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Qualities of Native Apple Cultivar Juices Characteristic of Central Europe

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Abstract

During the last century due to the changes in landscape management, in country people's lives and with intensive fruit-growing the native cultivars of apple fruit trees have been gradually disappearing. The aim of the study was to compare the juices made of native apple cider cultivars. The cultivars-'Boikovo', 'Jadernicka moravska', 'Kardinal zihany', 'Panenske ceske', 'Parmena zlata zimni', 'Strymka' growing in the locality of the Litencicke Hills in the eastern part of the Czech Republic were selected. For comparison, the fruits of commercial 'Idared' cultivar were also included. The chemical composition, antioxidant capacity, phenols, flavonoids and ascorbic acid content and the influence of juices on scavenging activity of nitric oxide and hydroxyl radical were measured. The mutual ratio of examined properties which were found, provide information about qualities and possibilities of use of native cultivars. High antioxidant properties characterize the juice of native apple cultivars. In particular, the 'Strymka' cultivar contained 2,637.34 mg of AAE (ascorbic acid equivalent) per litre in case of antioxidant capacity. In the juice of this cultivar the value of 144.05 mg of ascorbic acid per litre was recorded. As regards the 'Panenske ceske' cultivar, antioxidant capacity was 2,548.38 mg of AAE l^{-1} and in relation to ascorbic acid, the value was 145.35 mg l^{-1}. Similarly, high values were observed in both cultivars concerning the scavenging effect of apple juices on hydroxyl radical and nitric oxide (the 'Strymka' cultivar 16.38% and 19.26%, the 'Panenske ceske' cultivar 16.31% and 18.60%).

Keywords: antioxidant properties, native cider cultivars, phenolics, reactive oxygen species

Introduction

For many years the native cultivars of apple fruit trees have been important sources of living as well as a source of financial income. However, interest in their use has recently considerably dropped. Many works bring knowledge about the necessity of their conservation (Rop *et al.,* 2011; Tetera, 2003; Wojdylo *et al.,* 2008). Native apple cultivars have been adapting to local conditions for centuries and thus they are equipped with specific genofond (Tetera, 2006). Genetic uniqueness of fruits of native apple cultivars represents an irreplaceable ecological wealth, for that reason these native cultivars could become a good and outstanding source of nutrients. Today, they can be used not only for direct consumption or in food industry, but also as a potential material for further breeding and selection (Hricovsky *et al.,* 2003). Moreover, they can be used for extensive growing in places not suitable for intensive agricultural production. As parts of orchards, alleys, windbreaks, green spots as well as solitary plants they are impressive landscape elements and important parts of landscape ecosystems. With their disappearance the cultural heritage of the predecessors might be lost (Tetera, 2003).

Apple juice contains all biologically valuable substances from fruits (Karaman *et al.,* 2010) although it is without insoluble fruit matter (Kyzlink, 1990). The chemical composition of apple juice enables direct consumption without dilutions or sugar added. The quality and a ratio of matters contained depend from the cultivar, environmental conditions and the grade of maturity of fruit (Rop and Hrabe, 2009).

From this point of view, apple cider cultivars are very valuable. They have a considerable amount of organic acids. Owing to the content of polyphenolic compounds and high antioxidant capacity the consumption of apple juice is recommended to prevent cardiovascular or oncogenic diseases (Wojdylo *et al.,* 2008) or as a dietetic source of food (Kader, 2008).

The aim of the study was to monitor selected technological (necessary for food industry) and antioxidant properties of apple juices from 7 typical Central European native apple cultivars used as apple cider cultivars. In juic-

es, the soluble solid content, titrable acidity, antioxidant capacity, the phenols, flavonoids, ascorbic acid content and the influence of juices on scavenging activity of nitric oxide and hydroxyl radical were evaluated. The juices of seven native apple cultivars mentioned in this study has not been described before thus the present study is really unique, especially as far as their chemical composition is concerned.

Materials and methods

Sample collection and juice processing

Fruit were harvested in the area of the Litencicke Hills (49° 17' N; 17° 23' E) near the town Kromeriz, the Czech Republic. The average altitude is 293 m above sea level, and the mean annual temperature and precipitation are 7.5°C and 703 mm, respectively. The soil type was classified as the Mesotrophic Cambisol (Anonymous, 2007).

