Effect of hypobaric treatment on the quality and reactive oxygen species metabolism of blueberry fruit at storage

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ABSTRACT
This study investigated the effect of hypobaric treatment on the fruit quality, bioactive compound contents, and reactive oxygen species (ROS) metabolism of blueberry. Blueberry fruit cv. bluecrop were stored under hypobaric pressures of 0.025, 0.05, 0.075 and 0.1 MPa (control) at 20°C for 25 and 50 d, respectively. Fruit physiological changes, bioactive compound contents, pro- and anti-oxidant enzyme activities and antioxidant capacities were monitored. Results showed that hypobaric treatment had little influence on decay rate, firmness, respiratory rate, pH and soluble solid content (SSC). However, the contents of bioactive compounds such as anthocyanins, total phenols, and total flavonoids increased significantly, especially at 0.025 MPa. The effect of hypobaric storage on the bioactive compounds of blueberries was through (i) promoting ROS degradation through catalase (CAT), (ii) reducing ROS and bioactive compounds degradation by polyphenol oxidase (PPO) and peroxidase (POD), (iii) increasing the ROS scavenging and antioxidant capacity of blueberries.

1. Introduction
Blueberry (Vaccinium spp.) is a delicious fruit with pronounced benefits on human health (Kazan, Sevimli-Gur, Yesil-Celiktas, & Dunford, 2017; Li, Li, & Geng, 2011; Norberto et al., 2013). It was suggested to be one of the richest sources of dietary antioxidants and health-promoting bioactive compounds such as anthocyanins, phenols, and flavonoids (Celik, Bozhuyuk, Ercisli, & Gundogdu, 2018; Colak, Kupe, Bozhuyuk, Ercisli, & Gundogdu, 2018; Jara-Palacios et al., 2019; Prior et al., 1998). However, blueberries usually have a short postharvest shelf-life at room temperature due to physiological deterioration or fungal decay (Wang & Chen, 2010). In the past years, a number of postharvest practices were developed to extend the shelf-life and increase the bioactive compound contents of blueberry, such as 1-MCP treatment, different temperature conditions, and Co-γ-irradiation (Delong, Prange, Bishop, Harrison, & Ryan, 2003; Hua, Wang, Wang, & Wei, 2003; Kalt, McDonald, & Donner, 2000; Srivastava, Akoh, Yi, Fischer, & Krewer, 2007; Wang & Meng, 2016; Wang, Tian, & Xu, 2005). Although these practices were beneficial for fruit quality maintenance, more effective storage methods still need to be developed.

Hypobaric storage, which refers to storage of fruit at sub-atmospheric pressure, is effective in maintaining fruit quality and preserving fruit from harmful metabolites, such as ethanol and acetaldehyde (Kou et al., 2016). Under hypobaric storage, the fungal decay of strawberries was reduced (Hashmi, East, Palmer, & Heyes, 2013) and the scald-related volatiles of apples were removed (Wang & Dilley, 2000). In addition, the bioactive compounds such as phenols and...
flavonoids were higher in tomatoes under hypobaric storage than those under atmospheric pressure (Kou et al., 2016). The reactive oxygen species (ROS) in bamboo shoots were reduced (H. Y. Chen et al., 2013), and the antioxidant defense of honey peach was enhanced under hypobaric storage (Wang et al., 2015). Although hypobaric treatment has been proved to be efficient in the quality maintenance of various fruits, limited information is available about its effects on blueberry fruit quality, bioactive compound contents, and ROS metabolism and their relationships.

Multivariable analysis is a powerful tool to clarify complex relationships and has been used to reveal the influence of fruit stalk on the quality maintenance and ROS metabolism of peaches (Li et al., 2019). It includes principal component analysis (PCA), partial least squares regression (PLSR) and many other statistical methods. PCA is a mainstay of modern data analysis to detect the most important basis to re-express a data set. By PCA, the relationship between harvest date and blueberry fruit quality has been clearly demonstrated (Lobos, Callow, & Hancock, 2014). PLSR can predict dependent factors from independent variables (Helland, 1990; Obando-Ulloa et al., 2008), and has been used to predict the changes of phenols, flavonoids and antioxidant activity in red bayberry juice during storage (Zheng et al., 2011).

