Anthocyanin content in mature/immature blueberry fruits under different storage temperature

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Abstract: The anthocyanins in blueberry ‘Brigitta’ mature and immature (purple) fruits stored in different temperature (4°C and 25°C) were analyzed by HPLC. The results showed that ‘Brigitta’ blueberry contained 9 anthocyanins. Anthocyanins contents in the purple fruit stored in 4°C showed a trend of previous rise and end fall. In the mature fruit under 25°C, anthocyanins contents kept on rising. In the mature fruits at 4°C and purple fruits at 25°C, same trends of decline first and then rose were revealed. This study suggested that in a certain storing during, anthocyanins contents in blueberry fruit fluctuated.

1. Introduction
Blueberry, the fruit of a perennial shrub of the genus Vaccinium, family Ericaceae, is popular worldwide because of its flavor and abundant active ingredients. Blueberry is rich in anthocyanin, which can improve vision, reduce dazzle, improve night vision, and improve dark adaptation ability [1-3]. The world health organization considers blueberry to be one of the most antioxidative fruit and is recommended by the FAO as one of the five healthy fruits [4]. Blueberry planting is rapidly expanded in south of China. Now consumers mainly eat fresh blueberries. However, since the ripening period of blueberries is from June to August, which is in the high temperature and humidity season in most south of China, the fruits are easy to rot after harvested. The shelf life of blueberry fruits is then shortened, which seriously affects the economic value of blueberries.

Anthocyanins are glycoside and acyl glycoside derivatives of antioxidative which are widely found in flowers, fruits and vegetables and give these plants bright colors [5]. The specie and content of anthocyanins in Blueberry fruits are different. The contents and dynamic changes of anthocyanins in different stored-blueberry fruits were investigated. It is to provide theoretical basis for predicting the change mechanism of the shelf life and anthocyanins of blueberry fruits.

2. Materials and methods
2.1 Plant materials
Purple fruit (immature) and mature fruit of ‘Brigitta’ blueberry were used. Fifty grams purple/mature fruits were stored in a refrigerator of 4°C, and another 50 g purple/mature fruits were placed in an incubator of 25°C. On the day 1, 3, 5 and 7 of storage, 10 g fruits were taken out for anthocyanin content determination. The treatments were performed 3 replications.
2.2 Anthocyanin extraction and measurement

The fruits were grinded, added 5 mL 1% hydrochloric acid-methanol leaching solution, and extracted at 4℃ by avoiding light overnight. The content of anthocyanin was determined by Agilent 1260 liquid chromatograph, the flow phase A was ultrapure water, the flow phase B was acetonitrile (HPLC grade), and the flow phase C was formic acid (HPLC grade). Gradient elution program were shown in Table 1. Twenty minutes with a flow rate of 0.5 mL·min⁻¹ and the column temperature set to 25℃. Injection volumes were 10 μL. Anthocyanins were detected by the absorbance at 525 nm.

The standard substances of 7 types of anthocyanins, delphinidin-3-O-galactoside (Del-gal), delphinidin-3-O-arabinoside (Del-ara), petunidin-3-o-galactosid (Pt-gal), petunidin-3-O-arabinosid (Pt-ara), malvidin-3-O-galactosil (Mv-gal), malvidin-3-O-arabinosid (Mv-ara) and cyanidin-3-O-arabinosid (Cy-ara), were prepared. External standard method of quantitative analysis of a single content of anthocyanin, according to a certain amount of standard substance, respectively dissolved in methanol, configuration into a single standard, diluted to different concentrations of methanol (0.5 ~ 70 μg·mL⁻¹) for HPLC analysis.

| Table 1 Gradient elution program of HPLC |
|----------------------------------------|
| **Time (min)** | **A (%)** | **B (%)** | **C (%)** |
| 0             | 90        | 8.4     | 1.6      |
| 4             | 90        | 8.4     | 1.6      |
| 13            | 75        | 23.4    | 1.6      |
| 20            | 60        | 38.4    | 1.6      |

2.3 Statistical analysis

One-way analysis of variance (ANOVA) and the Student–Newman–Keuls q test were performed at the 5% significance level with SPSS Statistics 19.0 software. Contrast analyses were used to separate the interactions.

