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EVALUATION OF CAROTENOIDS, POLYPHENOLS CONTENT AND ANTIOXIDANT ACTIVITY IN THE SEA BUCKTHORN FRUIT JUICE

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ABSTRACT

Due to the content of biologically active substances, sea buckthorn (*Hippophae rhamnoides* L.) has become the object of great interest of both, experts and the general public. It is appreciated particularly for the high content of vitamins and other biologically active substances, not only in berries but also in leaves and bark. The aim of the study was to evaluate the nutritional quality of sea buckthorn juice prepared from different varieties of sea buckthorn based on the content of total carotenoids, polyphenols and antioxidant activity. In this study we used varieties Hergo, Tytti, Vitaminaja, Raisa, Askola, Dorana, Slovan, Leikora, Bojan, Terhi and Masličnaja. Content of different components was quantified using spectrophotometry. The total carotenoids content expressed as β-carotene content in juice ranged from 50.63 mg.100 g^{-1} DM to 93.63 mg.100 g^{-1} DM, the highest content was in variety Askola and the lowest one in Terhi. Total polyphenols content determined by Folin-Ciocalteu method ranged from 13.03 mg GAE. dm^{-3} DM to 25.35 mg GAE. dm^{-3} DM. The highest content was identified in juice of variety Dorana and the lowest one in Raisa. The antioxidant activity quantified by the FOMO method ranges from 45.11 g AA. dm^{-3} DM to 108.77 g AA. dm^{-3} DM. The highest antioxidant activity was determined in juice of Dorana and the lowest in variety Bojan.

Keywords: sea buckthorn; sea buckthorn juice; carotenoids; polyphenols; antioxidant activity

INTRODUCTION

The first remarks about sea buckthorn are several centuries old. In traditional Chinese medicine it was used in the years 618-907 AD against cough, to improve blood circulation, to help with digestive problems, to relieve pain. Leaf extract was used in Mongolia to treat colitis **(Guliyev et al., 2004)**. Sea buckthorn caught considerable attention in Russia, where it has been seen as a very important plant for its healing and regenerative effects **(Bajer, 2014)**. Nowadays the public interest in sea buckthorn as a special dietary supplement is growing, mainly for its nutritional and health-related effects **(Yang and Kallio, 2002)**.

Sea buckthorn is native to Asia and very large Eurasian area at different altitudes. It is a unique plant that is currently domesticated in different countries, in particular China, Russia, Germany, Finland, Romania, France, Nepal, Pakistan and India **(Selvamuthukumaran et al., 2007)**. Sea buckthorn belongs to the less demanding timber species in terms of location requirements. The most suitable for its growth is light sandy clay soil but it thrives also in arid, semi-arid and fragile mountain areas. From the temperature point of view, it withstands high daytime temperatures in summer and severe winter frosts **(Letchamo et al., 2007)**.

Although almost all parts of the sea buckthorn plant are used, the fruit is the most valuable product, together with seeds. Sea buckthorn berries contain almost all water soluble vitamins and fat soluble vitamins and many other substances necessary for the human body. They contain pectin, essential oils, tannins, organic acids, oils, minerals and other substances. Significant sugars in sea buckthorn berries are glucose, fructose and xylose. From the organic acids there are mainly malic and quinic acids. Research has shown that Russian berries have relatively lower concentration of organic acids (2.1 to 3.2 g 100 mL^{-1} juice) than Finnish and Chinese genotypes holding the highest concentrations of organic acids (3.5 – 9.1 g 100 mL^{-1} juice) **(Bal et al., 2011)**. **Raffo et al., (2004)** state that the sour varieties contain predominantly malic acid (46.6 $mg.g^{-1}$) and quinic acid (28.2 $mg.g^{-1}$). **Bal et al., (2011)** reported that the fruits have a high proportion of aspartic acid and glutamic acid, but it is necessary to mention also the high content of essential amino acids, especially lysine, valine, threonine, methionine, leucine, izoleucine, tryptophan, phenylalanine.

