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THE EFFECT OF PLANT AGE AND HARVEST TIME ON THE CONTENT OF CHOSEN COMPONENTS AND ANTIOXIDATIVE POTENTIAL OF BLACK CHOKEBERRY FRUIT

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Abstract. The fruit of black chokeberry (*Aronia melanocarpa* (Michx.) Elliott) is valuable raw material for food and pharmaceutical industries due to the content of anthocyanins, but also other components. The aim of this study was to estimate the content of dry matter, reducing sugars, vitamin C, anthocyanins and the antioxidant potential in black chokeberry fruits depending on the age of plants and the time of fruit harvest. Material for the study was collected in 2011 and 2012 from 6–7, 11–12 and 16–17-year-old plants. Fruits for analyses were harvested on 10–12 August, 29 August – 1 September, 11–13 September, 26–27 September, 11–14 October, 25–27 October. Fruits from the oldest plants contained 16–18% less anthocyanins and 8% less vitamin C, but 2.7 percentage points more dry matter than the fruits from younger plants. On the 29 August–1 September fruits achieved the maximum content of reducing sugars, vitamin C and anthocyanins, 13.7, 8.2 and 715.5 mg 100 g^{-1}, respectively. The content of reducing sugars and anthocyanins stayed unchanged until the end of October, and the content of vitamin C decreased considerably. Antioxidative potential (DPPH) ranged from 77.0 to 85.2% inhibition and it did not depend on the age of harvest time of fruits. The most valuable chokeberry fruits were obtained from plants up to 12 years of age, harvested between 29 August and 13 September.

Key words: anthocyanins, *Aronia melanocarpa,* reducing sugars, DPPH, vitamin C

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INTRODUCTION

Blackberry is a shrub from the family Rosaceae, grown for fruits which are rich in biologically active compounds with antioxidative properties. Mature fruits are raw materials for food and pharmaceutical industries [Niedworok and Brzozowski 2001, Kokotkiewicz et al. 2010]. Components responsible for pharmacological properties are mostly compounds from the group of polyphenoles, including: anthocyanins, flavonoids and phenolic acids. Blackberry fruits belong to the richest natural sources of anthocyanins [Benvenuti et al. 2004, Szajdek and Borowska 2004, Mikulic-Petkovsek et al. 2012]. Anthocyanins present in black chokeberry are a mixture of cyaniding glycosides [Oszmiański and Sapis 1988]. These compounds have strong antioxidative, anti-inflammatory, hypotensive, antibacterial and anti-tumour properties as well as they have a positive effect on the lipid profile of plasma. Anthocyanins of blackberry fruits are also beneficial for the cardiovascular system [Broncel et al. 2007, Kulling and Ravel 2008, Sikora et al. 2009, Kokotkiewicz et al. 2010].

Blackberry fruits contain substantial amounts of organic acids, macro- and micro-elements and vitamins, including vitamin C regarded as an antioxidant [Sikora et al. 2009, Kokotkiewicz et al. 2010, Mikulic-Petkovsek et al. 2012]. Benvenuti et al. [2004] showed that the content of ascorbic acid in fresh fruits is similar to blackberry and raspberry, but it is considerably lower than in fruits of red currant and especially black currant.

Chokeberry fruits are characterized by a high concentration of tannins that stabilize anthocyanins [Szajdek and Borowska, 2004] but at the same time generate the vinegary taste of fruits. Due to that taste they are rarely eaten when fresh, and rather processed, among other things, into jams and juices. The taste of fruits and preserves is also determined by the concentration of reducing sugars – glucose and fructose. Mikulic-Petkovsek et al. [2012] report that their content in chokeberry fruits is higher than in red and black currant fruits. Dry matter content in chokeberry fruits ranges from 17 to 29% [Niedworok and Brzozowski 2001, Białek et al. 2012].

The distinctive feature of chokeberry is longevity, but a considerable reduction in yield is observed already from 14–15 year of age [Kawecki and Tomaszewska 2006]. From one shrub 5 to 20 kg fruits can be collected, depending on the growing conditions and the age of plants [Kawecki and Tomaszewska 2006]. There is no information in the scientific literature concerning the quality of raw material depending on the plant age. There is also very few data concerning determination of the optimal harvest time. It is known that the degree of fruit maturity affects the quantity and quality of phenolic compounds which largely determine the antioxidative activity of fruits [Szajdek and Borowska 2004]. In Poland chokeberry fruits can be harvested from mid-August to October [Kawecki and Tomaszewska 2006]. In Sweden Jeppsson and Johansson [2000] maximum chokeberry weight achieved by 22 August but optimal harvest time determined to 8 September when also anthocyanin content reached their maximum. During the period mid-August to mid-September anthocyanin content increased by 180% [Jeppsson and Johansson 2000]. In Poland the traditionally recommended harvest time falls at the end of August and the beginning of September [Rumińska 1991]. Such early time facilitate the organization of harvesting and processing but raw material of a better quality can probably be obtained from a later harvest, especially because mature fruits remain on shrubs until first frosts [Rogowski 2010].

