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INFLUENCE OF STORAGE CONDITIONS ON CHANGES IN PHYSICAL PARAMETERS AND CHEMICAL COMPOSITION OF HIGHBUSH BLUEBERRY (*VACCINIUM CORYMBOSUM* L.) FRUIT DURING STORAGE

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Abstract

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The research was carried out in the years 2009-2012 at the Fruit Farming Laboratory of the West Pomeranian University of Technology in Szczecin. The experiment was conducted in a production plantation where Sunrise cultivar bushes were planted in grey-brown podzolic soil, and collected fruit was stored for 6 weeks. The Palliflex storage system ensures specific atmosphere in a single pallet, which enables long- and short-term storage of fruit. In the research, fruit quality (physical parameters and chemical composition) was assessed depending on method of fruit preparation for storage (without shock cooling or shock cooling of fruit after harvest) and storage conditions (an ordinary cold store and CA cold store – the Palliflex system). The smallest changes in firmness (437 $G \cdot mm^{-1}$) and color when compared to fresh fruit were observed in a CA cold store after fruit pre-cooling (425 $G \cdot mm^{-1}$). The highest fruit weight loss was noted after fruit storage in normal atmosphere (1.9%). The storage resulted in a reduced content of ascorbic acid (59 and 72 $mg \cdot 1000\ mL^{-1}$) and polyphenols (175 and 207 $mg \cdot 100g^{-1}$), while the content of nitrates in fruit grew (47.4 and 41.2 $mg \cdot 1000\ mL^{-1}$). No changes were observed in the content of extract and organic acids.

Due to pre-cooling of fruit and its storage in a CA cold store, the fruit had the most advantageous physical and chemical parameters – similar to those of fresh fruit.

Key words: color, firmness, polyphenols, shock-cooled, storage of control atmosphere, *Vaccinium corymbosum*

Introduction

Poland and Germany are the greatest producers of highbush blueberry in Europe. In Poland research on highbush blueberry was started in 1946 (Smolarz, 2006). An important factor of highbush blueberry cultivation is providing it with humus-rich soil of 3.5-7% (Eck, 1988) with low pH (Starack et al., 2002). The berries are large with dark-blue skin (Kader et al., 1996), and have various anthocyanin compositions (Ochmian, 2013). Delphinidin and malvinidin are main anthocyanins of the fruit (Ścibisz and Mitek, 2007b). That has a beneficial influence on the circulatory system as well as an anti-carcinogenic effect (Zheng and Wang, 2003). Additionally, the fruit is rich in vitamins, minerals, pectin and fiber (Ehlenfeldt and Prior, 2000).

Storage of frozen fruit is a method of preservation because its antioxidant properties (Lohachoompol et al., 2004) and content of individual anthocyanins (Ścibisz and Mitek, 2007a; Grajkowski et al., 2007) are similar to those of fresh fruit. However, the content of vitamin C decreases during the storage (Skupień, 2006). Ripe highbush blueberry fruit is well stored in an ordinary cold store for more than a dozen days at a temperature of 0-2°C, while in a CA cold store – up to eight weeks (Krupa and Tomala, 2006). Conditions of fruit storage and fruit physiological condition have an effect on an increase in anthocyanin content (Ehlenfeld and Prior, 2001), especially in controlled atmosphere (Krupa and Tomala, 2006). The Palliflex system enables long- and short-term storage of fruit under ULO and CA conditions, in specific atmosphere which is maintained in an individual pallet

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(Kurubaş et al., 2013). Fruit is stored in special bags, hermetically sealed, where atmosphere composition is checked several times during a day (link 1). Gases: carbon dioxide and nitrogen, displacing oxygen from air, are delivered to the bags. Constant humidity is maintained, which should be 90-95% of air relative humidity. Fruit intended for such a storage system should be gathered when not fully ripe, even slightly pink (Connor et al., 2002).