Sea buckthorn is an excellent source of natural antioxidants **(Bal et al., 2011)**. The most important antioxidant in sea buckthorn juice is vitamin C **(Rosch et al., 2003)**. The authors report that one berry covers the recommended daily dose of vitamin C. The highest concentration of vitamin C is in Chinese subspecies *H. sinensis,* 2500 mg.100 g^{-1}. European subspecies of *H. rhamnoides* berries contain more than 360 mg.100 g^{-1} of vitamin C **(Bal et al., 2011)**. **Ercisli et al., (2007)** reported that there is lower content of ascorbic acid in varieties which are not cultivated. Vitamin E in sea buckthorn berries is found in the form α, β, γ, δ - tocopherols **(Yang and Kallio, 2002)**. The content of vitamin E varies from 8.0 mg to 16 mg 100 g^{-1} of fruit. Is is mainly in pulp oil (100 to 160 mg.100 g^{-1} of oil) and seed oil (105 to 120 mg.100 g^{-1}) **(Bajer, 2014)**. According to **Rosch et al., (2003)**, the content of vitamin E in the seed oil corresponds to 61-113 mg.100 g^{-1} and the content in the juice oil varies from 162 to 255 mg.100 g^{-1}.

Carotenoids give sea buckthorn typical yellow to orange colour. Therefore, the oil from the pulp contains more carotenoids than seed oil. The most active representative of carotenoids is β-carotene. In addition to β-carotene in sea buckthorn, there are also lycopene, zeaxanthin, β-kryptoxanthin **(Yang and Kallio, 2002b)**.

In sea buckthorn berries there were identified 12 types of flavonoids: quercetin, kaempferol, hesperidin, rutin, and others. Myricetin, citrine, catechin and others are also present. Those are secondary plant metabolites that are beneficial for the body due to its antioxidant character **(Chen et al., 2013; Bal et al., 2011)**. **Arimboor et al., (2006)** reported that sea buckthorn berries and leaves contain 9 phenolic acids: gallic acid, protocatechuic, salicylic, p-hydroxybenzoic, vanillic, caffeic, cinnamic, p-coumaric and ferulic.

The aim of this study was to determine the content of total carotenoids, polyphenols and antioxidant activity of sea buckthorn juice from selected varieties.

MATERIAL AND METHODOLOGY

The study reviewed 11 varieties of sea buckthorn – Hergo, Tytti, Vitaminaja, Raisa, Askola, Dorana, Slovan, Leikora, Bojan, Terhi, Masličnaja, which were obtained from the Central Control and Testing Institute for Agriculture - State department of fruit variety testing in Veľké Ripňany. German varietis were represented by Hergo, Askola, Dorana, Leikora. Russian originating were varieties Vitaminaja and Masličnaja, Finnish varieties were represented by varieties Tytti, Terhi and Raisa and Slovak by Bojan and Slovan.

Harvesting of berries for the analysis was carried out in the second half of September, when the fruits had characteristic deep orange colour. Due to fine surface structure of berries and their vulnerability, the full branches with berries were collected. The shoots were then frozen at -18 °C and were stored in the freezer until the analysis was run. Analyses were performed within 48 hours after harvesting. Before the analysis, we separated berries from the shoots in frozen state by gently shaking them off. Frozen sea buckthorn berries of different varieties were partially thawed and then pressed to obtain sea buckthorn juice, which was then subject to analysis.

Content of total carotenoids expressed in β- carotene was analysed at a wavelength of 455 nm by spectrophotometer UV VIS Jenway model 6405 UV / VIS in accordance with the methodology STN 12136 Determination of total carotenoids content and individual carotenoid fractions. Samples were extracted in acetone followed by capturing carotenoids in the petroleum ether solution.

Polyphenolic substance content was determined by spectrophotometry at a wavelength of 700 nm by Folin – Ciocalteu method **(Singleton and Rossi, 1965)** and was measured as equivalent content of gallic acid. The method is based on reaction of the Folin - Ciocalteu reagent with polyphenols, with leads to formation of blue colour product. The intensity of the blue colour is proportional to the content of the polyphenols.