3. Results

The content of different anthocyanin in different storage temperatures was showed as Table2. Content of all anthocyanins in the purple fruit 4℃ treatment was going to an up-down mode, in the purple fruit 25℃ treatment was going to an up mode, in the mature fruit 4℃ was going to a down-up mode, and in the mature fruit 25℃ treatment has different change modes. In particular, mature fruit 25℃ treatment was always higher than any other treatment. All anthocyanins were higher in treatment purple fruit 4℃ than in treatment purple fruit 25℃ and mature fruit 25℃ than in treatment mature fruit 4℃ on the first day, while 25℃ was always higher than 4℃ on the seventh day no matter in which maturity. Contents of Dp-gal, Dp-ara, Pt-gal and Cy-ara in 4℃ are lower on the seventh day than on the first day, and in 25℃ are higher on the seventh day than on the first day no matter in which maturity. Contents of Pt-ara and Mv-gal in purple fruit 4℃ treatment are lower on the seventh day than on the first day, and in the other three treatment are higher on the seventh day than on the first day. Contents of Mv-ara in all treatments are higher on the seventh day than on the first day. Among the seven anthocyanins, Dp-gal has the highest content which varied between 30.74±12.71 μg·mL⁻¹ and 61.50±12.44 μg·mL⁻¹, then Mv-gal has the second highest content which varied between 12.19±4.98 μg·mL⁻¹ and 69.69±13.72 μg·mL⁻¹, and Cy-ara has the lowest content which varied between 2.64±0.99 μg·mL⁻¹ and 4.49±0.21 μg·mL⁻¹.

| Table 2 Content of different anthocyanins after storage |
|---------------------------------------------------------|
| **Anthocyanin** | **Treatment** | **The day of storage** |
|-----------------|---------------|------------------------|
|                 | 1             | 3          | 5        | 7        |
| Dp-gal          | PF-4          | 38.32±10.75 | 49.36±0.69 | 37.10±3.27 | 36.90±3.78 |
|                 | PF-25         | 30.74±12.71 | 49.01±6.16 | 49.16±2.05 | 50.73±7.25 |
|                 | MF-4          | 55.79±31.03 | 40.95±13.12 | 40.12±5.07 | 49.91±2.54 |
|                 | MF-25         | 59.69±3.66  | 56.59±15.61 | 57.95±8.08 | 61.50±12.44 |
| Dp-ara          | PF-4          | 20.27±5.42  | 24.98±3.34 | 16.22±1.27 | 15.55±1.74 |


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\begin{array}{cccc}
\text{Pt-gal} & \text{PF-25} & 17.91 \pm 7.36 & 26.24 \pm 1.03 \\
 & \text{MF-4} & 30.15 \pm 16.83 & 23.03 \pm 7.43 \\
 & \text{MF-25} & 32.96 \pm 1.78 & 30.15 \pm 11.13 \\
\text{Pt-ara} & \text{PF-4} & 18.38 \pm 4.99 & 21.55 \pm 0.23 \\
 & \text{MF-4} & 13.85 \pm 5.64 & 24.82 \pm 2.80 \\
 & \text{MF-25} & 27.27 \pm 15.25 & 20.12 \pm 6.52 \\
\text{Mv-gal} & \text{PF-4} & 7.50 \pm 1.95 & 8.82 \pm 0.18 \\
 & \text{MF-4} & 6.07 \pm 2.38 & 11.65 \pm 1.42 \\
 & \text{MF-25} & 14.61 \pm 0.67 & 15.15 \pm 4.60 \\
\text{Mv-ara} & \text{PF-4} & 19.75 \pm 5.35 & 20.68 \pm 0.57 \\
 & \text{MF-4} & 12.19 \pm 4.98 & 32.39 \pm 3.75 \\
 & \text{MF-25} & 32.31 \pm 18.33 & 2586 \pm 8.58 \\
\text{Cy-ara} & \text{PF-4} & 53.77 \pm 1.51 & 57.75 \pm 18.49 \\
 & \text{MF-4} & 6.54 \pm 1.69 & 7.58 \pm 1.12 \\
 & \text{MF-25} & 4.66 \pm 1.70 & 13.60 \pm 1.54 \\
\end{array}
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Note: PF-4: purple fruits stored in 4°C; PF-25: purple fruits stored in 25°C; MF-4: mature fruits stored in 4°C; MF-25: mature fruits stored in 25°C

4. Conclusions

Previous researches conducted storage experiments with colored potato tubers\(^6\), and found that the content of total anthocyanins in one variety rose sharply at 7°C on the 5 d of storage, while waxberry\(^7\) reached the maximum at 1°C on the 2 d and 5°C on the 4 d. The results of this study showed that the anthocyanin content in blueberries stored for 7 days generally increased at 25°C purple fruit and 25°C mature fruit, most of which reached the maximum value at 7°C. The anthocyanin content reached the maximum value at 4°C purple fruit and 4°C mature fruit, which was consistent with previous research results. It was showed that the anthocyanin content of peony was significantly higher than 22°C at 4°C\(^8\), and the anthocyanin content of morning glory was significantly increased after 120h of low temperature stress\(^9\), indicating that low temperature can promote the rise of anthocyanin at the early stage of storage, which is consistent with the results of this experiment.

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