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CORRELATION OF ANTHOCYANIN AND TOTAL POLYPHENOL CONTENT OF BERRIES WITH THEIR ANTIOXIDANT CAPACITIES

Anna Mária Nagy[1], Meriem Serine Hamaidia[2], Lilla Szalóki-Dorkó[2], Zsuzsa Jókai[3], Eva Stefanovits-Bányai[3], Mónika Máté[2*]

[1]Holi-Medic Ltd., Fehérvári 44, 1117 Budapest, Hungary
[2]Department of Fruit and Vegetable Processing Technology, Institute of Food Science and Technology, Hungarian University of Agriculture and Life Sciences, Villányi 29-43,1118 Budapest, Hungary
[3]Department of Food Chemistry and Analytics, Institute of Food Science and Technology, Hungarian University of Agriculture and Life Sciences, Villányi 29-43, 1118 Budapest, Hungary

[*]e-mail: mate.monika.zsuzsanna@uni-mate.hu

Abstract

Widely known berries, such as blueberries (*Vaccinium myrtillus* L.) and cranberries (*Vaccinium oxycoccos* L.), are well-known functional foods. At the same time, berries that are less known to the general population and therefore consumed less often - such as Aronia (*Aronia melanocarpa* Micht.), black elderberry (*Sambucus nigra* L.), and black currant (*Ribes nigrum* L.) - although their nutritional values are also outstanding. Therefore, our research aimed to understand better the correlations between certain berries' dietary values and their antioxidant capacity properties and to broaden their therapeutic usability.

During our studies, we measured spectrophotometrically the total polyphenol content - TPC (by the Folin-Ciocalteu method), the anthocyanin monomer content (with pH differential method), and antioxidant capacity using the ferric reducing antioxidant power (FRAP) and Trolox-equivalent antioxidant capacity (TEAC) method, of the 65% concentrated juice made by vacuum evaporation from the berries mentioned earlier.

Our results confirmed a strong correlation between the total polyphenol content of berries and the antioxidant capacity measured by the TEAC method, but no correlation was measured by the FRAP method. However, the combined TPC and anthocyanin content were closely correlated with the antioxidant capacity measured by the FRAP method, but not by the TEAC method. This also highlighted that due to their different nutritional values, using several antioxidant capacity measurement methods together could more accurately determine the combined antioxidant capacities of individual fruits. For example, the antioxidant capacity of cranberries was highest using the TEAC method, but lowest with FRAP.

Overall, it can be stated that higher anthocyanin content and antioxidant capacity were measured (by FRAP method) in the juice concentrates of the lesser-known black elderberry, Aronia, and blackcurrant than the well-known blueberry and cranberry juice. Therefore, using lesser-known berries for a broader therapeutic purpose may be advisable.

Key words: *Antioxidant capacity, Polyphenols, Anthocyanins, Berries, Dietary nutrition.*

1. Introduction

In the last 40 years, numerous publications have proven the importance of the balance of free radicals and antioxidants in maintaining our health (Kehrer and Smith [1], Halliwell [2]). Fruits are the best sources of antioxidants in our food (Hegedűs *et al.*, [3]) therefore, their regular consumption contributes to maintaining the redox homeostasis of our body (Fraga *et al.*, [4]).

By regularly consuming vegetables and fruits, which are rich in polyphenols and antioxidants, the risk of civilization diseases with the highest morbidity and mortality rates can be significantly reduced (Martin *et al.*, [5], Boeing *et al.*, [6]) - such as cardiovascular diseases based on atherosclerosis (Wang and Goodman [7]), the development of certain tumor diseases (Hung *et al.*, [8]) and type 2 diabetes (Martineau *et al.*, [9]), since free radical reactions can be delayed or inhibited by antioxidants (Foito *et al.*, [10], Yang and Kortesniemi [11]).

Despite all this, a significant part of the population in Europe does not consume enough vegetables and fruits (the 5 times a day recommended by the WHO). An international study published in 2018, examining fruit consumption and related behaviors in 8 countries in North America, Europe, and East Asia, found that older, married adults who considered themselves healthy and physically active consumed fruit more often and in larger quantities than younger, physically inactive individuals living alone.

