ACTA SCIENTIARUM POLONORUM Acta Sci. Pol., Technol. Aliment. 6(4) 2007, 75-82

THE CHANGES OF ANTIOXIDANT PROPERTIES IN HIGHBUSH BLUEBERRIES (*VACCINIUM CORYMBOSUM* L.) DURING FREEZING AND LONG-TERM FROZEN STORAGE*

Iwona Ścibisz, Marta Mitek
Warsaw University of Life Sciences – SGGW

Abstract. The aim of this work was to investigate the effect of freezing and long-term frozen storage on total phenolic, total anthocyanin contents and antioxidant activity of highbush blueberries (*Vaccinium corymbosum* L.). In addition, a HPLC method was developed for the determination of individual anthocyanins and chlorogenic acid contents. Blueberries were frozen at –18°C and –35°C and were stored at these temperature conditions for six months. Derivatives of malvidin and delphinidin accounted for 61% and 22% of total anthocyanin contents in fruit, respectively. Measurements of the antioxidant activity and bioactive compounds contents of blueberries showed there were no significant differences between fresh and frozen fruits. Also, at the end of frozen storage period, antioxidant activity remained significantly unchanged compared with the values measured just after the freezing process.

Key words: highbush blueberry, antioxidant, freezing, frozen storage

INTRODUCTION

A large number of epidemiological investigations prove that consumption of fruits and vegetables is associated with lower risk of several oxidative stress diseases, including cardiovascular disease, cancer and stroke [Ames et al. 1993]. These health benefits are ascribed to phytochemicals in fruit and vegetables such as polyphenolics, carotenoids, vitamin C. Recent studies have found that blueberries compared to other fruits and vegetables have the highest amount of antioxidant activity [Wang et al. 1996].

Highbush blueberries are members of the Ericaceae family and are native to North America. Blueberries are extensively cultivated in the United States and more recently

*Research work financed by the Ministry of Education and Science in the years 2004-2006 (grant 2P06T05826).

Corresponding author – Adres do korespondencji: Dr inż. Iwona Ścibisz, Department of Food Technology of Warsaw University of Life Sciences – SGGW, Nowoursynowska 159 C, 02-776 Warsaw, Poland, e-mail: iwona_scibisz@sggw.pl

are also successfully grown in Australia, New Zealand, Chile and across Central Europe including Poland. In the year of 2004 Polish growers produced 3.9 thousand tons of highbush blueberries of which majority were marketed as fresh. Highbush blueberry varieties have very large berries compared to bilberries. The weight of fruits of highbush cultivars are between 1 and 3 g/fruit [Gough 1991, Wyniki... 2005].

The highbush blueberry harvest season in Central Europe is short, lasting from mid-July to mid-September. Freezing of blueberries will increase flexibility for consumers by extending the length of time in which fruits are available. Freezing and frozen storage is one of the best methods of preserving because the quality and antioxidant properties of frozen fruits is close to fresh berries. The literature provides many studies about the effects of freezing and long-term frozen storage on the retention of antioxidant in different kinds of berries [Kmiecik et al. 1995, Gonzàlez et al. 2003, Lohachoompol et al. 2004]. Little is known, however, about the effect of this process on the content of phenolic compounds of highbush blueberry.

The aim of this work was to investigate how freezing and long-term storage can affect the bioactive compounds in blueberries such as anthocyanins and chlorogenic acid.

MATERIALS AND METHODS

Blueberries used in this study were grown at plantation in Central Poland and harvested at commercial maturity stage in 2003. After harvest, fruits were washed, packaged in polyethylene bags, frozen in domestic freezers at –18°C and –35°C and stored for six months. The temperature of the blueberries centre was measured by digital thermometer. Before analysis berries were thawed at room temperature (22°C, 1.5 h). Analysis were conducted on fresh, just after freezing and on berries after two, four and six months of storage. The samples were analysed for total phenolic, total anthocyanins individual anthocyanins, chlorogenic acid and antioxidant activity.