The present research aimed to investigate the effects of different hypobaric treatment levels (0.025, 0.05, and 0.075 MPa) on fruit quality and bioactive compound contents of blueberry. In addition, the relationships between bioactive compounds and ROS metabolism were further analyzed.
2. Materials and methods

2.1. Fruit material

Blueberry fruit (Vaccinium spp. cv. Bluecrop) were harvested from an orchard in Qingdao (36 °N and 120 °E), Shandong province of China, and was transported immediately to Beijing Technology and Business University. Ripened fruit (blue in color) with uniform size and free from visible disease were selected for the study.

2.2. Sample preparation

The blueberries were randomly divided into four groups and placed into different containers. One container was used as the control and placed at ambient atmosphere at 20°C. The other three containers were set at 0.025, 0.05, and 0.075 MPa using pressure regulators. Samples were taken at 0, 25, and 50 d for quantification of physiological changes, bioactive compound contents, pro- and anti-oxidant enzyme activities and antioxidant capacities. For analysis, 5 g of fruit was homogenized in extract A (0.2 g poly-vinylpolypyrrolidone (PVPP), 10 mL water and 10 mL phosphate buffer (PBS)), extract B (20 mL methanol), extract C (20 mL acetone) and extract D (10 mL 10% TCA and 10 mL water), respectively. The homogenates were centrifuged at 10,000g for 60 min at 4°C, and the supernatant was collected for analysis. Sample extract A was used to determine the activity of superoxide dismutase (SOD), catalase (CAT), ascorbic acid peroxidase (APX), polyphenol oxidase (PPO), and peroxidase (POD). Sample extract B was used for the determination of ferric ion reducing antioxidant power (FRAP), 1,1-Diphenyl-2-picrylhydrazyl radical scavenging activity (DPPH), trolox equivalent capacity (TEAC), total reducing capacity, total phenolic, and total flavonoid content. Sample extract C was used to determine hydrogen peroxide and anthocyanin content. Sample extract D was used to determine malondialdehyde (MDA) content (Wang, Tian, Xu, Qin, & Yao, 2004). All the results were expressed on a fresh weight basis.

2.3. Fruit quality evaluation

The decay rate was evaluated by counting the visible decay and recorded as decay incidence per replicate. Decay blueberries were thrown away after counting. Fruit firmness was determined using an LFRA Texture Analyzer (Brookfield), and the results were expressed in Newton (N). For respiratory rate assay, blueberries were placed into airtight glass bottles for 1 h. Then, the air was collected and CO₂ measured. The respiratory rate was expressed as mg CO₂ kg⁻¹. Fruit juice was used for quantification of pH value and soluble solid content (SSC). The pH value was measured using a pH meter (PHS-3D), and SSC was measured using a digital refractometer (PR-201 REFRACTOMETER).

MDA was determined following the procedure of (Wang et al., 2004). Sample extract D (1 mL) and 0.5 % solution of thiobarbituric acid (TBA, 2 mL) were mixed and heated at 95°C C for 20 min, cooled by cold water, and centrifuged at 10,000 g for 10 min. The supernatant was transferred to another tube and the absorbance at 532 and 600 nm was

Figure 2. Effects of hypobaric treatment on bioactive compounds of blueberries. (a) Anthocyanins, (b) total phenols, and (c) total flavonoids. Data presented are the mean of three replicates and vertical bars indicate the standard errors. Different letters represented significant differences at the same sampling time (P < 0.05).

Figura 2. Efectos del tratamiento hipobárico en compuestos bioactivos de arándanos. (a) Antocianinas, (b) fenoles totales y (c) flavonoides totales. Los datos presentados constituyen la media de tres ensayos y las barras verticales indican los errores estándar. Las letras diferentes indican la presencia de diferencias significativas al mismo tiempo de muestreo (P < 0.05).
determined. The MDA content was calculated based on the extinction coefficient of 155 mM cm\(^{-1}\). The results were expressed as µM (MDA) kg\(^{-1}\).

