# Antioxidant properties of saskatoon berry (*Amelanchier alnifolia* Nutt.) fruits

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#### Antioxidant properties of saskatoon berry (Amelanchier alnifolia Nutt.) fruits.

Abstract-Introduction. Saskatoon berry (Amelanchier alnifolia Nutt.) is a promising fruit species originating from North America. Among pomaceous fruits, saskatoon berries are a valuable source of chemical compounds with an antioxidant effect. Materials and methods. The total phenolic content, total flavonoid content and their correlation associated with the total antioxidant capacity of fruit of five particular cultivars were ascertained. Reactive oxygen species (specifically nitric oxide. superoxide anion and hydroxyl radical) and antioxidant activity in the liver lipid system of their methanolic extracts were also assessed. Results and discusssion. In saskatoon berry cultivars the total contents of phenolic compounds ranged from (2.52 to 3.82) g gallic acid Eq kg<sup>-1</sup> of fresh mass, while the values of total antioxidant capacity were observed to be from (4.17 to 5.29) g of ascorbic acid Eq·kg<sup>-1</sup> of fresh mass. High correlation coefficients between phenolics as well as flavonoids and antioxidant capacity were calculated ( $r^2 = 0.8921$  and  $r^2 = 0.9901$ , respectively). Nitric oxide, superoxide anion, hydroxyl radical and antioxidant activity in the liver lipid system of saskatoon berry fruit methanolic extracts (10%) were provided for the first time. In the case of nitric oxide, the inhibitions were 21.08-27.52%; as regards superoxide anion, they were 25.14-30.73%; concerning hydroxyl radical, 18.25-21.18%, and in respect of antioxidant activity in the liver lipid system, 7.90–8.38%. These inhibitions are stronger than, e.g., in apples which are the most important species of pomaceous fruit worldwide. Conclusions. Saskatoon berry fruit could be a suitable supplement for modern human nutrition. Furthermore, our work contributes to the popularisation of this pomaceous species, with the focus on its potential in relation to high antioxidant strength.

### Czech Republic / *Amelanchier alnifolia* / fruits / antioxidants / phenolic content / flavonoids / reactive oxygen species

#### Propriétés antioxydantes des fruits de l'amélanchier (Amelanchier alnifolia Nutt.).

Résumé - Introduction. L'amélanchier (Amelanchier alnifolia Nutt.), originaire de l'Amérique du Nord, est une espèce fruitière prometteuse. Parmi les fruits à pépins, ceux de l'amélanchier sont une source précieuse de composés chimiques ayant un effet antioxydant. Matériel et méthodes. Les teneurs totales en composés phénoliques et en flavonoïdes et leur corrélation, associées à la capacité antioxydante totale, des fruits de cinq cultivars particuliers ont été évaluées. Les dérivés réactifs de l'oxygène (en particulier l'oxyde nitrique, l'anion superoxyde, le radical hydroxyle) et l'activité antioxydante dans le système lipidique du foie de leurs extraits méthanoliques ont également été évalués. Résultats et discusssion. Pour les fruits des cultivars d'amélanchiers étudiés, la teneur totale en composés phénoliques a varié de (2,52 à 3,82) g d'acide gallique Eq·kg<sup>-1</sup> de poids frais, tandis que leurs valeurs de capacité antioxydante totale ont été de (4,17 à 5,29) g d'acide ascorbique Eq kg<sup>-1</sup> de poids frais. De forts coefficients de corrélation ont été trouvés entre les composés phénoliques et les flavonoïdes et la capacité antioxydante ( $r^2 = 0.8921$  et  $r^2 = 0.9901$ , respectivement). L'oxyde nitrique, l'anion superoxyde, le radical hydroxyle et l'activité antioxydante dans le système lipidique du foie d'extraits méthanoliques (10 %) de fruits d'amélanchier ont été mesurés pour la première fois. Dans le cas de l'oxyde nitrique, les inhibitions ont été de 21,08 % à 27,52 %; en ce qui concerne l'anion superoxyde, elles ont été de 25,14 % à 30,73 %; pour le radical hydroxyle, de 18,25 % à 21,18 % ; et pour l'activité antioxydante dans le système lipidique du foie, de 7,90 % de 18,25 % à 21,18 % ; et pour l'activité antioxydante dans le système lipidique du foie, de 7,90 % de 18,25 % à 21,18 % ; et pour l'activité antioxydante dans le système lipidique du foie, de 7,90 % de 18,25 % à 21,18 % ; et pour l'activité antioxydante dans le système lipidique du foie, de 7,90 % de 18,25 % à 21,18 % ; et pour l'activité antioxydante dans le système lipidique du foie, de 7,90 % de 18,25 % à 21,18 % ; et pour l'activité antioxydante dans le système lipidique du foie, de 7,90 % de 18,25 % de 19,25 % de 1 à 8,38 %. Ces inhibitions sont plus fortes que celles de la pomme par exemple, qui est l'espèce la plus importante des fruits à pépins dans le monde. Conclusions. Le fruit de l'amélanchier pourrait apporter un complément approprié à la nutrition humaine moderne. Nos travaux contribuent en outre à vulgariser cette espèce à pépins en insistant sur son potentiel quant à à ses fortes propriétés

