a	38.71 ± 1.71 a	39.58 ± 2.44 a	20.72 ± 1.35 a
Buchlovicky	25.30 ± 1.98 a	32.50 ± 1.60 b	41.60 ± 2.79 a	18.14 ± 0.65 b
Hergo	30.56 ± 1.15 b	40.11 ± 1.21 a	38.72 ± 2.58 a	19.11 ± 1.89 ab
Leicora	27.11 ± 1.81 a	38.27 ± 1.93 a	40.70 ± 1.63 a	20.92 ± 1.23 a
Ljubitelna	35.86 ± 1.73 c	45.16 ± 1.64 c	49.10 ± 2.57 b	24.25 ± 1.15 c
Trofimovskij	34.03 ± 1.47 c	48.52 ± 1.29 d	49.65 ± 2.38 b	24.70 ± 1.26 c

Different letters in each column indicate significant differences in means at $P < 0.05$ according to Tukey's test.

Table 3. Correlation relationships between the total phenolic content (TPC), the total flavonoid content (TFC), the total antioxidant capacity (TAC), the ascorbic acid content, and the scavenging effect of sea buckthorn extracts on hydroxyl radical, nitric oxide, superoxide anion, and lipid peroxidation.

Correlation between	r^2	Equation
TPC and TAC	0.8904	y = 1.1859x + 0.8340
TFC and TAC	0.8345	y = 1.8479x + 3.2129
Ascorbic acid and TAC	0.9312	y = 3.7591x – 3.4806
TPC and hydroxyl radical	0.8755	y = 1.5750x + 12.8580
TFC and hydroxyl radical	0.8439	y = 2.4888x + 15.8200
Ascorbic acid and hydroxyl radical	0.8930	y = 4.9299x + 7.4150
TPC and nitric oxide	0.8574	y = 2.0928x + 17.6980
TFC and nitric oxide	0.9059	y = 3.4624x + 20.7460
Ascorbic acid and nitric oxide	0.7862	y = 6.2114x + 12.0240
TPC and superoxide anion	0.8959	y = 1.8499x + 23.0310
TFC and superoxide anion	0.8635	y = 2.9229x + 26.5110
Ascorbic acid and superoxide anion	0.9108	y = 5.7521x + 16.8130
TPC and lipid peroxidation	0.9066	y = 1.0193x + 10.1790
TFC and lipid peroxidation	0.9170	y = 1.6500x + 11.8720
Ascorbic acid and lipid peroxidation	0.8580	y = 3.0734x + 7.1944

Table 4. Pearson correlation coefficients between investigated chemical parameters. The mean values were used in the analyses of chemical parameters at levels *P < 0.05, **P < 0.01, ***P < 0.001.

Chemical parameter	TAC	Hydroxyl radical	Nitric oxide	Superoxide anion	Lipid peroxidation
TPC	0.8904**	0.8755**	0.8574**	0.8959**	0.9066**
TFC	0.8345**	0.8439**	0.9059**	0.8635**	0.9170**
AAC	0.9312***	0.8930**	0.7862*	0.9108**	0.8580**

4. Discussion

The major antioxidant in sea buckthorn berries is ascorbic acid; they also contain tocopherols, carotenoids, and flavonoids (Christaki, 2012).

Many genotypes originate in Russia. Among the Russian genotypes there exists great variability, which can be utilized during breeding for obtaining new cultivars characterized by, for example, high content of antioxidants. This variability may be caused by the fact that sea buckthorn is tolerant of abiotic stresses, and plants can grow in nutritionally poor environments where it is difficult to grow other crops (Kanayama et al., 2012). More intensive formation of antioxidants as a response of a plant to environmental stress conditions is typical of some fruit, in particular pomaceous fruit crops (Tetera, 2006). Tiitinen et al. (2006) drew attention to a high content of vitamin C and generally bioactive substances, such as polyphenols, in Russian cultivars in comparison with the Finnish Raisa cultivar. Moreover, they declared the Trofimovskij cultivar to be promising (Tiitinen et al., 2005), and this cultivar was also studied in this research.

