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ANTIOXIDANT CAPACITY, SCAVENGING RADICAL ACTIVITY AND SELECTED CHEMICAL COMPOSITION OF NATIVE APPLE CULTIVARS FROM CENTRAL EUROPE

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ABSTRACT

The main aim of this study was to focus on 10 typical native apple cultivars from Central Europe and to determine the basic characteristics such as dry matter, soluble solid content, titrable acidity, the content of pectins, phenolics, ascorbic acid, antioxidant capacity and the scavenging effect of 10% apple fruit extracts on hydroxyl radical, nitric oxide and superoxide radical in them. In our experiments, it was found out that the highest content of organic acids was shown by the "Jeptiska" cultivar (5.40 g/kg of FM). As far as the content of pectins was concerned, the highest levels were found out in the "Strymka" cultivar (32.60 g/kg FM). In case of total phenolic content, antioxidant capacity, ascorbic acid content and scavenging activity of reactive oxygen species (hydroxyl radical, nitric oxide and superoxide anion), high efficiency was determined in the local cultivars, namely, in "Matcino," "Panenske ceske" and "Strymka."

PRACTICAL APPLICATIONS

The results shown have wide use in the alimentary industry and human nutrition as apples belong to the most widespread core fruit. There exist many apple cultivars that have not been described in detail in literature and this study provides an insight into 10 not very common cultivars in the world; and although in Central Europe these cultivars are cultivated successfully, so far they have not been utilized commercially. However, their genetic uniqueness represents an irreplaceable ecological wealth and for that reason these local cultivars could become a new and outstanding source of nutrients and food. Today, they can also be used as a potential material for further breeding and selection.

INTRODUCTION

In Central Europe, apple growing has a long tradition and we can find tens of different local cultivars there. Because of their nutritional properties, apples are rather valued as fruit species (Gallus *et al*. 2005); so, e.g., in human nutrition some authors recommend them as a preventive tool against the occurrence of diseases (Kader 2008) or as a dietetic source of food (Kovacs and Meresz 2004). Thanks to the content of polyphenolic compounds, the consumption of apples is recommended to prevent cardiovascular or oncogenic diseases (Wojdylo *et al*. 2008). As compared with some other core fruit species (e.g., pears), apples contain less energy (Kopec 1997) and show a high content of minerals, pectins and vitamin C (Kyzlink 1990).

In association with the process of intensification of agricultural production, the last century was characterized by extensive growing and breeding of only a few selected and

nowadays commercially successful apple cultivars. Tens of others are only on the margin of general interest, and their occurrence is often confined to the regions of their original growing (Goland and Bauer 2004).

However, the genetic diversity of apple cultivars represents a unique, irreplaceable and invaluable natural resource (Biedrzycka and Amarowicz 2008). Just because of the uniqueness of their genetic base and its relationship to nutritional properties, some nearly forgotten and sometimes only locally grown cultivars should be brought to a wider market (Iwane 1991; Toth *et al.* 2004). Moreover, many of these older cultivars show a significant tolerance to pathogens and pests, a high adaptability to less favorable climatic and soil conditions, and a good resistance to stress (Tetera 2003).

The main objective of this paper is to nutritionally characterize 10 local apple cultivars typical of Central Europe. During the process of landscape cultivation and care, many of the recommended apple cultivars were spread in Central Europe in the past. This process was also accompanied by the selection of local apple cultivars. Many of the cultivars mentioned in this study were not described in detail in the past, and their description, as performed in this study, is really unique, especially as far as their chemical composition is concerned. The cultivars addressed in this study could partly find a wider application in human nutrition, food engineering and they could also be used in the breeding work and selection.

MATERIALS AND METHODS

Sample Collection and the Preparation for Chemical Analyses

Fruits were harvested in experimental orchards of Tomas Bata University in Zlin within the period of 2007–2009. These orchards are situated in the southwestern part of the White Carpathians near Zlin, the Czech Republic. The average altitude is 340 m above sea level, and the mean annual temperature and precipitation are 7.9C and 760 mm, respectively. The soil type was classified as the mesotrophic cambisol (Anon 2007).

