

## Effects of harvest dates on the physiology and quality attributes of blueberries during long-term postharvest storage

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Harvest time has an important influence on the storability of fruits. The present study was conducted to investigate the effect of harvest dates on the postharvest quality of Northland blueberries during 49 days of storage at  $4\pm 1$  °C. We examined fruit weight loss, decay rate, firmness, soluble solid content (SSC), titratable acidity (TA), vitamin C (VC), total phenol, flavonoid, anthocyanin and malondialdehyde (MDA) content, polyphenol oxidase (PPO) and peroxidase (POD) activities in five blueberry batches with different (early, middle, and late) harvest times. The results of analysis of variance indicated that after harvest, blueberries underwent deterioration of their qualities owing to fast ripening and senescence. Among the five examined batches, those harvested 60 and 65 days after flowering (middle harvest) were less affected by the storage period. Fruits from these batches maintained longer postharvest quality and showed extended storage life through the deceleration of weight loss, decay incidence, PPO activity, softness, TA, SSC, VC, and increase in the total POD activity, phenol, flavonoid, anthocyanin, and MDA content. Our data indicated that compared with other batches, the weight loss and decay rate of middle-harvested blueberries can be reduced 5%, and the storage period can be extended 14 days. Therefore, we believe that the early and late harvested fruits are better suited for fresh fruit sales, while fruits with intermediate harvest dates are optimal for long-term storage. Our study provides a reference framework for the postharvest storage of certain blueberry cultivars by taking into account their harvest dates.

**Keywords:** Blueberry, harvest dates, cold storage, fruit quality, physiological changes.

### INTRODUCTION

Blueberry (*Vaccinium* spp.), which is also named 'Golden berry', is currently one of the most valuable fruits worldwide. Blueberries have high antioxidant activity and numerous health benefits (Jara-Palacios *et al.*, 2019), which makes them popular among consumers. They are good sources of anthocyanins, flavonols, and have strong antioxidant capacity (Li *et al.*, 2017). Blueberries are also rich in micro-nutrients and have a wide range of bioactive phytochemicals with antidiabetic, intestinal microbiota-modulating, anti-carcinogenic, immunity-enhancing, and cardio-protective properties (Ge *et al.*, 2019). Due to their nutritional and health characteristics, there has been a vast increase in the consumption of berry fruits in general-blueberries in particular-in recent years. During the last decade, the global blueberry cultivation area has grown at an average annual rate of 20-30%, and blueberries have become the second most important soft fruit species after strawberries (Giongo *et al.*, 2013).

Due to their successive flowering, fruit setting and maturity characteristics, blueberries are usually harvested in batches. Most of their fruits ripen within a 20-30 day period, and the biochemical, physiological, and structural changes that happen during this time will determine fruit quality. Better fruit quality will be obtained if the fruits are harvested at the suitable time, and the fruits with the intensity of grooves around the stylar-scar end of fruits from 0.075 mls to 0.425 mls in different mango varieties often have better storage capacity (Abu *et al.*, 2021). Moreover, harvest dates can also determine consumer acceptance and the market potential of fruits (Guerra *et al.*, 2009).

The effects of harvest dates on the physiology and quality changes of blueberries during storage have not been reported until now. The aim of our work was to evaluate the effects of different harvest dates on selected quality parameters, and to determine the most suitable harvest date for the maintenance of postharvest quality. We evaluated physiological parameters of the berries of the Northland cultivar, stored at  $4\pm 1$  °C, from different harvest periods. Our results could



serve as an important guideline for determining suitable harvest dates and to extend the postharvest life of blueberries.

## MATERIALS AND METHODS

**Sample preparation:** Fruit selection, packaging and storage. Fruits of the northern highbush blueberry variety 'Northland' were collected from the experimental orchard of Xiongyue Town, Yingkou city, Liaoning province, China. Tests were applied on five batches of blueberries. Fully ripe blueberries were harvested 50, 55, 60, 65, and 70 days after full bloom (DAFB) at 25 °C to 30 °C. The picked fruits were marked as I, II, III, IV and V, which corresponded to batches representing early, middle, and late harvest dates. Only blueberries with uniform size, color, and without any mechanical damage, were selected for this analysis. On the day of the harvest, about 125 g of fruits were labelled, weighed, and put into polyethylene terephthalate (PET) boxes. The samples were stored randomly. Each batch was composed of 5 kg of blueberries, which was divided into 20 small boxes. The fruits were precooled at 4±1 °C for 24 hours, and then stored at 4±1 °C, under a relative humidity of 95%. Samples were taken for quality assessment following 0, 7, 14, 21, 28, 35, 42, and 49 days of storage. Fruit samples were used in triplicates for further experiments.