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#### 1. Introduction

Saskatoon berry (Amelanchier alnifolia Nutt.) belongs to the rose family (Rosaceae) [1] and it is actually a pome [2]. It grows as a deciduous shrub or small tree, reaching a height of 1 m to 8 m. This species is native to the southern Yukon, the Canadian prairies and the northern plains of the United States [3]. The mature fruit is a purple berry-like pome, 1–1.5 cm in diameter [4]. Early settlers encountered the fruit, and in North America even today this fruit can be gathered in the wild. Apart from this, it is also cultivated as a fruit crop plant, particularly in Canada [5]. However, it generally belongs to the lesser known fruit species. The fruits are eaten fresh or processed into jams, juices, syrups, wine and pie fillings [6].

As regards growing, the plants have low demands from the environment and are extremely frost-resistant [7]. Similarly, during the growing season they can cope with cold conditions. Although poor soil conditions do not pose a problem, they can be best grown on slightly alkaline soils [8].

The fruits of saskatoon berry are gaining in popularity and have been introduced into Europe as well. There they can be cultivated particularly in the conditions of the cool temperate zone [9]. The fruits are ripe at the beginning of July and more often they are utilised for their sour and sweet refreshing taste to vary the menu [10].

Lately, the fruits have been mentioned in relation to their high antioxidant capacity, which is conditioned by a whole range of bioactive substances belonging to polyphenolics (in particular, flavonoids). For their nutritional value the fruits are regarded as a new promising fruit species both for human nutrition and the pharmaceutical industry [11]. The consumption of saskatoon berry fruits is recommended for the prevention of many illnesses including tumorous diseases. Furthermore, they can be used as a raw material for fruit preservation since the contents of pectins, sugars and mineral substances, which can be comparable with, e.g., apples, are not low [12].

Although the antioxidant properties of the fruits have been described in other research [13], some aspects have not been published so far, in particular concerning the influence of fruit extracts on reactive oxygen species scavenging activity (ROS). In the human body reactive oxygen species emerge as a response to the immune system [14]. Their production is often connected with stressful situations and the modern lifestyle. The reactive oxygen species attack biomembranes and damage molecules (of lipids, DNA, proteins, amino acids, etc.) [15].

The aim of our work was to describe the effect of methanolic extracts of fruits on reactive oxygen species scavenging activity in five saskatoon berry cultivars during a two-year experiment. In concrete terms, it was the impact on reactive oxygen species such as hydroxyl radical, nitric oxide and superoxide anion. With respect to saskatoon berry, most of these analyses were performed for the first time. Moreover, the influence of methanolic extracts on antioxidant activity in the liver lipid system was monitored. For comparison, the above-mentioned parameters of the results obtained were correlated with antioxidant capacity (TAC) and the contents of total phenolics (TPC) and total flavonoids (TFC). These contents (TAC, TPC and TFC) were provided as another aim of the experiment in order to verify the new findings that emerged.

#### 2. Materials and methods

#### 2.1. Description of locality

Fruit were harvested in an experimental gene-fund orchard of Mendel University in Brno (Czech Republic) within the period of 2010-2011. This orchard is situated in the area of Zabcice village, approximately 20 km southwards from Brno. The altitude is 184 m a.s.l., 49°01' N lat., 16°36' E long. The average annual temperature was 9 °C (during the growing season 15.6 °C) and the fifty-year average sum of precipitation was 553 mm (during the growing season 356 mm). Genetically, soils are classified as gleved alluvial soils developed on the Holocene calciferous sediments with a marked accumulation of organic compounds. As regards the texture, the topsoil is loamy and the subsoil clayey-loamy [16].

Table I. Description of the attributes of the saskatoon berry (Amelanchier alnifolia Nutt.) cultivars used. For all cultivars, fruits are purplish-red (Brno, Czech Rep.).

Cultivar	Fruit size in diameter (mm)	Number of berries in a cluster	Flavour
Honeywood	up to 16	9 to 15	Slightly sour
Martin	up to 18	8 to 13	Pleasantly sweet and sour
Northline	up to 16	7 to 13	Sweet
Smoky	up to 14	7 to 11	Smooth, sweet and sour
Thiessen	up to 17	7 to 13	Juicy, slightly sour

#### 2.2. Collection and processing of samples for chemical analyses

Fruit were harvested at full ripeness from three plants of each cultivar under study in the course of June [4]. The age of experimental trees ranged from 8 to 10 years. Forty randomly chosen fruit from each of the three plants of each cultivar were mixed and processed immediately together after the harvest (not later than within two days) in a laboratory grinder SJ500 (MEZOS, Hradec Kralove, Czech Republic) and used for analyses (i.e., altogether 120 berries per cultivar). Each parameter was measured in five replications for each cultivar. The results were expressed as the average of a two-year experiment (every year, there were measurements in five replications, thus  $2 \times 5 =$ 10 replications, *i.e.*, n = 10).

