The antioxidant capacity of sea buckthorn (*Hippophae rhamnoides* L.) berries depends on the genotype and harvest time

Makovics-Zsohár, N.[1], Hegedűs, A.[2], Stefanovits-Bányai, É.[1], Rédei, R.[1], Papp N.[1]

[1]*Corvinus University of Budapest, Faculty of Food Science, Department of Applied Chemistry, 1118 Budapest, Villányi út 29., Hungary; E-mail: nora.papp@uni-corvinus.hu*

[2]*Corvinus University of Budapest, Faculty of Horticultural Science, Department of Genetics and Plant Breeding, 1118 Budapest, Villányi út 29., Hungary;*

***Key words**:* antioxidants, genotype, harvest time, *Hippophae rhamnoides*, sea buckthorn, total phenolic content

Summary: Berries of sea buckthorn (*Hippophae rhamnoides* L.) are characterized by increasing popularity due to their presumable health-effects. The aim of this study was to compare the antioxidant capacity and total polyphenolic content in the berries of six Hungarian grown sea buckthorn genotypes and characterize the genetic variability in this trait. The harvest time of sea buckthorn berries affects the antioxidant capacity and total phenolic contents in berries of three popular cultivars of German origin. Berries harvested in October had higher antioxidant capacity compared with those harvested one month later. The extent of the difference was genotype-specific. Our analysis revealed a nearly 3-fold difference between the lowest and highest antioxidant capacities of the 6 tested genotypes with 'Leikora' showing the highest ferric reducing antioxidant power and total phenolic content. The TEAC values ranged between 1.76 and 3.13 mmol Trolox/100g fresh weight with Pető 1 and 'Frugana' having the highest values. The results presented in this study demonstrated that *Hippophae rhamnoides* berries possess *in vitro* antioxidant activity strongly determined by genotype but also influenced by harvest time.

Introduction

Sea buckthorn (*Hippophae rhamnoides* L. of the family Elaeagnaceae), a unique and valuable plant has recently gained worldwide attention, mainly for its medicinal and nutritional potential (*Schmidt* & *Tóth*, 2006; *Lalit* et al., 2011; *Negi* et al., 2005). Sea buckthorn is a native species of sunny areas of sand dunes such as river banks. It is a dioecious species, with separate male and female plants, the latter can be easily recognized in early autumn by their orange coloured berries. Sea buckthorn leaf has flourishing epidermis hair, thicker cuticle and developed palisade tissue (Dai-Qiong et al., 2004) characteristic for plants facing with sun exposure and limited water availability (*Jäger* et al., 2011).

Berries contain more than 100 different nutrients and bio-active substances and are rich sources of vitamins (A, C, E, K, riboflavin, folic acid), carotenoids (α, β, δ-carotene, lycopene), flavonoids, organic acids (malic acid, oxalic acid) minerals, essential oils, and essential fatty acids. The fruit of sea buckthorn is a good source of vitamin C (500-900 mg/100g), which is 4-100-times higher than any vegetable and fruit (*Farkas*, 2011; *Yan-Jun* et al., 2011; *Lalit* et al., 2011; *Vinay* et al., 2012, *Negi* et al., 2005; *Baoru* & *Heikki*, 2002).

Sea buckthorn leaves contain nutrients and bioactive substances which mainly include flavonoids, carotenoids, free and esterified sterols, triterpenols, and isoprenols. The leaves are equally rich sources of important antioxidants including β-carotene, vitamin E, catechins, ellagic acid, ferulic acid, folic acid and also significant amounts of calcium, magnesium and potassium are accumulated (*Al*, 2001, *Suryakumar* & *Gupta*, 2011).

A wide spectrum of pharmacological effects of sea buckthorn has been recently reported including antioxidant, immunomodulatory, anti-atherogenic, anti-stress, hepatoprotective, radioprotective as well as tissue repair promoting effects (*Suryakumar* & *Gupta*, 2011; *Eccleston* et al., 2002).