The aim of the present research was to assess influence of preparation method of highbush blueberry fruit for storage and storage conditions on fruit quality, physical parameters and chemical composition.

Materials and Methods

The studies were carried out in the years 2009–2012 in the Laboratory of Orcharding at the Department of Horticulture West Pomeranian University of Technology in Szczecin (Poland). The aim of the experiment was to determine the firmness, chemical composition and fruit weight-loss depending on application or not of fruit shock-cooling after the harvest and a method used for highbush blueberries 'Sunrise' storage. Both the shock-cooled berries (temperature drop to 3-4˚C within 2 hours after picking) and not shock-cooled berries were then stored in a cold room with a control atmosphere Palliflex system (5; 15) or in a normal atmosphere. Berries were stored for 6 weeks at the temperature 2-3˚C and relative air humidity of about 96%. The experiment was performed in five repetitions, each for 1.25 kg of berries.

Scheme of the experiment

- 2 methods of sample preparation – shock cooling, without shock cooling
- 2 methods of fruit storage – controlled atmosphere (CA), normal atmosphere

The experiment was conducted at a 60 ha production plantation located in the area of Szczecin. Blueberry bushes were planted in the spacing of 1.2 x 2.0 in the podzolic soil of the VI valuation class. The content analysis of the soil minerals showed a very high level of magnesium, medium levels of phosphorus and calcium, and a low level of potassium.

Physical features of fruits (firmness, puncture of the skin) and soluble solids, titratable acidity, L–ascorbic acid and color were measured on fresh berries. Phenolics samples that were kept frozen (-27˚C) in polyethylene bags (3 x 250 g) until analyzed.

Firmness and puncture resistance of the skin was measured with a FirmTech2 apparatus (BioWorks, USA) of 100 randomly selected berries from each replicate was expressed as a gram-force causing fruit surface to bend 1 mm. Puncture were made using a stamp with a diameter of 3 mm. To obtain juice, the berries (three replicate of 150 g) were macerated at 50˚C with the addition of the PT 400 Pektopol enzyme at a dose of 400 mg per kg of fruits for 60 minutes. After the completion of the enzymatic processing, the pulp was pressed using a hydraulic press at a pressure of 3 MPa (Oszmiański and Wojdyło, 2005). Titratable acidity was determined by titration of a water extract of juice with 0.1 N NaOH to an end point of pH 8.1 (measured with a pH meter Elmetron 501) (PN). Soluble solids content was determined with a PAL1 KonicaMinolta refractometer. L-ascorbic acid content was measured with RQflex 10 reflectometer (Merck) (Ochmian et al., 2012). Fruit color was measured in a transmitted mode through Konica Minolta CM-700d spectrophotometer. Measurements were conducted in CIE L*a*b* system - the full nomenclature is 1976 CIE L*a*b* Space, International Commission on Illumination in Vienna [L* white (100) black (0), a* green (-100) red (+100), b* blue (-100) yellow (+100)] (Hunterlab, 2012), through a 10˚ observer type and D65 illuminant, with the aperture diameter measuring 3 mm. The HPLC analyses of polyphenols were carried out with HPLC apparatus consisting of a Merck-Hitachi L-7455 diode array detector (DAD) and quaternary pump L-119 7100 equipped with D-7000 HSM Multisolvent Delivery System (Merck-Hitachi, Tokyo, Japan). The runs were monitored for phenolic acids at 320 nm, flavonols and luteolin glycoside at 360 nm, and anthocyanin glycosides at 520 nm. Retention times and spectra were compared to that of pure standards and total polyphenols content was expressed as mg per 100 g fruit tissue. Standards of anthocyanidin glycosides were obtained from Polyphenols Laboratories (Norway), while, for phenolic acids, flavonols and from Extrasynthese (France).

In order to determine the significance of differences, a two-factor analysis of variance was carried out, followed by the assessment of the significance of differences using the Tukey's test. The statistical analyses were performed using the Statistica software.