Five cultivars of saskatoon berry (Amelanchier alnifolia Nutt.) originating in North America were used: 'Honeywood', 'Martin', 'Northline', 'Smoky' and 'Thiessen' (table I).

#### 2.3. Sample preparation

The extraction and the total phenolic content assay were performed according to the method described by Kim et al. [17], using the following procedure: 10 g of a fresh sample were homogenised for 10 s in 100 mL of methanol. The resulting paste was placed into Erlenmeyer flasks (120 mL) and left to stand in a water bath with a temperature of +25 °C for a period of 24 h. The methanolic extracts were stored at +4 °C for further use.

To measure the total contents of phenolic substances, an aliquot of 0.5 mL of the sample was taken and diluted with water in a 50-mL volumetric flask. Thereafter, 2.5 mL of Folin-Ciocalteu reagent and 7.5 mL of a 20% solution of Na<sub>2</sub>CO<sub>3</sub> were added. The resulting absorbance was measured using a LIBRA S6 spectrophotometer (Biochrom Ltd., Cambridge, UK) at the wavelength of 765 nm against a blind sample, which was used as a reference. The results were expressed as g of gallic acid Eq·kg<sup>-1</sup> of fresh mass (fm).

#### 2.4. Antioxidant activity by the DPPH assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was conducted according to the method of Thaipong et al. [18] by dissolving 24 mg of DPPH radical with 100 mL of methanol and then storing it at -20 °C until needed. The working solution was obtained by mixing 10 mL of the stock solution with 45 mL of methanol to obtain the absorbance of  $(1.1 \pm 0.02)$  units at 515 nm using the LIBRA S6 spectrophotometer (Biochrom Ltd., Cambridge, UK). Fruit extracts (150 µL) were allowed to react with 2,850 µL of the DPPH solution for 1 h in the dark, then the absorbance was taken at 515 nm. Antioxidant activity was calculated as a decrease in the absorbance value using the formula: [antioxidant activity (%) =  $(A_0 - A_1/A_0) \times$ 100], where  $A_0$  is the absorbance of the control (without the sample) and A1 is the absorbance of the mixture containing the sample.

The results of absorbance were converted using a calibration curve of the standard and expressed as g of ascorbic acid Eq·kg<sup>-1</sup> of fresh mass [19].

#### 2.5. Total flavonoid content assay

The total flavonoid content was determined following Park *et al.* [20]. In a 10-mL Eppendorf tube, 0.3 mL of the fruit extract, 3.4 mL of 30% ethanol, 0.15 mL of NaNO<sub>2</sub> (0.5 M) and 0.15 mL of AlCl<sub>3</sub>.6H<sub>2</sub>O (0.3 M) were added and mixed. After 5 min, 1 mL of NaOH (1 M) was added, and 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 rutin Eq·kg<sup>-1</sup> of fresh mass.

### 2.6. Reactive oxygen species scavenging activity assay

For the measurement of reactive oxygen species activity, a 10% extract was prepared in a phosphate buffer (50 mM, pH 7.0). The hydroxyl radical scavenging activity was assayed according to the method by Ghiselli et al. [21]. One mL of the extract was mixed with 0.8 mL of the reaction buffer (KH<sub>2</sub>PO<sub>4</sub>.KOH, 0.2 M, pH 7.4; deoxyribose, 1.75 µM; iron ammonium sulphate, 0.1 µM; and EDTA, 0.1 µM). A quantity of 0.1 mL of H<sub>2</sub>O<sub>2</sub> (0.01 M) was then added to the reaction solution. The solution was incubated for 10 min at +37 °C prior to the addition of 0.5 mL of 1% thiobarbituric acid and 1 mL of 2.8% trichloracetic acid. The mixture was boiled for 10 min and cooled rapidly. The absorbance of the mixture was measured at 532 nm using the LIBRA S6 apparatus (Biochrom Ltd., Cambridge, UK).

The assay of nitric oxide scavenging activity was performed according to the method described by Green *et al.* [22]. One mL of the extract was mixed with 1 mL of the reaction solution containing sodium nitroprusside (10 mM) in phosphate buffer (50 mM, pH 7.0). This was followed by incubation at +37 °C for 1 h and an aliquot of 0.5 mL was then mixed with 0.5 mL of Griess reagent. The absorbance was measured at 540 nm.