The aim of this study was to compare the antioxidant capacity and total polyphenolics content in the berries of six Hungarian grown sea buckthorn genotypes and characterize the genetic variability in this trait. We also wanted to monitor the effect of harvest time on the antioxidant parameters in case of three popular cultivars of German origin.

Materials and methods

Plant material

The berries of German sea buckthorn (*Hippophae rhamnoides* L.) cultivars 'Leikora', 'Hergo' and 'Frugana' were collected in Budapest, Hungary two times (16. 10. 2012 and 26. 11. 2012) and unknown genotypes of Russian origin labelled Pető (1, 2, 3) were harvested in Jászapáti, Hungary.

Sample preparation

After sorting, washing, cleaning, the fresh pomace was stored frozen (-32 °C) until freeze drying (ScanVac, Denmark). The freeze-dried samples was ground to a fine powder in a mortar, and sample solution of 20 mg/ml was prepared in Milli-Q (MQ) water (18.2 MΩcm). Subsequently, the resulting suspension was placed in an ultrasonic water bath for one hour, and then the solutions were centrifuged (Micro 22 R, Hettich centrifuges, Tuttlingen, Germany; 6000 rpm, 20 min, 23 °C). Finally, the extracts were stored at -32°C until the determination of *in vitro* antioxidant parameters. The measurements were performed using a Nicolet Evolution 300 BB (Thermo Electron Corporation, Cambridge, UK) spectrophotometer.

Ferric reducing antioxidant power (FRAP) assay

FRAP assay was performed according to the methods of *Benzie* & *Strain* (1999). The values obtained were expressed as mmol ascorbic acid equivalent (AS)/100g of fresh weight.

Total phenolic content determination

Total phenolic contents of samples were determined using the Folin-Ciocalteau (FC) assay as described by *Singleton* & *Rossi* (1956). Results were expressed as mmol gallic acid equivalents (GA)/100g of fresh weight.

Measurement of total antioxidant activity

Miller et al. (1993) described the Trolox-equivalent antioxidant capacity (TEAC) method. The method is based on the formation of ABTS•+ cation [2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] and its scavenging by antioxidant sample constituents measured by spectrophotometry (decay of green/blue chromophore absorbance is inversely associated with antioxidant content. The standard antioxidant compound used for the quantification is Trolox, a hydrophilic analog of vitamin E.

Results and discussion

The harvest time of sea buckthorn berries seems to affect the antioxidant capacity and total phenolic contents in berries of three popular cultivars of German origin. Berries harvested on 16th October 2012 had higher antioxidant capacity compared with berries harvested one month later. The extent of the difference was genotype-specific with 'Hergo' and 'Frugana' showing a marked decrease while 'Leikora' had only a slightly reduced antioxidant capacity. It indicates an association between the antioxidant capacity of berries and harvest time at the overripe stage. Our analysis revealed a nearly 3-fold difference between the lowest and highest antioxidant capacities of the 6 tested genotypes (*Figure 1*). Pető 3 showed the lowest FRAP value, while the highest values were measured in berries of 'Leikora'.

Total phenolics content showed smaller differences (slightly smaller than two-fold variations) between the lowest and the highest values determined in the accessions Pető 3 and Pető 1 and 'Leikora', respectively (*Figure 2*). The FRAP and TPC data showed a close correlation, indicating that a crucial part of the sea buckthorn antioxidant capacity is attributable to a wide range of polyphenols present in berry skin and flesh. The high levels of phenolic compounds in plant extracts indicate radical scavenging activity. Polyphenols are very important compounds in berries because of their radical scavenging ability due to their several phenolic hydroxyl groups. They inhibit lipid peroxidation and are associated with antioxidant activity (*Pfeiffer* & *Hegedűs*, 2011).

Sea buckthorn extracts have been reported to possess strong antioxidant activity measured in terms of TEAC (1.76-3.13 mmol trolox/100g FW) using 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) assays (*Figure 3*). Pető 1 and 'Frugana' had the highest TEAC values. The values of 'Leikora' cultivar were not so outstanding compared with those determined in other antioxidant assays. In conclusion, the results presented in this study demonstrated that *Hippophae rhamnoides* berries possess strong *in vitro* antioxidant activity and hence may have cytoprotective effects. Sea buckthorn berries may improve the antioxidant defence system of cells by increasing the intracellular GSH levels and inhibiting ROS production (*Vijayaraghavan* et al., 2006; *Manickam* et al., 2014).

