



## Article

# Short-Term Storage in a Modified Atmosphere Affects the Chemical Profile of Blueberry (*Vaccinium corymbosum* L.) Fruit

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**Abstract:** Short-term modified-atmosphere storage with an adjusted CO<sub>2</sub> and/or O<sub>2</sub> concentration could recover blueberry fruit infested with the larvae of quarantine pests. However, this could significantly affect the fruit quality. In our experiment we investigated the performance of highbush blueberry ‘Bluecrop’ fruit (firmness, peel color, individual phenolics, sugars, and organic acids) under four short-term storage regimes: (1) a regular atmosphere with 0.03% CO<sub>2</sub>, 21% O<sub>2</sub>, and 78% N<sub>2</sub> at 22 °C, i.e., the control; (2) a regular atmosphere with 0.03% CO<sub>2</sub>, 21% O<sub>2</sub>, and 78% N<sub>2</sub> at 2 °C; (3) a modified atmosphere with 10% CO<sub>2</sub>, 5% O<sub>2</sub>, and 85% N<sub>2</sub> at 2 °C; and (4) a modified atmosphere with 100% CO<sub>2</sub> at 2 °C. Fruit sampling took place after 24 h, 48 h, and 72 h. Fruit firmness was not significantly altered by storage regime or duration, while some significant, but minor, changes were detected in the color parameters. Regarding the primary metabolites, the sugar/organic acid ratio stagnated in the first 48 h in all storage regimes. The content of the majority of the individual phenolics was significantly higher in the fruit stored under control conditions. From our results, we can conclude that the short-term storage of highbush blueberry ‘Bluecrop’ fruit for 24 h in a cold atmosphere does not affect the phenolic content, and storage for 48 h does not affect the total sugar/organic acid ratio, regardless of the atmosphere composition.

**Keywords:** bluecrop; peel color; primary metabolites; phenolics



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## 1. Introduction

Highbush blueberry (*Vaccinium corymbosum* L.) fruit is considered one of most popular fruit species among consumers due to its recognizable taste and high nutrient content [1]. On the other hand, it is highly perishable, since its fruit quality parameters and visual appearance are preserved at a high level for only 20 days when stored at 0 °C at 90% to 95% relative humidity [2]. Later, shriveling, softening, fruit decay, and the degradation of metabolites may occur [3,4]. Therefore, the manipulation of air composition, i.e., a decreased O<sub>2</sub> and/or increased CO<sub>2</sub> concentration in storage, in order to preserve the fruit firmness, color, and high nutritional value of blueberries for several weeks instead of days has already been examined [4].

In recent years, in addition to fruit-quality preservation, modified-atmosphere storage has been reported to be an effective method for the development of strategies to inhibit quarantine pests, which develop in various fruit species. Infested fruit is considered unmarketable, causing significant economic losses.

From a consumer perspective, there is zero tolerance for any larvae from quarantine pests or any other pests found in fresh fruit. However, the eggs or larvae may remain

undetected in harvested fruit. Consequently, growers are risking the potential discovery of infested fruit by consumers, or the further spread of insects in storage or later on the store shelves. Therefore, short-term storage in a modified atmosphere could be an effective postharvest practice with the ability to recover infested fruit [5,6].

In modified-atmosphere storage, reduced O<sub>2</sub> and/or increased CO<sub>2</sub> is used [7,8]. However, the optimal O<sub>2</sub>/CO<sub>2</sub> ratio in storage that does not impair the inner and outer fruit quality differs between cultivars. Based on the results published so far, the lower limit for the O<sub>2</sub> concentration for blueberries is 1%, below which fermentation is likely to occur. The optimal O<sub>2</sub> concentration ranges between 2% and 3% [8]. On the other hand, the upper limit regarding the effect of CO<sub>2</sub> concentration in storage on blueberry fruit quality strongly differs between cultivars [7]. Besides air composition, the stability of fruit quality also depends on the duration of exposure. Mostafa et al. (2021) [6] showed significant variability in sweet cherry fruit firmness, total soluble solids content, and titratable acidity at harvest after 10 days and 20 days in a modified atmosphere with 50% CO<sub>2</sub>. In blueberries, the maintenance of peel color and fruit firmness and, at the same time, primary and secondary metabolites is one of the top priorities from when the fruit is harvested to when it reaches the consumer [9]. All of the properties listed greatly depend on genotype, environmental conditions before harvest, ripening stage at harvest, and storage conditions [10].

As mentioned above, long-term modified-atmosphere storage with altered O<sub>2</sub> and/or CO<sub>2</sub> levels has already been examined in the transportation and storage of blueberry fruit, since it has been reported to prolong their shelf life [4,9,11]. On the other hand, elevated CO<sub>2</sub> levels, approximately 21.5% and 50% [6,12] in storage, for a shorter period of time, may increase the mortality of SWD in sweet cherries. Husain et al. (2015) [13] successfully used 100% CO<sub>2</sub> for almond moth (*Ephestia cautella* Walker) larvae destruction in date fruit after 72 h of exposure. However, studies regarding the effect of short-term storage in a modified atmosphere with elevated CO<sub>2</sub> levels, or even without O<sub>2</sub>, on the quality parameters and exact chemical profile of blueberry fruit are lacking. Consequently, in the present study, we aimed to determine whether blueberry fruit firmness, peel color, and individual phenolics, sugars, and organic acid content change after 24 h, 48 h, and 72 h of exposure to 10% CO<sub>2</sub> and 100% CO<sub>2</sub>.

## 2. Materials and Methods

### 2.1. Fruit Material and Storage Treatments

Blueberry fruit (*Vaccinium corymbosum* L. cv. 'Bluecrop') was harvested from a blueberry orchard located in an experimental field at the Agricultural Institute of Slovenia in Brdo pri Lukovici (latitude: 46°17', longitude: 14°69'; altitude: 380 m a.s.l.). The fruit was harvested at its full maturity stage, i.e., when the fruit is fully dark blue and immediately transported to the laboratory.