The superoxide anion scavenging activity was assessed by the method described by Beissenhirtz *et al.* [23]; it is based on the reduction of cytochrome c. One mL of the extract was mixed with 1 mL of the solution containing 0.07 units per ml of xanthine oxidase, xanthine (100  $\mu$ M) and cytochrome c (50  $\mu$ M). After incubation at +20 °C for 3 min, the absorbance was determined at 550 nm.

All tests were performed in triplicate. The scavenging activities of nitric oxide, super-oxide anion and hydroxyl radical were calculated as: [scavenging activity (%) =  $(A_0 - A_1/A_0) \times 100$ ], where  $A_0$  is the absorbance of the control (without the sample) and  $A_1$  is the absorbance of the mixture containing the sample.

## 2.7. Antioxidant activity in the liver lipid system

The antioxidant activity in the liver lipid system was assayed by the method of Srivastava et al. [24]. Five µg of rat liver were homogenised in 20 mL of tris-HCl buffer (40 mM, pH 7.0). An aliquot of 0.1 mL of the liver homogenate was incubated with the sample (0.2 mL of a 5% extract), 0.1 mL of KCl (30 mM), 0.1 mL of FeSO<sub>4</sub> (0.16 mM) and 0.1 mL of ascorbic acid (0.06 mM) at +37 °C for 1 h. Thereafter, we added 1 mL of 1% thiobarbituric acid (TBA) and 1 mL of 15% trichloracetic acid. The final solution was heated at +100 °C in a boiling water bath for 15 min, cooled with ice for 10 min, and then centrifuged at 5,000 rpm for 10 min. The absorbance of the supernatant was measured at 532 nm, using the LIBRA S6 spectrophotometer. The blank was performed by substituting tris-HCl buffer (50 mM, pH 7.0) for the sample. The inhibition percentage of the formation of TBAreactive substances was calculated as: [inhibition activity (%) =  $(A_0 - A_1/A_0) \times 100$ ], where A<sub>0</sub> is the absorbance of the control (without the sample) and A<sub>1</sub> is the absorbance of the mixture containing the sample.

#### 2.8. Statistical analysis

The data obtained were analysed statistically by an analysis of variance (ANOVA)

Table II. Total phenolic content, total antioxidant capacity and total flavonoid content of fruits of particular saskatoon berry (Amelanchier alnifolia Nutt.) cultivars (n = 10) (Brno, Czech Rep.).

Cultivar	Total phenolic content (g gallic acid Eq·kg <sup>-1</sup> fm)	Antioxidant capacity (g ascorbic acid Eq·kg <sup>-1</sup> fm)	Total flavonoid content (mg rutin Eq·kg <sup>-1</sup> fm)			
Honeywood	$2.52 \pm 0.19$ a	4.17 ± 0.15 a	461.62 ± 21.78 a			
Martin	$3.01 \pm 0.14 b$	4.24 ± 0.17 a	470.09 ± 16.55 a			
Northline	$2.98 \pm 0.14 b$	4.25 ± 0.15 a	474.53 ± 19.00 a			
Smoky	$3.82 \pm 0.26 \mathrm{c}$	$5.29 \pm 0.20  \mathrm{b}$	554.11 ± 15.71 b			
Thiessen	3.57 ± 0.11 c	$5.10 \pm 0.18  b$	530.28 ± 12.92 b			
fm: fresh mass.  The letters a, b, c in each column indicate the significant differences in the mean at $P \le 0.05$ .						

and Tukey's multiple range test for comparison of means [25]. Correlation functions were calculated using the statistical package Unistat, v. 6.1 (Unistat Ltd., London, UK) and Office Excel® Microsoft v. 2010.

#### 3. Results and discussion

The results were expressed as an average of a two-year experiment since there was no statistically significant difference among the years in any parameter investigated. In the case of saskatoon berries, Zatylny et al. [26] also confirmed the influence of the year has little impact.

In the berries studied, the total contents of phenolic substances ranged from (2.52 to 3.82) g gallic acid Eq·kg<sup>-1</sup> of fresh mass (table II). These values were comparable with those found in common kinds of fruit; for example, plums (3.48-4.95 g gallic acid Eq·kg<sup>-1</sup> of fresh mass [27]), blueberries  $(3.00-4.89 \text{ g gallic acid Eq·kg}^{-1} \text{ of fresh mass})$ [28]) and black currants (on average 5.33 g gallic acid Eq·kg<sup>-1</sup> of fresh mass [29]). Regarding apples, which are the most important species of pomaceous fruit [30], the values of total phenolic content usually ranged from (1.40 to 3.30) g gallic acid Eq·kg<sup>-1</sup> of fresh mass [31]. In respect of total phenolic content, the fruits of saskatoon berry fruits are interesting owing to a significant presence of neuroprotective phenolics such as gallic acid, rutin and quercetin [32].

