ACTA SCIENTIARUM POLONORUM *Acta Sci. Pol., Technol. Aliment. 6(2) 2007, 49-55*

CHANGES IN ANTIOXIDANT ACTIVITY OF BLACK CHOKEBERRY JUICE CONCENTRATE SOLUTIONS DURING STORAGE

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Abstract. The aim of the study was to determine the effect of pasteurization and storage conditions of black chokeberry juice concentrate solutions on their antioxidant activity. The study investigated the effect of solution pH (3-5), temperature (10-30°C) and storage time (0-20 days), as well as oxygen availability. Solutions were obtained by diluting concentrate with buffers according to McIlvaine to anthocyanin contents of 20 mg%. They were next pasteurized and stored in the dark under conditions specified in the experimental design. Antioxidant activity was determined colorimetrically using the cation-radical of 2'2-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS). Antioxidant activity decreased in all samples, both after pasteurization and after storage. The biggest effect on the lowering of antioxidant activity was found for oxygen availability rate during pasteurization and storage, and for storage temperature.

Key words: antioxidant activity, black chokeberry juice, storage, ABTS cation-radical

INTRODUCTION

In recent years consumers have shown increasing interest in foodstuffs rich in natural ingredients, including also natural pigments. Thus, for processing purposes raw materials with original sensory attributes are searched for, making it possible to obtain products with a rich, natural taste and colour. In this respect black chokeberry (*Aronia melanocarpa*), containing a lot of flavonoids, including anthocyanins with probiotic properties proved to be especially interesting [Valcheva-Kuzmanova et al. 2004, Oszmiański and Wojdylo 2005]. Its juice is used in the production of drinks, nectars, wines, liqueurs and other products, providing them with a characteristic, rich taste and a beautiful colour.

Black chokeberry is a fruit especially rich in polyphenols. According to literature data, there is a high correlation between contents of polyphenols in fruits and their antioxidant activity [Bermudez-Soto and Tomas-Barberan 2004, Cevallos-Casals et al. 2006]. In recent years a general trend has been observed to search for raw materials and

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food products either being sources of natural antioxidants or exhibiting other probiotic activity. Numerous studies confirm an advantageous effect of a diet rich in those ingredients on the organism, by lowering blood pressure, improving blood lipid profile, decreasing cancer incidence, enhancing the function of the alimentary tract; moreover, such a diet promotes also the treatment of arthritis, atherosclerosis, cataract and many other diseases and disorders [Utsunomiya et al. 2002, Kris-Etherton et al. 2002, Lucas et al. 2004, Mateos et al. 2005].

During fruit storage and processing polyphenol content and thus also antioxidant activity decrease. However, at the same time there are some reports on an increase of polyphenol concentration as a result of thermal processing of raw material, e.g. in broccoli, peppers, spinach or beans [Turkmen et al. 2005]. The most valuable source of natural antioxidants are fresh fruit and vegetables, since during juice production such procedures as fruit comminution, thermal processing, pressing, clarification, filtration and storage, may considerably lower antioxidant activity of the product in relation to that of the raw material [Borowska et al. 2005, Dietrich et al. 2004]. Thus, advantageous processing and storage conditions need to be provided for processed fruit products, e.g. by cutting off oxygen access and light, or lowering storage temperature in order to preserve valuable ingredients of the applied raw material also in processed products.

The aim of the study was to determine the effect of pH value, time and temperature, as well as oxygen availability during pasteurization and storage of black chokeberry juice concentrate solutions on their antioxidant activity.

MATERIALS AND METHODS

Material. The experimental material in the study was black chokeberry juice concentrate produced by O.K. Owocowe Koncentraty Sp. z o.o. in Przeworsk, with initial extract concentration of 66% and anthocyanin concentration of 588 mg%. Concentrate was diluted with buffers according to McIlvaine (Na_2HPO_4 – ascorbic acid) with pH of 3, 4 and 5, producing solutions with anthocyanin concentration of 20 mg%. Samples of solutions of 10 ml each were poured to glass vials, sealed, pasteurized (85°C, 10 min), cooled to room temperature and stored under conditions defined in the experimental design, with measurements being taken before storage, and after 10- and 20-day storage.

Description of the experiment. The following parameters were adopted as experimental factors: pH of solutions (3, 4, 5), storage temperature (10, 20, 30°C), storage time (0, 10, 20 days) and oxygen availability (aerobic or facultative anaerobic conditions). Oxygen availability was regulated by a degree to which the vial was filled with the analysed solution. Vials with a capacity of 10 and 20 ml were used, into which 10 ml solution were poured, thus generating facultative anaerobic conditions in the smaller vials and aerobic in the bigger vials. Samples were stored in the dark for 10 or 20 days in electric thermostats at 10, 20 and 30°C.