The antioxidant activity was determined by the FOMO method **(Prieto et al., 1999)**. The principle of the method is the reduction of Molybdenum (VI^+) to Molybdenum (V^+) by activity of the reducing component in the phosphorus presence. There is a green Phosphomolybdic complex which colour intensity is measured at a wavelength of 695 nm by spectrophotometer. The reductive ability of compounds can be expressed as ascorbic acid (AA) content, which is needed to achieve the same reduction effect.

The results were processed by the statistical package Statistica 8.0 (Statsoft, Inc., Tulsa, USA). The differences between the samples were followed by Tukey's HSD test and correlation dependence between evaluation indicators by using Pearson‘s correlation coefficient.

RESULTS AND DISCUSSION

Sea buckthorn fruits are rich in carotenoids, the most active representative is β-carotene, whose average content was mentioned by **Bajer (2014)** 1.8-3.9 g mg.100 g^{-1} fruit. In our samples the content of carotenoids in sea buckthorn juice ranged from 5.87 mg.100 g^{-1} in variety Terhi to 12.07 mg.100 g^{-1} in Askola (Table 1).

After the conversion of carotenoids to 100 % dry mater, the content of carotenoids ranged from 50.63 mg.100 g^{-1} dry matter in variety Terhi to 93.63 mg.100 g^{-1} dry matter in Askola. Carotenoid content in samples decreased in the following order Askola > Vitaminaja > Hergo > Leikora > Doran > Tytti > Slovan > Bojan > Raisa > Masličnaja > Terhi. Tukey's HSD test determined the lowest content of carotenoids in the juice of varieties Terhi and Masličnaja, among which there was no statistically significant difference. The highest content of carotenoids was in juice of German variety Askola, which was statistically significantly different from any other samples. High levels of carotenoids content over 70 mg.100 g^{-1} dry matter we also found in samples of juices from German varieties Dorana, Leikora, Hergo. From Russian varieties a high content of carotenoids in juice was evaluated variety Vitaminaja. Slovak varieties Slovan and Bojan were lower in content of carotenoids.

Yang and Kallio (2002) state the total content of carotenoids in sea buckthorn berries from 1.0 to 200.0 mg.100 g^{-1} and β-carotene content from 0.2 to 17.0 mg.100 g^{-1}. **Eccleston et al., (2002)** determined the total content of carotenoids in sea buckthorn juice at the amount of 7.3 ml mg.100 mL^{-1} and β-carotene formed 3.3 mg. 100 mL^{-1}. **Kuruczek et al., (2012)** analyzed nine Russian varieties of sea buckthorn and the maximum levels of carotenoids were found in varieties Aromatnaya (28.97 mg.100 g^{-1} fresh weight), Arumnyj (21.51 mg.100 g^{-1}) and Botanicheskaya (14.2 mg.100 g^{-1}). In the study of **Raffo et al., (2004)** in German varieties of sea buckthorn Askola, Hergo and Leikora a presence of major carotenoid zeaxanthin (3-15 mg.100 g^{-1}), β-carotene (0.3-5 mg.100 g^{-1}) and ß-cryptoxanthin (0.5-1.9 g mg.100 g^{-1}) were found.

Table 1 Rating of carotenoids content in sea buckthorn juice.

Varieties	Total carotenoids (mg.100 g^{-1})	Total carotenoids (mg.100 g^{-1} DM)
Terhi	5.87	50.63^{a}
Masličnaja	6.23	53.23^{b}
Raisa	9.89	64,20^{b}
Bojan	8.29	64.27^{b}
Slovan	8.56	67,92^{c}
Tytti	8.98	69.64^{cd}
Dorana	8.21	71.40^{de}
Leikora	9.66	73.76^{e}
Hergo	10.56	77.07^{f}
Vitaminaja	11.99	87.55^{g}
Askola	12.08	93.63^{h}

NOTE: $^{a-h}$ means indicated by the same letter are insignificantly different at P >0.05; DM – dry matter.