**Determination of fruit weight loss, decay rate, and firmness** Fruit weight loss, decay rate, and firmness. Fruit weight loss was calculated by the following equation:

$$\text{Fruit weight loss (\%)} = \frac{\text{Initial fruit weight} - \text{final fruit weight}}{\text{Initial fruit weight}} \times 100$$

Decay rate was calculated by the following equation:

$$\text{Decay rate (\%)} = \frac{\text{Number of decayed fruits}}{\text{Total number of fruits}} \times 100$$

Firmness was measured as follows: twenty fruits from each batch were analyzed using a digital fruit sclerometer (GY-4, Handpi, China) fitted with a plunger (2 mm diameter). Values were expressed in kilogram per square centimeter (kg cm<sup>-2</sup>).

**Determination of soluble solid content (SSC), titratable acidity (TA) and vitamin C (VC) content:** SSC, TA and VC content. SSC and TA of each fruit sample were measured from the pressed juice using a digital Brix-Acidity Meter (PAL-BX/ACID 5, Atago, Japan). SSC and TA were expressed as grams of per 100 grams fresh fruit (g 100g<sup>-1</sup>) of Brix and malic acid, respectively.

VC was determined following the procedure of Yang *et al.* (2010) by the 2, 6-dichloroindophenol titrimetric method, and it was expressed as milligrams of ascorbic acid per 100 grams of fresh fruit (mg 100g<sup>-1</sup>).

**Determination of total phenol, flavonoid, and relative anthocyanin content:** Total phenol, flavonoid, and relative anthocyanin content. Total phenol, flavonoid and relative anthocyanin content was determined according to Cao *et al.* (2011), with some modifications. Briefly, two grams fruit

flesh was put into the pre-cooled 1% hydrochloric acid-methanol solution, homogenized, and diluted to 20 mL in a scaled test tube. The samples were extracted at 4 °C in the dark with shaking for 20 min, and then centrifuged for the supernatant with high speed freezing centrifuge (5430R, Eppendorf, Germany). Absorbance values were recorded at 280 nm (total phenolic content), 325 nm (flavonoid content), 530 and 600 nm (anthocyanin content), respectively, using a UV-2550 spectrophotometer (Beijing Purkinje General Instrument Co. Ltd., Beijing, China). We used the 1% hydrochloric acid-methanol solution as a blank reference calibrator. Values were expressed per gram of the extract (U g<sup>-1</sup>).

**Determination of polyphenol oxidase (PPO), peroxidase (POD) activity and malondialdehyde (MDA) content:** For each sample, two grams of blueberries were homogenized (on ice) in 0.1 M of phosphate buffer. The mixtures were centrifuged for 15 min at low temperature. Supernatants were used for assaying the PPO and POD activities. PPO activity was determined by a colorimetric assay using catechol. Changes in the absorbance were recorded at 420 nm (Zhao *et al.*, 2019). POD activity was assayed by measuring the change of absorbance at 470 nm, using guaiacol colorimetry. PPO and POD activity was defined as a 0.01 min<sup>-1</sup> increase in the absorbance under standard reaction conditions. Values were expressed per minute and gram of the enzyme extract (U min g<sup>-1</sup>).

MDA content was determined by the thiobarbituric acid (TBA) chromogenic method described by Liu *et al.* (2018). Briefly, two grams of sample material were thoroughly mixed with 100 g L<sup>-1</sup> trichloroacetic acid (TCA), brought to a total volume of 25 mL, and subjected to centrifugation at 4 °C for 15 min. Then, 2 mL of TBA was put into 2 mL supernatant, and the mixture was boiled for 15 min. After cooling the mixture on ice, it was centrifuged again. Absorbance of the supernatant was recorded at 600, 532, and 450 nm. MDA content was expressed as μmol g<sup>-1</sup> mF and calculated as: MDA (μmol g<sup>-1</sup> mF) = [6.45 × (A<sub>532</sub> - A<sub>600</sub>) - 0.56 × A<sub>450</sub>] / 160.

