https://doi.org/10.17221/305/2018-CJFS

L-Ascorbic acid content and antioxidant capacity in less-known fruit juices

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Citation: Soural I., Šnurkovič P., Bieniasz M. (2019): L-Ascorbic acid content and antioxidant capacity in less-known fruit juices. Czech J. Food Sci., 37: 359–365.

Abstract: Eight less-known juices are characterised and contents are monitored of selected substances and nutritional parameters. 100% juices were produced of Aloe Vera, Aronia, Blackcurrant, Black elder, Cranberry, Malpighia, Pomegranate and Seaberry by pressing, including the flesh. Total phenolic compounds (TPC) and ascorbic acid (AA) content were analysed as other parameters. The juices were measured on total antioxidant capacity (TAC) as the content of trolox, where the highest levels were found for Malpighia 63.2 mM, when the value was significantly different ($P < 0.05$) compared with all the other juices, while Aloe Vera had lowest levels with 0.4 mM. The Aronia juice possessed the highest level of TPC (8297 mg of GAE/l). TAC levels very well corresponded with TPC levels while AA content actually did not correlate with TAC values. In addition to the objective analysis, the juices were subjected to a sensory evaluation. Blackcurrant presents an attractive product from the sensorial aspect as well as in terms of the nutritional value.

Keywords: antioxidant capacity TAC; polyphenols TPC; sensory aspect; vitamin C; 100% juices

In the recent years, there has been a growing consumer interest in foods on natural basis, *i.e.* without the addition of synthetic substances such as colouring, preserving or flavouring agents, *etc.*). In addition, consumers focus on a rather high content of fruit ingredients in beverages such as syrups, where 10% to 50% are standard figures. Juices are another option with the 100% content of the respective fruit. Orange and apple juices are the best known types; these can be distributed directly or prepared from concentrates as part of transport. Juices made from other types of fruit have been increasingly present on the market, such as those made from Aloe Vera, Aronia, Blackcurrant, Black elder, Cranberry, Malpighia, Pomegranate and Seaberry. Such less-known products may be attractive for consumers not only in terms of sensorial assessment, but also through high levels of beneficial substances such as vitamin C, antioxidants, polyphenols, and others (Nagamine *et al.*, 2004; Kikas *et al.* 2017). Vitamin C plays an important role in the organism (Fain 2004); its effect on the human body has been observed for dozens of years (schorah *et al.* 1965). Very recently, similar observations have been achieved in the field of sports (Dutra *et al.* 2018). Antioxidants are known to help influence – in a positive manner – the oxidative stress to reduce the amount of free radicals in the body (Jensen *et al.* 2008). Polyphenols are substances that are widely suggested to protect health due to their biological properties, which include anti-atherogenic,

Supported by the Operational Programme Research, Development and Education, Grant No. CZ.02.1.01/0.0/0.0/16_017/0002334.

https://doi.org/10.17221/305/2018-CJFS

anti-inflammatory and anti-tumour activities, inhibition of blood clots, antimicrobial activity and strong antioxidant activity (Aruoma *et al.* 2012).

MATERIAL AND METHODS

Materials. The analysis made use of less-known 100% commercial juices produced by direct pressing, without filtering, from the following crops: Aloe Vera, Aronia, Blackcurrant, Black elder, Cranberry, Malpighia, Pomegranate and Seaberry, all sourced as part of the sales chain.

Total antioxidant capacity (TAC). Determination by the ferric reducing antioxidant power (FRAP) method was done in a pH 3.6 acetate buffer (34 mM sodium acetate in solution of 281 mM acetic acid). The reaction mixture contained 12 mM $FeCl_3$ solution, 10 mM 2,4,6-tris(2-pyridyl)-s-triazin in 40 mM HCl solution, and buffer in a ratio of 1 : 1 : 10. Two mililitres of the reaction mixture was mixed with 25 ml of diluted sample with deionized water in a disposable plastic cuvette (10 mm) and the obtained solution was measured after 10 min at a wavelength of 593 nm using a spectrophotometer (Specord 50 Plus; Analytik Jena, Germany). Blank was prepared in the same way but 25 ml of deionized water was used instead of 25 ml of diluted sample with deionized water. The antioxidant capacity was calculated from the calibration curve using Trolox. The same method was used in the previous study (Hic *et al.* 2017).