Flavonoids, with anthocyanidins being their subclass, are one of the main groups of phenolic substances in fruits [13]. Saskatoon berries are known because of anthocyanins, which are glycosides of anthocyanidins associated with attractive, colourful and flavourful fruits [33]. Flavonoids are important as a nutritional factor in relation to the antioxidant efficiency of plants [34]. Saskatoon berries are referred to as an alternative source of currently available flavonoids in human nutrition [35]. In our work, the highest content of flavonoids was measured in the 'Smoky' and 'Thiessen' cultivars [(554.11 and 530.28) mg rutin Eq·kg<sup>-1</sup> of fresh mass, respectively]. These values are similar to those that Mazza [2] and Rop et al. [10] noted in saskatoon berry cultivars. Furthermore, other pomaceous species (e.g., apples) have a similar flavonoid content [13] which, of course, depends on the cultivar [31], as was also confirmed in our research. In our study, high values of total antioxidant capacity content were determined [(4.17 to 5.29) g ascorbic acid Eq·kg<sup>-1</sup> of fresh mass] in saskatoon berries. In apples, which are a typical representative of pomaceous fruit [12], the measured average values of antioxidant capacity are approximately 2.50 g ascorbic acid Eq·kg<sup>-1</sup> of fresh mass [31], while, in cherries, this value is 0.90 g ascorbic acid Eq kg<sup>-1</sup> of fresh mass on average [36]. On the contrary, e.g., plums are rich in total antioxidant capacity with an average of 5.00 g ascorbic acid Eq·kg<sup>-1</sup> of fresh mass [27]. Generally, it can be stated that saskatoon

**Table III.** Scavenging effect of particular saskatoon berry (*Amelanchier alnifolia* Nutt.) fruit methanolic extract (10%) on nitric oxide, superoxide anion, hydroxyl radica and antioxidant activity in the liver lipid system (n = 10) (Brno, Czech Rep.).

Cultivar	Nitric oxide	Superoxide anion	Hydroxyl radical	Lipid peroxidation	
	(% of inhibition)				
Honeywood	21.72 ± 0.89 a	25.14±0.65 a	18.25 ± 0.50 a	7.98 ± 0.45 a	
Martin	22.65 ± 0.53 a	26.46±0.78 a	19.01 ± 0.59 a	8.05 ± 0.61 a	
Northline	21.08 ± 0.88 a	26.51±0.59 a	20.41 ± 0.39 b	$7.90 \pm 0.52$ a	
Smoky	27.52 ± 0.85 b	30.73±0.60 b	$24.39 \pm 0.75$ c	8.35 ± 0.61 a	
Thiessen	$26.74 \pm 0.67  b$	29.29±0.71 b	21.18 ± 0.60 b	8.38 ± 0.58 a	
The letters a, b, c in each column indicate the significant differences in the mean at $P \le 0.05$ .					

berries are a valuable source of antioxidants among pomaceous fruits [3, 31].

Quite a number of authors [19, 37, 38] refer to the high correlation dependence of total phenolic content/total flavonoid content and total antioxidant activity in different fruit species, including saskatoon berry [39]. The coefficients of correlations existing between the total contents of phenolics, flavonoids and antioxidant capacity and the scavenging effect on reactive oxygen species and antioxidant activity in the liver lipid system were calculated (*table IV*); the results show that the correlation coefficients between the measured chemical parameters expressing the antioxidant properties of saskatoon berry are significant.

This is an important fact since it is evident that the total phenolic content and total flavonoid content of saskatoon berries have an influence not only on total antioxidant capacity, but also on reactive oxygen species and antioxidant activity in the liver lipid system. Moreover, looking at *tables II* and *III* in relation to *table IV*, it is apparent that the higher antioxidant efficiency of saskatoon berries corresponds to reactive oxygen species and antioxidant activity in the liver lipid system.

The fruit extracts (10%) showed a moderate inhibitory ability on nitric oxide (21.08% to 27.52%), superoxide anion (25.14% to 30.73%), hydroxyl radical (18.25% to 21.18%) and antioxidant activity in the liver lipid system (7.90% to 8.38%). Using extracts of saskatoon berry, the fruit

was more effective than those of other fruit species, e.g., mulberry [40] or apples [41]. In the case of apples, Rop et al. [13] observed that, studying 12 cultivars, % of inhibition for nitric oxide was between 12.78% and 21.36%; superoxide anion, from 17.10% to 24.99%; and hydroxyl radical, from 9.47% to 18.12%. In saskatoon berry fruits, scavenging activity is associated with phenolics [42]. Wang and Mazza [43] noted that an extract of saskatoon berry fruits can inhibit reactive oxygen species and special nitric oxide production in activated macrophages, indicating a potential protective role against cardiovascular disease and chronic inflammation. Hu et al. [42] found the fruits of saskatoon berries to be strong scavengers of free radicals without reducing cell viability.