For the storage treatments, 15 randomly chosen and uniform fruits were put into plastic containers with a ventilation hole and nylon mesh on the cover (insect breeding dish, square, 72 × 72 × 100 mm, Himedia, India) to enable circulation. Then, they were put into transparent polyethylene vacuum bags with reduced permeability and thoroughly heat-sealed (Besser Vacuum srl, Smart, Dignano, Italy). The fruits were stored under four different storage regimes: (1) a regular atmosphere with 0.03% CO<sub>2</sub>, 21% O<sub>2</sub>, and 78% N<sub>2</sub> in room temperature at 22 °C, i.e., the control; (2) a regular atmosphere with 0.03% CO<sub>2</sub>, 21% O<sub>2</sub>, and 78% N<sub>2</sub> in cold storage at 2 °C; (3) a modified atmosphere with 10% CO<sub>2</sub>, 5% O<sub>2</sub>, and 85% N<sub>2</sub> in cold storage at 2 °C; and (4) a modified atmosphere with 100% CO<sub>2</sub> in cold storage at 2 °C. Modified atmospheres were achieved with already prepared mixtures, purchased from TPJ Jesenice, Slovenia (50 L tank, 200 bar, 14 kg). Each treatment included six bags containing 15 fruits. The samples were removed from storage after 24 h, 48 h, and 72 h, with two bags used at each sampling time (therefore, 15 fruits for maturity parameters and 15 fruits for chemical analyses). Since the fruits were put into polyethylene bags for only three days, no weight loss, shriveling, or fungal decay occurred. Weight loss

was assessed at harvest and after each sampling time. Shriveling and fungal decay were estimated after each sampling time.

## 2.2. Fruit Firmness and Peel Color Measurements

Both maturity parameters were measured once on each fruit, on a fruit equator, and on 15 fruits after each sampling time (24 h, 48 h, and 72 h). Additionally, measurements were taken for 15 fruits directly after the harvest. Measurements of fruit firmness (digital penetrometer, TR, Turin, Italy; N) were made manually, once on each fruit and on the fruit equator, using a 1 mm diameter tip with cylindrical flat surface. Measurements present the maximum force required to penetrate blueberry fruit peel. Firmness was expressed in N.

The blueberry peel color was determined using a Konica Minolta portable colorimeter (CR-10 Chroma, Tokyo, Japan). The parameters that determine color are  $L^*$ ,  $a^*$ ,  $b^*$ ,  $C^*$ , and  $h^\circ$ . The  $L^*$  signifies lightness on a scale from 0 to 100, where 0 stands for black and 100 for white. Values  $a^*$  and  $b^*$  range from  $-128$  to  $127$ , where a negative value of  $a^*$  denotes green and positive denotes red, while a negative value of  $b^*$  characterizes blue and positive denotes yellow. The  $C^*$  value stands for chroma, with a higher value signifying a more intense color. The hue angle ( $h^\circ$ ) represents color in degrees from  $0^\circ$  to  $360^\circ$  ( $0^\circ$ , red;  $90^\circ$ , yellow;  $180^\circ$ , green;  $270^\circ$ , blue).

## 2.3. Individual Sugars and Organic Acids in Blueberry Fruit

For extractions, 1 g of finely chopped, fresh fruit material was mixed with 5 mL of bi-distilled water. In order to create 1 g, 3 fruits were used. Each treatment was made in four replicates. The samples were thoroughly mixed by vortexing and then left at room temperature for 30 min with constant agitation (Unimax 1010; Heidolph, Schwabach, Germany). The samples were then centrifuged for 10 min at  $9000 \times g$  and  $4^\circ\text{C}$ . The supernatants were filtered through cellulose filters (Chromafil A-20/25; Macherey-Nagel, Düren, Germany) into vials, which were stored at  $-20^\circ\text{C}$  until HPLC analyses [14].

The individual sugars were separated on an HPLC system (Vanquish, Thermo Scientific, Waltham, MA, USA) linked to a refractive index detector (RI plus, RefractoMax520, Thermo Scientific, Waltham, MA, USA). Separation took place on a Rezex RCM-monosaccharide Ca+ 2% column ( $150\text{ mm} \times 7.8\text{ mm}$ ; Phenomenex, Los Angeles, CA, USA), operated at  $85^\circ\text{C}$ , with a constant flow at  $0.8\text{ mL min}^{-1}$ . Individual samples were analyzed for 15 min. The mobile phase used was bi-distilled water. The identity of individual sugars was confirmed by comparing their retention times with external standards. Their contents were calculated from peak areas using standard curve equations and expressed as  $\text{mg g}^{-1}\text{ FW}$  [15].

Organic acid separation and identification took place using an HPLC system (Vanquish, Thermo Scientific, Waltham, MA, USA) on a Rezex ROA-Organic acid H+ 8% ( $150\text{ mm} \times 7.8\text{ mm}$ ) column, made by Phenomenex, CA, USA, operating at  $65^\circ\text{C}$ . Compounds were detected using a UV detector set at 210 nm. Each sample ( $20\text{ }\mu\text{L}$ ) was analyzed for 15 min, at a flow rate of  $0.6\text{ mL min}^{-1}$ . The mobile phase was 4 mM sulfuric acid in bi-distilled water. Organic acid identities were confirmed through comparison with external standards and the contents were calculated using standard curve equations. They were expressed as  $\text{mg g}^{-1}\text{ FW}$  [15].

## 2.4. Individual Phenolic Compounds in Blueberry Fruit

Phenolic compounds were extracted from freshly thawed fruit in four replicates per treatment. For individual extraction, 2 g of homogeneous fruit sample was mixed with 4 mL of extraction solution (70% MeOH and 3% formic acid in bi-distilled water) in labeled 10 mL test tubes. The samples were mixed on a vortex, put into a cooled ultrasonic bath ( $0^\circ\text{C}$ ) for 1 h, and then centrifuged (5810 R; Eppendorf, Hamburg, Germany) for 10 min at  $9000 \times g$  and  $4^\circ\text{C}$ . The supernatants were filtered through  $0.2\text{ }\mu\text{m}$  polyamide filters (Chromafil AO-20/25; Macherey-Nagel, Düren, Germany) into labeled vials. They were stored at  $-20^\circ\text{C}$  until the analyses [14].

The separation and detection of individual phenolic compounds took place on a high-performance liquid chromatograph (HPLC; Dionex, UltiMate 3000; Thermo Scientific, Waltham, MA, USA) with a diode array detector at absorbances of 280 nm (phenolic acids, flavan-3-ols), 350 nm (flavonols), and 530 nm (anthocyanins). An individual sample was analyzed for 50 min on a Gemini column (C18, 150 × 4.6 mm; 3 µm; Phenomenex, Torrance, CA, USA) set to 25 °C. The flow rate was maintained at 0.6 mL min<sup>-1</sup> and the injection volume was 20 µL. The autosampler temperature was kept at 10 °C. The mobile phase A was 3% acetonitrile and 0.1% formic acid in bi-distilled water (*v/v/v*) and mobile phase B was 3% bi-distilled water and 0.1% formic acid in acetonitrile. The analysis was performed according to the following gradient: 0–15 min, 5% B; 15–20 min, 5–20% B; 20–30 min, 20–30% B; 30–35 min, 30–90% B; 35–45 min, 90–100% B; 45–50 min, 100–5% B [14].