Every year, apples were harvested from five trees of each cultivar under study in the stage of harvest ripeness. Each of these samples involved three replications (three fruits) from each tree (i.e., altogether 15). The age of experimental trees ranged from 12 to 15 years. The results were expressed as the average of a 3-year experiment. The samples were stored in a controlled environment at the temperature of +2C and under conditions of 85% of relative humidity (Kyzlink 1990). Chemical analyses were performed in the stage of consume ripeness specific for each cultivar (Tetera 2006). For analyses, all fruits without core were used, i.e., peel and flesh together. The average samples were obtained by dividing into quarters after a thorough homogenization in a laboratory grinder SJ500 (MEZOS, Hradec Kralove, Czech Republic).

The samples were harvested in the area of Valasske Klobouky, the White Carpathians, and, in the past, the White Carpathians were declared as a protected landscape area and the cultivars described are quite typical of that region (Tetera 2003). Ten native apple cultivars were used: "Albrechtovo," "Bernske ruzove," "Hvezdnata reneta," "Jadernicka moravska," "Jeptiska," "Kratkostopka kralovska," "Lebelovo," "Matcino," "Panenske ceske" and "Strymka" (see the characteristics in Table 1). For comparison, two cultivars grown worldwide, "Spartan" and "Starkrimson" (see Table 1), were used from the same locality (Kutina 1991).

Chemical Analyses

The dry matter content was measured after drying off to a constant weight at the standard temperature of 105C ± 2C – the apparatus VENTICELL 111 (BMT, Brno, Czech Republic). The soluble solid content (SSC) was determined by means of polarimetric measurements in juice obtained after

TABLE 1. DESCRIPTION OF THE ATTRIBUTES OF APPLE CULTIVARS USED

Cultivar	Autumn/winter	Shape	Size	Color
Albrechtovo	Autumn	Flat globular	Medium	Red striped with yellow
Bernske ruzove	Autumn	Oval	Smaller	Red
Hvezdnata reneta	Winter	Flat globular	Smaller	Red
Jadernicka moravska	Winter	Oval	Smaller	Yellow with small areas of red blushing
Jeptiska	Winter	Conical	Medium	Deep red
Kratkostopka kralovska	Winter	Round	Smaller	Yellow with small areas of red blushing
Lebelovo	Autumn	Flat globular	Smaller	Yellow
Matcino	Autumn	Conical	Medium to large	Red
Panenske ceske	Winter	Conical	Smaller	Red
Spartan	Winter	Globular	Medium	Red
Starkrimson	Winter	Conical	Medium to large	Deep red
Strymka	Winter	Oval	Medium	Red mottled over a yellow background

squeezing the fruit and the results were expressed as % Brix. For the measurement of SSC, a digital instrument HI 96801 (Hanna Instruments, Woonsocket, RI) was used. The content of total acids was measured by potentiometric titration; 20 g of the homogenized sample was extracted for 30 min in a shaker in 200 mL of redistilled water at the temperature of 80C. The obtained extract was filtered and titrated with sodium hydroxide to the pH value of 8.1 by using the apparatus pH 211 (Hanna Instruments). The obtained result was converted to the content of acids (expressed as malic acid) in grams per kilogram of FM (Novotny 2000). The method described for the measurement of titrable acidity and the units used in our work are taken as the only exact and comparative method for core fruit (The methodology of the Ministry of Agriculture of the Czech Republic), and it was used as the cultivars originating from this country were compared. Of course, there exist much quicker methods in which, e.g., a direct titration of fresh juice with sodium hydroxide (Vendramini and Trugo 2000) is used.

Pectin Content Assay

The content of pectins was measured by means of a modified method described by Rop *et al.* (2008). The fruit sample (10 g) was extracted at the temperature of 80C in a shaker with hydrochloric acid $c = 1$ mol/dm for a period of 90 min. The obtained hydrolyzate was quantitatively transferred into a 250-mL volumetric flask and refilled to the volume with water. Pectins were thereafter measured photometrically as a colored complex consisting of the product of thermal decomposition of galacturonic acid with *m*-hydroxybiphenyl in concentrated H_2SO_4. The samples of 5 mL were gradually taken off and put into 50-mL flasks; thereafter, they were mixed with 6 mL of sodium tetraborate ($c = 0.013$ mol/dm) dissolved in concentrated sulphuric acid, filled up to the volume with distilled water and boiled for 5 min. Boiled samples were let to stand for 20 min and thereafter they were measured (at 520 nm) together with the standards in the apparatus LIBRA S6 (Biochrom Ltd, Cambridge, U.K.). The content of pectins was expressed in g/kg FM.

