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Study of the effects of 1-MCP to blueberry under cold storage

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Abstract. Blueberry is one of the thinnest exocarp fruits in the world, which is difficult to keep fresh due to the special structure of its skin. 1-Methylecyclopropene (1-MCP) is able to combine with ethylene(ETH) receptor. In this study we investigated the effect of 1-MCP on rotting rate, weight loss ratio, soluble sugar content, titratable acid content, antioxidant enzyme activities and malondialdehyde (MDA) content in blueberry (Vaccinium corymbosum 'O'Neal' and 'North Road') under cold storage. 1-MCP reduced the rotting rate, weight loss ratio and MDA content, while keeping high-leveled stability in antioxidant enzyme activities, soluble sugar content and titratable acid content. These results showed the role of 1-MCP in alleviating the negative effects of blueberry and suggested that 1-MCP could be used as a preservative for keeping thin exocarp fruit in fresh.

1. Introduction

Blueberry (Vaccinium corymbosum), a member of Ericaceae family, is a well-known fruit worldwide and mainly distributed in moist areas with acid soil. The fruit of blueberry is small but rich in vitamins, minerals, carbohydrates and anthocyanins, and it is regarded as one of the five healthy fruits by the Food and Agriculture Organization of the United Nations due to rich nutrients [1]. There are more than 100 varieties cultivated in China, of which “Bluette”, “O’Neal”, “Misty” and “Northland” are the main cultivars in Zhejiang Province in the eastern part of China. While blueberry is a kind of berry, it is hard to store for a long time because of the thin exocarp and juice.

1-Methylecyclopropene is a kind of active chemicals, which exists in the stage of gas under normal temperature. 1-MCP can combine with the ethylene receptor specificity, which effectively prevents the generation of endogenous ethylene and the inducing effect of exogenous ethylene [2]. The study found that, 1-MCP can reduce the happening of brown rot in ‘Los Angeles’ plum [3]; improve the storage quality of loquat during the ripening; affect the active oxygen metabolism related enzymes activity in melon [4].

Many studies on biological, physiological and biochemical, and molecular biological characteristics of blueberry [5-7] have been carried out, but little knows about the effects of 1-MCP on the blueberry under cold storage. Thus, the objective of this study was to investigate whether 1-MCP could alleviate the effect of fruit senescence of blueberry and improve the ability of storage of blueberry.
2. Materials and methods

2.1 Materials

In early June 2016, blueberry fruits “O’Neal” and “North Land” were collected from the ‘Mu shan nong’ blueberry base. Before being transported to the lab, all fruits were needed to be precooled at 10°C. After being transported to the lab, divided the blueberry into several retail-used transparent plastic boxes (about 125g per box). Each type of blueberry contained 4 groups (see the following table), and then every group needs to be put into the seal plastic box with same volume. After finishing that, the seal boxes were all placed in the refrigerator at 4°C (with 60% of humidity), at Zhejiang A & F University (N 30°23′, E 119°72′), China. After 48 hours, transfer the blueberry out of the seal box to the refrigerator.

| Group     | T1    | T2    | T3    | T4    |
|-----------|-------|-------|-------|-------|
| O’Neal    | Control | 10ul/L 1-MCP | 20ul/L 1-MCP | 30ul/L 1-MCP |
| North Land| Control | 10ul/L 1-MCP | 20ul/L 1-MCP | 30ul/L 1-MCP |

Every 5 days, a box of blueberry was used for the determination of soluble sugar content, titratable acid content, soluble solid, enzyme activities and the MDA content analysis.

2.2 Methods

2.2.1 Determination of rotting rate. About 125g of fresh blueberry were carried out for the detection of the rotting rate. Every 5 days, pick out and record the number of broken and mildew blueberry.

Rotting rate = (0 day’s number - other day’s number) / 0 day’s number *100%

2.2.2 Determination of weight loss ratio. About 125g of fresh blueberry were carried out for the detection of the weight loss ratio. Record the weight of the blueberry on the 0th, 5th, 10th, 15th, 20th, 25th, 30th day, and the weight loss would be the 0 day’s weight minus the other day’s weight.

Weight loss ratio = (0 day’s weight - other day’s weight) / 0 day’s weight *100%

2.2.3 Determination of soluble solid. We used the saccharimeter (HR108ATC) to measure the soluble solid content [8]. 10g of fresh blueberry was grounded at 4°C with an ice-chilled pestle and grinding fully. After filtration with gauze, use the pipette take 0.1ml of the liquid to the saccharimeter and read the soluble solid content.

2.2.4 Determination of MDA contents and anti-oxidant enzymes activities. For the MDA content, SOD, CAT and POD activities, 5g of fresh blueberry was ground at 4°C with an ice-chilled pestle and mortar in 10 ml of 50mM phosphate buffer (pH7.8) containing 1% polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 8000rpm at 4°C for 15min and the supernatants were collected for measuring MDA content and enzyme activities according to Ying[9].

