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INVESTIGATION OF NUTRITIONAL PARAMETERS IN DIFFERENT PARTS OF SEA BUCKTHORN (*HIPPOPHAE RHAMNOIDES* L.) BERRIES

ISTRAŽIVANJE NUTRITIVNIH PARAMETARA U RAZLIČITIM DELOVIMA BOBICA VUČJEG TRNA (*HIPPOPHAE RHAMNOIDES L.)*

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ABSTRACT

Sea buckthorn (SB) (Hippophae rhamnoides L.) excels as an ingredient of functional foods, being outstandingly suitable due to its biologically active compounds. During the processing of fruits, large amounts of waste materials are produced such as peels and seeds, which are not further processed although containing substantial amounts of valuable components. The purpose of our research is to compare the nutritional values of different parts of SB to determine the location of antioxidant components within the berry. Separated sea buckthorn parts (juice, shell, seed+shell) were examined in the study. The total polyphenol content and antioxidant capacity (FRAP) were determined using the spectrophotometric methods, whereas the colour parameters (Konica Minolta Chroma Meter-400) were measured using digital equipment. According to our results, shells with seeds had the largest amount of polyphenol and the highest antioxidant content compared to fruit juice and seedless shells. Therefore, further processing possibilities of SB pomace should be investigated.

Key words: *sea buckthorn, pomace, antioxidant, FRAP, polyphenol content*

REZIME

Bobice vučjeg trna (SB) (Hippophae rhamnoides L.) odlikuje se kao odličan sastojak funkcionalne hrane , koje su izuzetno pogodne zbog svojih biološki aktivnih jedinjenja . Tokom prerade voća , proizvede se velike količine otpadnih materija, kao što su ljuska i seme, koji se dalje ne obrađuju iako sadrže značajne količine vrednih sastojaka. Svrha ovog istraživanja je upoređivanje nutritivnih vrijednosti različitih dijelova SB kako bi se utvrdila lokacija antioksidativnih komponenti unutar ovog bobičastog voća. U istraživanju su ispitivani posebni delovi ovog voća (sok, ljuska, seme + ljuska). Ukupan sadržaj polifenola i antioksidativnog potencijala (FRAP) su određeni korišćenjem spektrofotometrijskih metoda , dok su parametri boje mereni pomoću digitalne opreme (Konica Minolta Chroma Meter-400). Prema rezultatima, ljuske sa semenom imale su najveću količinu polifenola i najveći sadržaj antioksidansa u poređenju sa voćnim sokovima i ljuskama bez semena . Zbog toga treba u budućnosti istražiti mogućnosti daljeg prerade kljuka od SB.

Ključne reči: *vučji trn, kljuk, antioksidant, FRAP, sadržaj polifenola*

INTRODUCTION

Although several possible sources of nutrients are known (namely plants, animals, microorganisms, and even artificial sources), plant materials, especially fruits and vegetables, are the most relevant (Guaadaoui et al. 2014; Chalalai et al., 2015; Wani et al. 2016; Tulin et al., 2017.).

Sea buckthorn (*Hippophae rhamnoides* L.) excels as an ingredient of functional foods, being outstandingly suitable due to its biologically active compounds. First and foremost, it exerts a positive impact on human health as a result of its high C-vitamin, flavonoid, carotenoid, and tocopherol content. In addition, it is rich in unsaturated fatty acids, proteins, and other vitamins (Surykumar and Gupta, 2011; Christaki, 2012; Krejcarová et al. 2015). The wound healing effect of sea buckthorn has been confirmed by a number of contemporary studies (Upadhyay et al. 2009; Upadhyay et al. 2011; Edraki et al. 2014).

In China, India, Mongolia, and several other Asian countries, sea buckthorn (partly, but not exclusively due to its outstanding ecological adaptability) plays a key role in landscape protection and is of great economic importance. Nevertheless, relatively low attention is paid to this promising species in Hungary. Considering that the fruit is unsuitable for fresh consumption, improvements in sea buckthorn cultivars are closely associated with the processing technology. Sea buckthorn juice, pulp, peel, and seed oil are used as a primary commodity in the industry. The proportion of useful components within different SB fruit parts has been reviewed by Krejcarová et al. (2015). However, little is known about the important parameters of the fruit at the cultivar level.