The aim of this study was to determine the content of dry matter, reducing sugars, anthocyanins, vitamin C, and antioxidative potential of chokeberry fruits depending on the age of plants and the fruit harvest time.

MATERIALS AND METHODS

Raw material for analyses was obtained in 2011 and 2012 from a chokeberry plantation located in the north part of the Wielkopolska region (Poland), in the village Czajcze (17º10' E, 53º16' N). The production plantation was established in 1995, 2000 and 2005 and was managed according to the principles of organic farming. The cultivar 'Galicianka' (Polish name 'Galicjanka') was grown.

The first experimental factor was the age of plantation: 6–7 years, 11–12 years, 16–17 years, and the second experimental factor was the fruit harvest time: 10–12 August, 29 August – 1 September, 11–13 September, 26–27 September, 11–14 October, 25–27 October.

The aggregate sample comprised one kilogram of fruits collected from different chokeberry shrubs at the proper age. Samples for analyses were randomly taken from the aggregate sample. They were mixed for a homogenous pulp from which weighed samples were collected for analyses of reducing sugars, the contents of anthocyanins and antioxidative potential. The sample for vitamin C content was taken and prepared separately.

The dry matter content in fruits was determined with the process of lyophilization for 3.5 days (temp. -40ºC, pressure 0.042 mBar), using a lyophiliser produced by Labconco.

The content of reducing sugars was analyzed using the DNP reagent according to the Talburt and Smith method [1987]. The weighed sample was 2 g of pulp. The analysis was performered by spectrophotometer by Bio-Rad SmartSpec Plus, at the wavelength 600 nm and compared with the standard reference curve.

The content of vitamin C was analyzed with the Tillmans method acc. to PN-A-04019 [1998], using the reagent 2,6-dichlorophenol-indophenol. Immediately after obtaining the sample, a randomly taken portion of fruits was fragmented in a ceramic mortar and after thorough crushing 1 g of chokeberry pulp was weighed. Titration was made by the same team under the same lighting conditions.

Determination of the anthocyanins content was performed with the Fuleka and Francis method [1968], which involved determination of the difference in absorbance of two buffer solutions with pH 1 and pH 4.5 at the wavelengths of 510 and 700 nm acc. to Giusti and Wrolstad [2001]. The initial material consisted of 2 g of pulp from which anthocyanins were extracted by a mixture of ethanol with HCl (85:15 0,1 N HCl). The results of the study was given for cyanidin-3-glucoside.

Antioxidative potential was examined using the synthetic radical DPPH (1,1-difenylo-2-pikrohydrazyl), and the wave absorbance was measured at $\lambda = 517$ nm based on the method described by Sanchez-Moreno et al. [1998]. The initial material consisted of 2 g of pulp which was treated with methanol. Antioxidative activity was expressed as the percent of inhibition.

All the analyses were performed in three replications. The results were worked out statistically with the analysis of variance, using the software ANALWAR-5.2-FR. Significance of differences were evaluated with Tukey's test at $p \leq 0.05$. Since the effect of interaction of experimental factors on the value of any of the evaluated characters was not statistically proved, the effect of age and the effect of the harvest time on the quality traits of the chokeberry fruits were described separately. Correlation coefficients between the assessed features were calculated using the program Excel 2010.

RESULTS

The course of the weather in the years of the study during sample collecting was generally similar (tab. 1). The exception was heavy rainfalls that occurred in 2011 between 10 and 20 August, hence in the period of the first harvest of chokeberry fruits. Moreover, September and October in 2011were respectively by 0.9 and 1.0°C warmer than in 2012.