2.4. Bioactive compound contents determination

Anthocyanin content was determined according to the method described Wang, Chen, and Wang (2009). A total of 0.5 mL sample extract C, 2.5 mL KCl (0.025 M, pH 1.0) and 2.5 mL NaAc (0.4 M, pH 4.5) were mixed and allowed to stay for 20 min at room temperature. The absorbance was measured at 520 and 700 nm. The anthocyanin content was calculated based on the extinction coefficient of 3-o-glucoside and was expressed as g (anthocyanins) kg\(^{-1}\).

Total phenolic was determined by Folin-Ciocalteu’s method (Singleton & Rossi, 1965). A total of 0.05 mL sample extracts B, 0.85 mL H\(_2\)O, 0.04 mL Folin-Ciocalteu reagent, and 0.06 mL saturated Na\(_2\)CO\(_3\) solution were mixed. The mixture was placed in a dark place at 20°C for 1 h, thereafter, the absorbance at 760 nm was measured. Gallic acid was used to construct a calibration curve, and the results were expressed as mg GAE (gallic acid equivalents) kg\(^{-1}\).

Total flavonoid content determination was based on the colorimetric method as described by Jia, Tang, and Wu (1999). Briefly, 0.05 mL sample extract B, 0.13 mL NaNO\(_2\) (5%), 0.13 mL Al(NO\(_3\))\(_3\) (10%) and 0.69 mL NaOH (1 M) were mixed and incubated in a dark place for 1 h. The absorbance was measured at 510 nm and water used as the blank. A calibration curve was prepared using rutin. Results were expressed as mg (rutin) kg\(^{-1}\).
2.5. Hydrogen peroxide (H₂O₂), pro- and anti-oxidant enzyme activity analysis

The H₂O₂ content determination was referred to Brennan and Frenkel (1977). In brief, 0.5 mL sample extract C, 0.1 mL titanium sulfate (5%) and 0.2 mL concentrated ammonia were mixed and centrifuged at 3,000 g for 10 min. The precipitates were washed by 3 mL acetone three times, and the residues dissolved in 3 mL H₂SO₄ (2 M). The absorbance at 415 nm was recorded and the results were expressed as μM (H₂O₂) kg⁻¹.

The activity of SOD was determined according to the method described by previously (Li et al., 2019). In briefly, a reaction mixture containing 0.1 mL sample extract A, 50 mM phosphate (pH 7.8), 75 μM nitroblue tetrazolium (NBT), 10 μM ethylenediaminetetraacetic acid (EDTA), 2 μM riboflavin and 13 mM dl-methionine were mixed, incubated for 20 min, and its absorbance was measured at 560 nm. One unit (U) of the enzyme activity was defined as inhibition of the photo-reduction of NBT by 50%. SOD activity was expressed as U g⁻¹.

The CAT activity was assayed using the method described by Wang and Tian (2008). The reaction mixture included 0.3 mL sample extract A, 0.6 mL H₂O₂ (50 mM), and 2.1 mL phosphate buffer (50 mM, pH 7.0). The mixture was incubated at 30°C, and the absorbance was traced at 234 nm for 1 min. The specific activity was calculated as U g⁻¹. One unit (U) of catalase refers to the conversion of 1 μM H₂O₂ per min.

The APX activity was detected according to the method described by Jimenez, Hernandez, Del Rio, and Sevilla (1997). The reaction was done by mixing 0.3 mL sample extract A,
2.7 mL PBS (50 mM, pH 7.0), 0.1 mL H$_2$O$_2$ (20 mM), and 0.1 mL ascorbic acid (6.25 mM). The mixture was incubated at 30°C, and the absorbance at 290 nm was traced for 1 min. The APX activity was expressed as U g$^{-1}$, where one unit (U) of APX was defined as 1 μM ascorbic acid oxidized by enzyme per min.

Determination of POD activity followed the protocol of Jiang, Tian, and Xu (2002). For the assay, 0.3 mL sample extract A was mixed with 2 mL of guaiacol (0.1 %) and 1 mL of 0.1 mM H$_2$O$_2$. The reaction was carried out at 30°C for 5 min, and the absorbance at 460 nm was traced. The specific activity was expressed as U g$^{-1}$. One unit (U) refers to the amount of enzyme required to increase the absorbance by 0.001 per min.