Determination of Ascorbic Acid

The determination of ascorbic acid content was carried out according to a modified method by Miki (1981). Five grams of the sample was weighed in an Erlenmeyer flask by adding 25 mL of extractant methanol: $H_2O : H_3PO_4$ in the ratio 99:0.5:0.5. The flask with the samples was placed into a water bath with a temperature of 25C, where the samples were extracted for 15 min. To keep out the samples of daylight, the flask was covered with aluminum foil during the preparation. After the extraction the content of the flask was filtrated through paper Filtrapak no. 390 (Munktell, Bärenstein, Germany). The filtrate prepared in this way before injection was diluted in ration of extractant and filtrated again through a membrane filter Nylon-0.45-μm Nylon filter disk (Labicom, Olomouc, Czech Republic). The instrument used for ascorbic acid analysis consisted of a solvent delivery pump (Model 582, ESA Inc., Chelmsford, MA), a guard cell (Model 5010A, with a working electrode potential K1 = 600 mV, K2 = 650 mV, ESA Inc.), a chromatographic column – Model Supelcosil LC8 (150.0 × 4.6 mm), 5-μm particle size and an electrochemical detector Coulochem III (ESA Inc.). The chromatographic conditions were constant: 30C, as a mobile phase was used methanol: $H_2O : H_3PO_4 = 99:0.5:0.5$ (filtrated through a filter Nylon, 0.2 μm); the type of elution was isocratic; the flow rate of the mobile phase was 1.1 mL/min; the retention time 1.9–2.0 min. The content of ascorbic acid was calculated as mg/100 g of FM.

Total Phenolic Content and Antioxidant Capacity Assay

The extraction was performed according to the method described by Kim *et al.* (2003), using 10 g of the fresh sample, which was homogenized for 10 s in an extraction mixture hydrochloric acid: methanol : water in the ratio 2:80:18. For the measurement of total phenolic content (TPC), Folin–Ciocalteau reagent was used. The resulting absorbance was measured in the spectrophotometer LIBRA S6 (Biochrom Ltd.) at a wavelength of 765 nm against a blind sample, which was used as reference. The results were expressed as grams of gallic acid (GAE) per kilogram of fresh mass.

Antioxidant capacity was measured using the method described by Sulc *et al.* (2007). This test is based on the monitoring of the course of inactivation of the cation $ABTS^+$, which is produced during the oxidation of 2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonate). $ABTS^+$ shows a strong absorbance in the visible region of the electromagnetic spectrum (600–750 nm); this solution is green and its antioxidant capacity can be measured easily by means of spectrophotometry. This method is standardly used when measuring antioxidant capacity and its great advantage consists in reproducibility and a good fit with other methods also used for measuring antioxidant capacity (Thaipong *et al.* 2006).

Altogether, 54.9 mg of ABTS was dissolved in 20 mL of a phosphate buffer (pH 7.0; 5 mM) and activated on cation radical of $ABTS^+$ by means of an addition of 1 g of MnO_2^+; the resulting solution was intermittently stirred for an activation period of 30 min. Thereafter, the solution was centrifuged for 5 min and at 7,000 rpm and filtered through a syringe filter (0.25 μm), and 2 mL of the filtrate was diluted with the phosphate buffer to the absorbance (t_0) of 0.500 ± 0.01, which was measured at a wavelength of 734 nm. After the absorbance measured in time t_0, 0.5 mL of the sample was added and the new absorbance value was measured in time t_{20}, i.e., after

20 min – using the apparatus LIBRA S6 (Biochrom Ltd.). Antioxidant capacity was calculated as a decrease in the absorbance value using the formula:

$$(\%) = 100 - [(A_{t20}/A_{t0}) \times 100]$$

The calculated capacity was converted using a calibration curve of the standard and expressed in ascorbic acid equivalents (AAE) (Rupasinghe *et al.* 2006).

