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BIOACTIVE COMPOUNDS IN ORGANIC APPLE JUICES ENRICHED WITH CHOKEBERRY AND GREEN TEA EXTRACT

Summary

The availability of organic fruit juices on the European market is increasing. There is also a growing range of combinations of various fruit species, as well as fruit juice supplements, such as e.g. herbal infusions, which not only enrich the juices in new flavors, but also can significantly increase their health benefits. Consumers are increasingly looking for natural, organic, high-quality products, so choosing the right combination of ingredients to ensure the health benefits of juice is extremely important. The aim of this study was to evaluate the content of selected bioactive compounds in organic apple juice enriched with chokeberry and green tea extract. Chokeberry fruit are known to be a valuable source of vitamin C and numerous polyphenols, especially anthocyanins, with high biological activity. At the same time the green tea extract is rich in phenolic acids and flavonoids. Most of these compounds are characterized by strong antioxidant properties, thus protect the body against the adverse effects of free radicals involved in pathogenesis of various diseases, i.e. heart diseases, cancers, neurodegenerative diseases, and aging-related processes. The results of the study indicate that the addition of chokeberry juice to apple juice enriched the juice with vitamin C, phenolic acids (in total) and anthocyanins, in particular 3,5-di-O-pelargonidine glycoside. At the same time the content of flavonoids (primarily quercetin and kaempferol) was lower in apple-chokeberry juice compared to the apple juice. The addition of green tea extract resulted in a significant enrichment of apple juice with phenolic acids (>2-fold) and flavonoids (>10-fold), including compounds found typically in tea leaves, such as catechin, epicatechin, epigallocatechin and epigallocatechin gallate. Organic apple-chokeberry juice and apple juice with green tea extract may be recommended as a part of a healthy diet, due to the high concentrations of antioxidants with beneficial health effects.
***Key words**: organic juice, bioactive compounds, polyphenols, anthocyanins, apple, aronia, chokeberry, green tea extract*

ZWIĄZKI BIOAKTYWNE W EKOLOGICZNYCH SOKACH JABŁKOWYCH WZBOGACONYCH DODATKIEM ARONII I EKSTRAKTU ZIELONEJ HERBATY

Streszczenie

Dostępność ekologicznych soków owocowych na europejskim rynku rośnie. Zwiększa się również wachlarz połączeń różnych gatunków owoców, a także nieowocowych dodatków do soków, m.in. naparów ziołowych, które nie tylko nadają sokom nowe walory smakowe, ale też mogą zwiększać znacząco ich wartość prozdrowotną. Konsumenci coraz częściej poszukują naturalnych, ekologicznych produktów o wysokich walorach prozdrowotnych, dlatego dobór odpowiedniej kombinacji składników, które zapewnią efekt prozdrowotny soku, jest niezwykle ważny. Celem niniejszej pracy była ocena zawartości wybranych związków bioaktywnych w ekologicznych sokach jabłkowych wzbogaconych dodatkiem aronii i ekstraktu zielonej herbaty. Owoce aronii stanowią cenne źródło witaminy C i licznych składników polifenolowych, przede wszystkim antocyjanów, odznaczających się dużą aktywnością biologiczną. Ekstrakt zielonej herbaty cechuje się ponadto bogactwem kwasów fenolowych i flawonoidów. Większość spośród wymienionych związków charakteryzuje się silnymi właściwościami przeciwutleniającymi, dzięki czemu chronią one organizm przed niekorzystnym działaniem wolnych rodników zaangażowanych w patogenezę m.in. chorób serca, nowotworów, chorób neurodegeneracyjnych, oraz w procesy związane ze starzeniem się organizmu. Wyniki przeprowadzonych badań wskazują, że dodatek soku z aronii do soku jabłkowego istotnie wzbogacił sok w witaminę C, kwasy fenolowe (ogółem) oraz antocyjany, a w szczególności glikozyd-3,5-di-O-pelargonidyny. Mniejsza była natomiast zawartość flawonoidów (przede wszystkim kwercetyny i kemferolu) w soku jabłkowo-aroniowym w porównaniu do soku jabłkowego. Dodatek ekstraktu z zielonej herbaty skutkował natomiast istotnym wzbogaceniem soku jabłkowego w kwasy fenolowe (>2-krotnie) oraz flawonoidy (>10-krotnie), w tym typowe dla naparu herbacianego związki, tj. katechinę, epikatechinę, epigalokatechinę oraz galusan epigalokatechiny. Ekologiczny sok jabłkowo-aroniowy oraz soki jabłkowe z dodatkiem ekstraktu zielonej herbaty mogą być polecane w profilaktyce zdrowotnej z uwagi na wysoką (istotnie wyższą w porównaniu do soku z jabłek) zawartość antyoksydantów o dobroczynnym wpływie na organizm.
***Słowa kluczowe**: sok ekologiczny, związki bioaktywne, polifenole, antocyjany, jabłko, aronia, ekstrakt zielonej herbaty*

