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High antioxidant – and anthocyanin contents of sour cherry cultivars may benefit the human health: international and Hungarian achievements on phytochemicals

Veres, Zs.[1], Holb, I.[2], Nyéki, J.[3], Szabó, Z.[3], Remenyik, J.[1] & Fári, M. G.[1]

[1]*Centre of Agricultural Sciences, University of Debrecen, Department of Vegetable Crops and Horticultural Biotechnology, 138 Böszörményi Str., 4032 Debrecen, Hungary;*
[2]*Centre of Agricultural Sciences, University of Debrecen, Department of Plant Protection, 138 Böszörményi Str., 4032 Debrecen, Hungary;*
[3]*Centre of Agricultural Sciences, University of Debrecen, Institute of Extension and Development, 138 Böszörményi Str., 4032 Debrecen, Hungary;*

Summary: Evidence suggests that a diet with high fruit and vegetable consumption may decrease the risk of chronic diseases, such as cardiovascular diseases and cancer, and phytochemicals including phenolics, flavonoids and carotenoids from fruits and vegetables may play a key role in reducing chronic disease risk. Recent research has proved that sour cherry (*Prunus cerasus* L.) is a valuable natural source of some bioactive compounds important in human health preservation. According to the published data, the most important biological effects of sour cherry are connected – directly or indirectly – to their endogenous antioxidant behaviour as well as to their specific pattern of anthocyanin components. In the present work, we measured the total antioxidant capacity of some Hungarian sour cherry varieties in combination with their anthocyanin-, and vitamin-C content. In 2003, twelve clones were selected and grafted from a local sour cherry population called "Bosnyák" sour cherry grown in small home gardens and farms of the village Csengőd (Great-Plain Region, South Hungary). Other Hungarian sour cherry varieties, i.e. cv. Újfehértói fürtös, cv. Érdi bőtermő, cv. Debreceni bőtermő, cv. Csengődi and cv. Kántorjánosi served as a control.

***Key words*:** phytochemicals, sour cherry, anthocyanin, total antioxidant capacity, ascorbic acid, water soluble antioxidants, lipid soluble antioxidants,

Introduction

By consuming high amounts of fruits and vegetables, the risk of some chronic diseases can be reduced, which is attributed to the antioxidant compounds (certain vitamins, polyphenols etc.) they contain (*Engelhart*, 2002). Sour cherry (P*runus cerasus* L.) is a valuable natural source of some bioactive compounds important in human health preservation. In this research, extremely high anthocyanin, bioflavonoid, melatonin and some other bioactive compounds have been identified as phytochemicals of high anti-inflammation, anti-cancer properties. In 1999, *Wang* et al. reported on 17 antioxidant compounds which can be found in sour cherry and on their role in human health. Following biochemical investigations based mainly on two sour cherry varieties, „Montmorency" and „Balaton", 10 patents have been registered about cherry phytochemicals since 1999 only in the USA. The cultivar „Balaton" is of Hungarian breeding, named „Újfehértói fürtös". In this latter cultivar, a higher anthocyanin content was measured than in Montmorency, the major cultivar of the USA (*Wang* et. al.,1999).

In plants, as in animals, melatonin is believed to function as a free radical scavenger and possibly plays a role in photoperiodism. Melatonin was detected and quantified in fresh-frozen Balaton and Montmorency tart cherries (*Prunus cerasus*) in the USA using high-performance liquid chromatography. Both cherry species contain high levels of melatonin compared to the melatonin concentrations in the blood of mammals. Montmorency cherries (13.46 +/– 1.10 ng/g) contain approximately 6 times more melatonin than Balaton cherries (2.06 +/– 0.17 ng/g). Scientists in the USA stated, that consuming cherries could be an important source of dietary melatonin since melatonin is readily absorbed when being consumed (*Burkhardt* et. al, 2001). Under normal conditions, the human blood plasma contains 2–10 – 100–200 pg/ml melatonin, the amount of which is the highest between 11 and 3 o'clock in the night (*Wurtman*, 2005).

Spanisch researchers found that the free radical scavenger properties of melatonin are mainly specific to the superoxide anion (*Cabeza* et al., 2001). According to the published data, the most important biological effects of sour cherry are connected – directly or indirectly – to their endogenous antioxidant behaviour as well as to their specific pattern of anthocyanin components. It is well known, that superoxide anion is considered to be one of the most important free radicals in organisms (*Békési & Fehér*, 2001). Though this radical alone shows only limited reactivity, it induces peroxidation processes in the lecithin-cholesterol membranes (*Seligman* et al., 1979). When reacting with hydrogen-peroxide, hydroxyl radical and singlet oxygen forms from it, and these are the main causes of the free-radical transformation of lipids, lipid peroxidation (*Kellog* et al., 1977). The superoxide anion reacts with numerous nitrogen-oxides of positive effect (e.g. inhibition of thrombocyte aggregation) and the superoxide anion neutralises the nitrogen-oxide. The resulting peroxinitrite also oxidates the lipoproteins, and is transformed into other highly reactive radicals (*Van Der Vliet* et al., 1994). In this study, the total antioxidant capacity of the sample was investigated as well as all the lipid-soluble (ACL) and water-soluble antioxidants (ACW) were detected which could react directly with the superoxide anions generated by the given biological sample. The aim of the present study was to determine the total lipid-, and water soluble anioxidant capacity and establish the correlation between ACL activity and total anthocyanin content as well as between ACW activity and vitamin-C content of some widely cultivated sour cherry varieties of Hungarian breeding.