2.2.5 Determination of total soluble sugar content. The total soluble sugar content was determined using anthrone colorimetry [10]. Briefly, 2g of smashed fresh fruit was placed in a large tube containing 15ml pure water, and increased to a volume of 100ml after 30min in a boiling water bath. Then, 1ml solution was mixed with 5ml anthrone reagent, and measured at 620nm after 10min in a boiling water bath.

2.2.6 Determination of titratable acid content. For the titratable acid content, we used the phenolphalein acid-base neutralization titration, in this research the concentration of NaOH was 0.1%. 5g of fresh blueberry was ground at 4°C with an ice-chilled pestle and mortar in 5ml of water. The homogenate was centrifuged at 8000 rpm at 4°C for 15min and the supernatants were collected for measuring titratable acid content.
Titratable acid content = 0.1% * Volume of NaOH / Volume of extracting solution (0.5ml)

3. Statistical analysis
The graphs and analysis of variance were performed using Excel and SPSS Institute Inc. (Version 19.0) software (USA).

4. Results

4.1 Rotting rate of blueberry during 1-MCP treatments under cold storage

![Graph showing rotting rate of blueberry](image)

Rotting rate is one of the most intuitive indicators of the fruits’ quality (Fig.1). Rotting rate was increasing from 0d to 30d, and “O’Neal” has a lower rotting rate than the “North Land”. At 30d, the rotting rate of the “O’Neal” control was risen to 44.44%, while 1-MCP treated groups (T2-T4) were at 33.85%, 27.69% and 38.10%, and has a 10.59%,16.75% and 6.34% lower than control, respectively; The rotting rate of “North Land” control were at 63.16%, while 1-MCP treated groups (T2-T4) were at 54.55%, 47.5% and 54.55%, with 8.61%, 15.66% and 8.61% lower than control, respectively.

4.2 Weight loss ratio of blueberry during 1-MCP treatments under cold storage

![Graph showing weight loss ratio of blueberry](image)

After the harvest, the fruits will gradually lose the weight, because of the water loss and dry matter loss caused by respiration (Fig.2). The weight loss ratio was increasing from 0d-30d, and “O’Neal” has a lower weight loss ratio than “North Land”. At 30d in “O’Neal” the control of the weight loss ratio increasing by 27.31% comparing to the 0d, while the 1-MCP treated groups (T2-T4) increased by 25.17%, 23.36% and 25.40%, comparing to 0d respectively; And in “North Land”, at 30d the weight
loss of control has increasing by 33.36%, while the 1-MCP treated groups (T2-T4) were increased by 29.81%, 24.90% and 26.31%, and has 3.55%, 8.46% and 7.05% lower than control respectively.

4.3 Soluble solid, soluble sugar and titratable acid content of blueberry during 1-MCP treatments under cold storage

![Graphs showing changes in soluble solid, sugar, and titratable acid content](image)

In order to investigate the internal quality of blueberry, we have measured the soluble solid, soluble sugar and titratable acid content (Fig.3A-Fig.3C). From 0d to 30d, soluble solid content was decreased...
and decreased again after the increased (Fig.3). Comparing to the control, 1-MCP treated groups(T2-T4) were in a more stable variation both in “O’Neal” and “North Land”, while “O’Neal” has a lower variation than “North Land” during the storage.

Soluble sugar content was in a decline both in “O’Neal” and “North Land” (Fig.3B). At 30d, the soluble sugar content in “O’Neal” were decreasing by 2.79%, 3.20%, 2.75% and 3.10% comparing to the 0d respectively; While in “North Land”, the soluble sugar content decreased by 3.51%, 2.44%, 2.54% and 2.44% comparing to the 0d respectively; Comparing to the “O’Neal”, “North Land” has been more effected by the 1-MCP treatments.

Titratable acid content is a way to judge the quality stability of blueberry. Fig.3C showed that the titratable acid content decreased with the increase of storage days; While 1-MCP treatment groups has a more gently variation than the control both in “O’Neal” and “North Land”; In the meantime, the titratable acid content in “O’Neal” were higher than that in “North Land”.

4.4 MDA content of blueberry during 1-MCP treatments under cold storage

![Fig 4. Changes in the MDA content of blueberry](image)

The MDA levels under different treatments were analyzed (Fig 4). MDA increased gradually with lasting of days. The treatments (T1-T4) in MDA was as follows: 433.1%, 324.5%, 334.4% and 307.6% from 0d to 20d in “O’Neal” respectively. While in “North Land” from od to 30d the MDA content of treatments (T1-T4) was as follows: 718.3%, 560.5%, 634.2% and 591.8%, respectively. Under 1-MCP treatments the amounts of MDA in blueberry were lower than that of control.