1. Introduction

Fruit juice and nectar consumption in the EU reached 9.6 billion liters in 2015, which means per capita consumption of 18.9 liters per year [1]. Poland remains the key driver of fruit juices and nectars in the Eastern European contingent of the EU. At the same time, in Western Europe volume of consumption is impacted by the on-going trend towards healthy and sustainable living, thus the shift from fruit juices and nectars towards other beverages that are

perceived healthier by the consumers can be currently observed [1]. Therefore, choosing the right combination of ingredients to ensure the health benefits of juice is extremely important. Moreover, for consumers who increasingly look for natural, high-quality, sustainable products, organic foods seem to be such a sustainable choice - they perceive 'organic' as healthier, more environmentally friendly, more tasty and more authentic. They believe it arouses more trust, has a better quality, is subject to more strict controls, and is produced in a more traditional way [2]. As shown in recently published international meta-analysis study based on more than 300 original research papers, plant based foods produced according to organic farming standards are characterized by significantly higher contents of many groups of antioxidant compounds and are less frequently contaminated with pesticide residues as compared to conventionally produced foods [3].

Following high consumers' interest, the availability of organic fruit juices on the European market is increasing. There is also a growing range of combinations of various fruit species, as well as fruit juice supplements, such as e.g. herbal infusions, which not only enrich the juices in new flavors, but also can significantly increase their health benefits. The aim of this study was to evaluate the content of selected bioactive compounds in organic apple juice enriched with chokeberry and green tea extract, as potential healthier alternatives to the apple juice. Black chokeberry (*Aronia melanocarpa*) is a fruit species utilized mainly as juices, jams, purees and wine, as important food colorant or nutritional supplement. Chokeberry fruit is known to be a valuable source of vitamin C and numerous polyphenols, especially phenolic acids and anthocyanins, with high biological activity [4]. Due to the presence and the high concentrations of these bioactive components, chokeberry fruits exhibit a wide range of positive effects, such as strong antioxidant activity and potential therapeutic benefits (gastroprotective, hepatoprotective, antiproliferative or anti-inflammatory activities). They can be recommended in prevention of chronic diseases including metabolic disorders, diabetes and cardiovascular diseases [4].

At the same time the green tea extract is rich in phenolic acids and flavonoids such as catechin, epicatechin, epigallocatechin and epigallocatechin gallate [5]. Most of these compounds are characterized by strong antioxidant properties, thus protect the body against the adverse effects of free radicals involved in pathogenesis of various diseases, i.e. heart diseases, neurodegenerative diseases, and aging-related processes [5]. Several studies have reported that green tea extracts possess antioxidant, antibacterial, antiviral, anti-carcinogenic, anti-apoptotic, and anti-mutagenic functions [6].

2. Materials and methods

The research was carried out at the Warsaw University of Life Sciences. Research material consisted of (1) organic apple juice, (2) organic chokeberry juice, (3) organic apple juice enriched with chokeberry and (4) organic apple juice enriched with green tea extract. All four juices were provided by one company producing organic juices for the Polish market.