Scavenging Activity of Reactive Oxygen Species Assay

To support the results of antioxidant capacity values, scavenging activity of reactive oxygen species (ROS) (superoxide anion, hydroxyl radical and nitric oxide) was determined. For this purpose, the extract was diluted with the phosphate buffer ($c = 50$ mmol/dm, pH 7.0) and prepared as a 10% solution of the primary extract (Beissenhirtz *et al.* 2004). The hydroxyl radical scavenging activity was assayed according to the method of Ghiselli *et al.* (1998). One milliliter of the extract was mixed with 0.8 mL of a reaction buffer (KH_2PO_4.KOH, $c = 0.2$ mol/dm, pH 7.4; deoxyribose, $c = 1.75$ µmol/dm; iron ammonium sulphate, $c = 0.1$ µmol/dm; and ethylenediaminetetraacetic acid, $c = 0.1$ µmol/dm). H_2O_2 of 0.1 mL ($c = 0.01$ mol/dm) was then added to the reaction solution. The solution was incubated for 10 min at 37C 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.

The assay of nitric oxide scavenging activity was done by the method described by Green *et al.* (1982). One milliliter of the extract was mixed with 1 mL of the reaction solution containing sodium nitroprusside ($c = 10$ mmol/dm) in the phosphate buffer ($c = 50$ mmol/dm, pH 7.0). The incubation at 37C for 1 h followed and 0.5 mL of aliquot was then mixed with 0.5 mL of the Griess reagent. The absorbance was measured at 540 nm.

The superoxide anion scavenging activity was done according to the method described by Beissenhirtz *et al.* (2004), and it is based on the reduction of cytochrome *c*. One milliliter of the extract was mixed with 1 mL of the solution containing 0.07 units per mL of xanthine oxidase, xanthine ($c = 100$ µmol/dm) and cytochrome *c* ($c = 50$ µmol/dm). After the incubation at 20C for 3 min, the absorbance at 550 nm was determined.

All tests were performed in triplicate. The scavenging activities of hydroxyl radical, nitric oxide and superoxide anion were calculated as follows:

$$\text{Scavenging activity}\,(\%) = (A_0 - A_1/A_0) \times 100\% \qquad (1)$$

where A_0 is the absorbance of the control (without the sample) and A_1 is the absorbance of the mixture containing the sample. All of the absorbances mentioned above were measured in the spectrophotometer LIBRA S6 (Biochrom Ltd.).

Statistical Evaluation

The data obtained were analyzed statistically by the analysis of variance and Tukey's multiple range test for comparison of means at $P < 0.05$ (Snedecor and Cochran 1967). Correlation functions were calculated using the statistical package Unistat, v. 5.1 (Unistat Ltd., London, U.K.) and Office Excel Microsoft.

RESULTS

The "Jadernicka moravska" cultivar showed the highest content of dry matter (17.57%). More than 16% of dry matter was also recorded in the "Strymka" and "Hvezdnata reneta" cultivars. The "Strymka" cultivar also showed a high content of soluble solid content (15.98% Brix). In the "Panenske ceske" cultivar this value was 14.86% Brix. More than 14% Brix of soluble solid content were also observed in the "Matcino" and "Starkrimson" cultivars (Table 2).

The titration acidity and the content of pectin substances were other important parameters determined in altogether 12 samples of cultivars under study. In this case, there were considerable differences among individual cultivars. As far as the total content of acids was concerned, the measured values ranged from 1.50 g/kg FM ("Jadernicka moravska") to 5.40 g/kg FM ("Jeptiska"). The differences were also noticed in the contents of pectins. The lowest values were found in the "Jeptiska" cultivar (11.55 g/kg FM), while the highest one was in the "Strymka" cultivar (32.60 g/kg FM).

Regarding TPC, the highest content was in the "Strymka" cultivar, which contained 3.29 g of gallic acid/kg FM (Table 3). High contents of TPC were also found in the "Matcino" or "Panenske ceske" cultivars. Furthermore, in these cultivars there were high contents of ascorbic acid and high values of total antioxidant capacity (TAC). So, e.g., a high content of ascorbic acid was found in the cultivar "Matcino" (152.64 mg/kg FM). The highest values of TAC (ranging from 2.70 to 2.91 AAE/kg FM) were observed in the "Panenske ceske," "Matcino" and "Strymka" cultivars. When estimating the correlations existing between TPC and TAC of all the cultivars, the calculated correlation coefficient was $r^2 = 0.9928$; $y = 0.9624x - 0.1725$. In addition, an interesting correlation coefficient was found between TAC and ascorbic acid ($r^2 = 0.9623$; $y = 37.41x + 41.023$).