The content of polyphenols in the sea buckthorn fruit is, above all, a matter of cultivars, and it can be very variable (Ercisli et al., 2007). Generally, sea buckthorn has a high content of total phenolics (Papuc et al., 2009). In sea buckthorn fruit, TPC ranges from around 1 to 6 g of GAE kg^{-1} FM (Korekar et al., 2011; Perino-Issartier et al., 2011), although the values can be even higher (Korekar

et al., 2011; Perino-Issartier et al., 2011; Varshneya et al., 2012). Velioglu et al. (2008) noticed 11.12 g of GAE kg^{-1} FM in sea buckthorn fruits. The assayed sea buckthorn cultivars had higher TPC values, from 8 to 14 GAE kg^{-1} FM. For comparison, in apples, these values range from 1.46 to 3.29 g of GAE kg^{-1} FM (Rop et al., 2011), in plums from 3.48 to 4.95 g of GAE kg^{-1} FM (Rop et al., 2009), and in blackcurrants on average around 5.33 g of GAE kg^{-1} FM (Lugasi et al., 2011).

Flavonoids, a large group of phenolic compounds (Sabir et al., 2005) with several well-documented biological activities beneficial to human health, are one of several important constituents of sea buckthorn berries (Barl et al., 2003). The significant position of flavonoids in our study was documented by high correlation coefficients (TFC–TAC, 0.8345; TFC–hydroxyl radical, 0.8930; TFC–nitric oxide, 0.9053; TFC–superoxide anion, 0.8635). According to Chen et al. (2013), the most effective flavonol glycosides in term of scavenging activity are isorhamnetin, quercetin, and kaempferol. When sea buckthorn is consumed, the flavonoid content is connected with the prevention of the occurrence of cholesterol (Larmo et al., 2009), high blood pressure, and high blood sugar (Xu et al., 2011).

In the literature, sea buckthorn is mentioned as one of the richest fruit sources of ascorbic acid (Kallio et al., 2002). In general, sea buckthorn has a high content of vitamin C (Papuc et al., 2009). In Turkish genotypes, the vitamin C content was found to be from 0.19 to 1.21 g kg^{-1} FM (Ercisli et al., 2007). Nevertheless, in sea buckthorn the vitamin C content is chiefly a matter of genetics and great variability exists in particular cultivars (Tiitinen et al., 2006). The content of vitamin C is mostly between 0.93 and 4.06 g kg^{-1} (Zadernowski et al., 2012). Stobdan et al. (2010) observed values on average of 2.75 g kg^{-1} in randomly collected sea buckthorn fruit growing in the mountainous regions of Pakistan. Sabir et al. (2005) mention vitamin C values between 2.50 and 3.33 g kg^{-1} FM. In the German Hergo and Leicora cultivars, also used in our research, Raffo et al. (2004) determined the vitamin C content to be between 1.80 and 3.70 g kg^{-1} FM, and even during the ripening stage the content remained stable (Gutzeit et al., 2008). In all mentioned studies, the values of ascorbic acid of other fruits were lower than in the observed cultivars. For example, in apples, plums, or blackcurrants the average contents of this vitamin are approximately 0.36 g kg^{-1} FM, 0.23 g kg^{-1} FM, and 3.25 g kg^{-1} FM, respectively; blackcurrant is considered to be one of the most valuable sources of ascorbic acid for humans. In addition, kiwi fruits or oranges are listed as fruits with high contents of vitamin C (1.00–2.95 g kg^{-1} FM). Among vegetables, green peppers are one of the richest sources of ascorbic acid, with 3.98 g kg^{-1} FM (Kováčiková et al., 1997).

Sea buckthorn is an excellent source of antioxidants (Papuc et al., 2008; Buyukuroglu and Gulcin, 2009), which was confirmed in our study, as well (11.26–18.11 g of AAE kg^{-1} FM). For example, black chokeberry had higher contents of AAE than sea buckthorn (Rop et al., 2010), but on the other hand, most fruit species had lower values: apples at around 2–4 g of AAE kg^{-1} FM, while in cherries this value is on average 0.9 g of AAE kg^{-1} FM (Usenik et al., 2008).

Phenolic fractions made a major contribution to the total antioxidant capacity in sea buckthorn berries (Gao et al., 2000), which is in accordance with the results of our experiment (r^2 = 0.9804). Korekar et al. (2011) also noticed high correlations, and generally they are typical of fruit (Moyer et al., 2002; Rupasinghe et al., 2006; Pokorná-Juríková and Matuškovič, 2007; Jurikova et al., 2012a). On the contrary, Ercisli et al. (2007) observed that the correlations between TPC and TAC measured by the DPPH method reached a value of only r^2 = 0.688 in Turkish genotypes of sea buckthorn fruit.