All juice samples were carefully analyzed in terms of selected important parameters of their nutritional value. All chemical analyses were carried out in the analytical laboratory of the Chair of Organic Food, Faculty of Human Nutrition and Consumer Sciences, using validated published protocols and methods. Dry matter content of the juices was determined using gravimetric method, according to the Polish standard [7]. The content of polyphenols (flavonoids and phenolic acids) in fresh juices was determined by HPLC method, with identification of individual phenolic compounds according to the Fluca and Sigma Aldrich standards with a purity of 99.98% (Shimazu equipment, USA Manufacturing Inc, USA: two pumps LC-20AD, controller CBM-20A, column oven SIL-20AC, spectrometer UV/Vis SPD-20 AV) [8]. Concentration of vitamin C (L-ascorbic acid) was determined using a spectrophotometric method according to the relevant Polish standard [9]. The method involves the oxidation of L-ascorbic acid to dehydroascorbic acid in the acidic environment using 2,6-Dichlorofenoloindofenol. This dye is reduced to the colourless form and at pH 4.2 is pink; the reaction proceeds quantitatively. The weighed juice samples were extracted in 2% oxalic acid. The solution was filtered. The filtrate was collected and then the acetate buffer (pH 4.0) and xylene were added to the sample. Organic layers were separated and upper layer was transferred to spectrometric cuvette. The light absorbance was measured with wavelength of 500 nm. The result was read from the standard curve.

Four replicates of each juice were analyzed. The results are presented as mean per 100 ml of fresh juice weight (f.w.) ± standard deviation (SD). The data were subject to a one-way analysis of variance ANOVA, followed by the parametric post-hoc Tukey's test ($\alpha = 0.05$), using Statgraphics 5.1. software (StatPoint Technologies, Inc, Warrenton, Virginia, USA). The *p*-values are given in the tables. When the result of the analysis was not statistically significant, it was designated as n.s.

3. Results and discussion

Phenolic acids and flavonoids

Results concerning the selected flavonoids and phenolic acids concentrations in the tested juices are presented in Table 1. Apples (*Malus domestica* Borkh) contain significant quantities of phenolic compounds, which are responsible for various sensory attributes of fruits and their products, such as color, bitterness and astringency [10, 11]. In the fruit, phenolic compounds are distributed in various tissues and fractions, however, epicarp is richer in most of phenolic compounds compared to the other apple tissues. Some groups of flavonoids, such as anthocyanins and flavonols (glycosides of quercetin) are almost exclusively found in epicarp. On the other hand, flavan-3-ols, representing about 60% of the total phenolic compounds in apples, are the major phenolics of epicarp and mesocarp [12].

Organic apple juice tested in the study contained 9.4 mg of phenolic acids in 100 ml, in that chlorogenic acid and p-coumaric acid. The flavonoids concentration reached at the same time 24.7 mg/100 ml and was dominated by quercetin and kaempferol D-glycoside. A chokeberry juice was the second product analyzed in the study. As already mentioned, nowadays chokeberry fruit is valued as a great source of numerous polyphenols, including anthocyanins and phenolic acids [4] considered to be important dietary antioxidants [13, 14]. Total polyphenols in chokeberry fruit were determined in the range of 690–2560 mg gallic acid equivalents (GAE) in 100 g f.w. [14-17]. The chokeberry juice tested in the study was found to contain nearly 75 mg of phenolic acids and 40 mg of flavonoids per 100 ml. The

main phenolic acids detected in this juice were p-coumaric and ferulic acid, while the most abundant flavonoids were quercetin D-glycoside and kaempferol D-glycoside. Chokeberry juice was 10 times richer in p-coumaric acid, more than 3 times richer in rutin and nearly 2 times richer in luteolin and kaempferol when compared to the apple juice. Moreover, some compounds not found in apple juice were detected in significant concentrations in the chokeberry juice (i.e. ferulic and cinnamic acid, quercetin D-glycoside, myricetin, apigenin). Addition of chokeberry juice to the apple juice significantly modified its phenolic compounds profile and enriched the juice in phenolic acids (in total). At the same time the content of flavonoids (primarily quercetin and kaempferol) was lower in apple-chokeberry juice compared to the apple juice.