Fruit were harvested within the period of 2010-2011. Every year apples were collected from five trees of each cultivar studied in the stage of harvest ripeness. Twenty randomly chosen fruit from each cultivar were mixed together and used for analyses (i.e. altogether 100 per each cultivar). The age of experimental trees ranged from 12 to 15 years. The samples were stored in a controlled environment at the temperature of +2°C and under conditions of 85% of relative humidity (Kyzlink, 1990). For processing, the fruits were taken within the period of consume ripeness. This stage is specific for each cultivar, it is described accurately for each cultivar (Tetera, 2006) and it depends entirely on apple harvest time. The juice from raw fruits was obtained by crushing and pressing the fruits by the fruit press machine Voran 100 P2 (Voran Maschinen GmbH., Pichl, Austria). Neither enzymatic treatment nor centrifugal clarification were applied to raw fruit which are common applied in case of native apple cultivars. The evaluation of particular cultivars was performed in five replications (n=10) per year.

Seven native cider apple cultivars were used-'Boikovo', 'Jadernicka moravska', 'Kardinal zihany', 'Panenske ceske', 'Parmena zlata zimni' and 'Strymka'. Furthermore, for comparison the juice of the worldwide 'Idared' cider cultivar, commercially grown in that region was analysed for comparison (Kutina, 1991). The characteristics of particular cultivars are given in Tab. 1.

Chemical analysis

The soluble solid content (SSC) was determined by using of polarimetric measurement in juice and the results were expressed as % Brix. For the measurement of SSC a digital instrument HI 96801 (Hanna Instruments, Woonsocket, RI, USA) was used. The total acid content was ascertained according to Vendramini and Trugo (2000), applying the direct titration of fresh juice with sodium hydroxide in order to reach the pH value of 8.1 using the digital potentiometer pH211 (Hanna Instruments, Woonsocket, RI, USA)-first, titrable acidity was determined and then the results obtained were converted to the content of acids (expressed as malic acid) in g l^{-1} (Novotny, 2000).

Total phenolic content assay

To measure total contents of phenolic (TPC) substances Folin-Ciocalteau reagent was used. Five hundred µl of the juice was taken and mixed with water in a 50-ml volumetric flask. Thereafter, 2.5 ml of Folin-Ciocalteau reagent and 7.5 ml of 20% solution of Na_2CO_3 were added. The resulting absorbance was measured in the spectrophotometer LIBRA S6 (Biochrom Ltd., Cambridge, UK) at the wavelength of 765 nm a blank of water was used as reference (Thaipong *et al.,* 2006). The results were expressed as grams of gallic acid (GAE) per kg of fresh mass (FM).

Total flavonoid content assay

The total flavonoid content (TFC) was determined using three hundred milliliters of juice were mixed with 3.4 ml of 30% ethanol, 0.15 ml of $NaNO_2$ (c = 0.5 mol

Tab. 1. The characteristics of particular cultivars used

Cultivar	Taste	Size	Colour of flesh	Autumn/ winter
'Boikovo'	slightly sour, good, juicy	large	greenish yellow, with red flush	winter
'Jadernicka moravska'	sweetly sourish, spicy, juicy	small to medium	yellow with small areas of red blushing	autumn
'Kardinal zihany'	slightly sour, juicy	large	yellow white, striped with red, mottled	autumn
'Panenske ceske'	sweetly sourish, spicy, slightly juicy	small	carmine red	autumn
'Parmena zlata zimni'	sweetly sourish, juicy	medium	golden-yellow, with orange flush and striped	autumn
'Strymka'	sweetly sour, very juicy	small to medium	red mottled over a yellow background	winter
'Idared'	sweetly sourish, juicy	medium to large	yellow-green with tinted rosy pink	winter

dm^{-3}) and 0.15 ml of $AlCl_3.6H_2O$ (c = 0.3 mol dm^{-3}) as described by Park *et al.* (2008). The mixture was measured at the wavelength of 506 nm-the spectrophotometer LIBRA S6 (Biochrom Ltd., Cambridge, UK). Total flavonoid content was calculated from a calibration curve using rutin as standard. The results were expressed in mg kg^{-1} of FM.