The activity of PPO was detected according to the method described by (Wang et al., 2004). Briefly, 0.3 mL sample extract A and 2.5 mL catechol (0.5 M) were mixed, and the absorbance at 398 nm was measured. The PPO activity was expressed as U g$^{-1}$.

2.6. Antioxidant capacity assay

Ferric reducing antioxidant power (FRAP) was performed according to the method of Benzie and Strain (1996). In brief, 0.2 mL B sample extract, 3 mL of Tri-2-pyridyl-s-triazine (TPTZ) working solution and 0.3 mL of acetate buffer (0.3 M, pH 3.6) were mixed and allowed to stay at 37°C for 45 min. The absorbance at 593 nm was recorded. The antioxidant capacity of the sample was calculated using a FeSO$_4$ standard curve and was expressed as U g$^{-1}$.

DPPH radical scavenging activity was detected following the method of Blois (1958). The reaction mixture consisted of 0.1 mL B sample extract and 2.8 mL DPPH solution (0.5 mM). The mixture was incubated at room temperature for 60 min, thereafter its absorbance was determined at 517 nm. The results were presented as U g$^{-1}$, and one unit (U) referred to the amount of sample required for 50% DPPH scavenging.

Trolox equivalent capacity (TEAC) determination was expressed as U g$^{-1}$, where one unit (U) was defined as 50% free radical clearance.

The reduction capacity assay was based on the reduction of ferric ions in potassium ferricyanide to ferrous ions. Briefly, 2 mL B sample extract, 1 mL PBS (0.2 mol L$^{-1}$, pH 6.6), 1 mL 1% potassium ferricyanide and 1 mL trichloroacetic acid (TCA) (10%) were mixed, incubated at 50°C for 20 min and centrifuged. The supernatant (1 mL) was mixed with 2 mL H$_2$O and 0.3 mL FeCl$_3$ and incubated for 10 min. After incubation, the absorbance at 700 nm was measured. The total reducing capacity of the sample was determined using the Vitamin C standard curve and was expressed as U g$^{-1}$.

Superoxide radical (O$_2^-$) scavenging activity was determined according to the method described by Giannopolitis and Ries (1977). A total of 0.3 mL B sample extract was incubated in PBS buffer (pH 7.8, 0.1 mM), containing riboflavin aqueous solution (20 μM), L-methionine (26 mM), and nitroblue tetrazolium (0.75 mM) at room temperature for 30 min, and the absorbance at 560 nm was measured. The O$_2^-$ scavenging activity was expressed as U g$^{-1}$, where 50% inhibition of the photochemical reduction of NBT was defined as One unit (U).

Hydroxyl radical (-OH) scavenging capacity assay was performed according to the methods described by Avellar et al. (2004). Briefly, 0.1 mL B sample extract, 0.2 mL crystal violet (0.2 M), 1.7 mL potassium phosphate buffer (0.2 M, pH 7.4), 0.5 mL FeSO$_4$ (1 M) and 0.5 mL H$_2$O$_2$ (1 %) were mixed and incubated in dark at room temperature for 1 h. The absorption changes at 584 nm were recorded and the results were expressed as U g$^{-1}$. One unit (U) was defined as the amount of sample required to give 50% scavenging rate of the hydroxyl radical.

2.7. Statistical analysis

All the experiments were repeated at least three times. Statistical differences between hypobaric treatments were determined.
with the SPSS software (SPSS Inc., Chicago, IL, USA). One-way ANOVA and Tukey’s test was used, and $P < 0.05$ was considered as significant. The line charts were constructed with Origin9. The PCA and PLSR models were constructed using Unscrambler software (CAMO AS., Norway), and all the data used were normalized before PCA and PLSR model construction.