Sea buckthorn fruit extracts are strong scavengers of nitric oxide and superoxide anion, although they have the lowest values in relation to hydroxyl radical (Varshneya et al., 2012), which was also confirmed in our measurements. In addition, Yang et al. (2007) drew attention to sea buckthorn fruits as a significant inhibitor of nitric oxide. Nitric oxide has many physiological functions, including vasodilatation or synaptic plasticity in the central nervous system. On the other hand, the nitric oxide radical is implicated in the pathogenesis of several diseases (Sumanont et al., 2004). Nitric oxide belongs to ROS, including free radicals such as superoxide anion (O_2^-) and hydroxyl radical species (OH•) (Wang et al., 2009). These ROS are known to cause aging, cancer, and many other negative effects on the human body (Aruoma et al., 1994). The scavenging effect on nitric oxide of sea buckthorn methanolic extract in assayed cultivars was 32.50%–48.52%.

In the present paper, the sea buckthorn fruit extract was also evaluated for its high ability to scavenge hydroxyl radical (25.30%–35.86%) using the deoxyribose degradation assay. Even in the process of inhibition of hydroxyl radical, sea buckthorn fruits have greater efficiency than other common fruit species (Papuc et al., 2009). Sea buckthorn methanolic extract exhibited the strongest scavenging activity against superoxide anion (38.72%–49.65%), which is in accordance with Papuc et al. (2008), although the aforementioned authors determined lower values of scavenging activity (25.5 ± 2.4%). In this way, extracts of sea buckthorn fruit were more effective than those of other fruit species, e.g., pome (Rop et al., 2011) or fruits of *Prunus* species (Jung et al., 2002).

For comparison, in selected apple cultivars the scavenging effect of 10% methanolic extracts on nitric oxide ranges from 12.78% to 21.36%, on superoxide anion from 17.10% to 24.99%, and on hydroxyl radical from 9.47% to 18.12% (Rop et al., 2011). Sea buckthorn is a suitable fruit species with enormous influence on the inhibition of lipid peroxidation (Zadernowski et al., 2012). This effect might be attributed to its high content of polyphenolic (Saggu and Kumar, 2008). According to the degree of dilution and the extract type, on average the peroxidation may reach the level of 36%–69% (Buyukokuroglu and Gulcin, 2009), which represented higher values than in the assayed cultivars (18.14%–24.70%). On the other hand, lipid peroxidation is higher than in other common fruit species (Maffei et al., 2007).

Comparing all of the above results with those of other fruit species (Velíšek, 2002), the measured values of antioxidant capacity, scavenging activity of ROS, and lipid peroxidation were outstanding. Antioxidant capacity is conditioned by a high content of phenolics, or, in concrete terms, of flavonoid substances (Jurikova et al., 2012b). Examining common species of pomaceous, stone, or berry fruit species (Kopec and Balík, 2008), these contents are again relatively high.

The results obtained explicitly indicate the value of this fruit species. Sea buckthorn berries can be used as a raw material for the production of various food products, e.g., oils or fruit spreads (Kyzlink, 1990). High nutritional value, antioxidant capacity, and processing ability of the fruit predetermine sea buckthorn cultivars to be a promising fruit species for wider use in human nutrition. Moreover, the advantages of cultivars suitable for growing in Central Europe consist of the modest requirements of the plants and their low demands as regards environment and locality; other positive features involve high resistance to frosts, diseases, and pests.

In conclusion, in our study, the uniqueness of cultivars of sea buckthorn suitable for cultivation in Central Europe with respect to antioxidant properties was fully corroborated. Compared with other, more regularly yielding fruit species, the economic and commercial aspects of its cultivation remain issues that require further research. However, under more adverse climatic conditions sea buckthorn is generally known as a very suitable, plastic, and adaptable fruit-bearing species. The results presented compare antioxidant properties of the cultivars studied, and in this work they have been described for these cultivars for the first time. The results obtained should contribute to the popularization of sea buckthorn as a promising source for the food industry as well as an object of further breeding work. Antioxidant properties make sea buckthorn extracts applicable for use as natural antioxidants in the medical and pharmaceutical industries. In the future, it will be very interesting to see the results of studies on the cytoprotective effects of sea buckthorn fruits in relation to flavonoids.

Acknowledgments

This work was kindly supported by funding from the Ministry of Education, Youth, and Sports of the Czech Republic (Grant LH 11134), the Ministry of Agriculture of the Czech Republic (QH 82232), and the Ministry of Culture of the Czech Republic (DF 11P01OVV006).

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