Analytical methods. Antioxidant activity was determined colorimetrically using the cation-radical of 2,'2-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) [Re et al. 1999]. The ABTS cation-radical was generated in a $K_2O_8S_2$ solution, while for dilutions a phosphate buffer PBS with pH = 7.4 (NaCl, KH_2PO_4, Na_2HPO_4, KCl) was used. Absorbance was measured at 734 nm and results were expressed in equivalents of µmol Trolox/cm^3.

Results were subjected to the analysis of variance for principal effects using Statistica 7.1 software.

RESULTS AND DISCUSSION

Antioxidant activity in solutions of black chokeberry juice concentrate was determined before and after pasteurization and after 10- and 20-day storage. Changes in antioxidant activity of the analysed solutions were observed both during pasteurization and storage. They were dependent primarily on oxygen availability and storage temperature. As a result of pasteurization antioxidant activity of the analysed solutions decreased by 1.2-5.8% in case of samples heated under facultative anaerobic conditions and by 6.0-15.8% in case of samples pasteurized under aerobic conditions (Table 1).

Table 1. Changes in antioxidant activity of black chokeberry juice concentrate solutions during pasteurization
Tabela 1. Zmiany aktywności antyutleniającej w roztworach koncentratu soku aroniowego podczas pasteryzacji

pH	Antioxidant activity – Aktywność antyutleniająca (TEAC) µmol Trolox/1 cm^3	
	before pasteurization przed pasteryzacją	after pasteurization po pasteryzacji
	Facultative anaerobic conditions – Warunki względnie beztlenowe	
3	15.5	14.6
4	15.9	15.5
5	16.5	16.3
	Aerobic conditions – Warunki tlenowe	
3	15.0	14.1
4	15.2	13.7
5	15.1	12.7

During storage antioxidant activity was observed to decrease in all cases. Under facultative anaerobic conditions after 20 days of sample storage antioxidant activity decreased by 7-12% at 10°C, 12-15% at 20°C and by 16-35% at 30°C (Table 2). Under aerobic conditions these changes were much bigger and ranged from 63 to 76% after 10 days and from 64 to 79% after 20-day storage (Table 3). The decrease of antioxidant activity increased with increasing storage temperature. Analysis of variance of principal effects showed a significant effect of all analyzed factors on changes in antioxidant activity ($p \leq 0.05$). Numerous publications confirm changes in antioxidant activity during heating and storage of plant products. Heating of fresh noni juice (*Morinda citrifolia* L.) for 1 h at 75°C resulted in a slight increase of free radical scavenging activity, while at 65°C no significant changes were reported [Yang et al. 2007]. In turn, when storing the juice for 30 days at the temperature of 4°C or 24°C, free radical scavenging

Table 2. Changes in antioxidant activity of black chokeberry juice concentrate solutions during storage in facultative anaerobic conditions
Tabela 2. Zmiany aktywności antyutleniającej w roztworach koncentratu aroniowego podczas przechowywania w warunkach względnie beztlenowych

Storage time days Czas przechowywania dni	Storage temperature Temperatura przechowywania °C	Antioxidant activity Aktywność antyutleniająca (TEAC) µmol Trolox/1 cm^3			Lowering of antioxidant activity Obniżenie aktywności antyutleniającej %*		
		pH = 3	pH = 4	pH = 5	pH = 3	pH = 4	pH = 5
0	–	14.6 ±0.18	15.5 ±0.19	16.3 ±0.14		–	
10	10	13.8 ±0.22	15.1 ±0.26	15.8 ±0.11	5.5	2.9	2.9
	20	13.3 ±0.22	14.6 ±0.18	14.5 ±0.38	8.7	5.9	10.9
	30	13.0 ±0.22	14.5 ±0.52	11.8 ±0.30	10.8	6.6	27.4
20	10	13.0 ±0.10	14.4 ±0.14	14.4 ±0.43	10.6	7.1	12.0
	20	12.3 ±0.72	13.5 ±0.14	13.8 ±0.41	15.7	12.9	15.3
	30	12.2 ±0.27	13.0 ±0.23	10.5 ±0.10	16.0	16.0	35.2

*Changes (%) were calculated in relation to original samples after pasteurization.
± – standard deviation for three replications.
*Procentowe zmiany obliczono w odniesieniu do prób wyjściowych po pasteryzacji.
± – odchylenie standardowe z trzech powtórzeń.

