Changes in pectin components of different blueberry varieties during fruit development

Henggang Li¹, Jinqiu Huang¹, Yuhui Bie¹, Yingying Huang¹, Xun Wang²*

¹College of Horticulture, Sichuan Agricultural University, Chengdu, Sichuan, 611130, China
²Institute of Pomology and Olericulture, Sichuan Agricultural University, Chengdu, Sichuan, 611130, China
*Corresponding author’s e-mail: wx0104@sicau.edu.cn

Abstract: In order to reveal the mechanism of texture change during the fruit mature period of the main blueberry cultivars in Sichuan Province, this paper studied the content of different pectins in the cell wall of ‘Brigitta’, ‘O’Neal’, and ‘Britewell’ cultivars during the period of the 25th to 70th days after flowering period. It was found that the water-soluble pectin content (WSP) in ‘O’Neal’ and ‘Britewell’ increased continuously as the fruits matured. In contrast, the content of alkali-soluble pectin (SSP) continued to decrease. These may be related to changes in PG enzyme activity. In the pink fruit period (the 49th day after flowering), the chelant soluble pectin (CSP) content of the three varieties all reached the highest value, which may be due to the higher PME enzyme activity. In general, the pectin of the fruits of all three varieties gradually degrades as their maturation.

1. Introduction
Blueberry (Vaccinium spp.), also known as huckleberry and originated from North America, is rich in antioxidants such as anthocyanin, polyphenol and flavone. It is considered by the world health organization that blueberry is one of the most antioxidative fruit which is recommended by the FAO as one of the five healthy fruits[1].

Pectin is an acidic polysaccharide substance that exists in the primary cell wall and middle gum layer of higher plants, and its structural composition and content changes have an important effect on fruit texture change[2]. The fruit formation period after blueberry flowering and fruit setting period is the key period for blueberry cultivation, yield and quality formation. In this experiment, three blueberry varieties, ‘Brigitta’, ‘O’Neal’, and ‘Britewell’, were used to study the changes in the content of different pectins in the cell wall during the fruit development and maturation process, which will help reveal the mechanism of fruit texture change of blueberry before harvest.

2. Materials and methods
2.1 Plant materials and postharvest treatments
The test materials include the late maturity blueberry named ‘Brigitta’ from North highbush, the early maturity blueberry named ‘O’Neal’ of from South highbush, and special late maturity blueberry named ‘Britewell’ from Vaccinium ashei, which were planted in the blueberry planting base in QiongLai,
Sichuan Province. The test duration was from March to August in 2018. The fruits were obtained in 7 stages of the fruit development stage, including young fruit stage, green fruit stage, fruit expansion stage, pink fruit stage, purple fruit stage, mature stage, and overripe stage, which were the 25th, 35th, 42th, 49th, 56th, 63th, and 70th day after flowering respectively. Fruits were picked and selected for uniformity without decay and external injuries. After the samples were collected, they were stored at -20°C.

2.2 Pectin determination
Refer to Li Qianqian’s research method and modify it slightly[3]. 10 g blueberry pulp was grinded to homogenate with 35 mL 95% alcohol, and then water bath for 1 h at 75°C. The sample was filtered by vacuum suction, and the filter residue was rinsed by 95% alcohol, the mixture of chloroform and ethanol (v/v, 2:1), and acetone respectively to obtain alcohol insolubles (AIS), which should be dried at 37 °C for 1 d. Take 100 g of AIS to extract WSP, CSP, and SSP by water, 50 mmol/L EDTA-2Na solution, and 50 mmol/L Na2CO3 (containing 20 mmol/L Na2BH4) respectively. Finally, the content of different pectin was determined by the Carbazole Method[4].

2.3 Statistical analysis.
Excel 2010 was used to conduct data statistics and draw charts, SPSS Statistics 20.0 software was used for data analysis. The various pectin’s contents was measured in triplicate and data represent mean values ± Standard Error of Mean.

3. Results
As shown in table 1, from the 25th day to the 70th day after flowering, except for Brigitta’s WSP content, there was no significant difference, and the WSP content of ‘O’Neal’ and ‘Britewell’ increased continuously. There was no significant change in CSP content in ‘O’Neal’, and there was no significant difference in CSP content at the 49th day after flowering of ‘Brigitta’. For the three varieties, CSP content was higher at the 49th day after flowering compared to other periods. The SSP content in ‘Brigitta’ after flowering was significantly higher than that in the later period, and the SSP content in O’Neal was significantly higher than that in the later period.

| Variety | pectin | 25 | 35 | 42 | 49 | 56 | 63 | 70 |
|---------|--------|----|----|----|----|----|----|----|
| Brigitta | WSP  | 0.12±0.06a | 0.09±0.06a | 0.03±0.01a | 0.15±0.06a | 0.08±0.01a | 0.12±0.03a | 0.08±0.03a |
| | CSP   | 0.11±0.02b | 0.11±0.02b | 0.08±0.01b | 0.32±0.11a | 0.11±0.01b | 0.14±0.00b | 0.14±0.02b |
| | SSP   | 0.83±0.02a | 0.65±0.04a | 0.31±0.07b | 0.36±0.19b | 0.09±0.02b | 0.11±0.01b | 0.20±0.08b |
| O’Neal  | WSP  | 0.11±0.01b | 0.10±0.04b | 0.09±0.01b | 0.17±0.03ab | 0.15±0.01ab | 0.18±0.03ab | 0.25±0.08a |
| | CSP   | 0.10±0.01a | 0.15±0.09a | 0.13±0.02a | 0.19±0.01a | 0.12±0.03b | 0.22±0.01a | 0.19±0.05a |
| | SSP   | 0.97±0.03a | 0.72±0.34a | 0.84±0.07a | 0.25±0.02b | 0.12±0.03b | 0.15±0.02b | 0.14±0.03b |
| Britewell | WSP  | 0.07±0.00c | 0.09±0.03de | 0.04±0.01e | 0.15±0.01cde | 0.23±0.05bc | 0.33±0.02ab | 0.39±0.07a |
| | CSP   | 0.11±0.01c | 0.20±0.04bc | 0.19±0.02bc | 0.26±0.04ab | 0.22±0.04bc | 0.23±0.01bc | 0.26±0.06ab |
| | SSP   | 0.84±0.08a | 0.90±0.07a | 0.80±0.03a | 0.75±0.18a | 0.22±0.01b | 0.30±0.03b | 0.27±0.02b |