#### 4. Conclusions

Saskatoon berry (Amelanchier alnifolia Nutt.) is a less widespread fruit species. The aim of our work was to determine the phenolic content, the flavonoid content and antioxidant capacity in the fruit of the five most common cultivars of this deciduous shrub. Our research provides novel findings and, so far, unpublished results in these particular cultivars. Apart from the chemical parameters mentioned, it concerns the scavenging activities of methanolic extracts (10%) on reactive oxygen species (nitric oxide, superoxide anion and hydroxyl radical) and antioxidant activity in the liver lipid system, where the fruits appear to be strong

#### Table IV.

Correlation relationships between the total phenolic content, the total flavonoid content and the total antioxidant capacity, and the scavenging effect of saskatoon berry (Amelanchier alnifolia Nutt.) fruit methanolic extract (10%) on hydroxyl radical, nitric oxide, superoxide anion and antioxidant activity in the liver lipid system (Brno, Czech Rep.).

Parameter	Total phenolic content	Total flavonoid content
Total antioxidant capacity	0.8921 $Y = 0.9863 x + 0.4732$ $P < 0.001$	0.9901 $Y = 0.0130 x - 1.8503$ $P < 0.001$
Hydroxyl radical	0.8380 $Y = 4.2310 x + 7.1934$ $P < 0.001$	0.8579 $Y = 0.0534 x - 5.9688$ $P < 0.001$
Nitric oxide	0.8420 $Y = 5.2895 x + 7.1213$ $P < 0.001$	0.9312 $Y = 0.0694 x - 10.6340$ $P < 0.001$
Superoxide anion	0.9768 $Y = 4.4060 x + 13.6150$ $P < 0.001$	0.9804 $Y = 0.0551 x + 0.1811$ $P < 0.001$
Antioxidant activity in liver lipid system capacity	0.7668 Y = 0.3723 x + 6.9482 P < 0.01	0.8511 Y = 0.0049 x + 5.6936 P < 0.001

scavengers of free radicals – in particular the 'Smoky' and 'Thiessen' cultivars. This work should contribute to the popularisation of this pomaceous fruit species for worldwide use even in cooler temperate zones.

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#### References

- [1] Evans R.C., Dickinson T.A., Floral ontogeny and morphology in Gillenia (Spiraeoideae) and subfamily maloideae C. Weber (Rosaceae), Int. J. Plant Sci. 166 (2005) 427-447.
- [2] Mazza G., Composinational and functional properties of saskatoon berry and blueberry, Int. J. Fruit Sci. 5 (2005) 101-120.
- [3] Ozga J.A., Saeed A., Reinecke D.M., Anthocyanins and nutrient components of saskatoon fruits (Amelanchier alnifolia Nutt.), Can. J. Plant Sci. 86 (2006) 193-197.

- Rogiers S.Y., Knowles N.R., Physical and chemical changes during growth, maturation, and ripening of saskastoon (Amelanchier alnifolia) fruit, Can. J. Bot. 75 (1998) 1215-1225.
- St-Pierre R.G., Zatylny A.M., Tulloch H.R., Evaluation of growth and fruit production characteristics of 15 saskatoon (Amelanchier alnifolia Nutt.) cultivars at maturity, Can. J. Plant Sci. 85 (2005) 929-932.
- McGarry R., Ozga J.A., Reinecke D.M., The effects of ethephon on saskatoon (Amelanchier alnifolia Nutt.) fruit ripening, J. Am. Soc. Hortic. Sci. 130 (2005) 12-17.
- [7] Vertucci C.W., Stushnoff C., The state of water in acclimating vegetative buds from Malus and Amelanchier and its relationship to winter hardiness, Physiol. Plantarum 86 (1992) 503-511.
- Voeller P.J., Zamora B.J., Harsh J., Growth response of native shrubs to acid mine spoil and to proposed soil amendments, Plant Soil 198 (1998) 209-217.
- Suojala T., Linden L., Frost hardiness of Philadelphus and Hydrangea clones during ecodormancy, Acta Agr. Scand. B-S.P. 47 (1997) 58-63.