Table 1. Mean daily air temperature and rainfall in months of sample collection (acc. to the Experimental Station in Bobrowniki)

Date	Air temperature (C°)		Rainfall (mm)	
	2011	2012	2011	2012
1–10 August	18.7	19.1	25.9	26.9
11–20 August	18.1	18.0	82.8	4.2
21–31 August	17.8	17.9	17.3	26.3
Mean/Total	18.2	18.3	126.0	57.4
1–10 September	15.2	15.2	13.8	1.5
11–20 September	15.4	13.8	16.2	14.6
21–30 September	12.2	11.0	0.6	3.1
Mean/Total	14.3	13.4	30.6	19.2
1–10 October	12.5	9.9	10.0	17.0
11–20 October	5.9	8.3	3.6	1.1
21–31 October	7.1	4.9	2.2	3.3
Mean/Total	8.6	7.6	15.8	21.4

When comparing the mean values and their standard deviations, it should be stated that the contents of dry matter, reducing sugars and anthocyanins in the analyzed fruits were similar in both years of the study (tab. 2). The content of vitamin C in turn was higher in the second year of the study than in the first, whereas the antioxidative potential was higher in the first year of the study.

Table 2. Mean content of dry matter, some components in fresh matter of chokeberry fruits and on their antioxidative potential in the years of study (irrespective of experimental factors)

Year	Dry matter (%)	Reducing sugars (mg 100 g^{-1})	Vitamin C (mg 100 g^{-1})	Anthocyanins (mg 100 g^{-1})	Antioxidative potential DPPH (% of inhibition)
2011	24.5 ±2.2	8.8 ±0.8	7.4 ±0.41	678.4 ±96.2	83.2 ±3.6
2012	23.8 ±2.0	8.0 ±0.9	8.3 ±0.43	741.5 ±162.1	76.2 ±9.9

Age of plants had a significant effect on the content of all assessed components, but it had no effect on the antioxidative potential of chokeberry fruits (tab. 3). Fruits from the oldest plants (16–17 years) accumulated more dry matter than fruits from younger plants. Fruits from 6–7-year-old plants accumulated significantly more sugars than fruits from 11–12-year-old plants. The content of vitamin C and anthocyanins in fruits from 6–7-year-old and 11–12-year-old plants was similar, and in fruits from 16–17-year-old plants it was significantly lower.

Table 3. Effect of plant age on the content of dry matter, some components in the fresh matter of chokeberry fruits and on their antioxidative potential (mean 2011–2012)

Age of plants (years)	Dry matter (%)	Reducing sugars (mg 100 g^{-1})	Vitamin C (mg 100 g^{-1})	Anthocyanins (mg 100 g^{-1})	Antioxidative potential DPPH (% of inhibition)
6–7	23.2b	8.6a	8.1a	765.8a	79.3n.s.
11–12	23.5b	8.2b	8.0a	734.6a	77.1n.s.
16–17	26.0a	8.4ab	7.4b	629.5b	82.7n.s.

Values with different letters in a column differ statistically ($p \leq 0.05$), n.s. – statistically non-significant

The dry matter content increased along with fruit maturation (tab. 4) and statistically significant differences were noted between the first and each next harvest time, and between the second and the fourth and each next harvest time. From the end of September to the end of October there was no increase in dry matter content in fruits. The sugar content in fruits increased substantially to 11–13 September, and then it remained at the same level to the last harvest time. The highest content of vitamin C was recorded in fruits harvested at 29 August – 1 September. Each successive delay of harvest, particularly after 11–13 September, resulted in a considerable decrease in the content of vitamin C. In the period between the first and second harvest time there was a significant increase in the content of anthocyanins and this level, only in some downward tendency, remained until the end of October.

Table 4. Effect of harvest time on the content of dry matter, some components in fresh mass of chokeberry fruits and on their antioxidative potential (mean 2011–2012)

Age of plants (years)	Dry matter (%)	Reducing sugars (mg 100 g^{-1})	Vitamin C (mg 100 g^{-1})	Anthocyanins (mg 100 g^{-1})	Antioxidative potential DPPH (% of inhibition)
10–12 August	21.0c	6.8c	9.5c	503.7b	78.6 n.s.
29 Aug–1 Sept	23.5b	8.2ab	13.7a	715.5a	85.2 n.s.
11–13 September	24.8ab	9.0a	11.2b	802.9a	80.5 n.s.
26–27 September	25.1a	8.9a	6.7d	753.5a	77.1 n.s.
11–14 October	25.3a	8.8ab	3.7e	793.8a	79.7 n.s.
25–27 October	25.7a	8.8ab	2.3f	690.3a	77.0 n.s.