Content of total phenolic in acetone extract was determined using the Folin-Ciocalteu method [Peri and Pompei 1971], with absorbance measured at 700 nm. The results were expressed as milligrams chlorogenic acid in 100 g fruits. The total anthocyanin content was determined using the pH-differential method as described by Swain and Hillis [1959]. The content of individual anthocyanins was determined by reversed-phase HPLC method using a Shimadzu apparatus equipped with a UV/VIS detector. A Luna RP-18 (5 μm) column from Phenomenex was used. The mobile phase consisted of 10% aqueous formic acid (A) and acetonitrile (B). The flow rate was 1 ml/min and elution profile was used: 5.5% phase B in A, then 9% B in 7 min, 11% B in 18 min, 14% B in 21 min, 22% B in 26 min, 30% B in 30 min and 5.5% B for 7 min. Chlorogenic acid content was performed using the same HPLC conditions as described above, with absorption at 320 nm recorded for chlorogenic acid and 520 nm for anthocyanins. Chlorogenic acid was identified according to retention time by comparing with the standard. Standards of anthocyanins available from previous work [Cho et al. 2004, Gao and Mazza 1994, Kalt et al. 1999] were used to identify anthocyanins. Total antioxidant activity was measured using the ferrylmyoglobin/ABTS spectrophotometric assay [Miller and Rice-Evans 1996]. Trolox, a water-soluble tocopherol analog, was used as an antioxidant standard.

The statistical analyses were performed with Statgraphics Plus 4.1. To assess the effect of freezing, a one factor ANOVA model was used, compared fresh blueberries, just frozen berries at –18°C and at –35°C. The influence of time and storage temperature was determined by two-way analysis of variance. Significant differences (at p = 0.05) between the means were determined by Tukey method. Results of individual anthocyanin content was not estimated statistically. Mean of analysis of two replications of field samples create the individual data of anthocyanin content.

RESULTS AND DISCUSSION

The total phenolic content in blueberry in this study was much higher that the value reported by Prior et al. [1998], but very comparable to the quantity reported by Connor et al. [2002] and Moyer et al. [2002]. No significant changes in total phenolic content of highbush blueberries were found after freezing (Table 1). At the end of frozen storage period, total phenolic contents remained practically unchanged compared to the contents measured in berries just after freezing process (Table 2). The insignificant changes of total phenolic content observed during the freezing process and frozen storage of blueberry are consistent with the results reported by Ancos et al. [2000] for raspberry. Greatest losses of total phenolic (50%) in sweet cherries were monitored at six moths of frozen storage at –23°C [Chaovanalikit and Wrolstad 2004].

The anthocyanins analysis showed that blueberries contain all of the common anthocyanidin aglycons except for pelargonidin (Table 1). Quantitatively, the malvidin and delphinidin glycosides were present in the largest amount, accounted for 61% and 22% of the total anthocyanins, respectively. Derivates of peonidin were the least abundant (1%). In our studies only negligible differences in anthocyanins contents were found between the fresh and frozen fruits. The anthocyanins pigment profiles appear to be very similar, however analysis indicated slight increases of delphinidin and cyanidin glycosides and peonidin-3-galactoside contents in just frozen fruits. It is most probable that the anthocyanin in frozen fruits becomes more easily extractable. This might be due to degradation of cell structures in fruits. Previously, an increase in anthocyanins content during freezing has been reported in raspberry by Ancos et al. [2000].

Kmiecik et al. [1995] reported that the amount of anthocyanins in frozen fruits depended on the fruit species and method of thawing. Thawing is a crucial step for the overall quality of the frozen food. On this process, compounds which under normal conditions are kept apart in the intact cell, can mix and possibly react with each other. The highest anthocyanins content was assessed in fruit thawed at 2-4°C [Kmiecik et al. 1995]. Nevertheless, the differences of anthocyanin contents between bilberries thawed in conditions of domestic refrigerator (2-4°C) and fruit thawed in room temperature (18-20°C) were small, and did not exceed 10%.

In our study, the long-term frozen storage of blueberries did not induce significant changes in anthocyanin content (Table 2). Gonzàles et al. [2003] showed similar anthocyanins losses for frozen blackberry stored for 12 months at –24°C. Other investigations have demonstrated dramatic losses of anthocyanins during frozen storage. Chaovanalikit and Wrolstad [2004] reported 88% anthocyanins degradation in sweet cherries after 6 months of storage at –23°C.