A dry green tea extract was the second supplement added to the apple juice to enhance its bioactive compounds profile. Green tea is known as a rich source of phenolic acids and flavonoids, particularly catechin, epicatechin, epigallocatechin and epigallocatechin gallate [5]. This was confirmed by the results of the presented study. The tested green tea extract contained 3.54±0.06 g/100g flavonoids and 256±2.8 mg/100g phenolic acids and was especially rich in p-coumaric and gallic acid and all four previously mentioned flavonoids typically found in green tea leaves - catechin, epicatechin, epigallocatechin and epigallocatechin gallate. The addition of green tea extract to the tested apple juice resulted therefore in a significant enrichment of the juice in total flavonoids (>10-fold) and total phenolic acids (>2-fold), but especially catechin and its derivatives typically found in tea.

Anthocyanins (flavonoids)

Anthocyanins are water-soluble plant pigments that are responsible for the red/blue colour in plants, flowers, seeds and fruits [18]. Their concentrations of 0.1% up to 1.0% of dry weight can be found in a number of commonly consumed red, blue or purple fruits and vegetables such as berries (including chokeberry), blackcurrants, red grapes, plums, cherries, radishes and aubergine [19]. They are predominantly found in the skin of fruit, except for berries that contain anthocyanins in both skin and flesh. Also fruit-derived products such as red wine and juices can be rich dietary sources of anthocyanins. Anthocyanins are known to present high biological activity [4]. Acting as strong antioxidants, they exert gastroprotective, hepatoprotective, antiproliferative and anti-inflammatory properties in the body. Foods rich in anthocyanins are widely recommended in prevention of numerous non-communicable diseases. According to many studies [3] organic fruit and fruit-derived products are richer in anthocyanins compared to non-organically produced ones.

In apples, as mentioned, anthocyanins are almost exclusively found in epicarp, thus their concentration in apple fruit juices is negligible. Therefore, the addition of anthocyanin-rich chokeberry to the apple juice should ensure a significant enrichment in these valuable bioactive compounds. Results related to the anthocyanin content in the tested juices can be seen in Figure 1. The concentration of anthocyanins in the tested chokeberry juice amounted nearly to 350 mg/100ml, which was mainly due to a high concentration of 3,5-di-O-pelargonidine glycoside.

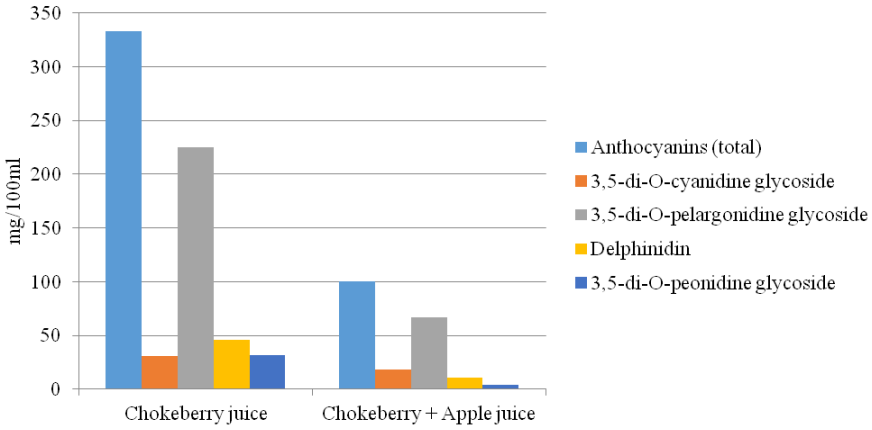
Table 1. Phenolic acids and flavonoids content in organic apple juice, organic chokeberry juice, organic apple juice enriched with chokeberry, green tea extract and organic apple juice enriched with green tea extract (mg 100 ml^{-1} of juice and mg 100 g^{-1} dry green tea extract) (average ± s.d.)*

Tab. 1. Zawartość kwasów fenolowych i flawonoidów w ekologicznym soku jabłkowym, soku z aronii, soku jabłkowym wzbogaconym sokiem z aronii, ekstrakcie zielonej herbaty i soku jabłkowym wzbogaconym ekstraktem zielonej herbaty (mg 100 ml^{-1} soku i mg 100 g suchego ekstraktu zielonej herbaty) (średnia ± s.d.)