Those who consume fruits more often make their decisions primarily not based on price but on the freshness, nutritional value, origin, and seasonal nature of the fruits (Heng and House [12]). In recent decades, the consumption of berries - mainly frozen berries - has increased significantly in the EU (Tavoschi *et al.*, [13]). At the same time, the latter hid an unexpected consequence, as epidemiological studies by the European Union highlighted frozen berries as carriers of norovirus (NoV), hepatitis A virus (HAV), and *Shigella sonnei* infections. 32 epidemiological events were identified from 1983 - 2013 (26 of them from 2004 - 2013). The most common of these (with 15,000 cases) was the norovirus, which was involved in 27 events (Tavoschi *et al.*, [13]).

At the same time, it should also be emphasized that the level of consumption of soft fruits (berries and currants) and berry-based products shows significant differences in individual European countries: e.g. in France, berry-based products are consumed in significantly larger quantities than in Romania or Turkey (Popa *et al.*, [14]).

Berries are a rich source of a wide variety of non-nutritive, nutritive, and bioactive compounds such as phenolics, flavonoids, anthocyanins, stilbenes, and tannins, as well as nutritive compounds such as sugars, essential oils, carotenoids, vitamins, and minerals. Bioactive compounds from berries have potent antioxidant, anticancer, antimutagenic, antimicrobial, anti-inflammatory, and anti-neurodegenerative properties, both *in vitro* and *in vivo* (Nile and Park [15]).

Many beneficial physiological effects of bilberry are known, such as it is powerful antimicrobial, antioxidant, and anti-inflammatory effects, it also has endothelial protective effects, strengthens arteries, decreases vascular permeability and capillary fragility, and enhances/improves circulation. Hypoglycemic, anti-diabetic, and anti-obesity effects would also be cardioprotective. Its neuroprotective effects are also known and can help prevent Alzheimer's disease. Numerous publications have confirmed its anticancer effects both *in vitro* and *in vivo* in animal experiments, as well as in human studies (Helmstädter and Schuster [16], Ghosh and Konishi [17], Matsunaga *et al.*, [18], and Thomasset *et al.*, [19]). One of the best-known physiological effects of the wild bilberry (*Vaccinium myrtillus* L.) and its widely cultivated variety, the blueberry (*Vaccinium corymbosum* L.), is the improvement of visual acuity through the natural replacement of lutein and zeaxanthin (Li *et al.*, [20], Arunkumar *et al.*, [21]). Cranberries are most commonly consumed due to their antibacterial, anti-inflammatory, and diuretic effects in recurrent urinary tract infections (Fu *et al.*, [22]). In addition, its cardiovascular (Nile and Park [15]) and digestive system-supporting effects have also been confirmed, for example**,** in gastritis (Nikbazm *et al.*, [23]). Elderberry has a long history as a medicinal plant and has become increasingly popular due to its antiviral and immunomodulatory effects (Sargin [24]). During the Covid-19 epidemic, due to their antiviral and anti-inflammatory effects, elderberry (Silveira *et al.*, [25]) and black chokeberry (Aronia) became increasingly widely known (Eggers *et al.*, [26]). Elderberry is a significant source of protein, free and bound amino acids, unsaturated fatty acids, fiber, vitamins, antioxidant compounds, and minerals (Nile and Park [15]). The measurements of Sidor and Gramza-Michałowska [27], showed that it contains components with a high biological effect, mainly polyphenols, among them primarily anthocyanins, flavonols, phenolic acids, and proanthocyanidins, as well as terpenes and lectins. The presence of the same compounds was also detected in large quantities in chokeberry berries. Aronia could alleviate oxidative stress, insulin resistance, inflammation, and tissue damage in the liver (King and Bolling [28], Chen and Meng [29]).