For the DPPH method, 1.9 ml of DPPH radical solution in methanol (0.1 mM) was mixed with 0.1 ml of diluted sample with deionized water in a disposable plastic cuvette (10 mm). Absorbance at 515 nm was measured after 30 min using the spectrophotometer Specord 50 Plus. Blank was prepared in the same way but 0.1 ml of deionized water was used instead of 0.1 ml of diluted sample with deionized water. The antioxidant capacity was calculated from the calibration curve using Trolox. The methodology was employed by Hic *et al.* (2017).

Antioxidant capacity measured by both methods was expressed as millimoles of Trolox per liter of juice.

Determination of total polyphenols (TPC). This method is based on spectrophotometric measuring of colour products occurring during the reaction of hydroxyl groups of phenolic compounds with Folin-Ciocalteau reagent (FCR). Sample preparation: The juice sample (0.1 ml) was pipetted into a 50 ml volumetric flask and mixed with 20 ml of distilled water and 1 ml of FCR. After 3 min, 5 ml of 20-percent Na_2CO_3 solution was added, the volumetric flask was filled with distilled water to the mark and stirred. Thirty minutes later, the absorbance was measured in a spectrophotometer at the wavelength of 700 nm using a 10-mm cuvette. The result was compared with absorbance of a blind sample (1 ml of FCR, 5 ml of 20% Na_2CO_3 solution, and distilled water up to 50 ml). The content of total polyphenols was thereafter converted to the fresh plant matter and expressed as milligrams of gallic acid per 1 l of juice (mg GAE/l). The same method was used in the previous study (Snurkovic 2013).

HPLC determination of ascorbic acid (AA). Juice sample (2 ml) was diluted with oxalic acid to the final volume of 10 ml. Using a nylon filter, the diluted sample was filtered (0.22 μm) into a brown vial. Condition of chromatographic analysis: Column: Prevail 5μm Organic Acid 110Å HPLC 250 × 4.6 mm, flow rate of mobile phase 25 mM KH_2PO_4 1 ml/min, UV detection at 210 nm, column temperature 30°C. The determination was carried out by means of a calibration curve on standard, *i.e.* L-ascorbic acid. The same method was used in the previous study (Snurkovic 2013).

Statistical method. Averages values and standard deviation were calculated for all measurements: TAC (FRAP and DPPH), TPC and AA. Statistical data were analysed by ANOVA, applying the Tukey multiple range test for making comparisons with Statistica Cz 12 and MS Excel 2010 software.

RESULTS AND DISCUSSION

The juices were analysed for the content of antioxidants using DPPH and FRAP methods; the resulting contents were expressed as TAC (total antioxidant capacity) using trolox as the standard; see Figure 1.

The highest average TAC values were identified for the Malpighia juice; here, they reached 63 mM and 53 mM for FRAP and DPPH methods, respectively; even a double amount113 mM was measured by Mezadri *et al.* (2008) in squeezed fruit using the DPPH method; in six different types of commercial flesh, however, they measured an average value of 59 mM, which very well corresponds to the value of 53 mM measured by us in the same type of juice. Mean values (20 to 50 mM) were achieved in the case of juices produced from Aronia, Blackcurrant and Black elder (as berries with black colour) along

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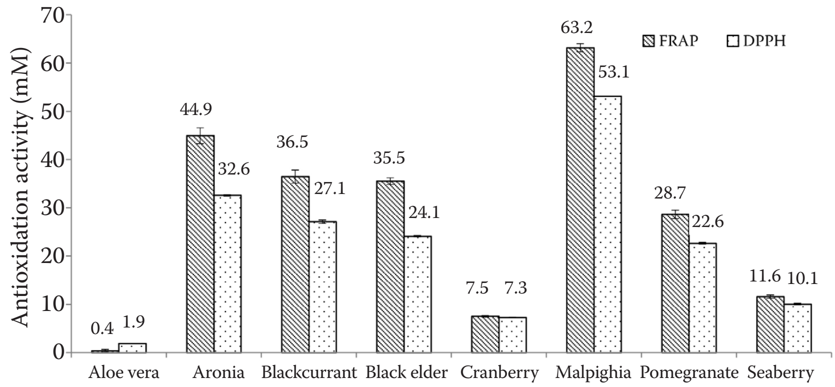

Figure 1. Total antioxidant capacity (TAC) in less-known 100% juices

with that made from Pomegranate, when the average TAC values ranged from 22.6 to 44.9 mM; the similar values (around 20 mM) were identified for Pomegranate juices in Gil *et al.* (2000). Low values, *i.e.* below 20 mM, were found for juices made from Seaberry, Cranberry and Aloe Vera (from 0.4 to 11.6 mM). The difference between Malpighia and Aloe Vera juices in terms of average values was about 150-fold for FRAP and 28-fold for DPPH.