Compounds	Apple juice	Chokeberry juice	Chokeberry + Apple juice	Green tea extract	Apple juice + Green tea extract
Phenolic acids (total)	9.4±0.3**e	74.8±1.3b	13.8±2.1d	256.0±2.8a	21.4±0.1c
Gallic acid	n.d.	n.d.	n.d.	59.9±1.1a	6.7±0.3b
Chlorogenic acid	4.6±0.2a	3.0±0.1bc	2.5±0.6c	n.d.	3.3±0.1b
Caffeic acid	n.d.	0.3±0.0c	0.2±0.0d	2.1±0.1a	0.6±0.0b
p-coumaric acid	4.7±0.0d	45.7±0.0b	7.4±1.4c	190.5±2.1a	4.6±0.0d
Ferulic acid	n.d.	22.2±1.3a	3.2±0.5c	3.5±0.2c	6.2±0.3b
Cinnamic acid	n.d.	3.5±0.1a	0.6±0.0b	n.d.	n.d.
Flavonoids (total)	24.7±0.6d	40.0±0.6c	10.0±0.5e	3542.3±58.1a	317.0±4.8b
Rutin	0.9±0.0c	3.2±0.2b	3.4±0.3b	11.3±0.5a	1.3±0.0c
Quercetin D-glycoside	n.d.	10.6±0.1a	1.8±0.2b	n.d.	n.d.
Kaempferol D-glycoside	7.7±0.3c	8.6±0.1b	1.2±0.2e	9.6±0.2a	1.9±0.0d
Myricetin	n.d.	3.5±0.1a	0.6±0.0b	n.d.	n.d.
Luteolin	2.0±0.0b	4.0±0.0a	0.7±0.0d	1.5±0.0c	4.1±0.1a
Quercetin	12.4±0.2a	4.4±0.1c	1.6±0.2d	n.d.	11.7±0.2b
Apigenin	n.d.	2.7±0.5a	0.4±0.0b	n.d.	n.d.
Kaempferol	1.7±0.1b	3.0±0.0a	0.4±0.0c	1.0±0.0bc	3.1±0.9a
Epigallocatechin	n.d.	n.d.	n.d.	392.9±18.2a	30.4±0.6b
Catechin	n.d.	n.d.	n.d.	2190.5±77.9a	178.7±6.8b
Epicatechin	n.d.	n.d.	n.d.	534.3±46.5a	54.9±4.1b
Epigallocatechin gallate	n.d.	n.d.	n.d.	401.2±16.4a	31.0±0.2b

* average ± standard deviation, ** means in a row followed by different letters are significantly different (Tukey's test, $p< 0.05$),

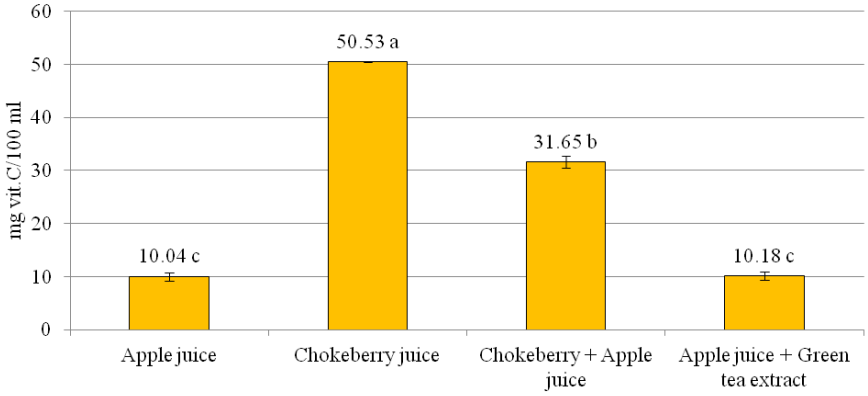
Source: own work / Źródło: opracowanie własne.