3. Results

3.1. Storage quality determination

Throughout storage, an increase in fruit decay rate and a decrease in fruit firmness were observed in the control blueberries. No significant difference ($P > 0.05$) of fruit decay rate were observed between the control and hypobaric treated blueberries on 25 d. However, a lower decay rate was recorded in the 0.025 MPa treated fruit on 50 d (Figure 1a). There was no significant difference ($P > 0.05$) of fruit firmness between the control and hypobaric treated blueberries on both 25 and 50 d (Figure 1b).

As shown in Figure 1c, the control blueberries showed an increased respiration rate on 25 d, and then decreased on 50 d of storage. A slightly lower respiration rate was observed in 0.025 MPa treated fruit, whereas respiration rate in the fruit treated with 0.05 MPa was higher than that in the control on both 25 and 50 d of storage. The 0.075 MPa
treated fruit indicated a lower and higher respiration rate than the control on 25 and 50 d of storage, respectively (Figure 1c).

Blueberries pH values displayed noticeable decrease during storage as compared with that at harvest. The pH value of 0.025 MPa treated fruit was lower, and that of 0.05 MPa treated fruit was higher than control on 50 d of storage. In contrast, 0.075 MPa treated blueberries indicated unchanged and higher pH value as compared to control on 25 and 50 d of storage, respectively (Figure 1d).

During storage, the highest SSC values were observed in the control (10.57% SSC) on 25 d and in 0.075 MPa treated fruit (10.67% SSC) on 50 d of storage, respectively. A slight decrease in SSC was observed in all the blueberries under hypobaric treatment on 25 d of storage. However, SSC showed a gentle increase on 50 d of storage under hypobaric treatment at 0.05 and 0.075 MPa (Figure 1e).

MDA content of the control and all the hypobaric treated fruit displayed a significant decrease on 25 d, followed by an increase on 50 d of storage. The 0.025 MPa treated fruit showed a lower and higher MDA content in comparison with the control on 25 and 50 d of storage, respectively. In contrast, significantly lower and higher MDA values of 0.05 and 0.075 MPa treated fruit were observed on both 25 and 50 d of storage, respectively (Figure 1f).

3.2. Bioactive compounds contents assay

Little change in anthocyanin content of the control blueberries was observed throughout storage. As compared to the control, the anthocyanin content of blueberries under 0.025 MPa was higher on 25 d, and lower on 50 d of storage. The 0.05 MPa treatment caused a decrease in anthocyanin content on both 25 and 50 d of storage. Also, anthocyanin content in the 0.075 MPa treated blueberries were insignificantly higher on 25 d, and significantly lower on 50 d of storage compared to that of control (Figure 2a).

Total phenols in the control blueberries decreased during storage. Hypobaric treatment significantly increased total phenol level in blueberries, with the most significant effect observed at 0.025 MPa on both 25 and 50 d of storage (Figure 2b).

Total flavonoids in the control blueberries showed a decreasing trend during storage. On 25 d of storage, total flavonoids in blueberries under all hypobaric treatments were higher than control, with the highest value in blueberries under 0.025 MPa treatment. In addition, the total flavonoids in blueberries under 0.025 MPa was higher, while that in blueberries under 0.05 and 0.075 MPa were lower than control on 50 d of storage. Blueberries under 0.075 MPa indicated the lowest values of total flavonoids on 50 d of storage (Figure 2c).

3.3. Hydrogen peroxide (H$_2$O$_2$), pro- and anti-oxidant enzyme activity analysis

As shown in Figure 3a, a significant increase of H$_2$O$_2$ content in blueberries under 0.025 MPa was observed on 25 d of storage. At the same time, a significant decrease was observed for the blueberries under 0.05 MPa on 25 d as well as under 0.075 MPa on 50 d of storage.

SOD activity in the control blueberries on 25 d of storage remained the stable, whereas a significant increase was observed on 50 d as compared with harvest time. Hypobaric treated fruit showed a slightly higher SOD activity on 25 d of storage, as compared to the control. On 50 d of storage, blueberry SOD activity under 0.025 and 0.05 MPa treatment was almost the same with that in control, and three folds higher than that at harvest. However, blueberry SOD activity under 0.075 MPa treatment was two folds lower than control on 50 d of storage, indicating a negative effect of 0.075 MPa hypobaric treatment on SOD activity on 50 d of storage (Figure 3b).