In these three varieties increased concentrations of carotenoids were observed while berry ripening. **Andersson (2009)** in his work examines the content of carotenoids in sea buckthorn berries. In his experiments he used varieties Ljubitelskaja, originating in Russia and BHi 72587, BHi 72588, BHi 727102 from the Swedish University of Agricultural Sciences Balsgard. The main carotenoids occurring in sea buckthorn berries include lutein, zeaxanthin, β cryptoxanthin, lycopene, γ-carotene, β-carotene. The author quantified that the total carotenoid content ranged from 1.5 to 18.5 g mg.100 g^{-1}, in variety Ljubitelskaja it was 5.9 mg.100 g^{-1}, in BHi 72587 15.1 mg.100 g^{-1}, in BHi 72588 13.8 mg.100 g^{-1}, in variety BHI 727102 9.4 mg.100 g^{-1}. **Mőrsel et al., (2014)** provided the total content of polyphenols and carotenoids in sea buckthorn and orange juices. The highest content of β-carotene (18.65 mg.100 mL^{-1}) and polyphenolic substances (156.65 mg.100 mL^{-1}) were found in sea buckthorn juice. In orange juice the content of β-carotene was 11.68 mg.100 mL^{-1} and polyphenols 140.73 mg.100 mL^{-1}.

While evaluating the polyphenols content we identified the lowest one expressed in mg GAE.dm^{-3} (gallic acid equivalent) in the juice of varieties Raisa (2.00 g GAE.dm^{-3}) and the highest in the juice of Dorana (2.92 g GAE.dm^{-3}) (Table 2). After conversion of polyphenols content into dry weight, monitored substances were found within the values from 13.3 g GAE.dm^{-3} dry matter in a sample of the varieties Raisa to 25.35 g GAE.dm^{-3} dry matter in the juice of Dorana. Polyphenols content in the studied samples of sea buckthorn juice declined in the following order of Dorana > Hergo > Leikora > Vitaminaja > Masličnaja > Tytti > Terhi > Slovan > Bojan > Askola > Raisa (Table 2).

Tukey's test proved statistically significant differences in polyphenols content in the evaluated juice samples. The difference was not statistically significant between the juices of Slovan and Terhi. Similarly like in the carotenoids evaluation, the high levels of polyphenols were found in juices of German varieties Dorana, Hergo,

Table 2 Rating of polyphenol content in sea buckthorn juice.

Varieties	Total polyphenols (g GAE.dm^{-3})	Total polyphenols (g GAE.dm^{-3} DM)
Raisa	2.00	13.03^{a}
Askola	2.05	15.88^{b}
Bojan	2.11	16.34^{c}
Slovan	2.24	17.81^{d}
Terhi	2.07	17.88^{d}
Tytti	2.36	18.31^{e}
Masličnaja	2.30	19.68^{f}
Vitaminaja	2.72	19.89^{g}
Leikora	2.64	20.13^{h}
Hergo	2.84	20.76^{i}
Dorana	2.92	25.35^{j}

NOTE: $^{a-h}$ means indicated by the same letter are insignificantly different at P >0.05, DM – dry matter.

Leikora. Variety Askola, reaching the highest levels of carotenoids did not belong to varieties with a high content of polyphenols. Correlation analysis proved that there is no correlation dependence between the polyphenol and carotenoiod contents in sea buckthorn juice.