- [10] Rop O., Reznicek V., Mlcek J., Jurikova T., Sochor J., Kizek R., Humpolicek P., Balik J., Nutritional values of new Czech cultivars of saskatoon berries (*Amelanchier alnifolia* Nutt.), Hortic. Sci. 39 (2012) 123–128.
- [11] Bakowska-Barczak A.M., Marianchuk M., Kolodziejczyk P., Survey of bioactive components in Western Canada berries, Can. J. Physiol. Pharm. 85 (2007) 1139–1152.
- [12] Kyzlink V., Principles of Food Preservation, Elsevier, Amst., Neth., 1990.
- [13] Velisek J., Chemie potravin, OSSIS, Tabor, Czech Rep., 2002.
- [14] McCord J.M., The evolution of free radicals and oxidative stress, Am. J. Med. 108 (2000) 652–659.
- [15] Aruoma O.I., Nutrition and health aspects of free radicals and antioxidants, Food. Chem. Toxicol. 62 (1994) 671–683.
- [16] Anon., Data from Central Institute for Supervising and Testing in Agriculture, UKZUZ, Brno, Czech Rep., 2008.
- [17] Kim D.O., Neony S.W., Lee C.Y., Antioxidant capacity of phenolic phytochemicals from various cultivars of plums, Food Chem. 51 (2003) 321–326.
- [18] Thaipong K., Boonprakob U., Crosby K., Cisneros-Zevallos L., Byrne D.H., Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts, J. Food Compos. Anal. 19 (2006) 669–675.
- [19] Rupasinghe V.H.P., Jayasankar S., Lay W., Variation in total phenolic and antioxidant capacity among European plum genotypes, Sci. Hortic. 108 (2006) 243–246.
- [20] Park Y.S., Jung S.T., Kang S.G., Heo B.G., Arancibia-Avila P., Toledo F., Drzewiecki J., Namiesnik J., Gorinstein S., Antioxidants and protein in ethylene-treated kiwifruits, Food Chem. 107 (2008) 640–648.
- [21] Ghiselli A., Nardini M., Baldi A., Scaccini C., Antioxidant activity of different phenolic fractions separated from an Italian red wine, J. Agric. Food Chem. 46 (1998) 361–367.
- [22] Green L.C., Wagner D.A., Glogowski J., Skipper P.L., Wishnok J.S., Tannenbaum S.R., Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids, Anal. Biochem. 126 (1982) 131–138.
- [23] Beissenhirtz M.K., Kwan R.C., Ko K.M., Renneberg R., Schiller F.W., Liskat F., Comparing an in vitro electrochemical measurement of superoxide scavenging activity with

- an *in vivo* assessment of antioxidant potential in Chinese tonifying herbs, Phytother. Res. 18 (2004) 149–153.
- [24] Srivastava A., Harish S.R., Shivanandappa T., Antioxidant activity of the roots of *Decale*pis hamiltonii, LWT-Food Sci. Technol. 36 (2006) 1059–1065.
- [25] Snedecor G.W., Cochran W.G., Statistical methods, Iowa State Univ., Ames, U.S.A., 1968.
- [26] Zatylny A.M., St-Pierre R.G., Tulloch H.P., Comparative agronomic performance of 15 saskatoon (Amelanchier alnifolia Nutt.) cultivars during their first seven years of growth, J. Am. Pomol. Soc. 56 (2002) 118– 128.
- [27] Rop O., Jurikova T., Mlcek J., Kramarova D., Sengee Z., Antioxidant activity and selected nutritional values of plums (*Prunus domes-tica* L.) typical of the White Carpathian Mountains, Sci. Hortic. 122 (2009) 545–549.
- [28] You Q., Wang B.W., Chen F., Huang Z.L., Wang X., Luo P.G., Comparison of anthocyanins and phenolics in originally and conventionally grown blueberries in selected cultivars, Food Chem. 125 (2011) 201–208.
- [29] Lugasi A., Hovari J., Kadar G., Denes S., Phenolics in raspberry, blackberry and currant cultivars grown in Hungary, Acta Aliment. 40 (2011) 52–64.
- [30] Magazin N., Gvozdenovic D., Keserovic Z., Milic B., Fruit quality of Granny Smith apples picked at different harvest times and treated with 1-MCP, Fruits 65 (2010) 191–197.
- [31] Rop O., Jurikova T., Sochor J., Mlcek J., Kramarova D., Antioxidant capacity, scavenging radical activity and selected chemical composition of native apple cultivars from Central Europe, J. Food Qual. 34 (2011) 187– 194.
- [32] Gazdik Z., Reznicek V., Adam V., Zitka V., Jurikova T., Krska B., Matuskovic J., Plsek J., Saloun J., Horna A., Kizek R., Use of liquid chromatography with electrochemical detection for the determination of antioxidants in less common fruits, Molecules 13 (2008) 2823–2836.
- [33] Seeram N.P., Schutzki R., Chandra A., Nair M.G., Characterization, quantification and bioactivities of anthocyanins in *Cornus* species, J. Agric. Food Chem. 50 (2002) 2519– 2523.
- [34] Stintzing F.C., Stintzing A.S., Carle R., Frei B., Wrolstad E., Colour and antioxidant properties of cyanidin-based anthocyanin