Conclusions

The antioxidant capacity of sea buckthorn is mainly genotype-dependent but harvest time also influences it. On the basis of the present study, it could be concluded that berries of sea buckthorn exert pronounced antioxidant properties and might be important as nutraceutical after conducting safety and toxicological studies. Their curative power against a range of diseases might be exploited, especially those associated with oxidative stress. Antioxidant capacity is an important fruit quality parameter together with the related parameters such as total phenolic content and antioxidant capacity (FRAP and TEAC).

Acknowledgements. This work was financed by the OTKA K84290 grant. Attila Hegedűs is grateful for receiving a János Bolyai Scholarship, Hungarian Academy of Sciences. The authors thank Ágnes Pető and Péter Karádi for kindly providing plant material.

References

Al, O. (2001): Sea Buckthorn. Special Crops Factsheet. Ministry of Agriculture, Food and Fisheries. British Columbia, Kamloops, 1-4.

Baoru, Y. & Heikki, K. (2002): Composition and physiological effects of sea buckthorn (*Hippophaë)* lipids. Trends Food Sci. Technol., 13: 160–167.

Benzie, I.F.F. & Strain, J.J. (1996): The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Anal. Biochem., 239: 70–76.

Dai-qiong, L.I., Liang, Y.M., Huang, J. & Jiang, J. (2004): Study on the characteristic of anatomy of seabuckthorn on the semiarid loess region [J]. *Seabuckthorn,* 1: 002.

Eccleston, C., Baoru, Y., Tahvonen, R., Kallio, H., Rimbach, H.G. & Minihane, M.A. (2002): Effects of an antioxidant-rich juice (sea buckthorn) on risk factors for coronary heart disease in humans. J. Nutr. Biochem., 13: 346–354.

Farkas, Á. (2011): Kicsit savanyú, kicsit keserű, de a miénk…. Farmakológiai Hírek, 6.21: 1.

Jäger, K., Fábián, A., Tompa, G., Deák, C., Höhn, M., Olmedilla, A., Barnabás, B. & Papp, I. (2011): New phenotypes of the drought-tolerant cbp20 *Arabidopsis thaliana* mutant have changed epidermal morphology. Plant Biol, 13: 78–84.

Lalit, M.B., Venkatesh, M., Naik, S.N. & Santosh, S. (2011): Sea buckthorn berries: A potential source of valuable nutrients for nutraceuticals and cosmoceuticals. Food Res. Int., 44: 1718–1727.

Manickam, M. & Tulsawani, R. (2014): Survival response of hippocampal neurons under low oxygen conditions induced by *Hippophae rhamnoides* is associated with JAK/STAT signaling. PloS One, 9: e87694.

Miller, N.J., Rice-Evans, C., Davies, M.J., Gopinathan, V. & Milner, A. (1993): A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clin. Sci., 84: 407–412.

Negi, P.S., Chauhan, A.S., Sadia, G.A., Rohinishree, Y.S. & Ramteke, R.S. (2005): Antioxidant and antibacterial activities of various seabuckthorn (*Hippoohae rhamnoides* L.) seed extracts. Rev. Chim. 59: 392–394.

Papuc, C., Diaconescu, C., Nicorescu, V. & Crivineanu, C. (2008): Antioxidant activity of polyphenols of sea buckthorn fruits (*Hippophae rhamnoides*). Roum. Biotechnol. Lett., 13: 4049–4053.

Pfeiffer, P. & Hegedűs, A. (2011): Review of the molecular genetics of flavonoid biosynthesis in fruits. Acta Aliment. Hung., 40: 150–163.

Schmidt, G. & Tóth, I. (2006): Kertészeti dendrológia.Mezőgazda Kiadó, Budapest, p. 240–242.