Bončiková et al., (2012) followed the content of total polyphenols in apple varieties Topaz, Pinova, Jonagold and Idared. Analyzing the detected value of 496.7 mg. kg^{-1} in a variety Idared after 842.2 mg. kg^{-1} in a variety Topaz. While determining the antioxidants in sea buckthorn juice **Eccleston et al., (2002)** declare flavonoids content in the amount of 1.182 mg.100 mL^{-1}, isorhamnetin-rutinoside formed 355 mg. 100 mL^{-1}, isorhamnetin-glucoside 142 mg. 100 mL^{-1}, guercetin-glycoside and guercetin-rutinoside 35 mg.100 mL^{-1}. **Arimboor et al., (2006)** dealt with processing of the fresh sea buckthorn berries and their chemical evaluation and indicated that the pure sea buckthorn juice containing no oil is characteristic by a high content of vitamin C (168.3-184.0 mg.100 g^{-1}) and polyphenols (2392-2821 mg.100 g^{-1}). **Raffo et al., (2004)** demonstrated the presence of flavonoids isorhamnetine (350-660 g mg.100 g^{-1}, quercetin (30-100 mg.100 g^{-1}) and kaempferol (2-5 mg.100 g^{-1}) in the German varieties Askola, Hergo and Leikora. **Gutzeit et al., (2007)** in their study isolated certain flavonoids from the juice of sea buckthorn using highspeed countercurrent liquid chromatography. Isorhamnetin-3-O-β-D-glucoside (95 mg), isorhamnetin -3-O-β-rutinoside (10 mg), guercetin-3-O-β-D-glucoside (5 mg) were separated from 4.1 g of the crude ethyl acetate extract. **Rop et al., (2014)** investigated and determined in the fruit of sea buckthorn the total content of polyphenols, flavonoids and antioxidant activity. The following samples were analyzed – varieties of Czech origin – Botanicky, Buchlovicky, of German origin – Hergo, Leikora and Russian origin – Ljubitelna, Trofimovskij. Polyphenolic substances content were measured spectrophotometrically by Folin – Ciocalteu method. Total polyphenol content was detected in the range from 8.62 g GAE. kg^{-1} dry matter in variety Buchlovicky to 14.17 g GAE. kg^{-1} dry matter in variety Trofimovskij. In variety Hergo it was 9.65 g GAE. kg^{-1} dry matter and in variety Leikora 9.74 g GAE. kg^{-1} dry matter, which are the values lower to what we have found in our work.

In the samples of sea buckthorn juice antioxidant activity was assessed by using the FOMO method. The antioxidant activity of the samples was expressed in mg.dm^{-3} equivalent of ascorbic acid (AA). The values of antioxidant activity of sea buckthorn juice samples ranged from 12.51 g AA.dm^{-3} in varieties Dorana to 5.82 g AA.dm^{-3} in Bojan (Table 3).

After conversion to dry weight we identified statistically the highest antioxidant activity in the juice of variety Dorana (107.88 g AA.dm^{-3} dry matter). Evaluated varieties according to antioxidant activity formed six homogeneous groups. Relatively high levels of antioxidant activity in addition to variety Dorana were also found in juices of Slovan Masličnaja. Also varieties Leikora, Terhi and Vitaminaja achieved higher levels of antioxidant activity. Average values we found in the juice of varieties. Hergo and Askola, with no statistically significant difference, and variety Tytti. The lowest antioxidant activity we found in the juice of varieties Raisa and Bojan.

Kuruczek et al., (2012) devoted their study to analysis of antioxidant activity of crude extracts from sea buckthorn berries. Analyses were performed using spectrophotometric methods FRAP and DPPH and nine Russian sea buckthorn varieties grown in Poland were examined. The highest values of antioxidant activity by DPPH method the authors found in varieties Avgustinka (45.78%), Aromatnaya (45.37%) Arumnyj (44.08%), Prozachnaya (39.06%) and by the FRAP method it was in the varieties Botanicheskaya (1892 μmol.L^{-1}) Avgustinka (819 μmol.L^{-1}), Luchistaya (676 μmol.L^{-1}), Aromatnaya (648 μmol.L^{-1}). **Rop et al., (2014)** investigated the antioxidant activity of botanical varieties Botanicky, Buchlovicky, Hergo, Leikora, Ljubitelna, Trofimovskij. The highest antioxidant activity using DPPH method was found in the Russian variety Ljubitelna (18.11 g TEAC kg^{-1}), the lowest in the Czech variety Botanicky (11.26 g TEAC kg^{-1}). In the German variety Hergo authors found antioxidant activity 11.58 g TEAC kg^{-1} and in Leikora 11,50 g TEAC kg^{-1}.