**Statistical analysis:** Data was checked for normality of errors and homogeneity of variances prior to the statistical analyses. The experimental data was preprocessed by Excel 2007 software, the figures were plotted by Origin 2018 software, statistical differences between the harvest batches were calculated with SPSS 18.0 software, Duncan's test was used to detect differences among batches. All of the statistical tests were two-tailed, and p < 0.05 was defined as the margin of significance.

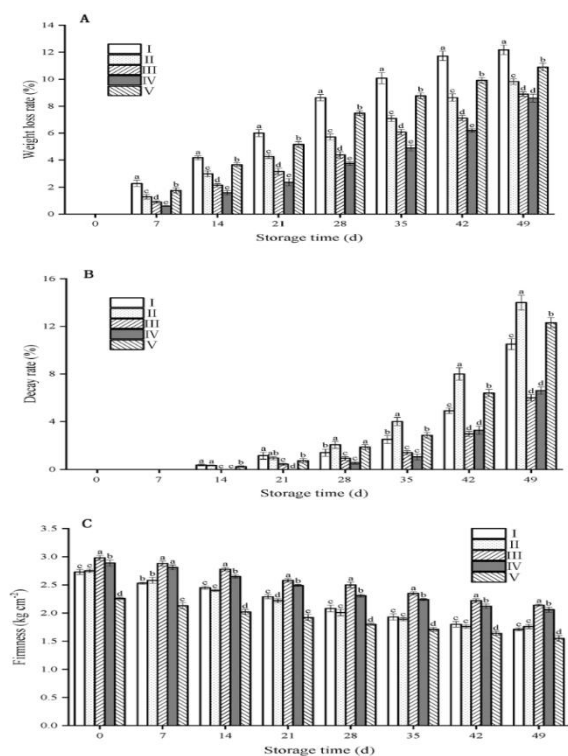
## RESULTS

**Effects of different harvest dates on fruit weight loss, decay rate, and fruit firmness in blueberries during storage:** The weight loss of blueberries increased until the end of storage for all harvest dates, as shown in Fig. 1A. After 49 days of

storage, the weight loss rates of batches I to V were 12.2%, 9.8%, 8.9%, 8.6%, and 10.9%, respectively. There were significant differences between the batches. Fruits from the middle batch (III, IV) had minimal changes compared with the early (I, II) and late (V) batches.

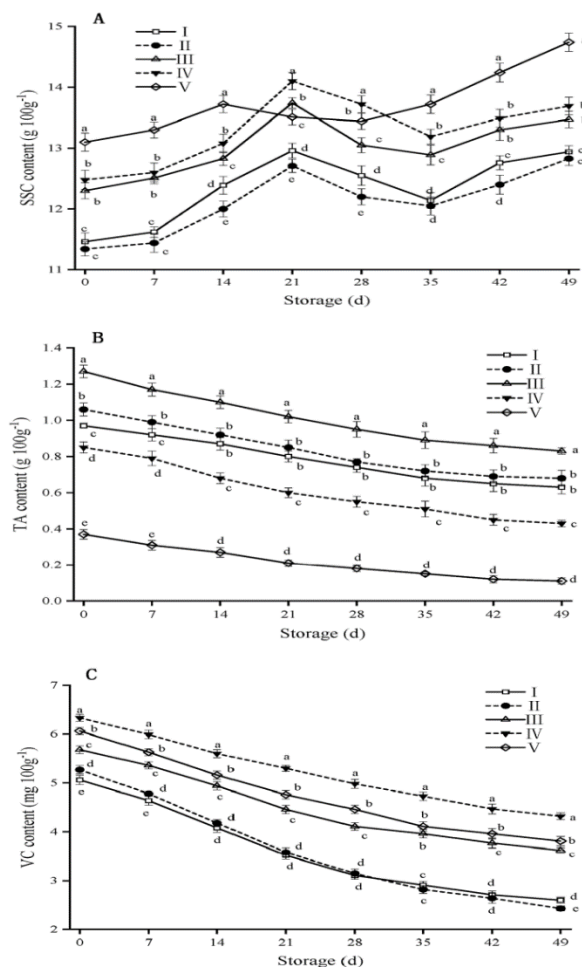
As shown in Fig. 1B, decay occurred when stored for 14 days for batches I, II, and V, 21 days for III, and 28 days for IV. Decay rates for each batch increased with storage time. After 49 days of storage the decay rates were 10.5%, 14.0%, 6.0%, 6.6%, and 12.3% for each batch, respectively. Differences were significant, except for batches III and IV, which had lower decay rates than the other groups.

