Nutritional values of new Czech cultivars of Saskatoon berries (*Amelanchier alnifolia* Nutt.)

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Abstract

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The Saskatoon berry (*Amelanchier alnifolia* Nutt.) belongs to less known cultivated pomaceous fruit. Over the last two decades new cultivars have been bred in the Czech Republic. In our work the fruit of those new cultivars were analysed as far as basic nutritional characteristics were concerned. Moreover, the content of phenolic substances, antioxidant capacity and flavonoid content were determined. For comparison, the fruit of selected North American cultivars grown in the conditions of Central Europe were analysed. Besides North American cultivars also the Tisnovsky cultivar seems to be promising since both the highest content of phenolic substances (3.80 g of gallic acid equivalent/kg of fresh mass) and the highest antioxidant capacity (5.05 g of ascorbic acid equivalent – measured by the ABTS test) were recorded. In Central European cultivars there were high contents not only of pectins, but also of phosphorus, calcium, magnesium, sodium and manganese as far as mineral elements were concerned.

Keywords: *Amelanchier* sp.; phenolics; antioxidant capacity; flavonoids; pectins; mineral elements

The Saskatoon berry (*Amelanchier alnifolia* Nutt.) is a lesser known fruit species belonging to members of the Rosaceae family (Catling, Mitrow 2006) and it is actually a pome (Mazza, Davidson

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1993). This species is native to the southern Yukon, the Canadian prairies and the northern plains of the United States of America. Saskatoon berries were originally used as a major food source by the native people and early settlers of the North American prairies and, until recently, could be picked mostly in the wild. Over the past decades, however, there was an increasing interest in utilizing this pome as a unique fruit crop (McGarry et al. 1998).

Due to a high content of antioxidant compounds (Bakowska-Barczak, Kolodziejczyk 2008), the dietary intake of Saskatoon fruit has a positive and profound impact on human health, performance and disease. However, little information is available on antioxidant capacity and mineral composition of Saskatoon berries, and further research in this field is necessary (Seeram 2008).

The aim of this work was to describe chosen nutritional parameters of 9 cultivars selected within the framework of the breeding work performed in the Czech Republic in the last decades. Furthermore, the focus was on the popularization of new fruit species, which is possible to cultivate thanks to the low requirements for the environment even in harsh conditions for fruit. Nonetheless, this fruit excels in tastiness and this work refers both to its nutritional and technological parameters. The main aim consisted in verifying the nutritional parameters of particular cultivars, their mutual comparison and the determination of the most suitable cultivars for further horticultural production.

MATERIAL AND METHODS

Description of locality. Fruits were harvested in an experimental gene-fund orchard of the Mendel University in Brno (MUB) within the period of 2009–2011. This orchard is situated in the area of the village Žabčice, approximately 20 km southwards from Brno, Czech Republic. The altitude is 184 m a.s.l.; 49°01'N, 16°36'E. The average annual temperature and a fifty-year average sum of precipitation are 9°C (during the growing season 15.6°C) and 553 mm (during the growing season 356 mm), respectively. Soils are classified as gleyed alluvial soils developed on the Holocene calciferous sediments with a marked accumulation of organic compounds. As far as the texture is concerned, the topsoil is loamy and the subsoil clayey-loamy. The value of pH/KCl (the primary use of the pH in c = 1 mol/dm of KCl solution as the test for the presence of exchangeable hydrogen) of topsoil is 6.39 (Anonymous 2008).

Collection and processing samples for chemical analyses. Fruits were harvested in full ripeness (Rogiers, Knowles 1998) from three plants of each cultivar under study in the course of June. Forty randomly chosen fruits from each cultivar were mixed together and used for analyses (i.e. altogether 120 per each cultivar).

Fruits of individual cultivars were processed immediately after the harvest (not later than within two days). Harvested fruits were made into a purée in a mixer and the average sample was obtained by dividing into quarters. Each parameter was measured in five replications.

Fresh samples were used for the measurement of the total content of phenolic substances and overall antioxidant capacity. In order to characterize the nutritional value of Saskatoon berries, these basic parameters were supplemented with data about the contents of flavonoids, selected mineral elements (phosphorus, potassium, calcium, magnesium, sodium, zinc, iron, copper and manganese), soluble solid content, titratable acidity, crude protein value and pectins.