Some of the juices presented a very good correlation of TAC and TPC results. The lowest average TPC values (below 3000 mg/l) were detected for Seaberry 2408 mg/l, Cranberry 1547 mg/l, and Aloe Vera 21 mg/l juices. Mean average TPC values (up to 6000 mg/l) were exhibited by juices made from Blackcurrant 5402 mg/l and Black elder 5881 mg/l as berries with black colour, along with those made from Pomegranate 3717 mg/l similarly as was seen in TAC. A similar value (2566 ± 131 mg/l) was determined in another study (Gil *et al.* 2000) in terms of numeric order except that the authors used *p*-coumaric acid instead of gallic acid. In terms of correlation, the juice from Aronia measured by us exhibited a deviation; in the case of TPC, it possessed the highest value of 8297 mg/l, which is approximately 15% higher than the value measured by Catana *et al.* (2017) in the fresh juice (6885 ± 228 mg/kg). In terms of polyphenols, the juice from Malpighia placed the second among the juices we analysed with 7362 mg/l; similar values were obtained by Mezadri *et al.* (2008) – they ranged from 4520 to 7510 mg/kg for six different types of commercial flesh of Malpighia. The ration between the average TPC values for the Aloe Vera a Aronia juices we measured was nearly up to 400-fold; see Figure 2.

An enormously high level of ascorbic acid (AA, referred to as vitamin C) was found in Malpighia 8528 mg/l; this makes around 0.85% and in fact the same level (8560 mg/l) was measured in squeezed fruit by Mezadri *et al.* (2008). Vendramini and Trugov (2000) found Malpighia to contain 1.07% vitamin C in ripe fruits, while unripe fruits exhibited even 2.16% (which equals 21600 mg/l). The other juices measured by us featured contents below 1200 mg/l, *i.e.* less than 0.12%), which was one-seventh of the content less than for Malpighia. Very low levels of ascorbic acids were found in juices made from Aronia 398 mg/l, Black elder 295 mg/l, Pomegranate 5 mg/l and Aloe Vera; for the last mentioned type, the values were even less than the detection threshold. Mean values (600–1200 mg/l) of ascorbic acid were found in the following juices: Blackcurrant 801 mg/l,