As shown in Fig. 1C, fruit firmness progressively decreased over time in all samples. Fruit firmness at harvest was 2.73 kg cm<sup>-2</sup> for I, 2.75 kg cm<sup>-2</sup> for II, 2.98 kg cm<sup>-2</sup> for III, 2.89 kg cm<sup>-2</sup> for IV and 2.26 kg cm<sup>-2</sup> for V. Significant differences could be observed between each batch, except for I and II. Firmness of III and IV decreased slowly and more steadily compared to the other batches. Concerning the weight loss values, after 49 days of storage these were 37.36%, 36.00%, 28.19%, 28.72% and 31.42%, for batches I to V, respectively.



**Figure 1. Effect on weight loss rate (A), decay rate (B), and fruit firmness (C) in blueberries during storage under batches I (50 DAFB), II (55 DAFB), III (60 DAFB), IV (65 DAFB) and V (70 DAFB).** Column shows mean values of three replicates and bar shows the standard error of means. Columns labeled with different alphabets show significant difference according to LSD test at 5% probability.

**Effect of different harvest time on the SSC, TA, and VC content of the blueberries during storage:** In our study, SSC increased initially and then decreased, but the TA and VC content kept decreasing during storage. As illustrated in Fig. 2A, fruit SSC content initially increased in all groups, but then decreased. The SSC values for groups I to V were 11.46 g 100g<sup>-1</sup>, 11.34 g 100g<sup>-1</sup>, 12.3 g 100g<sup>-1</sup>, 12.48 g 100g<sup>-1</sup>, and 13.1 g 100g<sup>-1</sup> when picking, respectively. For I, II, III and IV, these values reached a maximum on the 21st day of storage, after which they decreased. For batch V, however, the maximum was reached after only 14 days of storage. After the 49-day storage period, SSC increases were 12.91%, 13.14%, 9.51%, 9.70%, 12.52%, for batches I to V, respectively. The SSC exhibited a small increase for middle batch (III, IV) fruits.



**Figure 2. Effect on SSC content (A), TA content (B), VC content (C) in blueberries during storage under batches I (50 DAFB), II (55 DAFB), III (60 DAFB), IV (65 DAFB) and V (70 DAFB).** Column shows mean values of three replicates and bar shows the standard error of means. Columns labeled with different alphabets show significant difference according to LSD test at 5% probability.

TA showed a decreasing trend during storage (Fig. 2B), with statistical differences recorded between the different batches of blueberries. TA values were  $0.97 \text{ g } 100\text{g}^{-1}$  for I,  $1.06 \text{ g } 100\text{g}^{-1}$  for II,  $1.27 \text{ g } 100\text{g}^{-1}$  for III,  $0.85 \text{ g } 100\text{g}^{-1}$  for IV, and  $0.37 \text{ g } 100\text{g}^{-1}$  for V, indicating that TA values were lower in late-harvested fruits. The rates of TA decline were 35.05%, 35.85%, 34.65%, 49.41% and 70.27% for batches I-V, respectively, during the storage period. This decline was higher in the early-harvested fruits.