4.5 Antioxidant enzyme activities of blueberry during 1-MCP treatments under cold storage

![Fig 5A. Changes in the SOD activities of blueberry](image)
Fig 5B. Changes in the POD activities of blueberry

Fig 5C. Changes in the CAT activities of blueberry

The antioxidant enzyme activities in the blueberry are shown in Fig 5a - Fig 5c. The SOD activities of blueberry decreased and decreased after the increased (Fig. 5a). At the early stages of the treatments (0d–5d), there were in a decline in SOD activities, while at 20d, the SOD activities decreased by 7.01%, 13.58% and 9.41% in “O’Neal” (T2–T4) comparing to the control respectively; While in “North Land”, under the 1-MCP treatments (T2–T4) the SOD activities were lower than the control.

Under the 1-MCP treatments, the blueberry has a more stable variation of POD activities both in “O’Neal” and “North Land”. In “O’Neal”, under different 1-MCP treated (T2–T4), the POD activities were decreased by 19.70%, 15.47% and 14.84% in T2, decreased by 25.76%, 15.11% and 12.52% in T3, and decreased by 32.39%, 13.18% and 14.58% in T3, comparing to the control respectively at 15d, 20d and 25d. And at 30d, under 1-MCP treatments T1–T3 were have a 14.53%, 18.72% and 15.36% higher in POD activities than control; While in “North Land”, T1–T3 were decreased by 21.05% and 10.37%, 17.98% and 17.18%, 30.92% and 16.16% at 10d and 15d, comparing to the control respectively; And at 25d and 30d T1–T3 increased by 31.83% and 14.02%, 17.29% and 10.32%, 10.53% and 12.70%, comparing to the control, respectively.

The CAT activities of blueberry decreased after the increased (Fig. 5C). And under the 1-MCP treatments (T2–T4) the CAT activities were higher than control both in “O’Neal” and “North Land”; In “O’Neal”, under the 1-MCP treatments (T2–T4) the CAT activities increased by 27.5%, 12.5% and 27.5% at 20d, increased by 85.19%, 48.15% and 55.56% at 25d and increased by 73.08%, 42.31% and 65.39% at 30d, comparing to the control respectively; In “North Land”, under the 1-MCP treatments (T2–T4) the CAT activities increased by 16.28%, 11.63% and 25.58% at 20d, increased by 21.62%, 27.03% and 29.73% at 25d, comparing to the control, respectively.

5. Discussion

Blueberry is one of the most nutrition fruits in the world, while it has a very short period of storage time caused by the effects of the bacteria and mechanical damages [11]. Rotting rate and weight loss...
ratio are considered as the important indicator, showing the different quality of blueberry \cite{12}. In this study, we can see that: different varieties of blueberry have different quality of shelf-stable; “O’Neal” was better than the “North Land” both in rotting rate and weight loss ratio, while 1-MCP can help the blueberry keep in a good stage, especially when it comes to the concentration of 20μl/L. 1-MCP reduced the water loss, dry matter loss and decay of blueberry, this is congruence with other studies on apple, waxberry and pear \cite{13-14}.

Soluble solid content, soluble sugar content and titratable acid content are the important inclusions of blueberry. In the fruits, soluble solid content will be increasing during the mature period, while decreasing when it has started to decay \cite{8}. In this study, soluble solid content decreased from 0d to 10d, that was mainly because of the cold storage, while soluble solid content increased at 10d and decreased at 20d in “O’Neal” and at 25d in “North Land”, which shows that “North Land” stays well during the storage and 1-MCP largely help the blueberry keep in a good state. In this study, soluble sugar content and titratable acid content were changing during the storage, while 1-MCP can keep the soluble sugar content and titratable acid content in a more steady state.

The increasing of MDA content was symptoms of damage and deterioration \cite{15}. In this study, MDA content increased during the storage. However, the 1-MCP treatment significantly reduced the MDA content. To protect against oxidative damage, plants produce a variety of antioxidant enzymes, and lipid- and water-soluble molecules to scavenge ROS \cite{16}. In this study, the SOD, CAT and POD activities of blueberry were induced by the 1-MCP, which was in agreement with the results reported in apple and pear \cite{13-14}.

As one of the ethylene inhibitor, 1-MCP play an important role in controlling the ripening and senescence of the non-climacteric fruits \cite{17}. Other study also shows that 1-MCP can reduced the gas exchange, decreased the inside oxygen content and delaying senescence of the blueberry \cite{18}. In this study, 1-MCP could keep the blueberry in a good stage in rotting rate, weight loss, soluble substances, MDA content and antioxidant enzyme activities, while different varieties of blueberry showed different during the storage.

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