Individual phenolics were identified by comparing their retention times with external standards and by mass spectrometry analysis (LTQ XL; Thermo Scientific, Waltham, MA, USA) based on their mass fragmentation patterns. Fragmentation was reached with electrospray ionization, operated in negative or positive (anthocyanins) ion mode. The mass spectrometer conditions were set as follows: injection volume, 10 µL; capillary temperature, 250 °C; flow rate, 0.6 mL min<sup>-1</sup>; sheath gas, 20 units; auxiliary gas, 8 units; source voltage, 4 kV. The mass spectrometer was set to scan from *m/z* 115 to *m/z* 1600. Individual phenolic contents were calculated from the corresponding standard curves or similar compounds and expressed as mg kg<sup>-1</sup> fresh weight (FW) [14].

### 2.5. Statistics

Statistical analysis was performed in the statistical program R commander i386 4.3.0 via two-way analysis of variance, where significance of interaction between storage duration and CO<sub>2</sub> concentration was tested. One-way analysis of variance was used for significant differences determination of individual, sugars, organic acids, and phenolic compounds between storage durations within individual storage regimes and vice versa. Significant differences were estimated via Tukey's test (*p* < 0.05) and are presented with different letters.

## 3. Results

### 3.1. Fruit Firmness and Color

The fruit's firmness and peel color during storage are presented in Table 1. The interaction between storage duration and CO<sub>2</sub> concentration was not significant in any of the measured parameters. The fruit in all storage regimes remained equally firm from harvest until the end of short-term storage. Regarding the peel color parameters, all of them significantly changed during storage in the control and 10% CO<sub>2</sub>, while in 0.03% CO<sub>2</sub>, only a reduction in peel *L\** occurred. In storage with 100% CO<sub>2</sub>, *L\** and *C\** decreased significantly, while the *b\** value increased. All measured parameters decreased/increased to the same extent for all four storage regimes. This was confirmed by corresponding statistical analyses that provided us with non-significant results between storage regimes within individual storage durations.

**Table 1.** Fruit firmness and peel color parameters ( $L^*$ ,  $a^*$ ,  $b^*$ ,  $C^*$ , and  $h^\circ$ ) of blueberry fruit under different storage regimes (0.03% CO<sub>2</sub>, 21% O<sub>2</sub>, and 78% N<sub>2</sub> at 22 °C, i.e., control; 0.03% CO<sub>2</sub>, 21% O<sub>2</sub>, and 78% N<sub>2</sub> at 2 °C; 10% CO<sub>2</sub>, 5% O<sub>2</sub>, and 85% N<sub>2</sub> at 2 °C; 100% CO<sub>2</sub> at 2 °C) for each storage duration.

Storage Duration (h)	CO <sub>2</sub> , O <sub>2</sub> (%)	Temperature (°C)	Firmness (N)	$L^*$	$a^*$	$b^*$	$C^*$	$h^\circ$
Harvest	0.03%, 21%	Control 22 °C	20.0 ± 0.07	37.10 ± 3.32 a	−0.65 ± 1.12 b	−7.13 ± 0.94 b	7.23 ± 1.01 a	265.4 ± 8.02 b
24 h			0.16 ± 0.05	32.31 ± 3.26 b	0.82 ± 1.29 a	−5.33 ± 1.19 a	5.55 ± 1.07 b	280.1 ± 14.52 a
48 h			0.17 ± 0.04	34.23 ± 1.58 ab	−0.53 ± 1.05 b	−6.36 ± 0.72 ab	6.49 ± 0.74 ab	265.7 ± 9.40 b
72 h			0.18 ± 0.05	32.09 ± 0.44 b	−0.69 ± 0.81 b	−5.52 ± 1.38 a	5.61 ± 1.43 b	264.1 ± 7.74 b
Significance			NS	***	***	***	***	***
Harvest	0.03%, 21%	2 °C	20.0 ± 0.07	37.10 ± 3.32 a	−0.65 ± 1.12	−7.13 ± 0.94	7.23 ± 1.01	265.4 ± 8.02
24 h			0.18 ± 0.05	32.87 ± 2.67 b	−0.41 ± 0.89	−6.03 ± 1.05	6.12 ± 1.04	267.0 ± 8.54
48 h			0.17 ± 0.05	33.46 ± 3.31 b	−0.71 ± 0.74	−6.27 ± 1.33	6.36 ± 1.32	264.2 ± 7.62
72 h			0.15 ± 0.05	34.25 ± 3.69 ab	−0.73 ± 0.66	−6.35 ± 1.27	5.54 ± 3.60	262.8 ± 4.65
Significance			NS	**	NS	NS	NS	NS
Harvest	10%, 5%	2 °C	20.0 ± 0.07	37.10 ± 3.32 a	−0.65 ± 1.12 ab	−7.13 ± 0.94 b	7.23 ± 1.01 a	265.4 ± 8.02 ab
24 h			0.16 ± 0.04	32.47 ± 2.25 b	0.05 ± 0.98 a	−5.93 ± 0.86 a	6.01 ± 0.86 b	271.0 ± 9.65 a
48 h			0.16 ± 0.04	34.95 ± 2.70 ab	−0.84 ± 0.60 b	−6.75 ± 0.95 ab	6.81 ± 1.00 ab	263.1 ± 4.50 b
72 h			0.15 ± 0.04	34.86 ± 3.04 ab	−0.75 ± 0.71 ab	−6.51 ± 0.98 ab	6.58 ± 1.01 ab	264.3 ± 6.20 ab
Significance			NS	***	*	**	*	*
Harvest	100%	2 °C	20.0 ± 0.07	37.10 ± 3.32 a	−0.65 ± 1.12	−7.13 ± 0.94 b	7.23 ± 1.01 a	265.4 ± 8.02
24 h			0.17 ± 0.04	33.23 ± 2.89 b	0.14 ± 1.62	−6.03 ± 1.34 ab	6.29 ± 1.09 ab	273.8 ± 17.90
48 h			0.16 ± 0.04	33.55 ± 3.11 b	−0.21 ± 0.81	−5.90 ± 1.22 a	5.96 ± 1.24 b	268.7 ± 7.41
72 h			0.18 ± 0.08	32.46 ± 2.86 b	−0.57 ± 0.48	−5.84 ± 1.00 a	5.89 ± 1.04 b	264.6 ± 3.66
Significance			NS	***	NS	**	**	NS
Significance 24 h			NS	NS	NS	NS	NS	NS
Significance 48 h			NS	NS	NS	NS	NS	NS
Significance 72 h			NS	NS	NS	NS	NS	NS
Storage duration × CO <sub>2</sub>			NS	NS	NS	NS	NS	NS

Data are means with corresponding standard errors (15 replicates per storage regime and duration). Different letters (a,b) indicate significant differences between storage durations within each storage regime (Tukey’s test,  $\alpha < 0.05$ ). \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ ; NS, not significant). Significance 24 h, 48 h, and 72 h are related to the difference between the four storage regimes (0.03% CO<sub>2</sub> and 21% O<sub>2</sub> at 22 °C, 0.03% CO<sub>2</sub> and 21% O<sub>2</sub> at 2 °C, 10% CO<sub>2</sub> and 5% O<sub>2</sub> at 2 °C, and 100% CO<sub>2</sub> at 2 °C).