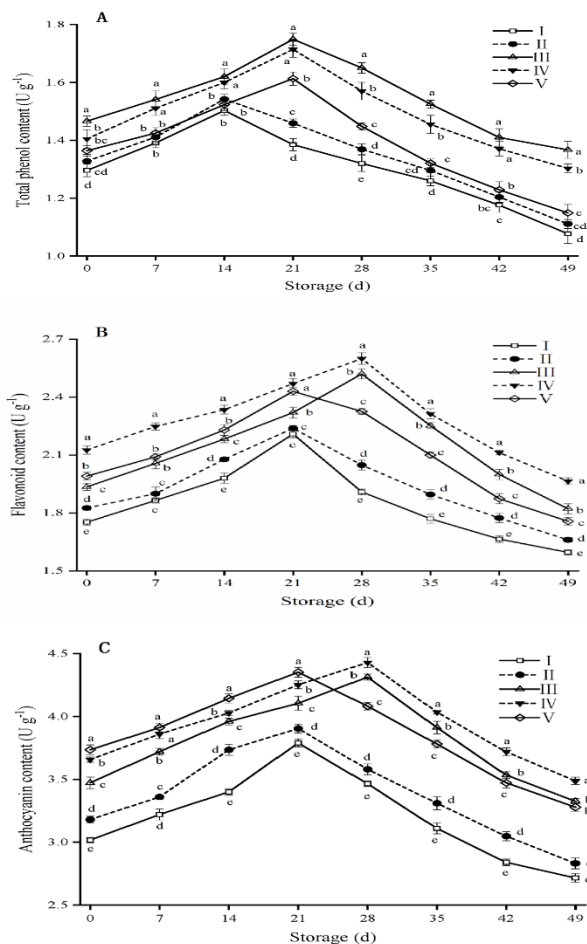
As shown in Fig. 2C, the original VC content of each group was  $5.06 \text{ mg } 100\text{g}^{-1}$ ,  $5.27 \text{ mg } 100\text{g}^{-1}$ ,  $5.68 \text{ mg } 100\text{g}^{-1}$ ,  $6.33 \text{ mg } 100\text{g}^{-1}$ , and  $6.06 \text{ mg } 100\text{g}^{-1}$ , respectively. These values decreased at varying rates after 49 days of storage (48.62%, 53.89%, 36.27%, 31.75% and 37.13%, for I-V, respectively). These results indicate that the VC amount was correlating with harvest dates. VC content was high at the time of harvest, and its decrease was more moderate in the late-harvested fruits, suggesting that late harvesting lengthened the preservation period of VC.

**Effect of different harvest time on the total phenol, flavonoid, and anthocyanin content in blueberries during storage:** The total phenol content of blueberries increased steadily in the first 14 (for batches I and II) and 21 days (for the rest), after which it went into a rapid decline until the end of the storage period. Total phenol content ranged between  $1.30 \text{ U g}^{-1}$  to  $1.47 \text{ U g}^{-1}$  at harvest, with significant differences between each batch. At the end of the storage period, however, total phenol content ranged between  $1.08 \text{ U g}^{-1}$  to  $1.37 \text{ U g}^{-1}$ , with batches III and IV being significantly higher than the others. The rates of decline in the total phenol content compared to the initial values were 16.90%, 16.34%, 6.75%, 7.26%, and 15.76%, for I-V, respectively, after 49 days of storage (Fig. 3A).

The flavonoid content changes of blueberries showed that there were significant differences in flavonoid content from different harvest dates blueberries in Fig. 3B. Their amounts increased initially, but then declined in the later stages of storage. Flavonoid values were between  $1.75 \text{ U g}^{-1}$  and  $2.13 \text{ U g}^{-1}$  at harvest, and between  $1.60 \text{ U g}^{-1}$  to  $1.97 \text{ U g}^{-1}$  after 49 days of storage. Declines in the total phenol content during the cold storage period were 8.90%, 9.04%, 5.84%, 7.57%, and 11.75% for each batch, respectively. There was a slow increase and a slight loss in flavonoid content in the blueberries harvested in the middle of the five harvesting periods (batches III and IV).

As shown in Fig. 3C, changes in anthocyanin content roughly corresponded with the level of total phenols and flavonoids. Values ranged from  $3.02 \text{ U g}^{-1}$  to  $2.72 \text{ U g}^{-1}$  for I, from  $3.18 \text{ U g}^{-1}$  to  $2.83 \text{ U g}^{-1}$  for II, from  $3.47 \text{ U g}^{-1}$  to  $3.33 \text{ U g}^{-1}$  for III, from  $3.66 \text{ U g}^{-1}$  to  $3.49 \text{ U g}^{-1}$  to IV and from  $3.74 \text{ U g}^{-1}$  to  $3.28 \text{ U g}^{-1}$  for V, from the beginning to the end of storage. The rates of decline for this parameter were 9.98%, 10.94%, 4.21%, 4.62%, and 12.21% for batches I-V, respectively. There was

also a slow increase and a slight loss in anthocyanin content in the middle-harvested blueberry fruits.