Table 1. Effect of freezing process on total phenolic, total anthocyanin, individual anthocyanins, chlorogenic acid contents and antioxidant activity in highbush blueberries
Tabela 1. Wpływ procesu mrożenia na zawartość polifenoli ogółem, antocyjanów ogółem, poszczególnych antocyjanów, kwasu chlorogenowego oraz pojemność przeciwutleniającą owoców borówki wysokiej

Chemical composition Skład chemiczny		Fresh fruits Owoce świeże	Frozen fruits Owoce mrożone temperature of freezing temperatura mrożenia	
			–18°C	–35°C
Total phenolic, mg/100 g Polifenole ogółem, mg/100 g		427.8a	427.0a	427.5a
Total anthocyanin, mg/100 g Antocyjany ogółem, mg/100 g		137.6a	140.6a	139.4a
Individual anthocyanins, mg/100 g Antocyjany, mg/100 g	Mv-3-gal	25.6	25.4	25.0
	Mv-3-glu	14.8	15.2	14.9
	Mv-3-ara	23.9	23.5	23.9
	Dp-3-gal	8.7	11.0	10.6
	Dp-3-glu	4.0	6.3	5.6
	Dp-3-ara	10.4	11.9	11.0
	Pt-3-gal	7.9	7.4	7.5
	Pt-3-glu	4.8	4.3	4.9
	Pt-3-ara	3.8	4.9	5.5
	Cy-3-gal	0.7	1.0	0.9
	Cy-3-glu	0.7	1.0	0.7
	Cy-3-ara	0.6	1.0	0.9
	Pn-3-gal	0.3	0.8	0.4
	Pn-3-glu	0.9	0.9	0.8
	total ogółem	107.1	114.6	112.6
Chlorogenic acid, mg/100 g Kwas chlorogenowy, mg/100 g		105.6a	104.8a	104.9a
Antioxidant activity, μmol Trolox eq/g fruits Aktywność przeciwutleniająca, μmole Troloxu/g owoców		33.5a	32.4a	32.7a

Values within rows followed by different letters are significant at $p \le 0.05$.
Średnie oznaczone tą samą literą w wierszach nie różnią się istotnie według na poziomie istotności $\alpha = 0,05$.

Chlorogenic acid is the main phenolic compound found in highbush blueberries. The chlorogenic acid content in blueberries of Bluecrop cultivar was in agreement with previous reports [Gao and Mazza 1994, Taruscio et al. 2004]. In our work little differences in chlorogenic acid content were observed in blueberry fruits during freezing process (Table 1) and storage of six months (Table 2).

Table 2. Effect of frozen storage time and temperature on total phenolic, total anthocyanin, individual anthocyanins, chlorogenic acid contents and antioxidant activity of frozen highbush blueberries
Tabela 2. Wpływ temperatury i czasu przechowywania na zawartość polifenoli ogółem, antocyjanów ogółem, kwasu chlorogenowego oraz pojemność przeciwutleniającą mrożonych owoców borówki wysokiej

Chemical composition Skład chemiczny		Temperature of storage Temperatura przechowywania							
		–18°C				–35°C			
		time of storage, months czas przechowywania, miesiące				time of storage, months czas przechowywania, miesiące			
		0	2	4	6	0	2	4	6
Total phenolic, mg/100 g Polifenole ogółem, mg/100 g		427.0a	425.6a	425.8a	428.6a	427.5a	426.8a	426.2a	427.7a
Total anthocyanin, mg/100 g Antocyjany ogółem, mg/100 g		140.6a	138.2a	136.2a	138.6a	139.4a	139.1a	138.1a	136.4a
Individual anthocyanins, mg/100 g Antocyjany, mg/100 g	Mv-3-gal	25.4	25.1	25.1	26.4	25.0	25.1	25.0	24.7
	Mv-3-glu	15.2	15.0	15.1	15.5	14.9	14.8	14.7	14.5
	Mv-3-ara	23.5	23.5	23.2	23.5	23.9	23.9	23.8	23.5
	Dp-3-gal	11.0	10.6	10.2	10.1	10.6	10.7	10.3	10.2
	Dp-3-glu	6.3	6.0	5.7	5.8	5.6	5.4	5.1	5.0
	Dp-3-ara	11.9	11.6	11.4	11.6	11.0	10.7	10.4	10.4
	Pt-3-gal	7.4	7.4	7.4	7.9	7.5	7.6	7.7	7.3
	Pt-3-glu	4.3	4.0	3.9	3.8	4.9	4.9	4.7	4.6
	Pt-3-ara	4.9	4.7	4.5	4.9	5.5	5.2	5.0	4.9
	Cy-3-gal	1.0	1.0	1.1	1.1	0.9	0.9	1.0	0.9
	Cy-3-glu	1.0	0.9	0.8	1.0	0.7	0.6	0.5	0.6
	Cy-3-ara	1.0	1.0	1.0	0.9	0.9	1.0	1.0	0.9
	Pn-3-gal	0.8	0.8	0.8	0.9	0.4	0.3	0.3	0.4
	Pn-3-glu	0.9	0.9	0.9	1.0	0.8	0.8	0.7	0.7
	ogółem total	114.6	112.5	111.1	114.4	112.6	111.9	110.2	108.6
Chlorogenic acid, mg/100 g Kwas chlorogenowy, mg/100 g		104.8a	103.0a	102.7a	102.6a	104.9a	104.3a	103.6a	103.5a
Antioxidant activity, µmol Trolox eq/g fruits Aktywność przeciwutleniająca, µmol Troloxu/g owoców		32.4a	30.8a	29.8a	30.6a	32.7a	31.4a	31.8a	30.8a