The following cultivars were analysed: Brnensky, Ostravsky, Skolsky, Tisnovsky, NS-1 and NS-2, which are of Czech origin (Anonymous 2008). For comparison, Martin, Smoky and Thiessen cultivars were analysed and these are Canadian in origin (St-Pierre et al. 2005).

Chemical analyses. The dry matter content was measured after drying off to a constant mass at the standard temperature of 105 ± 2°C with the apparatus VENTICELL 111 (BMT, Brno, Czech Republic). The soluble solid content (SSC) was determined by means of polarimetric measurements in juice obtained after squeezing the fruit and the results were expressed as % Brix. For the measurement of SSC, a digital instrument HI 96801 (Hanna Instruments, Woonsocket, USA) was used. The content of total acids was measured by potentiometric titration. The result obtained was converted to the content of acids (expressed as malic acid) in g/kg of fresh mass (FM) (Novotný 2000).

The determination of the total phenolic content (TPC), total flavonoid content (TFC) and antioxidant capacity (TAC) was performed by the extraction according to the method described by Kim et al. (2003), using the following procedure: 10 g of the fresh sample were homogenized for 2 h in an extraction mixture (hydrochloric acid:methanol:water in the ratio 2:80:18).

To measure total contents of phenolic substances Folin-Ciocalteau reagent was used. The resulting absorbance was measured in the spectrophotometer LIBRA S6 (Biochrom, Ltd., Cambridge, UK) at the wavelength of 765 nm against a blind sample, which was used as reference (Thaipong et al. 2006). The results were expressed as grams of gallic acid (GAE)/kg of FM. The total flavonoid content was determined according to Yong et al. (2008) using 30% ethanol, $NaNO_2$ and $AlCl_3 \cdot 6H_2O$. 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 mg/kg of FM.

For the purpose of mineral element content assay the sample was dried to a constant mass in a drier at 105 ± 2°C; thereafter, 1g of homogenized dry matter (with the particle size of 1 mm) was further mineralized in a mixture of concentrated sulphuric acid with 30% hydrogen peroxide. The resulting mineralizate was measured in an atomic absorption spectrometer (Philips PU 9200X; Philips, Eindhoven, the Netherlands). The amount of crude protein was estimated on the basis of total nitrogen in the Kjeldahl apparatus KJELTEC TM 2300 (FOSS, Hilleroed, Denmark) and the result was multiplied by the coefficient 6.25 (Novotný 2000).

The content of pectins was measured using the modified method described by Rop et al. (2008). The fruit material was extracted with hydrochloric acid. Pectins were thereafter measured by photometry as a coloured complex consisting of the product of thermal decomposition of galacturonic acid with *m*-hydroxybiphenyl in a medium containing concentrated H_2SO_4. The samples and the pectin standard were measured in the apparatus LIBRA S6 (Biochrom, Ltd., Cambridge, UK) mentioned above at the wavelength of 520 nm and the content of pectins was expressed in g/kg of FM.

Antioxidant capacity assay. The measurement of antioxidant capacity was based on monitoring the course of inactivation of the cation $ABTS^+$ [2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonate)] using the method described by Šulc et al. (2007). Antioxidant capacity was calculated as a decrease in absorbance (at the wavelength of 734 nm) after 20 min. As the other test for the determination of antioxidant capacity the DPPH test was used according to the method of Brand-Williams et al. (1995). The DPPH (1,1-diphenyl-2 picrylhydrazyl) test is based on the ability of stabile free radicals of 1,1-diphenyl-2 picrylhydrazyl to react with donors of hydrogen. In its radical form, $DPPH^+$ absorbs light at 515 nm, but upon reduction by an antioxidant or a radical species the absorption disappears. In both methods the calculated activity was converted using a calibration curve of the standard and expressed in ascorbic acid equivalents (AAE) (Rupasinghe et al. 2006).

Statistical analysis. The data obtained were analysed statistically by the analysis of variance (ANOVA) and the Tukey's multiple range test for comparison of means (Snedecor, Cochran 1968). Averages of three-year results were calculated – every year there were measurements in five replications, thus 3 × 5 = 15 replications. Correlation functions were calculated using the statistical package Unistat, v. 5.1 (Unistat, Ltd., London, UK) and MS Excel®.