### 3.2. Primary Metabolites

Figure 1 and Table 2 present the total and individual sugar content in the blueberry fruit from all storage regimes and storage durations, with corresponding statistical analysis. Statistical analysis showed a significant interaction between both factors examined in this study. A general trend in the total sugars for all storage conditions can be observed in Figure 1, with a significant increase from harvest until the first sampling time. The statistical analysis for individual sugars mostly corresponds to that of the total sugars. Further storage under control conditions (between 24 h and 72 h) led to a significant reduction in sucrose and the stagnation of glucose and fructose, which were also predominant sugars in the fruit in the current study. On the other hand, cold storage triggered a significant breakdown of all individual sugars for storage longer than 24 h. However, the difference between harvest and the end of storage was not significant in the 0.03% CO<sub>2</sub> and 10% CO<sub>2</sub> treatments. On the contrary, the fruit stored in 100% CO<sub>2</sub> contained the lowest contents of all three individual sugars at the end of short-term storage, and this was significant. All of these results show that, after 72 h of storage, fruit from the control conditions had the highest sugar content among all storage regimes.

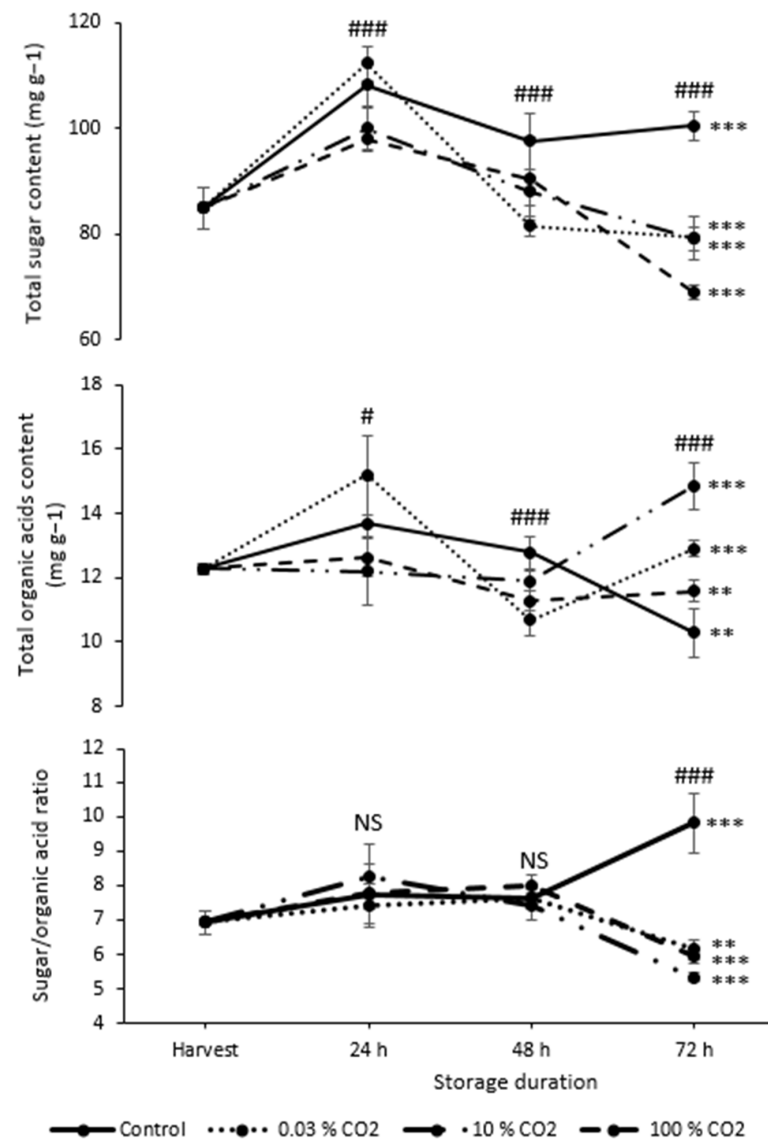
**Table 2.** Contents of individual sugars in blueberry fruits under different storage regimes (0.03% CO<sub>2</sub>, 21% O<sub>2</sub>, and 78% N<sub>2</sub> at 22 °C, i.e., control; 0.03% CO<sub>2</sub>, 21% O<sub>2</sub>, and 78% N<sub>2</sub> at 2 °C; 10% CO<sub>2</sub>, 5% O<sub>2</sub>, and 85% N<sub>2</sub> at 2 °C; 100% CO<sub>2</sub> at 2 °C) for each storage duration.

Storage Duration (h)	CO <sub>2</sub> , O <sub>2</sub> (%)	Temperature (°C)	Sucrose (mg g <sup>-1</sup> )	Glucose (mg g <sup>-1</sup> )	Fructose (mg g <sup>-1</sup> )
Harvest			12.20 ± 1.01 ab	35.81 ± 1.43 b	36.85 ± 1.85 b
24 h	0.03%, 21%	22 °C	13.73 ± 1.72 a	44.64 ± 1.37 a	46.72 ± 1.67 a
48 h			11.22 ± 0.72 b	42.02 ± 2.30 a	44.30 ± 2.75 a
72 h			8.95 ± 0.42 c	44.15 ± 1.21 a	47.33 ± 1.35 a
Significance			***	***	***
Harvest	0.03%, 21%	2 °C	12.20 ± 1.01 b	35.81 ± 1.43 b	36.85 ± 1.85 b
24 h			15.59 ± 0.43 a	47.25 ± 1.45 a	49.32 ± 1.43 a
48 h			10.42 ± 0.26 c	34.81 ± 1.08 bc	36.27 ± 0.58 b
72 h			12.18 ± 0.60 b	32.63 ± 1.69 c	34.49 ± 1.87 b
Significance	***	***	***		
Harvest	10%, 5%	2 °C	12.20 ± 1.01 ab	35.81 ± 1.43 b	36.85 ± 1.85 bc
24 h			11.03 ± 0.33 b	43.32 ± 2.62 a	45.67 ± 1.67 a
48 h			11.83 ± 0.56 ab	37.35 ± 1.08 b	38.82 ± 1.05 b
72 h			12.96 ± 0.67 a	32.15 ± 0.87 c	33.98 ± 0.89 c
Significance	*	***	***		
Harvest	100%	2 °C	12.20 ± 1.01 a	35.81 ± 1.43 c	36.85 ± 1.85 c
24 h			11.86 ± 0.74 a	42.01 ± 0.89 a	44.10 ± 1.04 a
48 h			11.52 ± 0.98 a	38.68 ± 0.72 b	40.22 ± 0.73 b
72 h			9.59 ± 0.50 b	28.53 ± 0.66 d	30.83 ± 0.62 d
Significance	**	***	***		
Significance 24 h			***	***	**
Significance 48 h			NS	***	***
Significance 72 h			***	***	***
Storage duration × CO <sub>2</sub>			***	***	***