With regard to SB, the examination of the berry composition is of particular importance because SB pomace (seed and shell), resulting from the extraction of juice from fruits (by pressing), has a very valuable composition. It can be used for therapeutic purposes and as a nutritional supplement. However, the largest amount of sea buckthorn is produced as animal feed, whereas the production residues are wasted (Van Dyk et al., 2013). From the food industry perspective, the development of the technology for extracting the most valuable components from SB pomace would revolutionise the use of SB berries and increase the amount of their biologically valuable ingredients. Therefore, it is indispensable to examine SB berry juice and pomace.

The main objective of our research is to examine the antioxidant properties of the ’Ascola’ and ’Leikora’ sea buckthorn cultivars, i.e. an analysis of their juice, seeds and shells.

MATERIAL AND METHOD

The berries of the sea buckthorn (*Hippophae rhamnoides* L.) cultivars 'Leikora' and 'Ascola' were collected from a commercial orchard (46° 57′ 28″ North Longitude, 18° 51′ 53″ East Latitude) near Nagyvenyim located in South Hungary. All analytical grade reagents were purchased from Sigma Aldrich Hungary Ltd. A Hitachi U-2900 UV-VIS spectrophotometer (Hitachi High-Technologies Europe GmbH, Krefeld, Germany) was used for photometric measurements.

During the separation of berry juice, seeds and shells, we followed the technological process used in the industrial practice. First, berries were heated to 80-85 °C, accompanied by continuous mixing to inactivate the enzymes. A small-scale berry pressing device (GM-POO) was subsequently used for the separation of juice and pomace. Since the further use of pomace is mainly in the dried form, the pomace obtained was dried at 80°C under atmospheric conditions until its moisture content reached 5 %. The dried pomace was stored in the vacuum-laminated package in an exicator to prevent backwatering and retain the original composition. In our research, the whole dried pomace (seed + shell) and the pomace without seed (shell) were investigated. The seeds were separated from the dried pomace manually using a sieve, and then ground using a Fagor Master Chef ML 2006 X INOX machine.

During the preparation, the berry parts were extracted using a 40 % acetone solution, with a 1:30 sample:solvent ratio (which ensures the most efficient yield based on previous experiments). The extraction time was 1 hour, during which the samples were placed in an ultrasonic water bath (BANDELIN SOMOREX RK52, U = 230 V, 50/60 Hz, I = 0.3 A, 35 kHz) for 30 minutes because ultrasounds accelerate the extraction, without decreasing the molecular mass, and preserve the chemical structure of the substance (Youssouf et al., 2017). Subsequently, the samples were centrifuged at 5000 rpm for 10 minutes, and the supernatant was used for the measurements. The measurements were performed in triplicate in each case.

Determination of the total polyphenol content

The quantity of the total polyphenol content (TPC) was determined as described by Singleton and Rossi (1965) using the Folin-Ciocalteu reagent. The colour change taking place during the reaction can be detected on 765 nm by a spectrophotometer, and the results can be expressed as the gallic acid equivalent (µg GAE/ml extract).

Determination of antioxidant capacity

The total antioxidant capacity was determined using the so-called FRAP value (Ferric Reducing Antioxidant Power), based on the Benzie and Strain method (1996). The iron ion ($Fe3^{+}$) reducing ability of the sample antioxidants is indicated by a blue colour change (λ=593 nm). The antioxidant capacity can be expressed as the ascorbic acid equivalent after calibration (µg AAE/ml extract).

Determination of colour measurements

The colour characteristics of the extracts were measured in the CIE colour measuring system using a Konica Minolta CR-400 tristimulus handheld colorimeter. To calibrate the instrument, distilled water was used. During the measurement, the L* (lightness factor), a* (red-green ratio) and b* (yellow-blue ratio) values were determined. The L* data is perceived as lightness approximately ranging from 0.0 for black to 100.0 for white. The a* data represents the red-green chroma perception. The b* data represents the yellow-blue chroma perception.

Statistical methods

Statistical evaluations were performed using the Statistica 9 (StatSoftInc., Tulsa, USA) software. The difference between the results obtained was evaluated by the Student's t-test at 95 % confidence.

RESULTS AND DISCUSSION

Relative to the polyphenol measurement (Figure 1), it can be generally stated that SB pomace with seeds contained the most polyphenols components, followed SB shells and SB juice. The differences between the fractions are significant within specific varieties. According to the pomace samples, the 'Ascola' cultivar was found to exhibit the higher values, whereas the polyphenol content of the 'Leikora' cultivar indicated higher juice values.