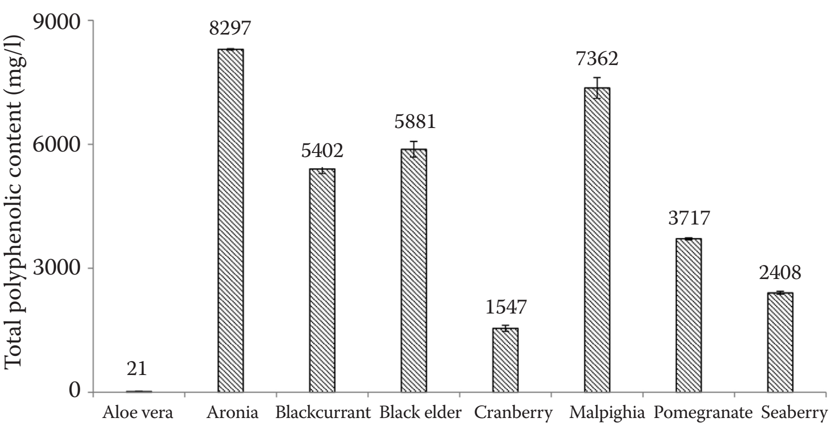

Figure 2. Total polyphenolic content (TPC) in less known 100% juices

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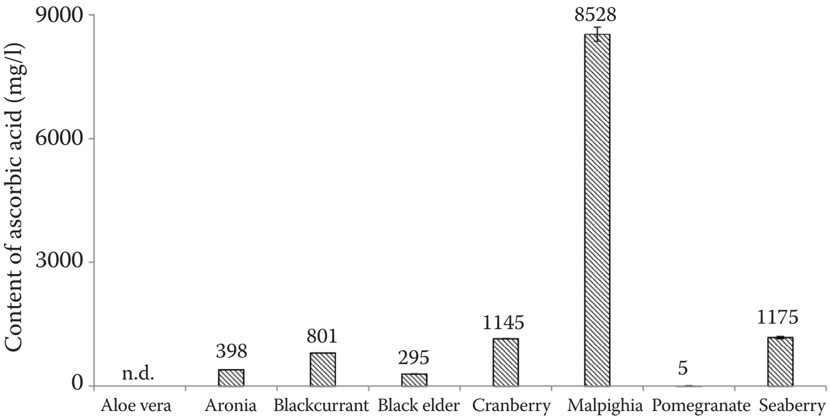

Figure 3. Ascorbic acid levels in less-known 100% juices

which matches the report by Zheng *et al.* (2009) who found 600 mg/l in the *Melalahti* variety and 1900 mg/l in the *Mortti* variety, as regards Blackcurrant juices; Cranberry 1145 mg/l and Seaberry 1175 mg/l (Figure 3). Identical levels (1220 ± 64 mg/l) or, statistically insignificant differences were measured by Rösch *et al.* (2003) in the case of Seaberry; Zeb (2004) provides various, subspecies-specific levels in juices and berries: 200–2500 mg/l in *Sinensis*; 28–310 mg/l in *Rhamnoides*; 460-1330 mg/l in *Fluviatilis* and 40–300 mg/l in *Mongolia*. As can be seen, the *Sinensis* subspecies stands out with the highest levels.

Significance of the differences for all kinds measurements (TAC, TPC, AA) are indicated in summary table (Table 1).

Measurements using both of the methods, *i.e.* FRAP and DPPH, correlated very well to each other (correlation coefficient R = 0.9890); this was however not true for the correlation of the content of ascorbic acid (AA) with TAC / TPC (R = 0.6314 for FRAP and 0.7299 for DPPH). Nonetheless, the AA and TPC summation correlated with TAC values (R = 0.9677 for DPPH and 0.9403 for FRAP) even better than TPC alone in correlation with TAC (R = 0.8879 for DPPH and 0.9402 for FRAP) (Figure 4). Although there are summed contents of different substances – polyphenols as GAE with a vitamin C, this 'plain sum' shows a better correlation than just TPC and makes also clear that 1 g/l of vitamin C is less involved in TAC than 1 g GAE/litre for TPC.

In addition to the objective analyses (TAC, TPC and AA), a sensory analysis was carried out on the most positive and the most negative sample of commercial juices.

As part of the overall sensory analysis 18 assessors selected the best sample from the following juices: Aloe Vera, Aronia, Blackcurrant, Black elder, Cranberry, Malpighia, Pomegranate and Seaberry. According to this analysis, juices made from Pomegranate and Blackcurrant placed the best, selected 7/6 evaluators out of a total of 18 (Figure 5). For the remainder of 5 evaluators, they selected Aronia, Black elder and Seaberry as the best samples of juices,

Table 1. Significance of the differences with averages for TAC (mM), TPC and AA (mg/l)

	TAC		TPC	AA
	DPPH	FRAP		
Aloe vera	1.9 ± 0.0^{a}	0.4 ± 0.3^{c}	21 ± 5^{b}	n.a.^{a}
Aronia	32.6 ± 0.2^{g}	44.9 ± 1.6^{e}	8297 ± 16^{g}	398 ± 2^{b}
Black elder	24.1 ± 0.1^{e}	35.5 ± 0.7^{b}	5881 ± 189^{a}	295 ± 5^{ab}
Blackcurrant	27.1 ± 0.4^{f}	36.5 ± 1.4^{b}	5402 ± 110^{a}	801 ± 1^{c}
Cranberry	7.3 ± 0.1^{b}	7.5 ± 0.2^{a}	1547 ± 74^{c}	1145 ± 5^{cd}
Malpighia	53.1 ± 0.0^{h}	63.2 ± 0.8^{f}	7362 ± 252^{f}	8528 ± 172^{e}
Pomegranate	22.6 ± 0.2^{d}	28.7 ± 0.9^{d}	3717 ± 29^{e}	5 ± 5^{a}
Seaberry	10.1 ± 0.2^{c}	11.6 ± 0.3^{a}	2408 ± 35^{d}	1175 ± 26^{d}