The CAT activity of blueberries increased with storage time. CAT activity was two folds higher in fruit under 0.025 and 0.05 MPa treatment compared to the control on 25 d of storage. At the same time, CAT activity was three folds lower in 0.075 MPa blueberries compared to the control. This indicated that the effect of hypobaric treatments on CAT activity...
was pressure dependent. On the 50 d of storage, a similar trend was observed. The maximum CAT activity was 260 U g\(^{-1}\) in 0.05 MPa and 395 U g\(^{-1}\) in 0.025 MPa blueberries on 25 d and 50 d of storage, respectively (Figure 3c).

APX activity was similar to that at harvest on 25 d and decreased to 0.97 U g\(^{-1}\) on 50 d of storage in control blueberries. All the blueberries under hypobaric treatments showed lower APX activity value on both 25 and 50 d of storage (Figure 3d).

The POD activity kept stable with storage in the control blueberries. A significant decrease (P < 0.05) in POD activity in blueberries under 0.025 MPa, as well as a significant increase (P < 0.05) under 0.05 MPa treatments were observed on 25 d of storage. On 50 d of storage, a significant increase (P < 0.05) in POD activity was recorded in 0.05 and 0.075 MPa treatments (Figure 3e).

The PPO activity displayed a continuous increase with storage in the control blueberries. All hypobaric treatment resulted in significantly reduced blueberry PPO activity compared to the control, as was shown in Figure 3f.

### 3.4. Antioxidant capacity determination

Ferric reducing antioxidant capacity (FRAP) in the control blueberries displayed a slight decrease and increase on 25 and 50 d of storage, respectively. As shown in Figure 4a, 0.025 MPa treated blueberries showed a significantly higher FRAP on both 25 and 50 d of storage. In addition, blueberries under 0.05 and 0.075 MPa treatment also exhibited higher FRAP on 25 d of storage. However, on 50 d of storage, the FRAP in blueberries under 0.075 MPa treatment was lower than in the control (Figure 4a).

DPPH scavenging capacity in the control blueberries was higher on 50 d of storage than the harvest time. The 0.025 MPa treated blueberries were significantly higher in DPPH content on 25 and 50 d of storage. However, the DPPH content of blueberries under 0.05 and 0.075 MPa treatment was higher on 25 d, and lower on 50 d of storage when compared with control (Figure 4b).

TEAC scavenging capacity values of fruit under all hypobaric treatments were lower than control, except for the 0.025 MPa treatment on 50 d of storage. In addition, 0.075 MPa treated fruit showed significant low TEAC activity on 25 and 50 d of storage (Figure 4c).

The reducing capacity in the control blueberries showed an overall decreasing trend with storage. However, the 0.025 and 0.05 MPa treated blueberries showed high reducing capacity on 25 d of storage. Also, significant differences were observed in blueberries reducing capacity on 50 d of storage, with the lowest and highest values recorded in 0.025 and 0.075 MPa treatments, respectively (Figure 4d).

The O\(_2^-\) and hydroxyl radical scavenging capacity decreased with storage time in control. Blueberries under hypobaric treatments (0.025, 0.05 and 0.075 MPa) showed higher O\(_2^-\) and hydroxyl radical scavenging capacity both at 25 d and 50 d of storage (Figure 4e,f).

### 3.5. Principal component analysis (PCA)

PCA was performed to separate hypobaric treatments and to indicate the most significant factors affected by hypobaric treatments. The storage quality parameters and bioactive compounds contents were used to construct the bi-plot PCA model. The PCA model explained 68% (46% for PC1 and 22% for PC2) of the variance in the data set (Figure 5). Hypobaric treatment at 0.025 MPa was well separated from other treatments and the control by PC2. This result indicated that 0.025 MPa hypobaric treatment play a significant role on fruit quality and bioactive compounds contents. As shown in Figure 5, anthocyanins, total phenols, and total flavonoids were the most significant positive contributors to separate 0.025 MPa from 0.05 MPa, 0.075 MPa, and the control.