The black currant's berry fruit is one of our most outstanding sources of iron and calcium among fruits (Nile and Park [15])**;** despite this, it has not been widely used in our country as a supplement to daily therapeutic practice in the complementary treatment of iron deficiency anemia and osteoporosis. Black currant possesses the highest non-heme iron content; its bioavailability to the body is increased due to the favorable vitamin C ratio, which is also a richly available resource (Rachtan-Janicka *et al.*, [30]).

Since previous studies have linked the beneficial physiological effects of the small berries we examined primarily to their antioxidant properties (Skrovankova *et al.*, [31]), our present studies aimed to look for connections and correlations between the content values of the small berries (total polyphenol content and anthocyanin monomer content) and their antioxidant capacity - measured by different analytical methods.

2. Materials and Methods

2.1 Materials

In our experiment, we used the juice concentrates of 5 berries: bilberry (*Vaccinium myrtillus* L.), cranberry (*Vaccinium oxycoccos* L.), black chokeberry (*Aronia melanocarpa* Micht)), elderberry (*Sambucus nigra* L.) and black currant (*Ribes nigrum* L.).

Since the content values of berries grown in different growing areas with different climatic conditions can differ significantly (Szalóki-Dorkó *et al.*, [32]), in our tests, we used juice preparations that consist of a mixture of raw materials from several different growing areas, so that it can be evaluated as the average of the individual landscape unit differences. We tested fruit juice concentrates with a dry matter content of 65% following food safety rules, strictly controlled, produced under the HACCP quality system, stored and distributed in an aseptic manner (Produced by Intercooperation Ltd.).

2.2 Analytical methods

2.2.1 Sample preparing for the measurements

For total polyphenol content (TPC) and antioxidant capacity methods, the 65% concentrates were tested as follows: 1 gram of juice extract was diluted with 10 mL of distilled water, then placed in an ultrasonic water bath for 1 hour, then centrifuged at 6,000 rpm for 20 minutes at 10 degrees Celsius and the supernatant was used for measurements after storage at minus 32 degrees.

When measuring the anthocyanin content, a mixture of 60% methanol, 39% distilled water, and 1% formic acid was used instead of distilled water.

2.2.2 Determination of total polyphenol content (TPC)

Total polyphenol content (TPC) was determined by the method of Singleton and Rossi [33], with the Folin-Ciocâlteu reagent. The measurements were performed at pH = 10.0. The electron transfer was based on assay and showed the reducing capacity, expressed as phenolic content. The color change during the Mo(VI) yellow → Mo(V) blue redox reaction was detected spectrophotometrically (λ = 760 nm). The results were expressed as gallic acid equivalent (mg GAE/100 g juice concentrate) based on the gallic acid (GA) standard curve.

2.2.3 Determination of total monomeric anthocyanin (TMAC) content

Total monomeric anthocyanin content was measured by a pH differential method at pH = 1.0, and pH = 4.5 spectrophotometrically at 520 nm and 700 nm, as described by Lee *et al.*, [34]. Results are expressed as mg cyanidin-3-glucoside/100 g of juice concentrate.

2.2.4 Determination of antioxidant capacities by TEAC method

The total antioxidant capacity was measured with the Trolox-equivalent antioxidant capacity (TEAC) method described by Miller *et al.*, [35]. The method is based on ABTS+ (2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) free radical scavenging by antioxidants measured by a spectrophotometer at 734 nm. Trolox (the hydrophilic analog of vitamin E) was used for the calibration. The values were expressed as Trolox equivalent antiradical capacity (TEAC) (μM Trolox/g juice concentrate).

2.2.5 Determination of antioxidant capacities by ferric reducing antioxidant power (FRAP) method

Measurement of ferric reducing antioxidant power of the peel extracts was carried out based on Benzie and Strain's procedure [36]. According to the measurement principles, the ferric-2,4,6-tris(2-pyridyl)-S-triazine (TPTZ) complexes were reduced by reductive compounds in a pH = 3.6 environment. The reaction causes a blue color shift, detectable spectrophotometrically at 593 nm. Ascorbic acid (AA) was used as a standard to prepare the calibration solutions. Results were expressed as μM AA/g of juice concentrate.