- pigments, J. Agric. Food Chem., 50, 6172-6181.
- [35] Adhikari P.D., Francis J.A., Schutzki R.E., Chandra A., Nair M.G., Quantification and characterisation of cyclooxygenase and lipid peroxidation inhibitory anthocyanins in fruit of Amelanchier, Phytochem. Anal. 16 (2005) 175-180.
- [36] Usenik V., Fabcic J., Stampar F., Sugars, organic acids, phenolic composition and antioxidant activity of sweet cherry (Prunus avium L.), Food Chem. 107 (2008) 185-192.
- [37] Moyer R.A., Hummer K.E., Finn C.E., Frei B., Wrostland R.E., Antocyanins, phenolics, and antioxidant capacity in diverse small fruits: Vaccinium, Rubus, and Ribes, J. Agric. Food Chem. 50 (2002) 519-525.
- [38] Jurikova T., Matuskovic J., The study of irrigation influence on nutritional value of Lonicera kamtschatica-cultivar Gerda 25 and Lonicera edulis berries under the Nitra conditions during 2001-2003, Hortic. Sci. 34 (2007) 1-6.
- [39] Bakowska-Barczak A.M., Kolodziejczyk P., Evaluation of saskatoon berry (Amelanchier

- alnifolia Nutt.) cultivars for their polyphenol content, antioxidant properties, and storage stability, J. Agric. Food Chem. 56 (2008), 9933-9940.
- [40] Bae S.H., Suh H.J, Antioxidant activities of five different mulberry cultivars in Korea, LWT-Food Sci. Technol. 40 (2007) 955-962.
- [41] Maffei F., Tarozzi A., Karbone F., Marchesi A., Hrelia S., Angeloni C., Forti G.C., Hrelia P., Relevance of apple consumption for protection against oxidative damage induced by hydrogen peroxide in human lymphocytes, Brit. J. Nutr. 97 (2007) 921-927.
- [42] Hu C., Kwok B.H.L., Kitts D.D., Saskatoon berries (Amelanchier alnifolia Nutt.) scavenge free radicals and inhibit intracellular oxidation, Food Res. Int. 38 (2005) 1079-
- [43] Wang J., Mazza G., Inhibitory effects of anthocyanins and other phenolic compounds on nitric oxide production in LPS/ IFN-gamma-activated RAW 264.7 macrophages, J. Agric. Food Chem. 50 (2002) 850-857.

### Propiedades antioxidantes del fruto del guillomo (Amelanchier alnifolia Nutt.).

Resumen - Introducción. El guillomo (Amelanchier alnifolia Nutt.), originario de Norteamérica, es una especie de fruta prometedora. Entre las frutas pomáceas, las del guillomo son una preciada fuente de compuestos químicos con efecto antioxidante. Material y métodos. Se estudiaron los contenidos totales en compuestos fenólicos y flavonoides y su correlación, asociados a la capacidad antioxidante total, de los frutos de cinco cultivares concretos. También se evaluaron los derivados reactivos del oxígeno (concretamente, el óxido nítrico, el anión superóxido y el radical hidroxilo) y la actividad antioxidante en el sistema lipídico del hígado de sus extractos metanólicos. Resultados y discusión. En el caso de los frutos de los cultivares de guillomos estudiados, el contenido total en compuestos fenólicos varió (2,52 a 3,82) g de ácido gálico Eq·kg<sup>-1</sup> de peso fresco, mientras que sus valores en capacidad antoxidante total fueron (4,17 a 5,29) g de ácido ascórbico Eq·kg<sup>-1</sup> de peso fresco. Se hallaron fuertes coeficientes de correlación entre los compuestos fenólicos y los flavonoides y la capacidad antioxidante ( $r^2 = 0.8921$  y  $r^2 = 0.9901$ , respectivamente). Se midieron por primera vez el óxido nítrico, el anión superóxido, el radical hidroxilo y la actividad antioxidante en el sistema lipídico del hígado de los extractos metanólicos (10%) de los frutos del guillomo. En el caso del óxido nítrico, las inhibiciones fueron del 21,08% al 27,52%; en el caso del anión superóxido, fueron del 25,14% al 30,73%; en el caso del radical hidroxilo, del 18,25% al 21,18%; y, en el caso de la actividad antioxidante en el sistema lipídico del hígado, del 7,90% al 8,38%. Dichas inhibiciones son mayores que, por ejemplo, las de la manzana que es la especie de frutas pomáceas más importante del mundo. Conclusión. El fruto del guillomo podría aportar un complemento apropiado para la nutrición humana moderna. Nuestros trabajos contribuyen asimismo a generalizar esta especie pomácea, insistiendo en su potencial en cuanto s sus elevadas propiedades antioxidantes.

República Checa / Amelanchier alnifolia / frutas / antioxidantes / contenido fenólico / flavonoides / especies de oxígeno reactivo