### 3.6. Partial least square regression analysis (PLSR)

To unveil the relationships between bioactive compounds and ROS metabolism parameters, a PLSR model was constructed. Anthocyanins, total phenols, and total flavonoids were respectively taken as regressors Y, whereas other ROS metabolism indexes were selected as regressors X. In the model with anthocyanins as Y-variable, the first two PLSR factors accounted for 41% of the X-variables and 97% of Y-variable. Anthocyanins were positively correlated with H\(_2\)O\(_2\), and negatively correlated with the POD (Figure 6a). Moreover, the first two PLSR factors in the model with total phenols as Y-variable accounted for 45% of the X-variables and 91% of Y-variable. Total phenols were positively correlated with O\(_2^-\) scavenging capacity (NBT) and negatively correlated with PPO (Figure 6b). In addition, as to the model with total flavonoids as Y-variable, the first two PLSR factors account for 41% of the X-variables and 96% of Y-variable. Total flavonoids were positively correlated with (HRSC), and negatively correlated with SOD (Figure 6c).

Taken together, the above results revealed that hypocaric storage at 0.025 MPa showed a significant increase in bioactive compounds and antioxidant capacity. As shown in Figure 7, more H\(_2\)O\(_2\) were degraded by CAT rather than PPO and POD, resulting in higher bioactive compounds in blueberries. Furthermore, the higher O\(_2^-\) and hydroxyl radical scavenging capacity implied less degradation of polyphenol compounds and higher antioxidant capacity of blueberries.

### 4. Discussion

Hypobaric storage had been reported to delay fruit decay of loquat and strawberry fruit (Gao et al., 2006; Hashmi et al., 2013). In the present study, there was no significant difference in the decay rate between hypobaric treated bluecrop blueberries and the control on 25 day of storage. However, decay rate was lower in 0.025 MPa treated fruit on 50 d of storage (Figure 1a). The firmness of blueberries in this study was not affected by hypobaric treatment (Figure 1b), which is consistent with the results of hypobaric treated strawberry fruit reported by Hashmi et al. (2013). Moreover, the respiration rate of mangoes was reported to increase with hypobaric treatment (Tovar, Montalvo, Damián, García, & Mata, 2011), whereas that of strawberry and curled lettuce was demonstrated to decrease under hypobaric storage (An, Park, & Dong, 2009). In this study, the respiration rate of blueberries was lower under 0.025 MPa and higher under 0.05 MPa treatments than control, respectively (Figure 1c). The changes in blueberry pH values under hypobaric storage corresponded with the respiration rate (Figure 1d). It is possible that respiration rate varied with the pressure and fruit cultivars, and the pH was affected by CO\(_2\) generated...
from respiration. Furthermore, a study conducted in China, reported insignificant changes in the SSC of the Chinese bayberry fruit under different hypobaric treatments (H. J. Chen et al., 2013), whereas that of Yali pear fruit exhibited an increase under 0.025 MPa treatment (Li et al., 2017). In this study, the SSC in hypobaric treated blue crop blueberries were lower on 25 d and slightly higher on 50 d when compared with the control. A gradual increase in SSC was observed in blueberries under hypobaric treatment toward 50 d (Figure 1e). In addition, the accumulation of MDA was reported to be inhibited by hypobaric treatment in Chinese bayberry fruit (H. J. Chen, et al., 2013). However, in the present study, MDA content was lower under 0.05 MPa treatment and higher under 0.075 MPa treatment than in the control (Figure 1f), perhaps due to the higher antioxidant activity under 0.05 MPa and the relatively lower antioxidant activity (indicated by FRAP and DPPH) under 0.075 MPa (Figure 4a,b).

Anthocyanins, total phenols, and total flavonoids contents were higher in hypobaric treated blueberries than in the control, except anthocyanins on 50 d of storage (Figure 2). Anthocyanins, total phenols and total flavonoids are bioactive compounds belonging to polyphenols, which could be oxidized into quinones by PPO or POD (Wang et al., 2004; Zhang, Pang, Xuewu, Ji, & Jiang, 2005). Polyphenol contents are positively correlated with antioxidant capacity (Kalt et al., 2000; Matthes & Schmitzberger, 2009). The high contents of bioactive compounds observed might be due to the lower PPO and POD activity (Figure 3e, f), and the higher antioxidant capacity as indicated by FRAP and DPPH (Figure 4a,b).