Total antioxidant capacity assay

For the determination of total antioxidant capacity (TAC) the DPPH (1,1-diphenyl-2 picrylhydrazyl) assay was used according to the work of Brand-Williams *et al.* (1995). The stock solution was prepared by dissolving 24 mg of DPPH with 100 ml of methanol and then stored at -20°C until needed. The absorbance of the DPPH radical without juice, i.e. control was measured daily. 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 ± 0.02 units at 515 nm using the spectrophotometer LIBRA S6 (Biochrom Ltd., Cambridge, UK). The juice (150 µl) was allowed to react with 2,850 µl DPPH solution for 1 hour in the dark. Thereafter, the absorbance was taken at 515 nm. Antioxidant capacity was calculated as a decrease in the absorbance value using the formula:

Antioxidant capacity (%) = $(A_0 - A_1/A_0)$ x 100%

where A_0 is the absorbance of a blank (without the sample) and A_1 is the absorbance of the mixture containing the sample. The calculated antioxidant capacity was converted using a calibration curve of the standard and expressed in ascorbic acid equivalents (AAE) (Rupasinghe *et al.,* 2006).

Determination of ascorbic acid

The determination of ascorbic acid content was conducted according to a modified method by Miki (1981). Samples were kept in dark, the flask with juice was covered with aluminium foil during the preparation. The sample was filtrated through paper Filtrapak No. 390. The filtrate before injection was diluted in ration of extractant and filtrated again through a Nylon membrane filter (0.45 µm). The instrument used for ascorbic acid analysis consisted of a solvent delivery pump (Model 582, ESA Inc., Chelmsford, USA), a guard cell (Model 5010A, with a working electrode potential K1 = 600 mV, K2 = 650 mV, ESA Inc., Chelmsford, USA), a chromatographic column-Model Supelcosil LC8 (150.0 x 4.6 mm), 5 µm particle size and an electrochemical detector Coulochem III (ESA Inc., Chelmsford, USA). The chromatographic conditions were constant: 30°C, a mobile phase comprising 100% methanol: redistilled water: 85% H_3PO_4 (in the proportion of 99:0.5:0.5) was used (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^{-1}. The content of ascorbic acid was calculated as milligrams of ascorbic acid kg^{-1} of fresh mass.

Scavenging activity of hydroxyl radical and nitric oxide assay

To support the results of antioxidant capacity values, scavenging activity of hydroxyl radical and nitric oxide, which belong to reactive oxygen species (ROS), was ascertained. For this purpose, juice was mixed with the phosphate buffer (c = 50 mmol dm^{-3}, pH 7.0) and prepared as a 10% solution of the primary juice (Beissenhirtz *et al.,* 2004). The hydroxyl radical scavenging activity was assayed according to the method of Ghiselli *et al.* (1998) using 1 ml of the 10% juice which was mixed with 0.8 ml of a reaction buffer (KH_2PO_4 KOH, c = 0.2 mol dm^{-3}, pH 7.4; deoxyribose, c = 1.75 µmol dm^{-3}; iron ammonium sulphate, c = 0.1 µmol dm^{-3}; EDTA, c = 0.1 µmol dm^{-3} and H_2O_2, c = 0.01 mol dm^{-3}). 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 then cooled rapidly. The absorbance was measured at 532 nm.

The assay of nitric oxide scavenging activity was applied by the method described by Green *et al.* (1982). One milliliter of the 10% juice was mixed with 1 ml of the reaction solution containing sodium nitroprusside (c = 10 mmol dm^{-3}) in the phosphate buffer (c = 50 mmol dm^{-3}, pH 7.0) and Griess reagent was added. The absorbance was measured at 540 nm.

Statistical analysis

The data obtained were analyzed statistically by the analysis of variance (ANOVA) and Tukey's multiple range test was used for comparison of means (Snedecor and Cochran, 1968). The correlation functions were calculated using the statistical package Unistat, v. 6.0.

Results and discussion

Chemical analyses are listed in Tab. 2 to Tab. 5 and the values of correlation relationships in Tab. 6. The results were expressed as an average of a two-year experiment. There was not statistical significant difference among the years in any parameter investigated, which can be typical of apple juices (Rop and Hrabe, 2009; Stracke *et al.,* 2009), therefore, the values are expressed as two-year averages of particular measurements. In the 'Jadernicka moravska', 'Panenske ceske' and 'Strymka' cultivars also high contents of SSC (16.65 - 16.95% Brix) were measured. The average content of SSC in apple juices ranges from 9 to 18% Brix (Valois *et al.,* 2006). The values of titrable acidity were also standard. The 'Idared' cultivar is known for high contents of malic acid (Kutina, 1991)-there was 5.10 g of malic acid per litre in the measurements. In native cultivars lower contents were observed (Tab. 2) with the exception of the 'Strymka' cultivar (5.11 g of malic acid l^{-1}). The average titrable acidity in apple juices is between 0.52 and 5.61 g of malic acid per litre (Chinnici *et al.,* 2005). SSC and