3. Results and Discussion

3.1 Results of total phenolic contents (TPC) and total monomeric anthocyanin content (TMAC)

The total polyphenol content (TPC) results of the five investigated fruit concentrates are shown in Figure 1.

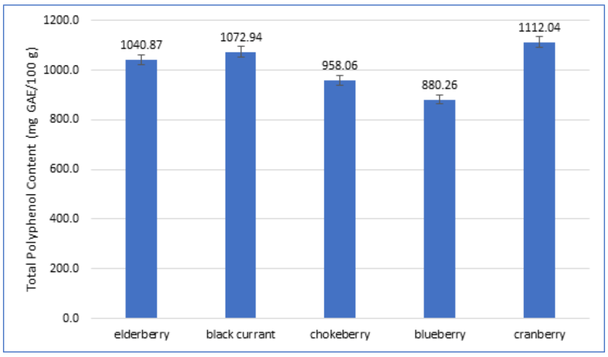

Figure 1. Total phenolic content of different fruit juice concentrates (mg GAE/100 g)

The results varied between 880.26 - 1112.04 mg GAE/100 g for the 65 Brix concentrate (135.4 - 171.1 mg GAE/100 g in the case of 10 Brix fruit juice). The two cranberries (red and black cranberries) had two extreme values, while the values of elderberry, black currant, and chokeberry juices were in between them. Cranberry has the highest total polyphenol content, but it should be noted that the values are relatively balanced for the five examined fruit species; the difference between the two extreme values is only 1.26 times.

However, a high polyphenol content does not mean a high anthocyanin content. Examining the data in Figure 2, it can be seen that the order of anthocyanin is completely different for the studied species. The values ranged from 242.1 to 775.7 mg/100 g (37.2 to 119.2 mg/100 g based on 10 Brix). The lowest value was for cranberry concentrate, while the highest was for elderberry. The concentration of the other three examined fruits showed almost the same values. In this case, the distribution moves on a much larger scale, the difference between the two extreme values is 3.2 times.

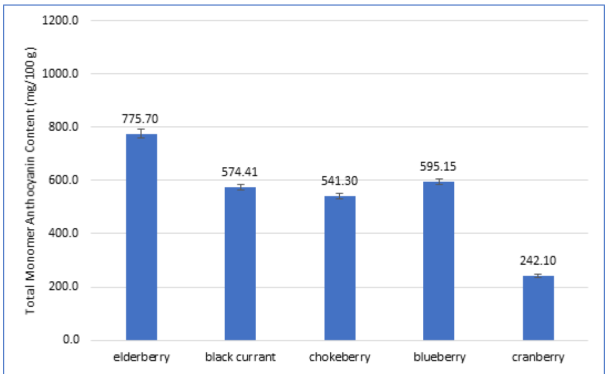

Figure 2. Total monomeric anthocyanin content of different fruit juice concentrates (mg/100 g)

Jakobek *et al.*, [37], examined black currant and elderberry varieties. Their results, although similar, were slightly higher, 636 mg/100 g in the case of elderberry and 277 mg/100 g in the case of black currant. Zhang *et al.*, [38], according to the comprehensive summary study on Aronia, their total polyphenol content was between 777 - 1,124 mg/100 g, which is five times the data we measured. At the same time, in terms of the total monomeric anthocyanin content, their results are the same (86 - 212 mg/100 g) as the results of the present study.

According to the Nile *et al.*, [15], in frequently consumed berries, blueberries contain predominantly proanthocyanidins, while blackberries, black and red raspberries, and strawberries contain ellagitannins. Therefore, it is important to consider the type and chemical structure of the tannins present in the given type of berry, because they can significantly contribute to the unique biological properties. For example, the anti-bacterial properties observed in blueberries appear unique among berries. This property is due to the oligomeric proanthocyanidins, which have an A-type structural bond. Similarly, the different biological effects observed in proanthocyanidin-rich blueberries and ellagitannin-rich strawberries on the nervous system function and behavior of aging animals may result from the effects of tannins in different regions (Szadljek Borowska [39]).

Figure 3 shows interesting results, where the TPC and TMAC content can be seen in relation to each other, as well as the Total monomer anthocyanin content in total polyphenol content %.