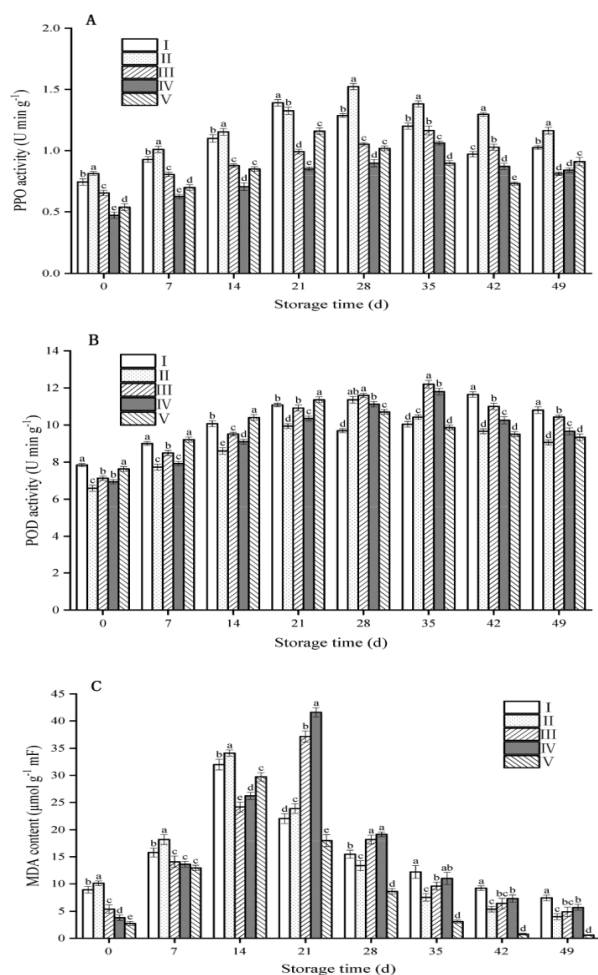


**Figure 3.** Effect on total phenol content (A), flavonoid content (B) and anthocyanin content (C) in blueberries during storage under batches I (50 DAFB), II (55 DAFB), III (60 DAFB), IV (65 DAFB) and V (70 DAFB). Column shows mean values of three replicates and bar shows the standard error of means. Columns labeled with different alphabets show significant difference according to LSD test at 5% probability.

**Effect of different harvest times on the PPO and POD activities and MDA content of blueberries during storage:** PPO activity reached peak value 21 days after storage for batches I and V, 28 days for batch II, and 35 days for batches III and IV, after which it continuously declined except for in batches III and IV (Fig. 4A). The peak values ranged from  $1.06 \text{ U min}^{-1}$  to  $1.52 \text{ U min}^{-1}$ . Interestingly, PPO activities were lower, and peaked later, in the middle-harvested fruits compared to the other groups.

POD activities of batches II, III, IV and V oscillated during the storage period (Fig. 4B). Middle-harvested blueberries had higher POD activities, with significantly higher peaks, which appeared later compared to the other batches.

Fig. 4C shows a gradual increase in the MDA content in all samples, followed by a decrease (after 14 and 21 days), indicating that a longer storage period aggravates the oxidative damages in the blueberries. MDA content at harvest ranged from 2.75  $\mu\text{mol g}^{-1}$  mF to 10.10  $\mu\text{mol g}^{-1}$  mF, but at the peak they measured 32.00  $\mu\text{mol g}^{-1}$  mF, 34.12  $\mu\text{mol g}^{-1}$  mF, 37.12  $\mu\text{mol g}^{-1}$  mF, 41.60  $\mu\text{mol g}^{-1}$  mF, and 29.73  $\mu\text{mol g}^{-1}$  mF, for batches I-V, respectively. Middle-harvested blueberries had higher peaks which appeared later compared with the early and late-harvested fruits.