Data are means with corresponding standard errors (4 replicates per storage regime and duration). Different letters (a–d) indicate significant differences between storage durations within each storage regime (Tukey’s test,  $\alpha < 0.05$ ). \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ ; NS, not significant. Significance 24 h, 48 h, and 72 h is related to the difference between the four storage regimes (0.03% CO<sub>2</sub> and 21% O<sub>2</sub> at 22 °C, 0.03% CO<sub>2</sub> and 21% O<sub>2</sub> at 2 °C, 10% CO<sub>2</sub> and 5% O<sub>2</sub> at 2 °C, and 100% CO<sub>2</sub> at 2 °C).

Similarly, as for sugars, the interaction between storage duration and CO<sub>2</sub> concentration also affected individual and total organic acids contents. The high variability in the total organic acids between storage conditions and also durations can be observed from Figure 1. The lowest fluctuations during storage were detected in fruit from the 100%

CO<sub>2</sub> treatment and the highest were found in fruit from the 0.03% CO<sub>2</sub> regime. However, the difference between the harvest and the 72 h time points was not significant, which also corresponds with the contents of citric, tartaric, and shikimic acid (Table 3). In the control, a significant breakdown of citric, tartaric, and shikimic acids occurred in the last 24 h of storage, reaching the lowest values among all storage conditions to a significant degree. The opposite happened in storage with 10% CO<sub>2</sub>, where the stagnation of all four individual organic acids was detected in the first 48 h, followed by a significant increase, thus reaching the highest values when compared with the control, 0.03% CO<sub>2</sub>, and 100% CO<sub>2</sub> treatments at 72 h.



**Figure 1.** Total sugar and organic acid contents and sugar/organic acid ratio in blueberry fruit under different storage regimes (0.03% CO<sub>2</sub>, 21% O<sub>2</sub>, and 78% N<sub>2</sub> at 22 °C, i.e., control; 0.03% CO<sub>2</sub>, 21% O<sub>2</sub>, and 78% N<sub>2</sub> at 2 °C; 10% CO<sub>2</sub>, 5% O<sub>2</sub>, and 85% N<sub>2</sub> at 2 °C; 100% CO<sub>2</sub> at 2 °C) for each storage duration. Data are means with corresponding standard errors (4 replicates per storage regime and duration). Significant differences between storage durations within each storage regime (\*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ ) and significant differences between storage regimes within each storage duration (#,  $p < 0.05$ ; ###,  $p < 0.001$ ; NS, not significant) were estimated using Tukey's test ( $\alpha < 0.05$ ).

**Table 3.** Contents of individual organic acids in blueberry fruits under different storage regimes (0.03% CO<sub>2</sub>, 21% O<sub>2</sub>, and 78% N<sub>2</sub> at 22 °C, i.e., control; 0.03% CO<sub>2</sub>, 21% O<sub>2</sub>, and 78% N<sub>2</sub> at 2 °C; 10% CO<sub>2</sub>, 5% O<sub>2</sub>, and 85% N<sub>2</sub> at 2 °C; 100% CO<sub>2</sub> at 2 °C) for each storage duration.

Storage Duration (h)	CO <sub>2</sub> , O <sub>2</sub> (%)	Temperature (°C)	Citric Acid (mg g <sup>-1</sup> )	Tartaric Acid (mg g <sup>-1</sup> )	Malic Acid (mg g <sup>-1</sup> )	Shikimic Acid (mg g <sup>-1</sup> )
Harvest	0.03%, 21%	Control 22 °C	10.97 ± 0.15 a	0.69 ± 0.03 c	0.60 ± 0.02 c	0.012 ± 0.001 a
24 h			11.43 ± 1.33 a	1.09 ± 0.12 a	1.15 ± 0.11 a	0.011 ± 0.001 a
48 h			10.70 ± 0.34 a	1.08 ± 0.08 a	0.99 ± 0.12 ab	0.011 ± 0.001 a
72 h			8.48 ± 0.72 b	0.87 ± 0.05 b	0.92 ± 0.02 b	0.008 ± 0.000 b
Significance			***	***	***	***
Harvest	0.03%, 21%	2 °C	10.97 ± 0.15 b	0.69 ± 0.03 b	0.60 ± 0.02 c	0.012 ± 0.001 b
24 h			12.73 ± 1.07 a	1.14 ± 0.12 a	1.30 ± 0.11 a	0.018 ± 0.001 a
48 h			9.45 ± 0.49 c	0.63 ± 0.02 b	0.61 ± 0.02 c	0.009 ± 0.001 c
72 h			11.43 ± 0.25 b	0.67 ± 0.02 b	0.79 ± 0.02 b	0.012 ± 0.000 b
Significance			***	***	***	***
Harvest	10%, 5%	2 °C	10.97 ± 0.15 b	0.69 ± 0.03 b	0.60 ± 0.02 c	0.012 ± 0.001 b
24 h			9.51 ± 0.92 c	1.34 ± 0.14 a	1.31 ± 0.08 a	0.017 ± 0.001 a
48 h			10.65 ± 0.33 bc	0.69 ± 0.03 b	0.55 ± 0.03 c	0.011 ± 0.001 b
72 h			13.20 ± 0.71 a	0.81 ± 0.01b	0.82 ± 0.03 b	0.012 ± 0.001 b
Significance			***	***	***	***
Harvest	100%	2 °C	10.97 ± 0.15 a	0.69 ± 0.03 c	0.60 ± 0.02 b	0.012 ± 0.001 b
24 h			10.51 ± 0.59 ab	1.11 ± 0.10 a	0.98 ± 0.15 a	0.017 ± 0.001 a
48 h			9.98 ± 0.29 b	0.69 ± 0.03 c	0.60 ± 0.01 b	0.010 ± 0.001 bc
72 h			9.98 ± 0.30 b	0.86 ± 0.07 b	0.74 ± 0.02 b	0.009 ± 0.001 c
Significance			**	***	***	***
Significance 24 h			**	*	**	***
Significance 48 h			**	***	***	**
Significance 72 h			***	***	***	***
Storage duration × CO <sub>2</sub>			***	***	***	***

Data are means with corresponding standard errors (4 replicates per storage regime and duration). Different letters (a–c) indicate significant differences between storage durations within each storage regime (Tukey's test,  $\alpha < 0.05$ ). \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ . Significance 24 h, 48 h, and 72 h are related to the differences between the four storage regimes (0.03% CO<sub>2</sub> and 21% O<sub>2</sub> at 22 °C, 0.03% CO<sub>2</sub> and 21% O<sub>2</sub> at 2 °C, 10% CO<sub>2</sub> and 5% O<sub>2</sub> at 2 °C, and 100% CO<sub>2</sub> at 2 °C).