Results and Discussion

Method of storage determines fruit quality. Blueberry fruit can be stored even up to 8 weeks in cold stores (Krupa and Tomala, 2006). Firmness is an indicator of freshness and also determines fruit resistance to damage during transport. Irrespective of method of storage, fresh fruit was more firm (both in diameter axis and height) as well as more resistant to damage (Table 1). The parameters were positively affected by shock pre-cooling and fruit storage in a CA cold store. After 6 weeks of storage, fruit firmness decreased from 7% (shock-cooled fruit stored in CA) to 21% (fruit without shock cooling and stored in an ordinary cold store). Also the lowest fruit weight losses were noted in a CA cold store, on average 0.8%. Similar firmness was exhibited by fruit of blue honeysuckle (*Lonicera caerulea* var. *kamtschatica*) (180-220 G·mm^{-1}), however, already after several days of storage in a cold store, a decrease in firmness amounted from 14 to 16%, and weight loss – ca. 3% (Ochmian et al., 2008). Similar relationships were observed for strawberry fruit, where a decrease in firmness ranged from 2 to 10% after 5-day storage in an ordinary cold store (Ochmian and Grajkowski, 2008).

During storage, changes in the basic color of fruit skin were observed. The fruit darkened, which was proved by the value of the L* parameter. The fruit also changed the color from green, when a* parameter directly after harvest was -3.02, to red, which was proved by a positive value of the parameter. Also an increase in the value of the b* parameter was found in all the examined objects. The greatest color changes were observed in fruit stored in an ordinary cold store without pre-cooling, and the parameters the most similar to those of fresh fruit were found for fruit stored in CA which underwent the process of pre-cooling. The L* parameter had similar values to those of fruit of several species of *Amelanchier* genus and small fruit of blackberry (< 13 mm), while a* and b* parameters considerably differed from those of fruit of these species (Ochmian et al., 2013a; Ochmian et al., 2013b). The a* parameter for apple "Braeburn" amounted to ca. 30, however, it was lower than that of highbush blueberry stored in normal atmosphere without shock cooling (42.76), (Ozturk et al., 2012).

Analysis of results showed no influence of method of fruit storage and preparation on the content of extract and acidity of berries, which were at a similar level as in fruit directly after harvest (Table 2). Extract content in highbush blueberry fruit, depending on cultivar, amounted from 11.7% to 14.45% under similar climate and soil conditions (Ochmian et al., 2009a; Ochmian et al., 2009b; Ochmian et al., 2010).

Ascorbic acid is a reduced form of vitamin C and the main biologically active form of the vitamin, an effective antioxidant (Jacob and Sotoudeh, 2002). The highest amount of ascorbic acid (AA) was found in berries directly after harvest (82 mg·1000 mL^{-1}). It is common knowledge that storage reduces the content of ascorbic acid in food (Uddin et al., 2002), which was observed also in the examined fruit. Degradation of ascorbic acid in food depends on many factors, such as oxygen, heat, light, water activity, occurrence of metallic ions, as well as temperature and time of storage (Santos and Silva, 2008; Marti et al., 2009). Methods of fruit preparation and

Table 1
Physical parameters of highbush blueberry fruit depending on method of storage

Method storage (A)	Preparation of fruit (B)	Puncture axis diameter, G mm	Firmness, G mm		Mass loss, %	Color CIE		
			axis diameter	axis height		L* white 100 black 0	a* green -100 red +100	b* blue -100 yellow +100
normal atmosphere	not shock-cooled	112	185	389	2.1	19.18	42.76	-29.22
control atmosphere	not shock-cooled	128	193	413	0.9	21.46	7.81	-12.19
	mean	120	189	401	1.5	20.32	25.29	-20.71
normal atmosphere	shock-cooled	127	195	409	1.8	22.13	6.94	-23.52
control atmosphere	shock-cooled	139	219	425	0.7	22.87	2.44	-10.11
	mean	133	207	417	1.2	22.50	4.69	-16.82
immediately fresh fruit after harvest		142	234	437	-	24.43	-3.02	-5.89
normal atmosphere	mean	120	190	399	1.9	20.66	24.85	-26.37
control atmosphere	mean	134	206	419	0.8	22.17	5.13	-11.15
$LSD_{0.05}$		A 9 B 11 AxB 14	A 8 B 11 AxB 113	A 11 B 13 AxB 16	-	A 3.53 B 4.05 AxB 4.97	A 5.64 B 7.19 AxB 8.76	A 4.25 B 5.99 AxB 7.48