$^{a–g}$different letters in the same line indicate significant difference by Tukey test (P = 0.05); n.a. – not analysed; TAC – total antioxidant capacity; TPC – total phenolic compounds; AA – ascorbic acid

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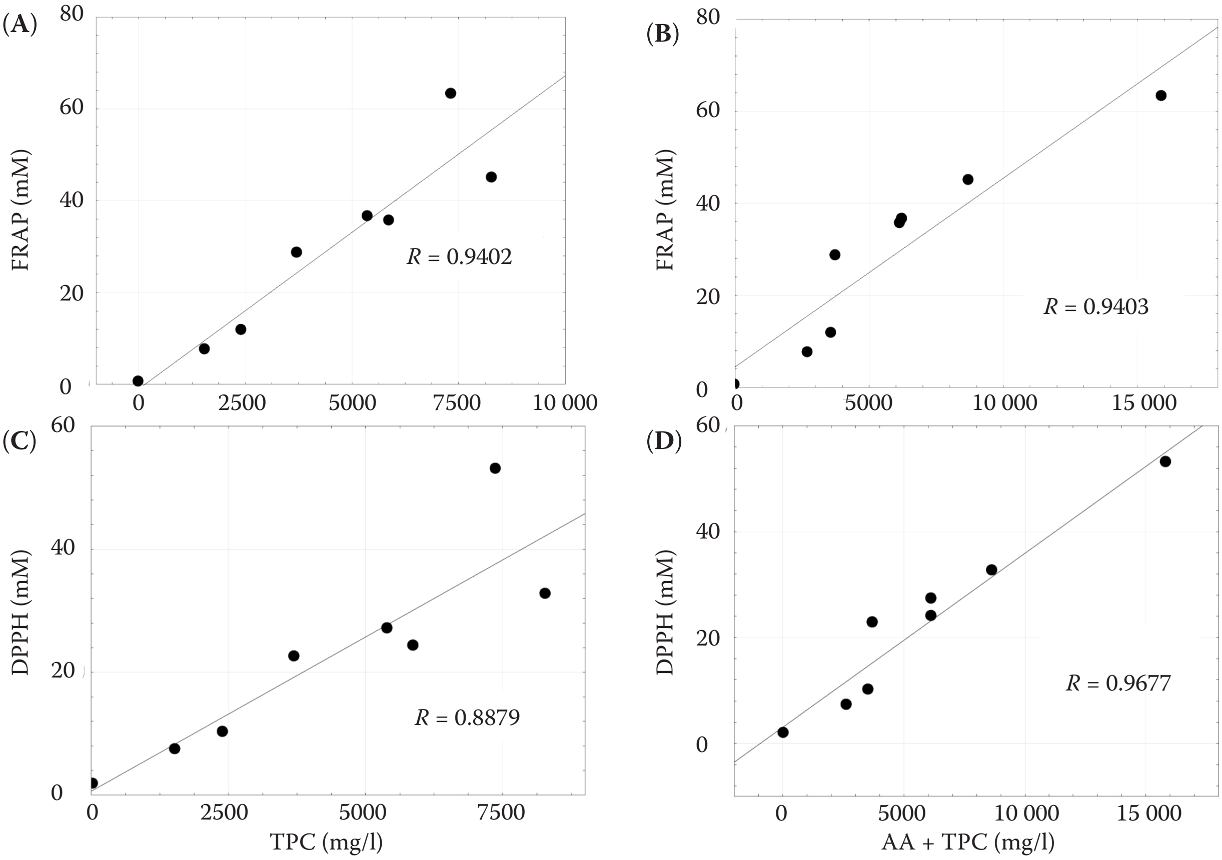

Figure 4. Total antioxidant capacity by FRAP and DPPH methods: (**A**) and (**C**) total phenolic content (TPC), (**B**) and (**D**) TPC and ascorbic acid (AA) summation correlation