RESULTS AND DISCUSSION

The results of chemical analyses of particular cultivars are given in Tables 1–4. No statistically significant differences were found in titratable acidity. On the other hand, statistically significant differences were found in the contents of SSC, crude protein, pectins (Table 1), TPC, TAC and TFC (Table 2) and mineral elements (Tables 3 and 4).

In terms of total phenolic content, the most promising cultivar seemed to be Tisnovsky (3.80 g GAE/kg FM). The highest total antioxidant capacity was also displayed in case of the ABTS test by the Tisnovsky cultivar (5.05 g AAE/kg FM).

The soluble solid content, which we determined, corresponds with tabular values (Kováčiková et al. 1997; Kopec 1998) for core fruit. In core fruit, the content of organic acids is represented mainly (90%) by malic acid (Kyzlink 1990). Regarding other core fruit species, the same values as we determined in Saskatoon berry fruits were observed by Kopec (1998). On the other hand, e.g. Zatylny et al. (2005) noticed the content of organic acids in Saskatoon berries reaching the values of 0.63 g/kg FM. These differences can be caused by other climatic and soil conditions of the locality (Juríková, Matuškovič 2007). Nevertheless, in case of Saskatoon berries the influence of the year has only little impact (Zatylny et al. 2002), which was confirmed in our measurement, too. We found no statistical significance among the years. Regarding the fact that all cultivars were grown under identical conditions and in the same locality, it is possible to conclude

Table 1. Average dry matter content (% w/w*), soluble solid content (% Brix), titratable acidity (g of malic acid/kg FM), pectin content (g/kg FM), crude protein content (g/kg FM) of fruits of different cultivars of *Amelanchier alnifolia* Nutt., $n = 15^{**}$

Cultivar	Dry matter	Soluble solid content	Titratable acidity	Pectins	Crude protein
Brnensky	22.37 ± 0.85^{a}	14.28 ± 0.10^{a}	1.37 ± 0.15^{a}	13.78 ± 0.85^{a}	7.34 ± 0.52^{a}
Martin	19.18 ± 0.79^{b}	18.78 ± 0.15^{b}	1.50 ± 0.16^{a}	10.36 ± 0.79^{b}	8.14 ± 0.56^{a}
NS-1	22.24 ± 0.89^{a}	14.55 ± 0.17^{a}	1.48 ± 0.19^{a}	12.49 ± 1.01^{a}	7.23 ± 0.61^{a}
NS-2	23.05 ± 1.26^{a}	14.17 ± 0.19^{a}	1.32 ± 0.24^{a}	12.88 ± 1.15^{a}	8.19 ± 0.43^{a}
Ostravsky	19.04 ± 1.13^{b}	15.39 ± 0.15^{c}	1.30 ± 0.18^{a}	10.31 ± 0.88^{b}	9.62 ± 0.40^{b}
Skolsky	21.93 ± 1.41^{ab}	16.25 ± 0.17^{d}	1.51 ± 0.13^{a}	13.80 ± 0.78^{a}	6.19 ± 0.51^{c}
Smoky	21.49 ± 0.85^{ab}	15.10 ± 0.18^{c}	1.43 ± 0.14^{a}	9.68 ± 1.17^{bc}	10.93 ± 0.58^{d}
Thiessen	21.18 ± 1.57^{ab}	15.56 ± 0.15^{c}	1.42 ± 0.14^{a}	9.95 ± 0.82^{bc}	10.24 ± 0.50^{bd}
Tisnovsky	21.97 ± 1.66^{ab}	16.29 ± 0.19^{d}	1.30 ± 0.20^{a}	8.12 ± 0.86^{c}	11.79 ± 0.47^{d}

*w/w is weight of dry matter in weight of the whole fresh sample; **it concerns averages of three-year results – every year there were measurements in 5 replications, therefore, 3 × 5 = 15; different superscripts in each column indicate the significant differences in the mean at $P < 0.05$

that one can clearly see the cultivar variability, which is quite typical of fruit (Kim et al. 2003).