storage were of significant importance for maintaining AA level in berries after harvest. Use of pre-cooling and storage of fruit in CA resulted in the smallest changes in the content of the compound (74 mg·1000 mL^{-1}). According to Skupień (2006), vitamin C losses during storage depend on cultivar. In another study, the content of vitamin C ranged from 98 to 279 mg·1000 mL^{-1} (Ochmian et al., 2009a; Ochmian, 2012).

A similar response to the employed methods was noted in generation of polyphenols in fruit. Likewise, the highest level of polyphenols was observed in fresh berries (264 mg·100 g^{-1}), and their storage in controlled atmosphere after pre-cooling proved to be the most favorable for maintaining a high content of these compounds (228 mg·100 g^{-1}). A range of biochemical, physical and microbiological processes takes place in stored plant products, resulting in changes in chemical composition, including nitrates (Lisiewska and Kmiecik, 1991). Analysis of experiment results demonstrated an increase in NO_3 in highbush blueberry fruit during its storage. A method significantly reducing a nitrate increase in berries was their storage in CA after pre-cooling (37.5 mg·1000 mL^{-1}).

Analysis of results of measurements of anthocyanin, chlorogenic acid and flavonol composition in fruit (Table 3) showed a significant decrease in their contents during storage. Fruit pre-cooling and storage in controlled atmosphere reduced a loss of anthocyanin and chlorogenic acid content. When compared to fresh fruit, the content of delphinidin 3-*O*-glucoside was the most reduced, especially in fruit stored in CA without pre-cooling – by 72%. According to Reque et al. (2013), 6-month storage of blueberry at a temperature of -18°C resulted in degradation of on average 59% of anthocyanins. Connor et al. (2002) came to a conclusion that fruit gathered prior to full ripeness can be stored in a refrigerator at a temperature of 5°C for seven weeks without loss of antioxidants, such as flavonols and anthocyanins. However, a study by Krupa and Tomala (2006) demonstrated a decrease in polyphenolic compounds during storage. Flavonoids are important nutrients with a wide-range biological effect and healthy properties (García-Salas et al., 2013). The conducted experiment demonstrated that storage of fruit in controlled atmosphere significantly reduced its losses.

Conclusions

The smallest changes in firmness and color were observed during fruit storage in a controlled atmosphere cold store when compared to fresh fruit. Shock pre-cooling of fruit had a positive effect on fruit firmness.

Fruit stored in normal atmosphere exhibited greater weight loss and color changes than fruit stored in a controlled atmosphere cold store.

Table 2
Chemical composition of highbush blueberry fruit depending on method of storage

Method storage (A)	Preparation of fruit (B)	Soluble solids, %	Titratable acidity, g·100mL^{-1}	Ascorbic acid, mg·1000 mL^{-1}	Polyphenols, mg·100 g^{-1}	NO_3, mg·1000 mL^{-1}
normal atmosphere	not shock-cooled	14.9	0.59	51	158	53.6
control atmosphere	not shock-cooled	14.7	0.62	69	185	44.8
	mean	14.8	0.61	60	172	49.2
normal atmosphere	shock-cooled	14.9	0.58	67	191	41.2
control atmosphere	shock-cooled	14.8	0.60	74	228	37.5
	mean	14.9	0.59	71	210	39.4
imediately fresh fruit after harvest		14.7	0.61	82	264	34.2
normal atmosphere	mean	14.9	0.59	59	175	47.4
control atmosphere	mean	14.8	0.61	72	207	41.2
$LSD_{0,05}$		A 0.2 B 0.2 AxB 0.3	A 0.05 B 0.08 AxB 0.11	A 7 B 9 AxB 12	A 13 B 19 AxB 23	A 4.3 B 5.2 AxB 6.7