The highest polyphenol concentration (1389.39 µg GAE / ml) was detected in the 'Ascola' seeds (extracted using a 40 % acetone), followed by the 'Leikora' cultivar with a 1125.18 µg GAE / ml concentration. Substantially fewer and nearly equal amounts of polyphenols were recorded in the shells of both cultivars ('Ascola': 428.64 µg GAE / ml, 'Leikora': 390.12 µg GAE / ml), with no significant difference.

The content of polyphenols in the juice is considerably lower than in its pomace ('Ascola': 15.35 µg GAE / ml, 'Leikora': 20.75 µg GAE / ml).

Nearly one hundred times more polyphenols were found in the 'Ascola' pomace than the 'Ascola' juice. However, 55 times more polyphenols were found in the 'Leikora' pomace than in the 'Leikora' juice.

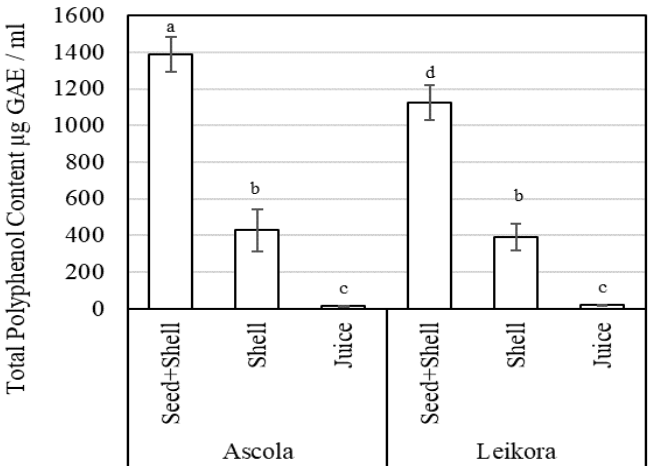

Fig. 1. Total polyphenol content of the sea buckthorn pomaces (seed+shell), shells and juices analyzed
[a. b. c.] etc. The same letter indicates that there is no significant difference at the 95 % confidence level between the sample results.

Regarding the antioxidant capacity results (Figure 2), it can be concluded that the pomace containing seeds and shells has the highest polyphenol content values. Of all the samples, the 'Leikora' pomace with seeds exhibited the highest values of the antioxidant capacity (2311.81 µg AAE / ml), which are considerably higher than the values recorded in the 'Leikora' pomace (1700.61 µg AAE / ml).

Seedless shells exhibited significantly less antioxidant compounds (Leikora 1071.78 µg AAE / ml, 'Ascola' 1108.28 µg

AAE / ml) compared to pomace with seeds, and there are almost no differences between the two cultivars examined. The antioxidant capacity of the examined SB juices (‘Leikora’ 65.19 μg AAE / ml, ‘Ascola’ 78.40 μg AAE / ml) was negligible compared to the pomace.

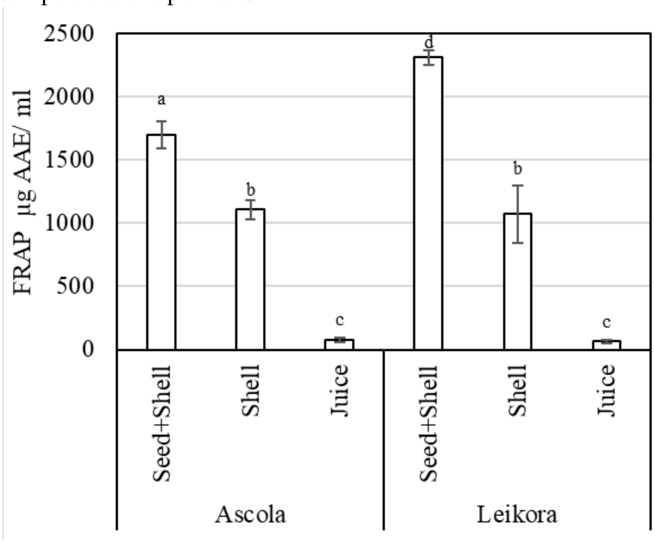

Fig. 2. FRAP results of the sea buckthorn pomaces (seed+shell), shells and juices analyzed.
[a. b. c.] etc. Same letter indicates that there is no significant difference at 95 % confidence between the sample results.