In addition, ROS scavenging activity was measured in the cultivars. The obtained results were the highest in the "Panenske ceske," "Matcino" and "Strymka" cultivars and were statistically significant in comparison with other cultivars

TABLE 2. DRY MATTER CONTENT (% w/w), SOLUBLE SOLID CONTENT (% BRIX), TITRABLE ACIDITY (g/kg FM) AND PECTIN CONTENT (g/kg FM) OF FRUIT OF DIFFERENT APPLE CULTIVARS

Cultivar	Dry matter	SSC	Titrable acidity	Pectins
Albrechtovo	11.14 ± 0.37^{e}	13.50 ± 0.26^{c}	3.80 ± 0.34^{b}	20.17 ± 2.11^{c}
Bernske ruzove	11.23 ± 0.43^{e}	11.23 ± 0.38^{d}	2.64 ± 0.41^{c}	21.90 ± 2.34^{bc}
Hvezdnata reneta	16.35 ± 0.51^{b}	11.60 ± 0.41^{d}	3.60 ± 0.42^{b}	13.33 ± 2.41^{d}
Jadernicka moravska	17.57 ± 0.60^{a}	13.30 ± 0.40^{c}	1.50 ± 0.38^{d}	28.41 ± 2.09^{ab}
Jeptiska	12.37 ± 0.32^{de}	11.80 ± 0.37^{d}	5.40 ± 0.36^{a}	11.55 ± 2.52^{d}
Kratkostopka kralovska	11.84 ± 0.49^{e}	11.95 ± 0.44^{d}	3.20 ± 0.40^{bc}	28.04 ± 2.85^{ab}
Lebelovo	12.97 ± 0.39^{d}	11.60 ± 0.31^{d}	4.90 ± 0.52^{a}	15.92 ± 1.91^{d}
Matcino	14.81 ± 0.39^{c}	14.21 ± 0.39^{b}	1.60 ± 0.29^{d}	14.80 ± 2.53^{d}
Panenske ceske	11.74 ± 0.53^{e}	14.86 ± 0.47^{b}	3.30 ± 0.34^{bc}	11.81 ± 2.12^{d}
Spartan	13.95 ± 0.67^{cd}	13.90 ± 0.38^{bc}	2.28 ± 0.39^{cd}	24.05 ± 2.55^{b}
Starkrimson	15.05 ± 0.38^{c}	14.01 ± 0.41^{bc}	1.70 ± 0.44^{d}	15.21 ± 2.87^{d}
Strymka	16.58 ± 0.61^{b}	15.98 ± 0.43^{a}	1.80 ± 0.31^{d}	32.60 ± 2.90^{a}

Different superscripts in each column indicate the significant differences in the mean at $P < 0.05$, $n = 45$.

(Table 4). The inhibition of hydroxyl radical was 17.19, 17.51 and 18.12%. In case of nitric oxide, these values were 21.36, 20.85 and 20.94% and concerning superoxide anion 25.03, 24.80 and 24.99%, respectively. As far as the correlation coefficients were concerned, they were found to be between TPC and hydroxyl radical $r^2 = 0.9478$; $y = 4.8497x + 1.6984$, in case of TPC and nitric oxide $r^2 = 0.9724$; $y = 5.3325 + 4.2449$ and regarding TPC and superoxide anion $r^2 = 0.9714$; $y = 4.7856 + 9.5637$.

DISCUSSION

Although it is necessary to consider some differences in the chemical composition resulting from the year of the harvest and influenced by the locality (Little and Taylor 1981), it can be said that local apple cultivars are well adapted to local climatic conditions (Melounova *et al*. 2004). It is also common knowledge that there are only slight differences in the contents of individual chemical compounds when comparing individual cultivars, and that they are determined by the genetic uniqueness of each of them (Goland and Bauer 2004).

As far as chemical parameters under study are concerned, the most important are the following: the soluble solid content, the content of acids and the content of pectins. In apples, the content of organic acids is represented mainly (90%) by malic acid (Kyzlink 1990). Among other organic acids it is possible to find malonic, shikimic and fumaric acids, some amino acids (with predominating aspartic acid) and polyphenolic chlorogenic acid (Stampar *et al*. 2002). So, for example, it was found, similarly as Suni *et al*. (2000) noticed, that the content of acids in the "Boskoopske" cultivar was low (1.9 g/kg FM). On the other hand, the "Jeptiska" cultivar contained 5.40 g/kg FM of organic acids.