Tab. 2. Soluble solid content (% Brix) and titrable acidity (expressed as grams of malic acid in one litre of the juice), n=10

Cultivar	Soluble solid content	Titrable acidity
'Boikovo'	15.38 ± 0.26 a	4.17 ± 0.10 a
'Jadernicka moravska'	16.95 ± 0.21 b	3.28 ± 0.12 b
'Kardinal zihany'	14.21 ± 0.28 c	3.38 ± 0.15 b
'Panenske ceske'	16.92 ± 0.30 b	3.25 ± 0.15 b
'Parmena zlata zimni'	14.38 ± 0.25 c	4.28 ± 0.14 a
'Strymka'	16.65 ± 0.34 b	5.11 ± 0.11 c
'Idared'	15.45 ± 0.29 a	5.10 ± 0.12 c

Different letters in each column indicate the significant differences (Tukey's test, P < 0.05)

Tab. 3. Total phenolic content (expressed as milligrams of gallic acid per litre) and total flavonoid content (expressed as milligrams of rutin per litre), n=10

Cultivar	Total phenolic content	Total flavonoid content
'Boikovo'	1,037.28 ± 117.25 a	479.34 ± 21.22 a
'Jadernicka moravska'	1,638.53 ± 108.60 b	892.62 ± 28.21 b
'Kardinal zihany'	1,659.41 ± 232.78 b	898.14 ± 31.05 b
'Panenske ceske'	1,731.50 ± 241.35 b	910.73 ± 27.85 b
'Parmena zlata zimni'	939.77 ± 72.18 a	311.29 ± 27.16 c
'Strymka'	1,789.29 ± 103.98 b	926.52 ± 22.32 b
'Idared'	569.09 ± 93.45 c	299.81 ± 19.65 d

Different letters in each column indicate the significant differences (Tukey's test, $p<0.05$)

Tab. 4. Total antioxidant capacity (expressed as milligrams of ascorbic acid per litre) and ascorbic acid content (milligrams of ascorbic acid per litre), n=10

Cultivar	Total antioxidant capacity	Ascorbic acid
'Boikovo'	1,985.44 ± 112.84 a	92.68 ± 2.73 a
'Jadernicka moravska'	2,541.38 ± 101.05 b	102.39 ± 3.82 b
'Kardinal zihany'	2,595.07 ± 95.45 b	105.11 ± 5.65 b
'Panenske ceske'	2,568.50 ± 106.28 b	145.35 ± 8.13 c
'Parmena zlata zimni'	1,287.26 ± 94.32 c	90.80 ± 4.37 a
'Strymka'	2,637.34 ± 109.90 b	144.05 ± 6.01 c
'Idared'	968.29 ± 92.56 d	101.62 ± 3.95 b

Different letters in each column indicate the significant differences (Tukey's test, $p<0.05$)

Tab. 5. Scavenging effect of apple juices on hydroxyl radical (percentage of inhibition) and nitric oxide (percentage of inhibition), n = 10

Cultivar	Hydroxyl radical	Nitric oxide
'Boikovo'	13.85 ± 0.12 a	15.11 ± 0.14 a
'Jadernicka moravska'	16.21 ± 0.10 b	18.55 ± 0.15 b
'Kardinal zihany'	15.56 ± 0.15 c	17.01 ± 0.14 c
'Panenske ceske'	16.31 ± 0.11 b	18.60 ± 0.16 b
'Parmena zlata zimni'	13.74 ± 0.16 a	13.27±0.14 d
'Strymka'	16.38 ± 0.25 b	19.62 ± 0.20 e
'Idared'	11.89 ± 0.14 d	12.98 ± 0.11 f

Different letters in each column indicate the significant differences (Tukey's test, $p<0.05$)

Tab. 6. Correlation relationships between total phenolic content, total flavonoid content, total antioxidant capacity, ascorbic acid content, and scavenging effect of apple juices on hydroxyl radical and nitric oxide