Values with different letters in a column differ statistically ($p \leq 0.05$), n.s. – statistically non-significant

No significant differences were found between the antioxidative potential of fruits from plants at different age and depending on different harvest times. The results only indicate a tendency to a higher antioxidative potential of fruits harvested at the second date, i.e. 29 August – 1 September.

Table 5. Correlation coefficients between studied components of chokeberry fruits and their antioxidative potential

Character	Dry matter	Reducing sugars	Vitamin C	Anthocyanins	Anti-oxidative potential
Dry matter	1.00	0.66*	-0.46*	0.16	0.24
Reducing sugars	–	1.00	-0.34	0.56*	0.28
Vitamin C	–	–	1.00	-0.03	0.20
Anthocyanins	–	–	–	1.00	-0.15
Antioxidative potential	–	–	–	–	1.00

* – statistically significant values ($p \leq 0.05$)

Correlation coefficients values show that along with an increase in dry matter content the reducing sugars increased also but at the same time the content of vitamin C decreased (tab. 5). Moreover when the content of reducing sugars increased then the content of anthocyanins increased as well. No relationship was observed between the antioxidative potential and the content of examined components of chokeberry fruits.

DISCUSSION

Like the most species from the family Rosaceae, chokeberry accumulates sugar in different forms in fruits, but the proportion of monosaccharides is always substantial [Mikulic-Petkovsek et al. 2012]. Mean content of reducing sugars was 8.4% and that level should be regarded as average, because e.g. chokeberry fruits studied by Mikulic-Petkovsek et al. [2012] contained two times less of those sugars, but Kulling and Rawel [2008] and Białek et al. [2012] report the values that range from 13.0 to 17 mg 100 g^{-1}. An increase in the content of reducing sugars affected dry matter accumulation in fruits, which is indicated by a significant correlation between those traits. Dry matter content in the studied fruits ranged from 20.0 to 27.7%, thus it also stayed within the range given by other authors [Niedworok and Brzozowski 2001, Białek et al. 2012].

The content of vitamin C stayed within the ranges given by other authors, e.g. Sikora et al. [2009] – 2.4 mg·100 g^{-1}, Benvenuti et al. [2004] and Gryszczyńska et al. [2011] – 13.1 mg·100 g^{-1}. Black currant fruits contain 10 times more of that vitamin than chokeberry fruits [Benvenuti et al. 2004]. In the results of the present study, first a considerable increase in vitamin C is noted and then, its intensive decrease after mid-September. Accumulation of ascorbic acid takes place in the period of maturation of vegetables and fruits, hence chokeberry fruits from the first harvest should be regarded as not mature enough, whereas in fruits harvested at the end of September and later, probably transformations of ascorbic acid decomposition already occurred [Yahia et al. 2001]. The content of vitamin C depends among other things on the light intensity during growth period [Lee and Kader 2000]. This parameter was not analyzed in the present study, but each successive date of fruit harvest undoubtedly fell in the period of lower sunlight intensity.

High content of anthocyanins is the character that differentiates the fruits of *A. melonocarpa* species from fruits of other cultivated and wild shrubs, e.g. *Rubus fruticosus* L., *R. ideaus* L., *Ribus rubrum* L. or *R. nigrum* L. [Benvenuti et al. 2004]. The level of anthocyanins in the analyzed chokeberry fruits of cv. 'Galicianka' should be assessed as very high, as the values given most often in the literature stay within the range 400–500 mg·100 g^{-1} [Strik et al. 2003, Benvenuti et al. 2004, Skupień and Oszmiański 2007, Jakobek et al. 2012]. Jakobek et al. [2012] indicated that the properties of genotype in the wide range reaching 100%, affect the accumulation of anthocyanins in chokeberry fruits. Of the chokeberry cultivars assessed by those authors, the fruits of the cultivar 'Galicianka' contained less anthocyanins than cultivars 'Viking' or 'Nero'. Jeppsson [2000] indicated that nitrogen fertilization resulted in a decrease in concentration of anthocyanins, and the raw material analyzed in the present study derived from an organic farming field where mineral nitrogen was not used.