Apples are important above all due to their content of pectins, which predetermine them for the processing to fruit spreads thanks to their capability of gelification in presence of saccharose under the conditions of low pH (Kyzlink 1990). Regarding other core fruit species, a high content of pectins can be found, for example, in quinces (Baker 1997), which may contain as much as 30 g/kg FM of pectins in FM (Kovacikova *et al*. 1997). In apples, however, the average content of pectins is about 11 g/kg FM (Kopec 1997). In our

TABLE 3. TOTAL PHENOLIC CONTENT (GRAMS OF GALLIC ACID/kg FM), ANTIOXIDANT CAPACITY (GRAMS OF ASCORBIC ACID/kg FM) AND ASCORBIC ACID CONTENT (mg/kg FM) OF FRUIT OF DIFFERENT APPLE CULTIVARS

Cultivar	TPC	TAC	Ascorbic acid
Albrechtovo	2.45 ± 0.27^{b}	2.17 ± 0.21^{b}	121.34 ± 5.83^{b}
Bernske ruzove	2.37 ± 0.18^{b}	2.20 ± 0.20^{b}	116.09 ± 6.11^{b}
Hvezdnata reneta	2.39 ± 0.23^{b}	2.15 ± 0.17^{b}	115.27 ± 5.02^{b}
Jadernicka moravska	1.56 ± 0.20^{c}	1.33 ± 0.17^{c}	91.41 ± 7.14^{c}
Jeptiska	2.41 ± 0.28^{b}	2.24 ± 0.22^{b}	117.84 ± 8.50^{b}
Kratkostopka kralovska	1.66 ± 0.25^{c}	1.45 ± 0.20^{c}	94.25 ± 4.94^{c}
Lebelovo	1.60 ± 0.26^{c}	1.32 ± 0.18^{c}	93.18 ± 4.89^{c}
Matcino	3.12 ± 0.20^{a}	2.86 ± 0.23^{a}	152.64 ± 5.27^{a}
Panenske ceske	3.03 ± 0.18^{a}	2.70 ± 0.25^{a}	148.25 ± 6.18^{a}
Spartan	1.55 ± 0.21^{c}	1.29 ± 0.20^{c}	88.91 ± 4.28^{c}
Starkrimson	1.46 ± 0.20^{c}	1.19 ± 0.17^{c}	91.53 ± 4.06^{c}
Strymka	3.29 ± 0.23^{a}	2.91 ± 0.18^{a}	152.31 ± 6.29^{a}

Different superscripts in each column indicate the significant differences in the mean at $P < 0.05$, $n = 45$.

TABLE 4. SCAVENGING EFFECT OF APPLE FRUIT EXTRACT (10%) ON HYDROXYL RADICAL (PERCENTAGE OF INHIBITION), NITRIC OXIDE (PERCENTAGE OF INHIBITION) AND SUPEROXIDE ANION (PERCENTAGE OF INHIBITION)

Cultivar	Hydroxyl radical	Nitric oxide	Superoxide anion
Albrechtovo	12.44 ± 0.85[b]	17.31 ± 1.42[b]	20.25 ± 1.11[b]
Bernske ruzove	12.50 ± 0.96[b]	16.99 ± 1.38[b]	21.11 ± 0.94[b]
Hvezdnata reneta	12.32 ± 0.81[b]	16.85 ± 1.12[b]	20.98 ± 1.04[b]
Jadernicka moravska	9.16 ± 1.12[c]	12.03 ± 1.31[c]	17.42 ± 1.73[c]
Jeptiska	12.79 ± 0.90[b]	17.45 ± 1.25[b]	20.55 ± 1.26[b]
Kratkostopka kralovska	9.50 ± 1.02[c]	11.90 ± 1.04[c]	16.98 ± 0.80[c]
Lebelovo	9.47 ± 1.16[c]	12.78 ± 1.15[c]	16.90 ± 0.84[c]
Matcino	17.19 ± 0.74[a]	21.36 ± 1.38[a]	25.03 ± 1.01[a]
Panenske ceske	17.51 ± 0.68[a]	20.85 ± 1.54[a]	24.80 ± 1.20[a]
Spartan	10.11 ± 0.95[c]	13.02 ± 1.22[c]	17.34 ± 0.98[c]
Starkrimson	9.68 ± 1.10[c]	12.85 ± 1.31[c]	17.10 ± 1.25[c]
Strymka	18.12 ± 1.31[a]	20.94 ± 1.40[a]	24.99 ± 1.14[a]

Different superscripts in each column indicate the significant differences in the mean at $P < 0.05$, $n = 45$.

experiments, this value was found in the cultivars with the lowest contents of pectin compounds (Table 2). The "Strymka" cultivar, which contained as much as 32 g of pectins/kg FM, was an interesting exception. This cultivar is frequently used in the region of the White Carpathians (Tetera 2006). However, this is not an extreme value because Bailoni *et al.* (2005) mentioned that some local cultivars may contain as much as 50 g of pectins/kg FM.