4. Conclusions
Pectin is an important component of plant cell walls, and its structure and content changes are closely related to fruit ripening, softening and hardness changes. There was study showing that the sharp decrease in the hardness of blueberry fruits from the fruit expansion stage to the mature stage may be related to the large deformation of the Parenchymatissues of ‘Bluecrop’ highbush blueberry, which made the blueberry fruits soften a lot during ripening. The increase in WSP was related to the decrease in acid-soluble pectin[5].
Pectinase can affect fruit firmness and the formation of fruit quality. The changes of pectin structure during fruit development and maturation are affected by the activities of related pectin-degrading enzymes. In this experiment, as the fruits matured, the WSP content in the two varieties of ‘O’Neal’ and ‘Britewell’ continued to increase, and in contrast, the SSP content continued to decrease. This is consistent with the findings of Wang Bin [6] and Qiu Zhihao [7]. Studies by Wei Jianmei [8] also showed that the change in fruit pectin content was dominated by SSP. During the ripening of blueberry fruits, insoluble SSP gradually degraded into WSP and CSP with smaller molecular weight[9]. Polygalacturonase(PG) could convert insoluble pectin into WSP. The study found that PG enzyme activity in ‘O’Neal’ first increased, then decreased, and then increased at the peak of overripe period and the PG enzyme activity in ‘Britewell’ continued to increase[10]. Although the changes were different, PG enzyme activity in ‘Brigitta’ was always lower than that of the other two varieties. These were consistent with the change of WSP of the three blueberry varieties in this experiment.

The pectin methyl esterase (PME) is a hydrolase that hydrolyzes the carboxyl group in the pectin molecule, making it negatively charged and free, and subsequently degraded by other hydrolases[10,11]. Chen Kaili[10] found that the changes in fruit development are relatively gentle, PME enzyme activity is higher during fruit setting stage, and PME enzyme activity increases slowly during the fruit expansion stage, and there will be a slow decline process during purple fruit stage and mature stage. The CSP content of the three varieties reached the highest value during the pink fruit stage (the 49th day after flowering), which may be due to the higher PME enzyme activity. Vaccinium ashei are different from the other two high clump varieties. Except for SSP, the changes of WSP and CSP are more complicated, which may be closely related to the characteristics of the splendid unique varieties.

References
[1] Wu, Z., Li, H., Wang, Y.D., Tan, H.J., Yang, Y., Zhan, Y., Wang, F.Q. (2019) Effects of different thermal-treated temperatures on polyphenols and flavonoids of blueberry juice during cold storage. Food and Fermentation Industries, 45(17): 209-215.
[2] Brummell, D.A., Labavitch, J.M. (1997) Effect of antisense suppression of endopolygalacturonase activity on polyuronide molecular weight in ripening tomato fruit and in fruit homogenates. Plant Physiology, 115(2): 717-725.
[3] Li, Q.Q., Li, W.S., Sang, W.N., Wang, L.M., Zhao, Y.M., Cao, J.K. (2018). Analysis of antioxidiant activity of pectin and hemicellulose extracted from tomato fruit. Science and Technology of Food Industry, 39(02) :17-21.
[4] Cao, J.K., Jiang, W.B., Zhao Y.M. (2007) Experiment guidance of postharvest physiology and biochemistry of fruits and vegetables. China Light Industry Press, Beijing.
[5] Chea, S., Yu, D. J., Park, J., Oh, H.D., Chung, S.W., Lee, H.J. (2019). Fruit softening correlates with enzymatic and compositional changes in fruit cell wall during ripening in ‘Bluecrop’ highbush blueberries. Scientia Horticulturae, 245, 163-170.
[6] Wang, B., Li, P.H., Dong, X.Y., Li, D.L., Duan, Y.X., Xin, P.P. (2013). Changes of pectin and firmness in peach fruit during maturation and after postharvest hormone treatments. Journal of Qingdao Agricultural University(Natural Science), 30(03): 174-178.
[7] Qiu, Z.H., Chen, J.Y., Peng, Y.Q., Li, S.X., Wu, S.T., She, W.Q. (2019) Changes in cell wall components and related enzyme activities of canarium album during fruit development stage. Journal of Tropical and Subtropical Botany, 27(06): 677-683.
[8] Wei, J.M., Ma, F.W. (2009). Relationship between storage property and cell wall components in apple during fruit development. Acta Botanica Boreali-Occidentalia Sinica, 29(2): 314-319.
[9] Xu, J., Li, J., Zhang, X.L., Li, P. (2015) Variations of cell wall components and hydrolase activity of ‘Korla fragrant pear’ during the fruit developmental stage. Journal of Fruit Science, 32(06): 1114-1117.
[10] Chen, K.L. (2018) Changes of main fruit quality and activity of key enzymes in pectin decomposition and their expression in blueberry fruit development process. Sichuan Agricultural University, Chengdu.

[11] Zhang, Y.W., Xin, Y., Chen, F.S. (2019) Research Progress of Pectin Degrading Enzymes and Related Genes in Fruit Softening. Storage and Process, 19(2):147-153.