ROS metabolism is a dynamic process in biological materials/cells. The $O_2^{-}$ generated from electronic leakage or other sources could be catalyzed by SOD to form $H_2O_2$ (Docampo & Moreno, 2017; Fukai & Ushiofuku, 2011) or reduced by other substances in the fruit matrix (Gulcin, Huyut, Elmastas, & Aboulenein, 2010). $H_2O_2$ would be further converted into other ROS forms such as hydroxyl radical (Florence, 1984). All ROS forms had the potential to oxidize functional molecules to form MDA (Shulaev & Oliver, 2006). In addition, $H_2O_2$ might also be scavenged through APX, CAT and/or POD enzymes (Nicolas, Billaud, Philippon, & Rouet-Mayer, 2003; Ozyigit et al., 2016; Yang & Poovaiah, 2002). As shown in Figure 3b, the SOD activity in blueberries under 0.025 MPa was higher than that of the control on 25 d, and no significant differences were observed on 50 d of storage. When blueberries were subjected to 0.075 MPa, the SOD activity of blueberries was lower than the control. A similar result was observed in the $H_2O_2$ contents (Figure 3a). However, high SOD activity and low $H_2O_2$ content were observed under 0.05 MPa treatment on 25 d of storage (Figure 3a,b). This perhaps due to the increased activity of CAT and POD, which lead to a fast rate of $H_2O_2$ hydrolysis (Figure 3c,e). The significant higher CAT activity and the lower APX and POD activity (Figure 3c–e) in blueberries under 0.025 and 0.05 MPa, suggested that more $H_2O_2$ could be degraded by CAT and fewer $H_2O_2$ degraded through APX and POD under hypobaric treatment. The lower POD activity might be lead to the high content of its substrates such as polyphenol (Figure 2). In addition, a significantly increased scavenging capacity of $O_2^{-}$ and hydroxyl radical was observed (Figure 4e,f). This was consistent with the increased antioxidant capacity indicated by FRAP and DPPH (Figure 4a,b).

In the present study, multivariable analysis was applied to unveil the effects of different hypobaric treatments on storage quality and bioactive compounds of blueberries. The PCA analysis indicated that 0.025 MPa treatment significantly influenced blueberry quality, antioxidant capacity and ROS metabolism (Figure 5). The major contributors to differentiate 0.025 MPa and other pressure treatments were anthocyanins, total phenols, and total flavonoids. PLSR results revealed that anthocyanins were positively related with $H_2O_2$, the substrates of the POD (Figure 6a). This relationship perhaps was due to the fact that anthocyanins and $H_2O_2$ were the substrates of the POD (Kader, Irmouli, Nicolas, & Metche, 2002; Zhang et al., 2005). Also, $H_2O_2$ had been reported to function in anthocyanin biosynthesis (Qi et al., 2016). Total phenols were positively correlated with $O_2^{-}$ scavenging capacity (NBT) and negatively corrected with PPO, as shown in Figure 6b. Higher $O_2^{-}$ scavenging capacity and lower PPO activities would contribute to phenol protection against oxidation (Matthes & Schmitzberger, 2009). Also, the PLSR analysis showed that total flavonoids were positively correlated with hydroxyl radical scavenging capacity (HRSC) and negatively corrected with the POD (Figure 6c). This result was consistent with previous studies, reported total flavonoids to scavenge hydroxyl radicals (Chang et al., 2002; Yamasaki, Sakihama, & Ikehara, 1997).

In conclusion, the present study showed that hypobaric treatment, especially at 0.025 MPa contributed to high values of bioactive compound contents and antioxidant capacity of blueberries. The 0.025 MPa treated fruit exhibited an increased CAT activity, a decreased PPO and POD activity, as well as an increased ROS scavenging capacity, which were responsible for high values of bioactive compound contents and antioxidant capacity. Therefore, 0.025 MPa hypobaric treatment was recommended for bluecrop blueberry storage.

Disclosure statement
No potential conflict of interest was reported by the authors.

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