Table 3 Evaluation of antioxidant activity of sea buckthorn juice.

Varieties	Total antioxidant activity (g AA.dm^{-3})	Total antioxidant activity (g AA.dm^{-3} DM)
Bojan	5.82	45.12^{a}
Raisa	7.01	45.53^{a}
Tytti	7.91	61.32^{b}
Hergo	9.04	65.98^{c}
Askola	7.80	68.21^{c}
Leikora	9.91	75.67^{d}
Terhi	8.84	76.22^{d}
Vitaminaja	10.51	77.95^{d}
Masličnaja	10.22	88.05^{e}
Slovan	11.16	89.06^{e}
Dorana	12.23	108.77^{f}

NOTE: $^{a-f}$ means indicated by the same letter are insignificantly different at P >0.05, DM – dry matter.

Table 4 Correlation matrix between the monitored parameters, depending on the results of the Pearson's correlation coefficient.

	Polyphenols	Antioxidant activity
carotenoids (mg.100 g^{-1} DM)	0.08	-0.02
polyphenols (g.dm^{-3} DM)		**0.79****

NOTE: ** Correlations are significant at the level *P* <0.01; n= 33, DM – dry matter.

Yildiz et al. (2012) analyzed samples of seven genotypes of sea buckthorn from Turkey. They assessed their polyphenols content and antioxidant activity. The total polyphenols content was between 213 mg GAE.100 g^{-1} and 262 mg GAE.100 g^{-1}. Analyses of antioxidant activity by DPPH method showed the high value in the sea buckthorn genotypes, on average 94.2% inhibition of DPPH radical. **Ivanišová et al., (2015)** analyzed antioxidant activity in sea buckthorn and its products (fruit, sea buckthorn tea, oil and juice. The highest activity by DPPH method was observed in oil 8.75 mg TEAC. g^{-1}. Antioxidant activity by phosphomolybdenum method was in the range of 111.59 mg TEAC. g^{-1} to 196.4 mg TEAC. g^{-1}, with higher rates in sea buckhorn tea.

Using Pearson correlation coefficients, we watched a relationship between assessed parameters of sea buckthorn juice quality. We found statistically significant positive correlation between polyphenols content and antioxidant activity. Between the polyphenols and carotenoids contents and carotenoids content and antioxidant activity of sea buckthorn juice we did not find correlation dependence (Table 4).

CONCLUSION

Sea buckthorn (Hippophae rhamnoides L.) has become a product of interest thanks to its content of biologically active substances. It is appreciated especially for the high content of vitamins not only in fruit but also in its leaves or bark which are characterized by healing effects. Sea buckthorn fruits are unique due to the large amount of vitamins, vitamin C, tocopherol, minerals, ß-carotene, flavonoids and organic acids. Sea buckthorn juice is rich in vitamin C, indicating its antioxidant effects. The goal of this research was to assess the content of total carotenoids, polyphenols and antioxidant activity in juice of selected sea buckthorn varieties In this work juice samples of 11 sea buckthorn varieties were evaluated – Hergo, Tytti, Vitaminaja, Raisa and Asko Doran Slovan, Leikora, Bojan, Terhi, Masličnaja. The total carotenoids expressed in β-carotene content in the evaluated sample juices ranged from 50.63 mg.100 g^{-1} dry matter in variety Terhi to 93.63 mg.100 g^{-1} dry matter in Askola. The lowest content of polyphenols we found in the juice of the variety Raisa (13.03 g GAE.dm^{-3} dry matter) and the highest in the sample of Dorana (25.35 g GAE.dm^{-3} dry matter). The values of antioxidant activity of sea buckthorn juice samples ranged from 45.12 g AA.dm^{-3} dry matter of the Bojan sample to 108.77 g AA.dm-^{3} dry matter in the sample of Dorana. Among evaluated varieties Dorana juice was characterized by a high quality of content of nutritionally important substances. It clearly reached the highest content of polyphenolic compounds and high antioxidant activity.

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