In our measurements, the highest values of TPC and TAC were determined in local cultivars. Drogoudi *et al.* (2008) emphasized high values of antioxidant capacity of local apple cultivars, which – as compared with commercial cultivars – contained considerable amounts of phenolic substances. Moreover, it was found out that there were high correlations between antioxidant capacity and the content of phenolic substances (Gil *et al.* 2002). It was also reported that the correlation between antioxidant capacity and the content of ascorbic acid was high (Schmitz-Eiberger *et al.* 2003). So, e.g., for apples Thaipong *et al.* (2006) mentioned correlation coefficients ranging from $r^2 = 0.81$ to 0.97. In our experiments, the value of correlation coefficient was $r^2 = 0.9928$. In our measurement, there was also considerable variability of the vitamin C contents (from 88.91 to 152.64 mg/kg FM) in individual cultivars, which may be considered typical of apple trees (Tetera 2006). Compared with other core fruit species, the contents of ascorbic acid are high in apples (Sanchez *et al.* 2003); nevertheless, e.g., the higher contents are described in quinces (Kopec 1997).

High scavenging activity was observed in case of hydroxyl radical, nitric oxide and superoxide anion using the extract of some local cultivars (see Table 4). For example, a 10% extract of the "Strymka" cultivar caused the percentage inhibition of hydroxyl radical by 18.12%, nitric oxide by 20.94% and superoxide anion by 24.99%. The nitric oxide radical has been implicated in pathogenesis of several diseases similar as other kinds of ROS including free radicals such as superoxide anion (O_2^-) and hydroxyl radical species (OH•) (Wang *et al.* 2009). Apple fruit extract has scavenging property against ROS (Maffei *et al.* 2007). Similar or even higher activity was noticed in some other fruit species, e.g., mulberry (Bae and Suh 2007) or fruits of *Prunus* species (Jung *et al.* 2002; Rop *et al.* 2009).

TPC and TAC influence the quality of apples and their products in appearance, flavor and nutritional properties (Zardo *et al.* 2009). Antioxidant composition of fruits varies among cultivars and genetics plays a significant role. Furthermore, the phytochemical content of the fruits not only increases the quality of the fruit, but it also has a major impact on shelf life and susceptibility to diseases (Khanizadeh *et al.* 2007). The work by, e.g., Glevitzky *et al.* (2008) draws attention to this fact when higher values of TAC act advantageously on higher shelf life of the fruit. Although there is a decrease in the TPC and TAC values during storage, initial high values of TPC as well as TAC have an influence on good shelf life of fruit (Matthes and Schmitz-Eiberger 2009). Muthuswamy and Rupasinghe (2007) notice a positive relationship between higher values of TAC and resistance of apple fruit to fungal diseases during storage. The native cultivars of apples not only show resistance to a disease with good shelf life, but also stimulate greater interest in the nutraceutical aspects of these fruits as well as in the processing (Tetera 2006).

CONCLUSIONS

All cultivars were characterized by the variability in the content of basic chemical components. From the technological and nutritional points of view, some of them (e.g., pectins) are rather important and their highest content was found in the "Strymka" cultivar. Thanks to their antioxidant capacity, local cultivars can be used in the prevention of many diseases. The highest antioxidant capacity was in "Strymka," "Matcino" and "Panenske ceske" cultivars. This fact was confirmed by using the extract of these cultivars and its effectivity on radical oxygen species scavenging activity. Many of the culti-

vars described in this study are not well known; practically, they have not been described in literature and the research results given here are unique, especially as far as their chemical composition is concerned.

NOMENCLATURE

FM	fresh mass
OH•	hydroxyl radical species
ROS	reactive oxygen species
SSC	soluble solid content
O_2^-	superoxide anion
TAC	total antioxidant capacity
TPC	total phenolic content
$ABTS^+$	2,2′-azinobis-3-ethylbenzothiazoline-6-sulphonate

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