In our experiments, the content of pectin substances ranged from 8.12 to 13.80 g/kg FM (Table 1). In other core fruit species, a high content of pectins can be found, for example, in quinces, which may contain as much as 30 g/kg of pectins in FM (Kováčiková et al. 1997). In apples, however, the average content of pectins is about 11.4 g/kg FM (Kopec 1998). These facts mentioned above predetermine Saskatoon berries for the processing to fruit spreads thanks to their capability of gelification (Kyzlink 1990). For the fermentation the content of crude protein and phosphorus is important. Similarly, other mineral elements can have an interesting influence, above all, due to their positive impact on human nutrition (Purves et al. 2004). Concerning all determined mineral elements, potassium was measured in the highest level in Saskatoon fruit (in the Thiessen and Tisnovsky cultivars 4,154.32 mg/kg FM and 4,311.72 mg/kg FM, respectively). Furthermore, the contents of other elements (phosphorus, calcium, magnesium, sodium) were relatively high in Saskatoon fruit, even when compared with the work of Mazza (2005). The differences in mineral contents among investigated cultivars are shown in Tables 3 and 4. The well-known fact that iron is a major microelement in fruit (Glew et al. 2007) was also confirmed in our measurement.

In Saskatoon fruit the similar contents of phenolics and antioxidant capacity as we found were measured by Ozga et al. (2007). On the other hand, Bakowska-Barczak and Kolodziejczyk (2008)

Table 2. Total phenolic contents (g of gallic acid/kg FM), antioxidant capacity (g of ascorbic acid/kg FM) and total flavonoid content (mg of rutin/kg FM) of fruits of different cultivars of *Amelanchier alnifolia* Nutt., $n = 15$

Cultivar	Total phenolic contents	Antioxidant capacity – the ABTS test	Antioxidant capacity – the DPPH test	Total flavonoid content
Brnensky	2.95 ± 0.24^{a}	4.04 ± 0.27^{a}	3.85 ± 0.25^{a}	361.31 ± 17.11^{a}
Martin	3.09 ± 0.27^{a}	4.11 ± 0.25^{a}	3.92 ± 0.19^{a}	475.04 ± 19.35^{b}
NS-1	2.87 ± 0.21^{a}	3.80 ± 0.31^{a}	3.61 ± 0.30^{a}	370.75 ± 21.70^{a}
NS-2	2.56 ± 0.32^{a}	3.81 ± 0.28^{a}	3.52 ± 0.31^{a}	344.61 ± 20.18^{a}
Ostravsky	3.15 ± 0.34^{ab}	4.07 ± 0.27^{a}	3.91 ± 0.21^{a}	424.71 ± 19.79^{c}
Skolsky	3.22 ± 0.26^{ab}	4.29 ± 0.29^{a}	4.02 ± 0.28^{a}	436.06 ± 22.61^{bc}
Smoky	3.56 ± 0.20^{b}	4.99 ± 0.20^{b}	4.63 ± 0.24^{b}	550.53 ± 20.45^{d}
Thiessen	3.45 ± 0.23^{b}	4.90 ± 0.31^{b}	4.60 ± 0.26^{b}	531.18 ± 17.73^{d}
Tisnovsky	3.80 ± 0.29^{b}	5.05 ± 0.21^{b}	4.81 ± 0.26^{b}	562.77 ± 19.97^{d}

different superscripts in each column indicate the significant differences in the mean at $P < 0.05$

Table 3. Average contents of mineral elements – macroelements (mg/kg FM) of fruits of different cultivars of *Amelanchier alnifolia* Nutt., n = 15

Cultivar	Phosphorus	Potassium	Calcium	Magnesium	Sodium
Brnensky	471.74 ± 15.29^{a}	3,119.77 ± 112.44^{a}	779.44 ± 26.17^{a}	243.00 ± 11.28^{a}	18.01 ± 1.66^{a}
Martin	410.72 ± 28.61^{b}	3,729.18 ± 151.06^{b}	656.36 ± 19.08^{b}	315.92 ± 16.15^{b}	22.08 ± 1.58^{b}
NS-1	480.33 ± 20.07^{a}	3,017.17 ± 203.44^{a}	780.12 ± 31.25^{a}	276.13 ± 9.77^{c}	19.33 ± 1.84^{ab}
NS-2	477.50 ± 19.55^{a}	3,094.68 ± 115.94^{ac}	745.74 ± 24.78^{a}	258.66 ± 12.46^{ac}	20.89 ± 1.65^{ab}
Ostravsky	414.56 ± 16.78^{b}	2,976.53 ± 169.74^{ac}	665.40 ± 23.11^{b}	210.09 ± 17.52^{d}	24.75 ± 1.37^{b}
Skolsky	364.68 ± 17.60^{c}	2,746.05 ± 118.56^{c}	620.71 ± 22.73^{b}	254.11 ± 12.09^{ac}	25.01 ± 1.75^{b}
Smoky	427.11 ± 12.39^{b}	4,049.35 ± 153.27^{d}	805.62 ± 29.69^{c}	231.80 ± 17.62^{ad}	13.87 ± 1.99^{c}
Thiessen	415.90 ± 18.15^{b}	4,154.32 ± 144.32^{d}	809.60 ± 31.78^{c}	223.31 ± 19.80^{ad}	14.16 ± 1.31^{c}
Tisnovsky	420.72 ± 27.40^{b}	4,311.72 ± 129.85^{d}	845.93 ± 28.36^{c}	296.17 ± 15.08^{bc}	15.89 ± 1.26^{ac}