Values within rows followed by different letters are significant at $p \leq 0.05$.
Średnie oznaczone tą samą literą w wierszach nie różnią się istotnie na poziomie istotności $\alpha = 0,05$.

Compared to our results, Moyer et al. [2002] reported higher value (50 µmol Trolox eq/g fruit) of antioxidant activity of highbush blueberry (Bluecrop). On the other hand, Elhlenfeldt and Prior [2001] reporter a considerably lower antioxidant activity of Bluecrop fruits (10.4 µmol Trolox eq/g fruit). Results in our work correspond with the one obtained by Connor et al. [2002] (26 µmol Trolox eq/g fruit).

Freezing process of blueberries did not result in a loss of antioxidant activity (Table 1). These results agreed with previous studies on antioxidant activity retentions in blueberry fruits during freezing and frozen storage [Lohachoompol et al. 2004]. Our work showed, that the temperature of storage of frozen blueberries did not influence the antioxidant activity (Table 2). This implies that storage in very low temperature (–35°C) is not necessary to maintain antioxidant activity of frozen fruits during storage. In conclusion, the freezing process followed by a six moths frozen storage is a very good preservative process to support almost unchanged antioxidant activity value and bioactive compounds content of highbush blueberry fruits.

CONCLUSIONS

1. The amount of bioactive compounds and antioxidant activity in the frozen blueberries was not significantly different from that in the fresh fruits.
2. Malvidin and delphinidin derivatives were the major anthocyanins present in Bluecrop blueberry.
3. The freezing process caused small changes in the contents of delphinidin and cyanidin glycosides and peonidin-3-galactoside in highbush blueberries. The slight increase of these anthocyanins after freezing of fruits might be explained by the rise of the anthocyanins extraction due to the cellular disruptions.
4. Long-term frozen storage of highbush blueberries did not significantly affect the total phenolic, total anthocyanin, individual anthocyanins, chlorogenic acid contents and antioxidant activity.

REFERENCES

Ames B.M., Shigena M.K., Hagen T.M., 1993. Oxidants, antioxidants and the degenerative diseases of aging. Proc. Natl. Acad. Sci. 90, 7915-7922.

Ancos B., Gonzalez E.M., Cano M.P., 2000. Ellagic acid, vitamin C, and total phenolic contents and radical scavenging capacity affected by freezing and frozen storage in raspberry fruit. J. Agric. Food Chem. 48, 4565-4570.

Chaovanalikit A., Wrolstad R.E., 2004. Anthocyanin and polyphenolic composition of fresh and processed cherries. J. Food Sci. 69, 1, FDT73-FCT83.

Cho M.J., Howard L.R., Prior R.L., Clark J.R., 2004. Flavonoid glycosides and antioxidant capacity of various blackberry, blueberry and red grape genotypes determined by high-performance liquid chromatography/mass spectrometry. J. Sci. Food Agric. 84, 1771-1782.

Connor A.M., Luby J.J., Tong C.B.S., 2002. Genotypic and environmental variation in antioxidant activity, total phenolic content, and anthocyanin content among blueberry cultivars. J. Amer. Soc. Hort. Sci. 127, 1, 89-97.