Singleton, V.L. & Rossi, J.A. (1965): Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Viticult., 16: 144–158.

Suryakumar, G. & Gupta, A. (2011): Medicinal and therapeutic potential of Sea buckthorn (*Hippophae rhamnoides* L.). J. Ethnopharm., 138.2: 268–278.

Vijayaraghavan, R., Gautam, A., Kumar, O., Pant, S.C., Sharma, M., Singh, S., Kumar, H.T.S., Singh, A.K., Nivsarkar, M., Kaushik, M.P., Sawhney, R.C., Chaurasia, O.P. & Prasad, G.B. (2006): Protective effect of ethanolic and water extracts of sea buckthorn (*Hippophae rhamnoides* L.) against the toxic effects of mustard gas. Ind. J. Exp. Biol., 44: 821.

Vinay, K., Madhuri, M. & Chandresh, V. (2012): Antioxidant potential and total phenolic contents of seabuckthorn (*Hippophae rhamnoide*) pomace. Free Rad. Antiox., 2: 79–86.

Yan-Jun, X., Meera, K., Reshvinder, S.D., Paramij, S.T. & Naranjan, S.D. (2011): Health benefits of sea buckthorn for the prevention of cardiovascular diseases. J. Funct. Food., 3: 2–12.

Figures

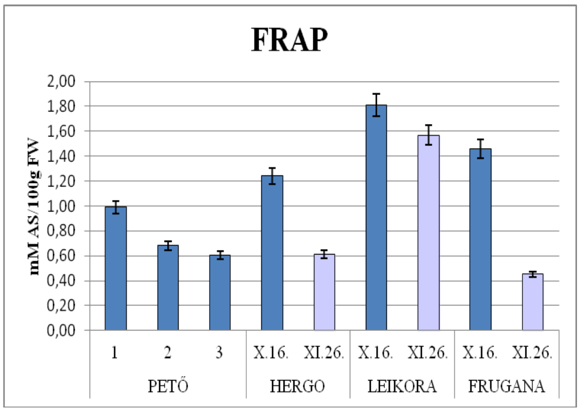

Figure 1. Antioxidant capacity in sea buckthorn berries according to genotypes and harvest times

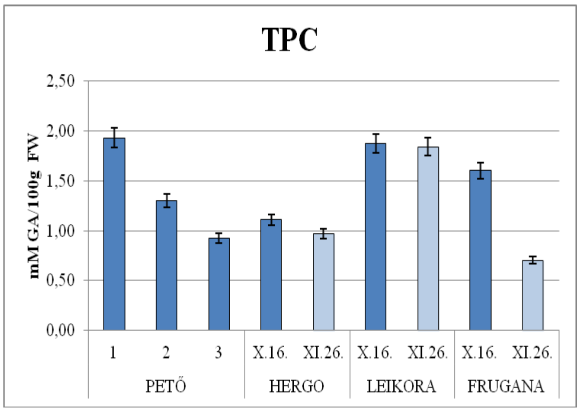

Figure 2. Total phenolic content in sea buckthorn berries according to genotypes and harvest times

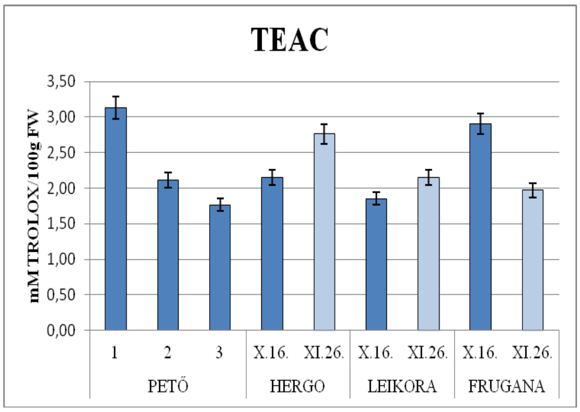

Figure 3. Antiradical capacity (TEAC assay) in sea buckthorn berries according to genotypes and harvest times