different superscripts in each column indicate the significant differences in the mean at $P < 0.05$

Table 4. Average contents of mineral elements – microelements (mg/kg FM) of fruits of different cultivars of *Amelanchier alnifolia* Nutt., n = 15

Cultivar	Zinc	Iron	Copper	Manganese
Brnensky	1.72 ± 0.15^{a}	13.62 ± 0.92^{a}	0.85 ± 0.05^{a}	6.23 ± 0.24^{a}
Martin	1.18 ± 0.17^{b}	13.07 ± 0.77^{a}	0.64 ± 0.08^{b}	4.83 ± 0.19^{b}
NS-1	1.19 ± 0.12^{b}	11.70 ± 0.54^{b}	0.92 ± 0.07^{a}	5.99 ± 0.25^{a}
NS-2	1.22 ± 0.15^{b}	12.41 ± 0.70^{ab}	0.81 ± 0.04^{a}	5.87 ± 0.20^{a}
Ostravsky	1.23 ± 0.14^{b}	13.09 ± 0.81^{a}	0.60 ± 0.06^{b}	4.75 ± 0.18^{b}
Skolsky	1.18 ± 0.13^{b}	12.58 ± 0.91^{ab}	0.64 ± 0.07^{b}	5.02 ± 0.26^{b}
Smoky	1.70 ± 0.15^{a}	15.96 ± 0.68^{c}	1.15 ± 0.07^{c}	4.97 ± 0.29^{b}
Thiessen	1.76 ± 0.16^{a}	14.51 ± 0.72^{ac}	1.11 ± 0.08^{c}	4.77 ± 0.25^{b}
Tisnovsky	1.74 ± 0.14^{a}	15.93 ± 0.80^{c}	0.97 ± 0.07^{ac}	4.94 ± 0.21^{b}

different superscripts in each column indicate the significant differences in the mean at $P < 0.05$

noticed higher contents of polyphenols (even up to 8.01 g/kg FM) in this fruit species. Quite a number of authors (e.g. Moyer et al. 2002; Rupasinghe et al. 2006; Rop et al. 2009) refer to high correlation dependence of polyphenols and antioxidant capacity in fruit, which was also shown in our measurement – in case of the ABTS test (R^2 = 0.8791; y = 0.7042 + 0.1272) and in case of the DPPH test (R^2 = 0.9292; y = 0.7802x – 0.0129). So, for example, for apples Thaipong et al. (2006) mentioned correlation coefficients ranging from R^2 = 0.81 to 0.97. The major phenolic components in Saskatoon berries are flavonoids (Ozga et al. 2007). We measured the highest contents of flavonoids in the Smoky, Thiessen and above all Tisnovsky cultivars (Table 2). For example, Mazza (2005) noticed the total flavonoid content of 530 mg/kg FM in the Smoky cultivar. We found very similar results in this cultivar (in case of flavonoids 550.53 mg/kg FM). Furthermore, the high correlation coefficient between total flavonoid content and antioxidant capacity (R^2 = 0.8870; y = 156.52x – 228.53) was observed.

CONCLUSION

The presented results may be used when popularizing this fruit species and also when studying the properties of new genetic resources. This means that in future some of the cultivars of Saskatoon berries could be used as a source of nutraceuticals when improving nutritional properties of European and worldwide fruit.

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