The analysis of cell wall degrading enzymes in ripening blueberry pulp in different post-harvest temperature

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Abstract. The ripening blueberry (in purple skin) were stored in refrigeration condition (4°C) and room temperature (25°C). By measuring the changes of PG, PME and β-Gal enzyme activities in blueberry over a period of time, we found that the changes in enzyme activities of PG and PME were negatively correlated with the softening of blueberry, and low temperature can inhibit the increase of PG and PME enzyme activity in the early stage.

1. Introduction

Blueberry is a small berry plant of the genus Vaccinium in family Ericales. The blueberry have high economic value and broad development prospects [1]. The fruit usually mature in summer. The high temperature and humidity are the factors leading to the decline of blueberry fruit quality. The fruit is seriously dehydrated and the flesh is soft [2]. The blueberry begin to rot after being placed at room temperature for 2-4 days [3]. If the fruits are quickly refrigerated, the freshness of the fruit can be maintained for a long time [4-6]. Studies showed that fruit softening is closely related to the activity of cell wall degrading enzymes [7]. Blueberry from purple skin (ripening fruit) are sourer and higher hardness than the ripe fruits. Fruit softening will continue in ripening fruits after harvested, and the enzymes revolved in decomposing cell wall will remain active. However, the activity pattern of these enzymes is an open question. Many enzymes involved in catalyzing pectin degradation, such as PG, PME, and β-Gal. PG is an enzyme that plays an important role in the alteration of cell wall structure. It can catalyze the cleavage of α-polygalacturonic acid in pectin molecules. PME enzyme can catalyze the transformation of pectin esters into pectin acids, which is more suitable for the depolymerization of PG. The β-Gal enzyme degrades pectin by degrading branched polyamic acid, resulting in destruction of cell wall integrity. They are all closely related to fruit softening. In this study, the effects of PG, PME and β-Gal enzyme activities on the softening of blueberry at different storage temperatures were studied in order to find out the method of prolonging the softening time of blueberry, which is of great significance for the storage of blueberry.

2. Material and methods

2.1. Material

The blueberry tested was the northern high bush variety 'Brigitta', collected from blueberry planting orchard in Qionglai country in Chengdu city, Sichuan province. Fresh, healthy and similarly
blueberry were selected from purple skin (ripening fruit), which are sourer and higher hardness than the ripe fruits.

The selected fruits were divided into two groups. One group was stored at 4°C and the other group was stored at 25°C. The fruits in each group were put in PU box (125g). One box was one experimental replication, and there were four replications in each experimental treatment. The enzymes activity measurement was performed four times. The first measurement was at the day when harvested fruits (Day 1). In the following days, enzymes activity measurement was performed every other day, i.e. Day 3, Day 5 and Day 7.

2.2 Test methods
Take one gram of blueberry pulp, add ethanol to stand, centrifuge, and pour off the supernatant. The acetic acid buffer was added to the precipitate and centrifuged after standing. The obtained supernatant is a crude enzyme solution. The absorbance of the relevant enzyme in the enzyme solution was determined by different methods, and the enzyme activity was calculated based on the absorbance.

3. Results and discussions

3.1. Changes of PG enzyme activity
The PG enzymes activity in both groups at 25°C and 4°C showed an upward trend (Fig. 1). Under normal temperature storage conditions, PG enzyme activity continued to rise, but after the fifth day, the upward trend was noticeably slower. The enzyme activity at 4°C decreased after D5, and the upward trend was more obvious when it rose under normal temperature conditions. The PG enzyme activity at 25°C was always higher than the PG enzyme activity at 4°C.

3.2. Changes of PME enzyme activity
The change of PME enzyme activity was similar to that of PG (Fig. 2). In the early stage (1-3d) of the experiment, two groups of PME activity showed markedly differences. At the storage temperatures of 25°C, PME enzyme activity was much higher than 4°C under the condition of enzyme activity. From the day three to the day five, the enzyme activity of PME was still rising at room temperature, but it rose slowly compared with the previous three days. Enzyme activity at 4°C reached its maximum on the fifth day and then declined.
3.3. Changes of $\beta$-Gal enzyme activity
The variation trend of $\beta$-Gal enzyme activity was basically consistent under the two groups of conditions (Fig. 3). The initial enzyme activity showed a downward trend, which began to rise after the third day and declined again after the fifth day. The enzymatic activity of blueberry stored at 4℃ was always higher than the enzyme activity at 25℃, the difference was particularly pronounced from the day three to the day five.

4. Conclusion
The experimental results showed that PG and PME enzyme activity increased throughout the storage period. In addition, the enzyme activities of PG and PME showed that the enzyme activity under room temperature (25℃) conditions was higher than that under refrigeration conditions in the present experiment. From this, we might infer that PG and PME were related to the initiation of softening of blueberry and have a negative correlation with fruit hardness. Low temperature can inhibit the increase of PG and PME enzyme activity[7], and that is benefit for prolonging the shelf life of blueberry.

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