Correlation between	r^2	Equation
TPC and TAC	0.9385	y = 1.3983x + 212.682
TFC and TAC	0.9331	y = 2.2651 + 556.380
Vitamin C and TAC	0.3445	y = 17.630 + 114.550
TPC and hydroxyl radical	0.9758	y = 0.0035x + 10.121
TFC and hydroxyl radical	0.8907	y = 0.055x + 11.151
Vitamin C and hydroxyl radical	0.4151	y = 0.048x + 9.492
TPC and nitric oxide	0.9158	y = 0.053x + 9.343
TFC and nitric oxide	0.9280	y = 0.0087 + 10.558
Vitamin C and nitric oxide	0.5789	y = 0.0875x + 6.643

titrable acidity are important for gustatory characteristics of juices, they can possibly have an impact on further processing such as fermentation (Garg *et al.,* 2008).

The consumption of apple juice has been shown to be more effective in the prevention of chronic diseases in comparison with other core fruit-pear or quince juices (Hricovsky *et al.,* 2003). In Central Europe, the consumption of juices made from native cultivars has a long tradition. For many centuries they have been used for drinking. The native apple cultivar juices are known for drinking without treatment by clarification (Tetera, 2006). That is due to medical reasons since clarification (enzymatic treatment, fining and filtration) decreases TPC, TAC and TFC in apple juices by around 50% (Will *et al.,* 2007; Zhang *et al.,* 2008). Moreover, also Mihalev *et al.* (2004) and Picouet *et al.* (2009) noticed a decrease in TAC in case of enzyme treatment of apple juices. Similarly, pasteurizing juices can lead up to a 50% lower phenolic content and radical scavenging activity (Al-Turki and Stushnoff, 2007). Typical native apple cultivars of Central Europe are a high valuable source of antioxidants, which was also observed in the results.

In juices of commercial cultivars the typical values of TPC range from 50 to 532 mg GAE l^{-1} and TAC from 64 to 1,280 mg AAE l^{-1} (Valois *et al.,* 2006), which is less than it was measured in most juices. The highest contents of TAC were observed in the 'Strymka' cultivar (2,637.34 AAE l^{-1}) and also in the 'Jadernicka moravska', 'Kardinal zihany' and 'Panenske ceske' cultivars and several times they exceeded the values found in the 'Idared' cultivar, which is the most widespread juice cultivar in the world (Tab. 4). Furthermore, Rop *et al.* (2011) draws attention to high antioxidant efficiency of methanolic extracts of native cultivars of the 'Panenske ceske' or 'Strymka'. It is obvious in whole fruits when the methanolic extract is used the contents of TPC, TFC as well as TAC are higher than in pressed juices (Kyzlink, 1990). In case of antioxidants there exist other scientific works which refer to the fact that high contents of TAC are typical of native cultivars and the consumption of juices is highly effective in human nutrition (Carbone *et al.,* 2011). For example, Kahle *et*

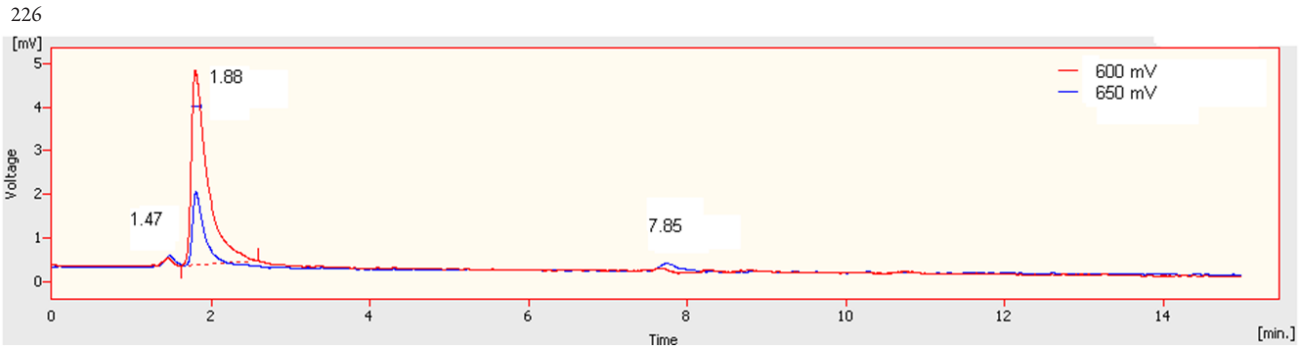

Fig. 1. Chromatogram representing the peak of ascorbic acid of the 'Strymka' cultivar (the retention time 1,88 min, 600 mV)