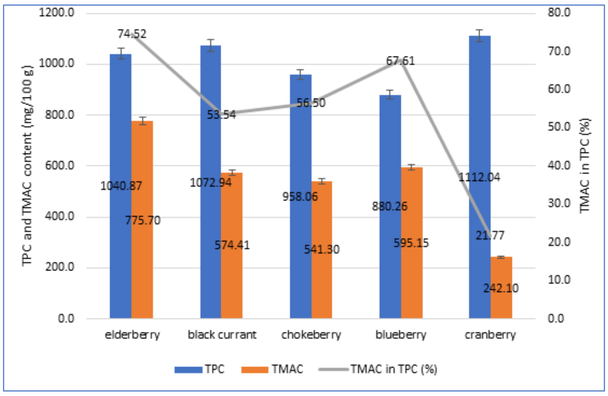

Figure 3. Total monomer anthocyanin content in total polyphenol content %.

In the case of elderberry, the value is the highest, where the anthocyanin content is 74.52% of the total polyphenol content, followed by black cranberry with 67.60%. In the case of black currant and Aronia, the values are 53.54 and 56.5%, while for cranberry it is the smallest, only 21.77%. However, it should be mentioned that the low anthocyanin content of the cranberry is combined with an extremely high polyphenol content, which means flavonoids, phenolic acids, and other polyphenolic compounds are outstanding in terms of health preservation and prevention based on numerous studies.

3.2 Results of antioxidant capacity measurements

The antioxidant status of the fruit juice concentrates included in the experiment was evaluated based on two aspects: their antioxidant effect is shaped by several groups of compounds and hundreds of compounds within them.

The results of FRAP based on iron-reducing capacity and related to ascorbic acid are shown in Figure 4.

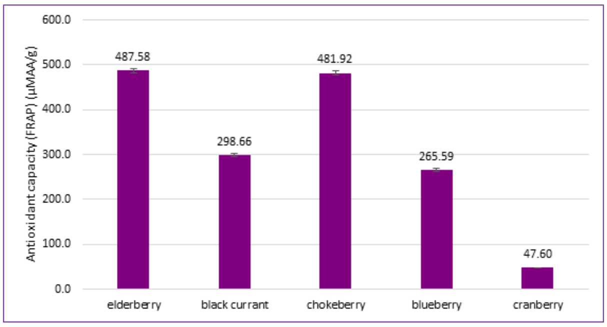

Figure 4. Antioxidant capacity of fruit juice concentrates measured by FRAP (µMAA/g) method

Values ranged from 47.60 to 487.48 µMAA/g for 65 Brix concentrate (7.32 to 74.9 µMAA/g for 10 Brix juice). The two extreme values were measured in black elderberries and cranberries, the difference is 10.2 times. Similar to the elderberry, we measured an outstanding FRAP value in the case of chokeberry, even though both TPC and anthocyanin content are lower than that of elderberry. The FRAP values of black currants and blueberries are almost similar, as is their anthocyanin monomer content.

Figure 5 shows the antioxidant capacity values related to Trolox.

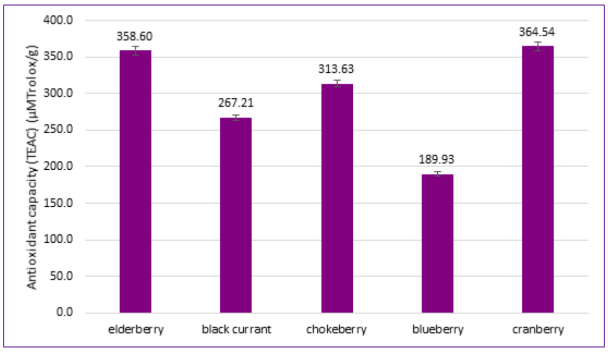

Figure 5. Antioxidant capacity of fruit juice concentrates measured by TEAC (µMTrolox/g) method

The values varied between 189.93 - 364.54 µMTrolox/g for 65 Brix concentrate (29.07 - 56.07 µMTrolox/g for 10 Brix juice). The outstanding value was mainly for the red cranberry, then the elderberry, while the black cranberry had the lowest value. Similar to TPC, the 2 extreme values were measured repeatedly for the 2 types of blueberries, the difference is 1.92 times.