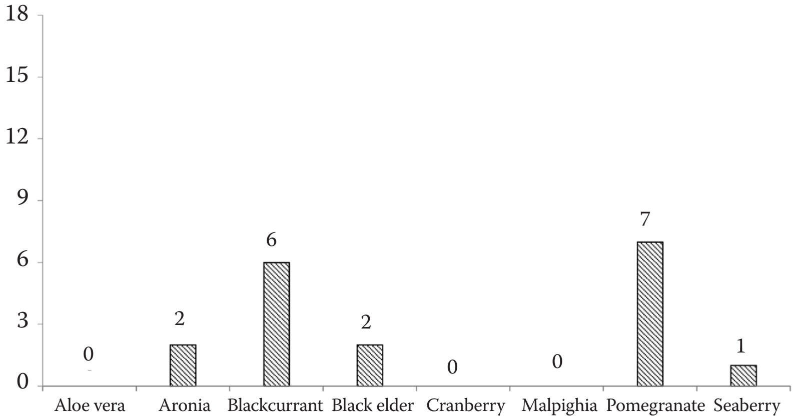

Figure 5. The overall assessment of juices – the best sample (18 evaluators)

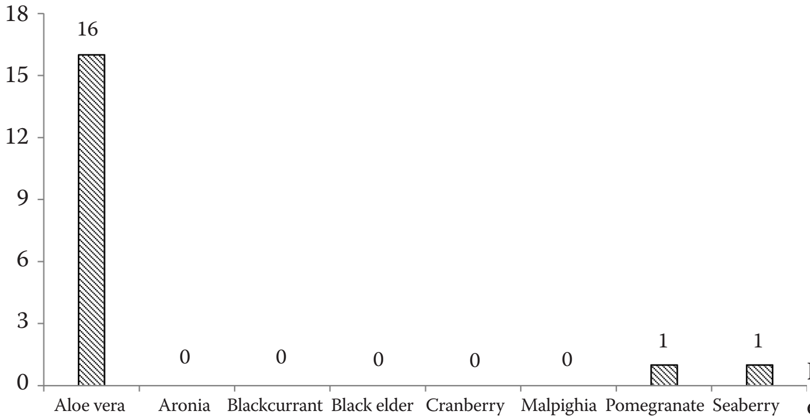

Figure 6. The overall assessment of juices – the worst sample (18 evaluators)

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where the two first-mentioned juices were selected by two assessors and the last-mentioned juice was selected by a single assessor. On the basis of these data, it can be concluded that sensorially, Blackcurrant and Pomegranate are the most attractive juices while consumers least preferred Aloe Vera, Cranberry and Malpighia when neither of them was selected as the best by a single assessor.

The same number and persons of assessors indicated the most negative samples of juices, selecting from 8 different types (Aloe Vera, Aronia, Blackcurrant, Black elder, Cranberry, Malpighia, Pomegranate and Seaberry). In this case, 16 from 18 assessors chose the juice made from Aloe Vera as the worst sample; the number represents around 89% of assessors (Figure 6). Juices made from Pomegranate and Seaberry were each indicated as the worst samples by one assessor. The remainder of 5 juices did not receive a negative assessment by any of the evaluators.

CONCLUSIONS

For these 8 juices, the best is one made from Malpighia in terms of the constituents; as part of sensory assessment, not one of the 18 evaluators selected it as the best or worst, while juices made from Blackcurrant and Pomegranate turned out to be sensorially attractive products. Since the Blackcurrant juice has even higher levels of studied substances, it presents an attractive product for consumers both sensorially and in terms of content. On the contrary, the juice made from Aloe Vera was found to be the worst of these 8 products from the same aspect. This is a noteworthy fact considering the increased interest of consumers in beverages containing this very type of fruit; this also opens wider opportunities for application of the other less-known juices, the remaining seven types, that surpassed Aloe Vera both sensorially and in terms of content.

Important factors to consider when choosing a commercial juice will be, among others, what particular subspecies and/or shares of multiple subspecies are involved since this can significantly influence the content of nutritional substances. Unfortunately, this information is not presented by producers. Nonetheless, the measured data show that some of these less-known juices form an interesting alternative to apple and orange juices.

As part of the measurements, there was a notable correlation between antioxidant capacity (TAC) and polyphenols (TPC), where DPPH and FRAP revealed correlation coefficients (R) of 0.8889 and 0.9402, respectively. Even better correlation was achieved through a simple sum of the ascorbic acid (AA) content and polyphenols (TPC) against antioxidant capacity (TAC) when the correlation coefficient was even R = 0.9677 for DPPH and 0.9403 for FRAP; however, ascorbic acid (AA) alone lacked sufficiently appropriate correlation with TAC (R below 0.7300) compared with TPC.

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Received: 2018–10–23
Accepted after corrections: 2019–07–25
Published online: 2019–09–26