Ehlenfeld M.K., Prior R.L., 2001. Oxygen radical absorbance capacity (ORAC) and phenolic and anthocyanin concentration in fruit and leaf tissues of highbush blueberry. J. Agric. Food Chem. 49, 2222-2227.

Gao L., Mazza G., 1994. Quantitation and distribution of simple and acylated anthocyanins and other phenolics in blueberries. J. Food Sci. 59, 5, 1057-1059.

Gonzàlez E., de Begoňa A., Cano M., 2003. Relation between bioactive compounds and free radical – scavenging capacity in berry fruits during frozen storage. J. Sci. Food Agric. 83, 722-726.

Gough R., 1991. The highbush blueberry and its management. FPP, Haworth Press New Yersey.

Kalt W., McDonald J., Ricker R., Lu X., 1999. Anthocyanin content and profile within and among blueberry species. Can. J. Plant Sci. 79, 617-623.

Kmiecik W., Jaworska G., Budnik A., 1995. Wpływ różnych technik rozmrażania mrożonek z owoców jagodowych na ich jakość [Influence of thawing methods of frozen berries fruits on their quality]. Rocz. PZH 46, 2, 135-143 [in Polish].

Lohachoompol V., Srzednicki G., Craske J., 2004. The change of total anthocyanins in blueberries and their antioxidant effect after drying and freezing. J. Biomed. Biotechnol. 5, 248-252.

Miller N.J., Rice-Evans C., 1996. Spectrophotometric determination of antioxidant activity. Redox Report 2, 3, 161-171.

Moyer A.R., Hummer E.K., Finn C.E., Frei B., Wrolstad R.E., 2002. Anthocyanins, phenolics and antioxidant capacity in diverse small fruits: *Vaccinium*, *Rubus* and *Ribes*. J. Agric. Food Chem. 50, 519-525.

Peri C., Pompei G., 1971. An assay different phenolic fraction in wines. Am. J. Enol. Vitic 22, 2.

Prior R.L., Cao G., Martin A., Sofic E., McEwen J., O`Brien C., Lischner N., Ehlenfeld M., Kalt W., Krewer G., Mainland C.M., 1998. Antioxidant capacity as influenced by total phenolics and anthocyanin content, maturity and variety of *Vaccinium* species. J. Agric. Food Chem. 46, 2686-2693.

Swain T., Hillis W.E., 1959. The phenolic constituents of *Prunus domestica*. The quantitative analysis of phenolic constituents. J. Sci Food Agric. 10, 1, 63-68.

Taruscio T., Barney D.L., Exon J., 2004. Content and profile of flavanoid and phenolic acid compounds in conjunction with the antioxidant capacity for a variety of Northwest Vaccinium berries. J. Agric. Food Chem. 52, 3169-3176.

Wang H., Cao G., Prior R.L., 1996. Total antioxidant capacity of fruits. J. Agric. Food Chem. 44, 701-705.

Wyniki produkcji roślinnej w 2004. Informacje i opracowania statystyczne [Results of plant production in 2004. Information and statistics]. Warszawa [in Polish].

ZMIANY WŁAŚCIWOŚCI PRZECIWUTLENIAJĄCYCH OWOCÓW BORÓWKI WYSOKIEJ (*VACCINIUM CORYMBOSUM* L.) PODCZAS PROCESU MROŻENIA ORAZ DŁUGOTERMINOWEGO PRZECHOWYWANIA ZAMRAŻALNICZEGO

Streszczenie. Celem pracy było zbadanie wpływu procesu mrożenia oraz długoterminowego zamrażalniczego przechowywania na zawartość polifenoli ogółem, antocyjanów ogółem oraz aktywność przeciwutleniającą owoców borówki wysokiej (*Vaccinium corymbosum* L.). Dodatkowo metodą wysokosprawnej chromatografii cieczowej (HPLC) oznaczono w owocach zmiany w zawartości poszczególnych antocyjanów oraz kwasu chlorogenowego. Owoce zamrażano w temperaturze –18°C i –35°C, a następnie przechowywano je w tych warunkach przez okres sześciu miesięcy. Stwierdzono, że pochodne malwidyny oraz delfinidyny są głównymi antocyjanami borówek i stanowią odpo-