Table 3
Content of polyphenolic compounds in highbush blueberry fruit depending on method of storage

	Preparation of fruits (B)						Fresh fruit
	not shock-cooled			shock-cooled			
Method storage (A)	normal atmosphere	control atmosphere	mean (B)	normal atmosphere	control atmosphere	mean (B)	
Del 3-*O*-gal	34.04	40.37	37.21	36.68	45.28	40.98	43.27
Del 3-*O*-glu	8.72	7.07	7.90	11.50	15.86	13.68	25.01
Del 3-*O*-ara	28.94	22.93	25.94	25.30	31.79	28.55	37.90
Cya 3-*O*-gal	3.98	5.98	4.98	6.47	8.81	7.64	10.08
Cya 3-*O*-glu	3.57	4.25	3.91	3.46	4.14	3.80	3.93
Cya 3-*O*-ara	2.85	1.91	2.38	2.50	2.63	2.57	2.34
Pet 3-*O*-gal	4.16	8.86	6.51	7.35	10.87	9.11	14.29
Pet 3-*O*-glu	5.17	5.43	5.30	2.41	6.49	4.45	7.71
Pet 3-*O*-ara	6.81	4.01	5.41	6.58	8.32	7.45	11.66
Peo 3-*O*-gal	8.24	15.96	12.10	10.58	9.81	10.20	10.11
Peo 3-*O*-glu	3.11	10.12	6.62	12.37	6.43	9.40	9.61
Peo 3-*O*-ara	0.64	0.05	0.35	0.84	0.23	0.54	1.12
Mal 3-*O*-gal	0.69	0.07	0.38	1.74	1.11	1.43	1.48
Mal 3-*O*-glu	2.84	0.06	1.45	1.33	0.87	1.10	0.89
Mal 3-*O*-ara	5.26	6.62	5.94	4.67	8.61	6.64	14.02
Total anthocyanins $LSD_{0.05}$; A 12 B 14 AxB 15	119.02	133.69	126.35	133.78	161.25	147.51	193. 42
Mean *(*A)	normal atmosphere 126.40			control atmosphere 147.47			
Chlorogenic acid $LSD_{0.05}$ A 4.7 B 5.9 AxB 7.1	26.98	34.86	30.92	46.00	48.91	47.46	51.37
Mean *(*A)	normal atmosphere 36.49			control atmosphere 41.89			
Que 3-*O*-gala	1.16	1.65	1.41	2.01	2.50	2.26	2.57
Que 3-*O*-glu	8.80	13.26	11.03	6.80	13.70	10.25	12.55
Que 3-*O*-ram	0.87	0.81	0.84	0.38	0.31	0.35	1.32
Kae 3-*O*-rut	1.09	0.77	0.93	2.20	1.07	1.64	2.76
Total flavonols $LSD_{0.05}$ A 2.3 B 2.6 AxB 3.0	11.92	16.49	14.21	11.39	17.58	14.50	19.20
Mean *(*A)	normal atmosphere 11.66			control atmosphere 17.04			

No influence of method of fruit preparation or storage on the content of extract and organic acids was found.

The content of ascorbic acid, polyphenolic compounds, anthocyanins, chlorogenic acid and flavonols decreased, while the content of nitrates increased in fruit during storage. The lowest losses in the content of organic compounds were demonstrated during fruit storage in controlled atmosphere after pre-cooling of fruit.

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