Source: own work / Źródło: opracowanie własne

Fig. 1. Anthocyanins concentration in organic chokeberry juice and organic apple juice enriched with chokeberry (mg 100 ml^{-1}) (average ± s.d.)

Rys. 1. Zawartość antocyjanów w ekologicznym soku z aronii oraz soku jabłkowym wzbogaconym sokiem z aronii (mg 100 ml^{-1} soku) (średnia ± s.d.)



Source: own work / Źródło: opracowanie własne

Fig. 2. Vitamin C concentration in organic apple juice, organic chokeberry juice, organic apple juice enriched with chokeberry and organic apple juice enriched with green tea extract (mg 100 ml^{-1}) (average ± s.d.)

Rys. 2. Zawartość witaminy C w ekologicznym soku jabłkowym, soku z aronii, soku jabłkowym wzbogaconym sokiem z aronii i soku jabłkowym wzbogaconym ekstraktem zielonej herbaty (mg 100 ml^{-1} soku) (średnia ± s.d.)

The mixture of chokeberry and apple juice contained 100 mg/100 ml anthocyanins, with the highest share of the same glycoside compound. Other anthocyanins found in both chokeberry- and apple-chokeberry juices were: Delphinidin (46.11±0.79 mg/100 ml and 11.04±0.43 mg/100 ml respectively), 3,5-di-O-peonidine glycoside (31.31±0.19 mg/100 ml and 4.05±0.09 mg/100 ml respectively) and 3,5-di-O-cyanidine glycoside (30.60±0.40 mg/100 ml and 17.85±0.34 mg/100 ml respectively).

Vitamin C

Vitamin C is another important antioxidant found in high concentrations in chokeberry fruit and in significantly lower concentrations in apple [4]. Results of the vitamin C content in the tested juices can be seen in Figure 2. Chokeberry juice tested in the presented study contained on average 50.53 mg/100ml vitamin C – 5 times more than the tested apple juice. In a consequence, supplementation of the apple juice with chokeberry juice resulted in a significant enrichment of the final product in vitamin C. At the same time addition of green tea extract to the apple juice did not increase the vitamin C concentration of the final product.

4. Summary and conclusions

The results of the study indicate that the addition of chokeberry juice to apple juice can result in enrichment of the juice with vitamin C, phenolic acids (in total) and anthocyanins, in particular 3,5-di-O-pelargonidine glycoside. At the same time the content of flavonoids (primarily quercetin and kaempferol) is lower in apple-chokeberry juice compared to the apple juice. The addition of green tea extract can result in a significant enrichment of apple juice with phenolic acids (>2-fold) and flavonoids (>10-fold), including compounds found typically in tea leaves, such as catechin, epicatechin, epigallocatechin and epigallocatechin gallate.

Most of the mentioned bioactive compounds found in chokeberry and tea leaves extracts are known for their strong antioxidant properties, thus can protect the body against the adverse effects of free radicals involved in pathogenesis of various diseases, i.e. heart diseases, cancers, neurodegenerative diseases, and aging-related processes. They also exhibit gastroprotective, hepatoprotective, antiproliferative or anti-inflammatory activities and can be recommended in prevention of metabolic disorders, diabetes and cardiovascular diseases. In addition, as already mentioned, it has been reported that green tea extracts possess antioxidant, antibacterial, antiviral, anti-carcinogenic, antiapoptotic, and anti-mutagenic activity. Thus, apple-chokeberry juice and apple juice with green tea extract, especially those organically produced, should be recommended as a part of a healthy diet.

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