The relationship between the sugars and organic acids is presented in Figure 1. Similar fluctuations in the sugar/organic acid ratio were detected between the storage regimes from harvest to the 48 h sampling time. Later, an increase occurred in the fruit in the control group, and a reduction occurred in the fruit kept in the cold storage conditions. The fluctuations in sugar/organic acids are the result of the interaction between both factors examined in the present study.

### 3.3. Individual Phenolic Compounds

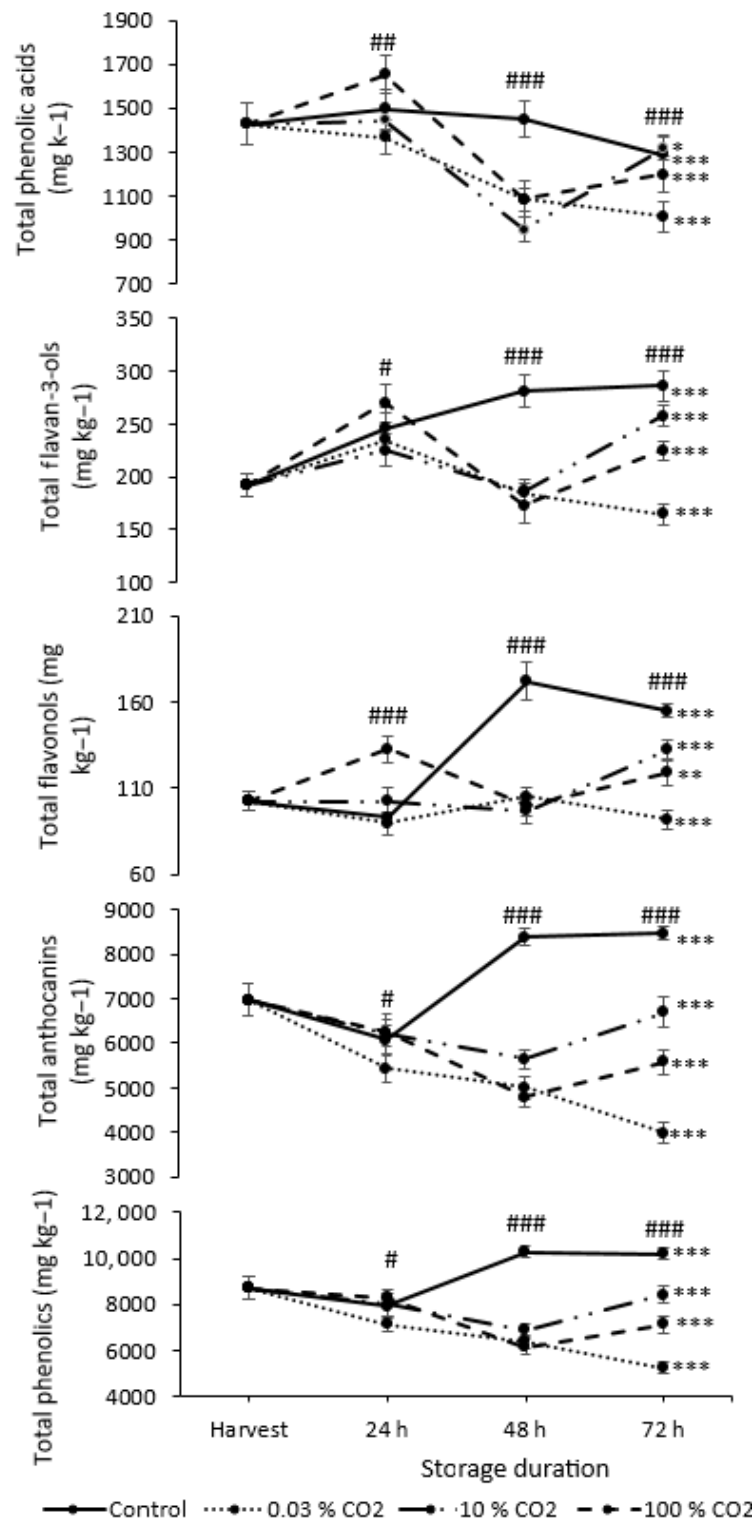
Significant differences during storage in all treatments were measured in individual (Supplementary Material Tables S1–S5) and total phenolic compounds (Figure 2) in the blueberry fruits, which resulted from significant interaction between storage duration and CO<sub>2</sub> concentration. In the control treatment, when the fruit was stored at room temperature in a regular atmosphere, a significant increase in the total phenolics occurred after 48 h. From then on, their content stagnated. On the other hand, in a regular atmosphere and 100% CO<sub>2</sub> storage at 2 °C, a significant breakdown of phenolics occurred after 24 h and 48 h, respectively. At 10% CO<sub>2</sub>, the total phenolic content did not differ between harvest and the end of short-term storage.

As anthocyanins represent the main share of the total phenolics, their content in the blueberry fruit during storage concur (Figure 2). Among the individual anthocyanins that were identified in the 'Bluecrop' blueberry fruit, malvidin derivatives predominated (91–94%), affecting the total anthocyanin content and, at the same time, phenolic compounds (Supplementary Material Tables S1–S4).

Similar to the anthocyanins, a sudden increase between 24 h and 48 h of storage was also observed for individual and, therefore total, flavonols in the fruit kept in the control conditions, where the highest values were, again, measured after 48 h and 72 h (Figure 2, Supplementary Material Tables S1–S4). Similarly, 10% CO<sub>2</sub> increased the contents of all individual flavonols in the last 24 h of storage, while at 100% CO<sub>2</sub>, most of the individual phenolics from the flavonol group increased. On the other hand, some significant fluctuations in individual flavonols were detected in the fruit kept in regular atmosphere cold storage; however, their content remained low, resulting in the most pronounced difference between storage conditions at the end of the storage period (Supplementary Material Table S5).