The ‘Leikora’ juice has the lowest value, whereas the highest value was recorded in the pomace of ‘Leikora’ berries (with almost a 35 times difference). Our results are consistent with the experimental results of Kruczek et al. (2012). In their paper, the antioxidant compounds of 9 sea buckthorn cultivars were studied, and their antioxidant capacity (FRAP) results in a 248-1892 μM / g of fresh fruit.

Table 1. The colour parameters of juice and pomace of ‘Ascola’ and ‘Leikora’sea buckthorn cultivars.

	‘Ascola’			‘Leikora’		
	Seed+Shell	Shell	Juice	Seed+Shell	Shell	Juice
L*	31.87 ± 5.01[a]	36.41 ± 2.96[b]	26.16 ± 0.34[c]	37.65 ± 0.83[b]	34.66 ± 3.55[b]	26.58 ± 0.46[c]
a*	3.54 ± 1.28[d]	5.78 ± 1.18[e]	2.45 ± 0.19[f]	2.79 ± 0.83[f]	4.95 ± 1.06[g]	1.44 ± 0.05[h]
b*	16.35 ± 4.49[i]	23.73 ± 2.90[j]	10.84 ± 0.65[k]	22.01 ± 0.10[l]	22.23 ± 3.14[l]	12.30 ± 0.40[k]

x±SD (x: mean, SD: standard deviation)
[a. b. c.] etc. Same letter indicates that there is no significant difference at 95 % confidence between the sample results.

Similar results have been obtained by several other researchers (Ercisli&Orhan, 2008). In case of sea buckthorn berries, the vitamin C and carotene content of the samples also contribute to the antioxidant capacity. This should be a subject of further research.

Table 1 shows the colour parameters of the sample extracts. The lightness factors of yellowish, orange-yellow extracts are in the lower third, i.e. the patterns are darker. The lowest L* value was measured in the ‘Ascola’ juice sample (26.16), whereas the highest result (37.65) was measured in the ‘Leikora’ pomace with seeds. In case of both cultivars of sea buckthorn, the juice was darker than the pomace samples. These differences are due to the carotenoid colorant in the juice (Breveridge et al., 1999). However, there is no difference between the colour values of shell and pomace with seeds.

In our research, the shell samples indicated the highest results (5.78 and 4.95), i.e. the most reddish shades. This is due to the presence of orange carotenoids in the shell. The lower a* value can be measured in the pomace with seeds (3.54 and 2.79), whereas the juices are the least red samples (2.45 and 1.44).

According to the results obtained, the highest values (23.73 and 22.23) were found in the seedless, shell sample of the ‘Ascola’ cultivar (which is the most yellowish-coloured sample), followed closely by the b* value of the ‘Leikora’ berry shell. The samples of the two SB juices analyzed are the least yellowish, and there is no significantly difference between the colours of the two cultivars.

CONCLUSION

Based on the research results, it can be stated that both sea buckthorn (SB) cultivars have extremely high antioxidant properties. The juice extracted from SB berries exhibits a significantly less antioxidant activity and polyphenol content than the pomace. The higher polyphenol content and the antioxidant capacity of the pomace are due to the compounds found in seeds.

Based on the results obtained, there is no significant difference between the juice and shell parameters of the two cultivars. However, a significant difference was found between the samples of different cultivars containing seeds.

Relative to the antioxidant capacity measurement, the ‘Leikora’ samples showed a higher value, whereas the polyphenol content was higher in the ‘Ascola’ cultivar, which likely suggests that the antioxidant content is not closely linked to the polyphenol content.

The results of our research suggest that sea buckthorn pomace, seeds, and shells contain valuable polyphenols and antioxidant components. An important field of research for the future is a further use of pomace in the juice production industry.

Extracts rich in valuable ingredients made from SB pomace could enrich certain foods, including fruit juices, jams, flour mixtures, and dietary supplements.

Another important issue entails examining the differences between SB cultivars in order to determine the best cultivar for processing. These results may also be of significant interest to those who cultivate sea buckthorn. Further examination could reveal whether the extracted antioxidant content of sea buckthorn, as a by-product of fruit processing, could be used as a natural food additive, i.e. bio-preservative, after the appropriate clarification processes.

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