Table 3. Changes in antioxidant activity of black chokeberry juice concentrate solutions during storage in aerobic conditions
Tabela 3. Zmiany aktywności antyutleniającej w roztworach koncentratu aroniowego podczas przechowywania w warunkach tlenowych

Storage time days Czas przechowywania dni	Storage temperature Temperatura przechowywania °C	Antioxidant activity Aktywność antyutleniająca (TEAC) µmol Trolox/1 cm^3			Lowering of antioxidant activity Obniżenie aktywności antyutleniającej %*		
		pH = 3	pH = 4	pH = 5	pH = 3	pH = 4	pH = 5
0	–	14.1 ±0.34	13.7 ±0.07	12.7 ±0.23		–	
10	10	5.1 ±0.11	4.9 ±0.09	4.7 ±0.09	63.8	64.2	63.0
	20	4.6 ±0.11	4.4 ±0.05	4.2 ±0.06	67.4	67.8	67.0
	30	3.5 ±0.03	3.3 ±0.07	3.0 ±0.09	75.1	75.9	76.4
20	10	4.6 ±0.06	4.6 ±0.03	4.5 ±0.06	67.4	66.5	64.6
	20	4.2 ±0.03	4.1 ±0.02	3.9 ±0.02	70.2	68.5	69.3
	30	3.1 ±0.06	2.8 ±0.02	2.6 ±0.06	78.0	79.6	79.6

Explanations as in Table 2.
Objaśnienia jak w tabeli 2.

capacity was found to decrease by 36 and 83%, respectively. According to literature data carrot (*Daucus carota*) proved to be a source of stable natural antioxidants, as the antioxidant activity of its extracts did not change during 15-day storage at 5 and 25°C, or during heating (15 min, 100°C) [Arabshahi et al. 2007]. High stability or increased antioxidant activity of foodstuffs during thermal processing results from the formation of compounds exhibiting antioxidant properties [Nicoli et al. 1999, Talcott et al. 2000].

Changes in antioxidant activity in solutions of black chokeberry concentrate were also connected with pH value, especially in samples stored at 30°C, in which the biggest drop in activity was recorded at pH 5.0. According to Azizah et al. [1999], the value of pH of the solution affects antioxidant activity of products, as it has an effect on the type of compounds extracted from the raw material and their changes during heating or storage. I this study, anthocyanin content decreased together with the increase of pH value during pasteurization and storage. Anthocyanins showed the highest stability at pH = 3.0 or lower, when occur in flavylium cation form, and the pH increase above this level resulted in a growing degradation of colorants, especially during heating or storage at non-refrigeration temperatures [Dyryby et al. 2001, Cevallos-Casals and Cisneros--Zevallos 2004]. Anthocyanins are interested not only from their colouring effect, but also from their antioxidising activity, especially flavylium cation is known to be more active as a free radical scavenger [Garcia-Viguera and Bridle 1999]. Therefore degradation of them reflected in decrease of antioxidant capacity of fruits and their products.

CONCLUSIONS

1. During pasteurization and storage of black chokeberry juice concentrate solutions their antioxidant activity decreased.
2. The decrease of antioxidant activity under facultative anaerobic conditions amounted to 7-35%, while under aerobic conditions it was 64-79%, depending on storage temperature.
3. The decrease of antioxidant activity enhanced together with the increase of solutions pH value, during pasteurization and storage.

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ZMIANY AKTYWNOŚCI ANTYUTLENIAJĄCEJ ROZTWORÓW KONCENTRATU SOKU ARONIOWEGO PODCZAS PRZECHOWYWANIA

Streszczenie. Celem pracy było określenie wpływu warunków pasteryzacji i przechowywania roztworów koncentratu soku aroniowego na ich aktywność antyutleniającą. Badano wpływ wartości pH roztworów (3-5), temperatury (10-30°C) i czasu przechowywania (0-20 dni) oraz dostępności tlenu. Roztwory otrzymywano przez rozcieńczenie koncentratu buforami według McIlvaine'a do zawartości antocyjanów 20 mg%, następnie pastery-

zowano i przechowywano w ciemności w warunkach zgodnych z planem doświadczenia. Aktywność antyutleniającą oznaczano kolorymetrycznie z użyciem kationorodnika kwasu 2'2-azynobis-3-etylobenzotiazolin-6-sulfonowego (ABTS). We wszystkich próbach, zarówno po pasteryzacji, jak i po przechowywaniu, stwierdzono spadek aktywności przeciwutleniającej. Największy wpływ na zmniejszenie aktywności antyutleniającej miał stopień dostępności tlenu w czasie pasteryzacji i przechowywania oraz temperatura przechowywania.

Słowa kluczowe: aktywność antyutleniająca, sok aroniowy, przechowywanie, kationorodnik ABTS

Accepted for print – Zaakceptowano do druku: 6.04.2007

For citation – Do cytowania: Walkowiak-Tomczak D., 2007. Changes in antioxidant activity of black chokeberry juice concentrate solutions during storage. Acta Sci. Pol., Technol. Aliment. 6(2), 49-55.