**Figure 4. Effect on PPO activity (A), POD activity (B) and MDA content (C) in blueberries during storage under batches I (50 DAFB), II (55 DAFB), III (60 DAFB), IV (65 DAFB) and V (70 DAFB).** Column shows mean values of three replicates and bar shows the standard error of means. Columns labeled with different alphabets show significant difference according to LSD test at 5% probability.

## DISCUSSION

The blooming period of northern highbush blueberries usually spans a period of 3-4 weeks, while fruit development takes 42-90 days. Fruit quality is closely related to harvest period. Poor fruit quality is found in early-harvested fruits, while fruit storage is poor for late-harvested fruits. Therefore, the harvest time has great significance for the storage of fruits (Liu *et al.*, 2018). These conclusions are generalized and require specific research for different varieties of fruits (Lobos, 2018). In this study, we found that harvest dates affected fruit quality, and overall, the middle-harvested blueberries had higher fruit firmness, SSC, VC, total phenol, flavonoid and anthocyanin content, as well as lower weight loss and decay rates compared with the early- or late-harvested batches.

Weight loss results from transpiration and respiration during storage and causes surface wilting and loss of commercial value of fruits (Gao *et al.*, 2013). Fruits and vegetables generally show loss of freshness at 3-10% of weight loss. Decay is caused by the corrosion of the fruit by microorganisms during storage (Chun *et al.*, 2013). Firmness refers to the resistance of the fruit when pressed, and it is an important indicator in the ripening and senescence of fruits. Weight loss, decay, and firmness are important external factors in the evaluation of fruit quality (Sun *et al.*, 2014). In this study, the weight loss rates of I and V were higher than 10% after 49 days of storage, while those of III and IV were significantly lower than the other batches. The decay rates of the different batches were similar until day 28, after which a gradual acceleration could be observed towards the end of the storage period (49 days). For batches III and IV, however, this acceleration was significantly slower (Fig. 1B). Similarly, a more gradual and slower decrease in fruit firmness was noted for III and IV compared to the other batches. This result is somewhat contradictory to an earlier study, where fruits that were picked too early stayed firmer over the whole storage period (Guerra and Casquero, 2008). The loss of berry moisture (measured as weight loss) usually coincides with decreased firmness (Cantín *et al.*, 2012). Treatments which restrict blueberry water loss to below 8% are likely to be beneficial in retaining acceptable berry firmness for storage up to three weeks (Paniagua *et al.*, 2013). Moisture loss due to reduced turgor-prompts the postharvest softening of blueberries (Angeletti *et al.*, 2010).

Soluble solids and titratable acidity are important determinants of fruit flavor. SSC showed a somewhat oscillating pattern during the storage period. Initially, an early increase-probably owing to a higher content of macromolecules like starch in the maturing fruits-was recorded. This was followed by a later, downward trend which was most likely owing to a partial degradation of these macromolecules. Finally, the last increasing trend during the later stages of storage could be explained by the transpiration-induced water loss and final events of the ripening process.

Since blueberries are expected to stop sugar accumulation once the fruits are picked (non-climacteric behavior), and there is no starch that can be converted into soluble sugar, an increase in carbohydrate levels may be due to cell wall degradation (Cheng *et al.*, 2020). Titratable acids are also indicators of ripening. TA levels declined throughout the storage period; these changes are not explained by the declining moisture content of the berries, but rather by the onset of senescence and the release of additional acids associated with fruit softening and cell wall breakdown (Tilahun *et al.*, 2020). Ascorbic acid is an antioxidant and an important marker of fruit metabolic status. Several factors (e.g., growing season, location, agricultural practices, cultivar, and crop ripeness) may affect ascorbic acid levels at harvest to varying degrees. In this study, we showed that an increase in VC when picking could also be an internal indicator of blueberry ripening, but that VC content progressively decreased during the storage period. This steady decrease could either be due to less effective recycling, or the presence of factors that promote the oxidation of the ascorbic acid pool.