In Sweden anthocyanin content reached maximum in the 8 of September [Jeppsson and Johansson 2000]. This was similar in our conditions. Interestingly, the level of anthocyanins obtained in September also remained in October, showing only a small downward tendency at the end of that month. The company buying fruits from the plantation in question also made determinations of anthocyanins in the years 2006, 2007, 2008 and 2009 and their contents amounted to 565, 477, 600 and 1064 mg 100 g^{-1} of fresh matter, respectively [Rogowski 2010]. In 2006–2008, the analyzed material was collected during fruit buying in bulk, i.e. between 15 and 25 August. In 2009, the material derived from fruits collected between 15 and 25 September [Rogowski 2010]. Additionally analyses made in 2006–2009 showed that total precipitations occurring in the area of the study did not affect the content of anthocyanins in chokeberry fruits [Rogowski 2010].

Antioxidative ability of chokeberry fruits can be compared with the extract from green tea (89.9%) and instant coffee (81.1%) [Zych and Krzepiłko 2010]. Analyzed fruits showed a very high antioxidative potential, irrespective of the harvest time and plant age. Similar values of DPPH inhibition (80–90%) were obtained in chokeberry snacks by Gramza-Michałowska and Człapka-Matyasik [2011]. However, a lower DPPH inhibition was shown in some studies, e.g. Skupień and Oszmiański [2007] received the value 38.1%. The study by Piasek [2010] indicated higher antioxidative properties of fruits derived from later harvests, i.e. from the end of September, in relation to the harvest from the beginning of the month.

The authors of this paper regard that the data about a decrease in values of some quality characters of fruits from the oldest plants, that is 16–17-year-old, is very important information from this study. This relationship was already observed on the plantation in question before, because in 2009 fruits from 5-year-old and 10-year-old plants contained by 25 and 14% more anthocyanins, respectively, than fruits from 15-year-old plants [Rogowski 2010]. Thus the results prove that the highest content of anthocyanins and vitamin C in fruits from 6–7-year old and 11–12-year-old plantations coincide with the peak of productivity that usually occur when the plants are 7–10 years of age [Rusnak 2013].

CONCLUSIONS

1. Analyzed chokeberry fruits were characterized by a high content of anthocyanins and high antioxidative potential. The antioxidative potential was not determined by the age of plants or the date of fruit harvest.

2. In fruits from 16–17-year-old plants, an increase in the content of dry matter and a decrease in the content of vitamin C and anthocyanins were determined in comparison with younger plants.

3. The fruits harvested between 29th August and 13th September contained the highest level of reducing sugars, vitamin C and anthocyanins. Fruits harvested later, until the end of October, still had a high content of reducing sugars and anthocyanins, but a low content of vitamin C.

4. The most valuable chokeberry fruits are obtained from plants up to 12 years of age, harvested in the first part of September.

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WPŁYW WIEKU ROŚLIN I TERMINU ZBIORU OWOCÓW NA ZAWARTOŚĆ WYBRANYCH SKŁADNIKÓW I POTENCJAŁ ANTYOKSYDACYJNY ARONII CZARNOOWOCOWEJ

Streszczenie. Owoce aronii czarnoowocowej (*Aronia melanocarpa* (Michx.) Elliott), ze względu na zawartość antocyjanów, ale także innych składników, są cennym surowcem spożywczym i farmaceutycznym. Celem pracy było określenie zawartości suchej masy, cukrów redukujących, witaminy C, antocyjanów oraz potencjału antyoksydacyjnego w owocach aronii w zależności od wieku roślin i terminu zbioru owoców. Materiał do badań pobierano w latach 2011 i 2012 z roślin 6–7-, 11–12- i 16–17-letnich. Owoce do analiz zbierano 10–12 sierpnia, 29 sierpnia – 1 wrzesńa, 11–13 września, 26–27 września, 11–14 października, 25–27 października. Owoce z najstarszych roślin zawierały o 2,7 punktów procentowych więcej suchej masy niż owoce z roślin młodszych, ale o 16–18% antocyjanów oraz o 8% mniej witaminy C. W terminie 29 sierpnia–1 września owoce osiągały maksymalną zawartość cukrów redukujących, witaminy C oraz antocyjanów, odpowiednio 715,5, 13,7 i 13,7 mg 100 g^{-1}. Zawartość cukrów redukujących i antocyjanów pozostawała bez zmian do końca października, a zawartość witaminy C znacząco spadała. Potencjał antyoksydacyjny wynosił od 77,0 do 85,2% inhibicji i nie zależał ani od wieku, ani od terminu zbioru owoców. Najbardziej wartościowe owoce aronii zebrano w terminie 29 sierpnia – 13września z roślin w wieku do 12 lat.

Słowa kluczowe: antocyjany, *Aronia melanocarpa,* cukry redukujące, DPPH, witamina C

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