Materials and methods

In 2003, twelve clones were selected and grafted from a local sour cherry population called „Bosnyák" sour cherry grown in small home gardens and farms of the village Csengőd (South-Hungarian Great-Plain Region). Other Hungarian sour cherry varieties, i.e. cv. „Újfehértói fürtös", cv. „Érdi bőtermő", cv. „Debreceni bőtermő", cv. „Csengődi" and cv. „Kántorjánosi" served as a control. The fruits were harvested in optimal ripening stage and were lyophilised (ALPHA 1–4 LSC, Germany) and stored at -18C for the biochemical measurements. The total antioxidant capacity of the sample was measured with a PHOTOCHEM chemiluminometer (Analytik Jena AG., Germany). The measurements of the anthocyanin content were performed with an Ultrospec 2100 pro spectrophotometer (Amershan, USA) according to the Hungarian National Standard method (MSZ 14881). Vitamin C content, belonging to the water-soluble antioxidants, was measured by the Boehringer-Mannheim UV Test method with a spectrophotometer.

Table 1 The water- (ACW) and the lipid (ACL) soluble antioxidant capacity in some Hungarian sour cherry varieties and clones

Varieties, clones	ACW (µmol vitamin C equivalent/100g fresh weight)		ACL (µmol TROLOX equivalent/100g fresh weight)		ACL in ACW (%)
		ORDER		ORDER	
Érdi bőtermő	**396.1**	**14**	**714.0**	**13**	**180.2**
Újfehértói fürtös	**511.7**	**13**	**608.6**	**14**	**118.9**
Debreceni bőtermő	**626.45**	**12**	**886.4**	**12**	**141.5**
Bosnyák-4	644.35	11	918.75	11	142.6
Bosnyák-1	693.8	10	1258.3	8	181.4
Bosnyák-3	717.5	9	1112.5	9	155
Bosnyák-8	778.2	8	1408.6	5	181
Bosnyák-10	930.6	7	1890.0	3	203.1
Bosnyák-12	983.4	6	1332.9	7	135.5
Kántorjánosi	1008.6	5	996.8	10	98.8
Bosnyák-11	1086.25	4	1900.2	2	174.9
Bosnyák-5	1361.5	3	1709.8	4	125.6
Csengődi	1625.4	2	1367.4	6	84.1
Bosnyák-6	**1767.3**	**1**	**2405.3**	**1**	**136.1**
Average	891.5		1284.4		144

Results and discussion

There was a significant difference between the lipid- (ACL) and the water- (ACW) soluble antioxidant fractions, with ACL being the higher for all varieties. On average, the lipid-soluble antioxidant content of the samples was 44% higher than the water-soluble antioxidant content. We measured the highest ACL value in clone "Bosnyák-6" (2,405.3 mol Trolox equivalent per 100 g fresh weight), which was 29.6% and 44.2% higher than that of cv. „Csengődi" and the average of the control cultivars, respectively (*Table 1*). The total anthocyanin content belonging to the lipid-soluble antioxidants was compared to the ACL content of the samples. This comparison showed how many percentages of the lipophyl antioxidant capacity of sour cherry cultivars are made up by anthocyanins (*Table 2*).

According to our results, anthoycanin content is 12% of the total ACL value on average. "Újfehértói fürtös" sour cherry being highly favoured in the USA belongs to the cultivars of lower anthocyanin content in Hungary, since there was a clone "Bosnyák-6" between the studied "Bosnyák" clones the red colour material content of which is

Table 2 The antocyanin content and the lipid (ACL) soluble antioxidant capacity in some Hungarian sour cherry varieties and clones

Varieties, clones	Anthocyanin content (mg/100 g fresh weight)	ACL (mg TROLOX equivalent/100 g fresh weight)	Anthocyanin content in ACL %
Érdi bőtermő	**27.90**	**178.7**	**15.6**
Újfehértói fürtös	**23.30**	**152.3**	**15.3**
Debreceni bőtermő	**27.00**	**221.9**	**12.2**
Kántorjánosi	26.40	249.5	10.6
Bosnyák-4	32.55	230.0	14.1
Bosnyák-1	30.75	315.0	9.8
Bosnyák-3	20.25	278.5	7.3
Bosnyák-8	31.95	352.6	9.06
Bosnyák-12	22.70	333.6	6.8
Bosnyák-11	72.70	475.6	15.3
Bosnyák-6	**99.45**	**602.0**	**16.5**
Average	36.0	298.2	12.07

4.3 times higher than that of "Újfehértói fürtös". The ACW capacity of 1 g fresh fruit of "Bosnyák-6" sour cherry clone was 17.7 (mol vitamin-C equivalents) that is the antioxidant value of 100 g sour cherry is equivalent to 311 mg of vitamin-C. Given that the average vitamin-C content in fresh sour cherry measured by the Boehringer-Mannheim UV-Test method is 0.197 mg per 100 g and that the total antioxidant capacity of 0.00197 mg vitamin-C (in 1 g of fresh sour cherry) is only 0.011, then almost all of the ACW antioxidant capacity in sour cherry must be due to water-soluble phytochemicals (*Tables 1 and 2*).

In our recent biochemical experiments with properly prepared, biologically mature "Bosnyák" sour cherry fruits, a significant melatonin accumulation was revealed by applying HPLC method. The retention time of the melatonin applied as a standard was the same as that of the material derived from the examined sour cherry clone (suspected to be melatonin) that has not been chemically identified as yet. For the precise chemical identification of this material, the MALDI-TOF MS technique was applied, with which the molecular weight of melatonin+hydrogen, melatonin+ potassium and melatonin+sodium was determined.

Our results indicate the immense, and almost unexplored genetic and biological potential of the sour cherry germplasm regarding the human health benefit.

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