Two chemical compounds were identified in the flavan-3-ols group, i.e., procyanidin dimer and epicatechin (Supplementary Material Tables S1–S4). According to the data in Figure 2, a significant increase in these two compounds was detected from harvest to the end of storage in the fruit kept under the control and 10% CO<sub>2</sub> treatments, while the opposite occurred in the fruit stored in 100% CO<sub>2</sub>. In cold storage with a regular atmosphere (0.03% CO<sub>2</sub>), the procyanidin dimer content increased significantly in the first 24 h, followed by a prominent breakdown. The fluctuations in procyanidin dimer coincide with the content of total flavan-3-ols, since it is the prevailing compound in the corresponding group of phenolics. On the contrary, the epicatechin content remained statistically the same throughout regular atmosphere short-term cold storage. The most noticeable discrepancy between the storage regimes regarding the flavan-3-ol content was detected after 48 h and 72 h, with the highest content being found in the fruit kept in the control conditions.

The predominant phenolic acid in blueberry fruit is chlorogenic acid, as it represents at least 75% of the total value (Supplementary Material Tables S1–S4). Therefore, the variations in their contents coincide. A different trend in the phenolic acid content fluctuations was observed in the fruit from all four storage regimes when compared with other groups of phenolics. A significant increase was only detected in fruit from the 10% CO<sub>2</sub> regime between 48 h and 72 h of storage and in fruit from the 100% CO<sub>2</sub> treatment in the first 24 h. The most pronounced difference was observed after 48 h, with the highest content found in the fruit from the control. At the end of storage, fruit from the 0.03% CO<sub>2</sub> cold storage condition contained the least phenolic acid content and the fruit from the 10% CO<sub>2</sub> and control conditions contained the most.



**Figure 2.** Contents of individual groups of phenolic compounds in blueberry fruits under different storage regimes (0.03% CO<sub>2</sub>, 21% O<sub>2</sub>, and 78% N<sub>2</sub> at 22 °C, i.e., control; 0.03% CO<sub>2</sub>, 21% O<sub>2</sub>, and 78% N<sub>2</sub> at 2 °C; 10% CO<sub>2</sub>, 5% O<sub>2</sub>, and 85% N<sub>2</sub> at 2 °C; 100% CO<sub>2</sub> at 2 °C) at each storage duration. Data are means with corresponding standard errors (4 replicates per storage regime and duration). Significant differences between storage durations within each storage regime (\*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ ) and significant differences between storage regimes within each storage duration (#,  $p < 0.05$ ; ##,  $p < 0.01$ ; ###,  $p < 0.001$ ) were estimated using Tukey's test ( $\alpha < 0.05$ ).

#### 4. Discussion

One of the main quality parameters of blueberry fruit is firmness, which is strongly dependent on the cultivar [14] and ripening stage [15]. In our experiment, all fruit remained firm during short-term storage, meaning that no pronounced loss in cell turgor occurred [16]. This reaffirmed the fact that a modified atmosphere preserves blueberry fruit quality in a state that is comparable to that at harvest [17]. Concha-Meyer et al. (2015) [18] came to similar conclusions, where highbush blueberry 'Ozark Blue' fruit firmness did not significantly change during four-day storage in regular air at 12 °C and 15% CO<sub>2</sub> at 4 °C. In our results, no significance was determined between the storage conditions within each sampling time. This suggests the independence of blueberry 'Bluecrop' fruit firmness from different storage regimes, including elevated air temperature, in such a short period of time [18].

The dark blue color of blueberry peel makes the fruit intriguing for the consumer. Therefore, one of the main goals of storage is to preserve the peel color to be comparable at harvest and after the end of storage with significant blueberry wax bloom. In all four storage regimes, a significant drop in peel lightness occurred in the first 24 h of storage, suggesting that the fruit became darker. Similar results were reported by Koort et al. (2018) [19], where *L\** decreased from 29.0 (pre-storage) to 26.0 in a regular atmosphere and to 27.0 in a modified atmosphere with 9.4% CO<sub>2</sub>. On the other hand, the increased *b\** value of the fruit from all storage conditions except those kept in 0.03% CO<sub>2</sub> indicates that the fruit became less blue. At the same time, the *C\** value decreased, meaning that the blueberry peel acquired a less intense color [19]. Both parameters could be correlated with a significant drop in total anthocyanins during storage.

The interaction between O<sub>2</sub> and CO<sub>2</sub> strongly affects blueberry fruit performance in storage, and the optimal ratio of these two gases differs between cultivars [7]. The lower limit regarding O<sub>2</sub> concentration is usually 1% (optimal is 2–3%), below which fermentation is likely to occur, while the upper CO<sub>2</sub> level depends on the cultivar and corresponding O<sub>2</sub> concentration. In our experiment, a significant breakdown of sucrose occurred in the control, and a reduction of glucose in 0.03% CO<sub>2</sub> and 10% CO<sub>2</sub> cold storage, and the degradation of all sugars in 100% CO<sub>2</sub> were observed. On the other hand, glucose and fructose, which are also the prevailing sugars in 'Bluecrop' fruit, increased significantly during storage, possibly resulting from sucrose degradation [8,20]. The highest amount of total sugars was, at the same time, measured in the fruit from the control conditions after the end of storage. In a regular cold atmosphere and storage at 10% CO<sub>2</sub>, the significant and equal drop in the amount of glucose indicates that it could be used for respiration processes, which is in agreement with the findings from our previous investigation [4]. However, in the regular atmosphere storage, the only limiting factor for respiration was temperature, while in the 10% CO<sub>2</sub> conditions, there was a combination of low temperature and changed atmosphere composition; since similar variations in individual sugars were measured (sucrose, glucose, and fructose), we can assume that 10% CO<sub>2</sub> combined with 5% O<sub>2</sub> is not an optimal gas composition for 'Bluecrop' blueberry fruit from the primary metabolite's, i.e., monosaccharide, point of view. After 72 h of storage, the lowest concentration of individual sugars was identified in the fruit from the 100% CO<sub>2</sub> treatment. As mentioned above, the prolonged exposure of fruit to elevated CO<sub>2</sub> levels may cause toxic damage to the fruit [8], and for that reason, it could be successfully used in the suppression of pest development [6,13,21]. At the same time, a lack of O<sub>2</sub> (usually under 1%) may accelerate fermentation [8].