al. (2005) states the values of TAC are six times higher in typical German native cultivars compared to those commercially grown such as 'Granny Smith'. The content of TPC has a fundamental importance for the TAC content (Sochor *et al.,* 2010), which is evident from high correlation coefficients between these factors (Tab. 6). High antioxidant efficiency belongs to one of protective factors for adaptation to harsh ecological conditions (Ercisli *et al.,* 2011; Kahle *et al.,* 2005; Tetera, 2006). In apple juices the most significant phenolic substances are flavonoids (Hertog *et al*., 1993), with quercetin being predominant (Gerhauser, 2008). In the measurements, TFC showed a high correlation in comparison to antioxidant properties (Tab. 4), which is, again, typical of apple juices (Soares *et al.,* 2008). On the contrary, the ascorbic acid content has a smaller impact on TAC in measurements (Gardner *et al.,* 2000; Gliszczynska-Swiglo and Tyrakowska, 2003) (Tab. 6).

In the cultivars of 'Panenske ceske' and 'Strymka' (the chromatogram for the vitamin C in juice of this cultivar is provided in Fig. 1), the highest contents of vitamin C were observed (145.35 and 144.05 mg l^{-1}, respectively). Native cultivars are not excellent sources of this vitamin (Tetera, 2006) and in general, apple juices do not belong to its rich sources in comparison with juices made from, for example, most species of berry plants (Oszmianski and Wojdylo, 2009).

For verifying antioxidant efficiency inhibitory effect on ROS was also monitored (Tab. 5). Particularly, hydroxyl radical (in the 'Idared' cultivar the inhibitory effect was only on average 11.89%, on the other hand, in native cultivars it was higher-for example, in the 'Strymka' cultivar up to 16.38%) and nitric oxide ('Idared' had the inhibitory effect only 12.98%, on the contrary, 'Strymka' as a native cultivar reached 19.62%). These inhibitory effects are quite high compared with juices of other species of pomaceous, but even berry fruit, although they do not reach the values of, for example, blackcurrant or blueberry juices (Rop and Hrabe, 2009). Results in Tab. 6 show that a high correlation between ROS and TAC, which corresponds to the measurements mentioned above. High inhibitory activity to ROS, as one of significant factors of cardiovascular and cancer diseases (Gerhauser, 2008), is typical of apple juices (Oh *et al.,* 2006). Regarding ROS, apple juices are the most effective to hydroxyl radical (Lichtenthaler and Marx, 2005) and in particular, nitric oxide (Jayakumar and Kanthimathi, 2011). Native cultivars are enormous potential for further utilization in the prevention of many diseases (Rop *et al.,* 2009). This research supports these facts and it can be used for the promotion of nutritional value of native apple cultivars in Central Europe. (Biedrzycka and Amarowicz, 2008; Tetera, 2003).

Conclusions

As regards technological parameters such as SSC and titrable acidity, in native apple cultivars high values were observed in juices of the 'Strymka' cultivar-16.65% of SSC and 5.11 g of malic acid l^{-1}. The 'Jadernicka moravska' cultivar contained 16.95% of SSC although the acid content was lower (3.28 g of malic acid l^{-1}). In addition, similar values were determined in the juices of the 'Panenske ceske' cultivar. With respect to antioxidant properties, the juices of native apple cultivars of 'Jadernicka moravska', 'Kardinal zihany', 'Panenske ceske' and 'Strymka' appear to be the most promising. The contents were found to be 2,541.38 mg of AAE l^{-1}; 2,595.07 mg of AAE l^{-1}; 2,568.50 mg of AAE l^{-1} and 2,637.34 mg of AAE l^{-1}, respectively. Between these values and the content of TPC and TFC there were significant correlations (r^2 = 0.9385 and r^2 = 0.9331) whereas between the ascorbic acid content and TAC the correlation was only r^2 = 0.3445. Similarly, in the cultivars mentioned above the highest scavenging effect on hydroxyl radical and nitric oxide was in the juice of the 'Panenske ceske' cultivar (16.31% and 18.60%) while in the commercially grown 'Idared' cultivar it was only 11.89% and 12.98%. Native apple cultivar juices can be a suitable supplement to modern human nutrition. Furthermore, the work contributes to their popularization and draws attention to their unique genetic potential.

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