Jakobek *et al.*, [37] reported similar results in their research: 30.1 µMTrolox/ml was measured for black currant, 62.1 µMTrolox/ml for elderberry, and 72.4 µMTrolox/ml for chokeberry. However, it is important to note that according to Zorzi *et al.*, [40] data, within the species, there can be multiple differences between the values of the tested varieties. Similar results were reported by Moyer *et al.*, [41] during the examination of the antioxidant compounds of various berries.

Table 1 illustrates the results of the correlation between each examined parameter.

Table 1. The results of the correlation of investigated parameters

Parameters	FRAP	TEAC	TPC	TMAC
FRAP		0.021	-0.373	0.838
TEAC			0.737	-0.225
TPC				-0.393

The highest correlation (R^2 = 0,806) was observed between the total monomer anthocyanin content and the antioxidant capacity measured by the FRAP method. A strong correlation (R^2 = 0.737) can also be shown between the total polyphenol content and the antioxidant capacity measured by the TEAC method.

No correlation between the total polyphenol content (TPC) and the monomeric anthocyanin content (TMAC) could be demonstrated, which is very well supported by Figure 3. There is also no correlation between the two methods representing antioxidant capacity (R^2 = 0.021). This may be due to the very different results of the two cranberry types in the case of TPC and TMAC, which also affected the antioxidant capacities. At the same time, it should be noted that if the correlation test is performed without the cranberry with extremely low anthocyanin content and only focused on the species with high anthocyanin content, the correlation between the two antioxidant methods is high: R^2 = 0.911.

In Table 2, we gave a comprehensive evaluation based on all the parameters of the 5 examined fruit juice concentrates. For each parameter, we set up an order and evaluated the results on a scale of 1 - 5, the highest value was given the number 1, while the lowest was the number 5.

Table 2. The ranking of the fruit concentrates based on the examined parameters

Parameters	TPC	TMAC	FRAP	TEAC
Elderberry	3	1	2	2
Black currant	2	3	3	4
Chokeberry	4	4	1	3
Blueberry	5	2	4	5
Cranberry	1	5	5	1

It should be emphasized that in the case of elderberry, there are 3 measured parameters for which hold the first (TMAC) or second (FRAP, TEAC) place in the rank, while the other fruits hold the first or second rank in the case of only 1 parameter.

The cranberry shows well the correlations measured during our tests, as it is at the top for 2 parameters

(TPC, TEAC), but at the same time, it is the last in terms of both anthocyanin content and FRAP.

4. Conclusions

- Our test results draw attention to the fact that the determination of antioxidant capacity based on a single measurement method does not necessarily provide thorough information and a satisfactory explanation regarding the effect of the tested fruits on health.
- Several evaluation methods for testing antioxidant compounds can reveal in more detail the relationships between the content values of the examined fruits and their antioxidant capacities, thus providing a more accurate understanding of their effects on health.
- In the case of fruits with a high anthocyanin content, it is more appropriate to choose the FRAP method, but not for those with a low content, because in the latter case, too low an antioxidant capacity value is obtained, such as e.g. in the case of cranberries. However, in the case of fruits with a high TPC, the TEAC method based on radical scavenging can be used as a reliably well-correlated measuring method.
- In the case of comparisons, it should be considered that in the case of plant materials, especially fruits, the amount of antioxidant compounds and their composition is strongly influenced by the growing area and the given microclimate, as well as the genetically determined variety. The use of different measurement and sample preparation methods further complicates the comparison of values.
- To eliminate these problems, the use of standardized measurement methods, especially sample preparation methods, should be considered. In this way, it would be possible to choose the measurement method that most accurately shows the content values of the given fruit and the resulting antioxidant capacity, since based on the latter, we can most reliably choose the fruit species and varieties that are most suitable for solving a given health problem.

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