A high air temperature is usually correlated with an elevated respiration rate, where individual sugars and organic acids are used as substrate compounds [8]. This could explain the outcomes of the current study, where, after the end of storage, the lowest content of organic acids was measured in the fruit kept under the control conditions. The highest amount of organic acids was detected after 48 h of storage in the fruit stored in 10% CO<sub>2</sub>, where the values were even higher than those at harvest. This corresponds to the fluctuations in citric acid. In fruit stored in an atmosphere without O<sub>2</sub>, a significant

breakdown of citric and shikimic acid occurred after 24 h of storage, which once more confirms the unsuitability of this atmosphere for the storage of 'Bluecrop' fruit for longer than 24 h.

The sugar/organic acid ratio determines the sweetness/acidity of the fruit. From our results, we can see that air temperature or composition did not affect the sweetness of the 'Bluecrop' fruit in the first 48 h of exposure, which could be a consequence of the increased sugar content and/or the reduction in organic acids. From the second sampling time onwards, a discrepancy between the control and cold storage regimes occurred due to the significant breakdown of citric and shikimic acids.

The optimal O<sub>2</sub>/CO<sub>2</sub> ratio that preserves blueberry fruit quality at a high level in storage differs between cultivars [17,22]. In the current study, changes in the individual and total phenolic compounds occurred between storage conditions and durations, which again confirms cultivar specificity regarding atmosphere composition in storage. At the same time, the role of air temperature on the fruit chemical profile during short-term storage should not be neglected.

An initial drop in anthocyanins at the beginning of storage occurred in all storage regimes, followed by greater variability in their fluctuations between treatments. The most pronounced discrepancy was noticed between the cold storage treatments and the control conditions (room temperature), since a significant increase occurred in the fruit in the latter. Kalt et al. (2003) [22] reported that anthocyanin synthesis still continues after the harvest in highbush blueberry 'Bergitta', 'Bluegold', and 'Nelson' fruit when stored at 20 °C. Secondary metabolites, including anthocyanins, can be enhanced in strawberry fruit by increasing the storage temperature up to 10 °C [23]. As already reported, the ambient temperature is an important factor affecting the synthesis of this particularly important quality parameter [24]. Among the cold storage conditions, 10% CO<sub>2</sub> preserved anthocyanins at the highest level and 0.03% CO<sub>2</sub> at the lowest, suggesting that the optimal value for CO<sub>2</sub> concentration in the current study for 'Bluecrop' fruit is 10%. An oxygen-free atmosphere proved to be unsuitable for the short-term storage of 'Bluecrop' regarding the anthocyanin content in the blueberry peel, indicating the high toxicity of elevated CO<sub>2</sub> concentrations on fruit in these storage conditions [25].

The fluctuations in the total flavonols in the cold storage regimes were not extremely pronounced; however, a significant increase was still detected after 72 h in 10% CO<sub>2</sub> and 100% CO<sub>2</sub>. Blanch et al. (2012) [25] reported that the contents of quercetin 3-glucuronide and kaempferol 3-glucuronide increased in strawberries after a 3-day exposure to 20% CO<sub>2</sub>. This could be a tolerance strategy of the fruit against CO<sub>2</sub>. The increased flavonols in the current study also suggest that the persistence of all individual flavonols, which were identified in the 'Bluecrop' fruit, was not interfered with due to the short exposure to the toxic behavior of high CO<sub>2</sub> levels or a lack of O<sub>2</sub> [5].

The procyanidin dimer content did not differ between the harvest and end of storage periods in the current study, which is in accordance with the results published by Blanch et al. (2012) [25]. In storage with 10% CO<sub>2</sub> and 100% CO<sub>2</sub>, the procyanidin dimer and epicatechin contents, which represent total flavan-3-ols, significantly increased. Similar results were obtained during the research on strawberries, where the total flavan-3-ols increased during 3-day storage under 20% CO<sub>2</sub> and remained statistically the same under 40% CO<sub>2</sub> [23]. According to Blanch et al. (2012) [25], an increased CO<sub>2</sub> concentration may enhance the flavan-3-ols content.

Phenolic acids are the second most abundant group of phenolic compounds in blueberry fruit, which is attributed to the high chlorogenic acid content [14]. Despite noticeable fluctuations in the amount of individual and total phenolic acids, significant differences were still detected at all sampling times. Nevertheless, a general trend for all storage conditions in content change could be observed, with initial stagnation (0.03% CO<sub>2</sub>, 10% CO<sub>2</sub>) or an increase (control, 100% CO<sub>2</sub>) in the first 24 h, followed by a significant drop. The sudden increase after the harvest indicates that the biosynthesis pathway of phenolic compounds is still active in picked fruit [26]. The significant breakdown until the end of short-term

storage suggests the poor persistence of individual phenolic acids in ‘Bluecrop’ fruit in storage for longer than 24 h. In our previous study, conducted on ‘Liberty’ fruit [4], the amount of total phenolic acids significantly increased from harvest until the end of 62-day storage in a regular atmosphere and a modified atmosphere with 5% CO<sub>2</sub>. This once more confirms that successful fruit storage largely depends on the cultivar.

## 5. Conclusions

Short-term, modified-atmosphere cold storage has recently been used to inhibit the development of various pest species [6,12]. The current research is the first to present the effect of the changed atmosphere in storage, especially the atmosphere without O<sub>2</sub> (100% CO<sub>2</sub>), on the exact maturity parameters and chemical profile of blueberry fruit. Fruit in all storage regimes remained equally firm from harvest until the end of short-term storage. Regarding the blueberry peel color parameters, significant, but still minor, differences that are hard to detect with the naked eye were observed between the storage regimes and durations. For the preservation of the sugar/organic acid ratio at the initial level, all three cold storage regimes would be appropriate, but only when the storage duration does not exceed 48 h. From then on, a significant reduction in the sugar/organic acid ratio may occur. The total phenolic content was more affected by air temperature than air composition since the highest contents during storage were measured in the control treatment. Thus, 10% CO<sub>2</sub> could be a suitable method for 72 h storage and 100% CO<sub>2</sub> suitable for 48 h storage, from the phenolics’ point of view.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/horticulturae10020194/s1>, Figure S1: Some examples of blueberry fruit in plastic containers and heat-sealed bags prepared for storage treatments. Table S1: Individual phenolic compounds in blueberry fruit stored in regular atmosphere at 22 °C (control) at each storage duration. Table S2: Individual phenolic compounds in blueberry fruit stored in regular atmosphere at 2 °C at each storage duration. Table S3: Individual phenolic compounds in blueberry fruit stored in modified atmosphere with 10 % CO<sub>2</sub> at 2 °C at each storage duration. Table S4: Individual phenolic compounds in blueberry fruit stored in modified atmosphere with 100 % CO<sub>2</sub> at 2 °C at each storage duration. Table S5: The results of one-way analysis of variance for individual phenolic compound at each storage duration.

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**Conflicts of Interest:** The authors declare no conflicts of interest.

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