Anthocyanins, total phenols, and total flavonoids, collectively known as polyphenols, are bioactive compounds. They exhibit antioxidant, cardioprotective, and anti-inflammatory properties, improve cardiovascular health, protect vision, and inhibit cancer (Roopchand *et al.*, 2013). Phenols also can maintain the nutritional qualities (e.g., stringency, color, bitterness, and flavor) of fruits and vegetables (Brahem *et al.*, 2017). In this study, there was an early increase in the total phenol, flavonoid and anthocyanin content during the storage period, followed by a decrease in storage time, which was mostly likely because of the oxidation by polyphenol oxidase and peroxidase. We attribute this initial increase to an early synthesis of bioactive compounds with antioxidant properties in the initial days after harvest. Our results were consistent with the conclusions of Sen *et al.* (2017) and Junxiang *et al.* (2015). Middle-harvested blueberries had higher total phenol, flavonoid, and anthocyanin content compared with the other batches, indicating that middle harvesting seems to have reduced the loss of bioactive compounds that could be oxidized into quinones by PPO or POD. In other words, the higher content of bioactive compounds in these batches (III and IV) might be due to a lower PPO and higher POD activities in these blueberries.

Enzymes play important roles in fruit ripening during postharvest storage. PPO activity is related to the variety and maturity of fruits, which can reflect the degree of fruit browning (Zhao *et al.*, 2019). POD can decompose the hydrogen peroxide in the fruit into oxygen and water, and can measure the ability of the system to scavenge free radicals. MDA is one of the important products of lipid peroxidation, and its amount can evaluate the degree of cell membrane damage and the preservation effect of fruits (Zhou *et al.*, 2014; Cheng *et al.*, 2020). In our study, blueberries from

batches III and IV had a lower PPO, but a higher POD activity (both peaking later) compared to the other batches. MDA, on the other hand, continuously increased during the storage period, and both PPO and POD activities peaked after the MDA levels reached their highest points. During the storage period, POD activity was induced by ripening, but MDA accumulation inhibited POD activity. This was in accordance with a previous study carried out by Wang *et al.* (2019), where POD had a protective effect on the prolonged storage of blueberries. Our results are also consistent with the high content of bioactive compounds reported by Li *et al.* (2019), which were postulated to have occurred due to lower PPO activity. However, Ming-xia *et al.* (2016) showed that appropriate treatment reduced the malondialdehyde content of the 'Baldwin' blueberry, which was inconsistent with the changes in our study.

Finding the suitable harvest date is very important for fruit storability (Shu-rui *et al.*, 2014). Fruits harvested at the right time not only taste good, but also maintain good quality during storage. Appropriate harvest dates should therefore be adjusted to the handling of fruits after picking. According to Zhi-hua *et al.* (2015), fruits that are stored for a long time should be harvested early, whereas fruits stored for only a limited time-or those that will be sold immediately-can be harvested late. However, the limitation of this study is that there is no research on harvest dates of other varieties, and there are no preservation technologies for blueberries. Further research is, therefore, necessary to explore whether the combination of low-temperature storage with other preservation technologies could yield better results in improving the quality of blueberries. Moreover, further studies into the molecular mechanisms of relevant metabolic pathways will also be needed to develop the future of postharvest research in blueberries.

**Conclusions:** In this study, fruits harvested too early or too late had serious losses in nutritional components and antioxidants, and had weak resistance to deterioration during the storage period. Early- and late-harvested fruits are best used for their fresh taste and for timely sales, while batches with intermediate harvest dates (fully ripe blueberries harvested at 60 and 65 days after full bloom) are optimal for long-term storage. However, the role of other preservation techniques in blueberries has not been verified and there is no research on harvest dates of other varieties. Therefore, our future research will focus on the effects of combining blueberries with other fresh-keeping techniques at different harvest periods under low-temperature storage.

**Conflict of Interest:** The Authors declare that there is no conflict of interest

**Authors' Contribution Statements:** Dan Guo and Xin Wei executed the laboratory analysing and article writing,

Hongguang Wang executed field research, whereas Cheng Liu conceived the idea and supervised the work.

**Acknowledgments:** This work was supported by the following projects: National Grape Industry Technology System, Supported by China Agriculture Research System of MOF and MARA (CARS-29) China-Poland Berry Research Center (2020030060-JH2/105); Discipline construction plan of Liaoning Academy of Agricultural Sciences: Small berry breeding and cultivation (2019DD164924).

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