Pectin Transitions During Blueberry Fruit Development and Ripening

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ABSTRACT

Quantitative and qualitative changes in the pectin composition of "Bluetta" blueberries were determined during fruit development between 30 and 57 days after full bloom. The contribution of alcohol insoluble solids (AIS) to the total fruit mass decreased threefold with maturation, while the pectin contribution decreased twofold in the fruits. Within the AIS fractions during ripening there was a reduction in dilute alkali soluble pectin (DASP) from about 65% to 20% of the AIS. A corresponding increase was observed in water soluble pectin (WSP), from about 20% to 60%, while chelator soluble pectin (CSP) increased slightly.

INTRODUCTION

BLUEBERRY PRODUCTION has increased by 90 million tons over the last ten years and many growers are considering blueberries as an alternative crop (Adams, 1987). Work has been done on some aspects of fruit quality including color and flavor (Sapers et al. 1984), whereas, little research has focused on changes in blueberry texture during their development, maturity, and senescence. Firmness of developing blueberries has been measured using the Instron Universal Testing Machine (Ballinger et al. 1973). Small, green, fruit are extremely firm with most softening occuring between the green and red stages. There is only a little softening after the red stage. At maturity the berry has reached maximum size and is entirely blue.

Alteration of the cell wall and middle lamella of fruits and vegetables is responsible for textural changes during development, senesence, postharvest storage and processing (Eskin, 1979; Huber, 1983). Pectic substances, hemicellulose, and cellulose are the major cell wall polysaccharides and they frequently undergo depolymerization in maturing fruit. Solubilization of cell wall components can be achieved due to the action of specific enzymes (Huber 1983) or possibly by free radical mechanisms (Miller 1986). Depolymerization of cell wall polymers contributes greatly to tissue softening (Eskin, 1979; Huber 1983). Pectin is the predominant component of the middle lamella which is depolymerized and solublized during the ripening of many fruits.

Blueberry cell wall components have not been studied extensively. Krause and Block (1973) reported the pectin composition of fresh blueberries to be 0.3%, on a fresh weight basis, but did not specify the stage of ripeness or variety. Woodruff et al. (1960) reported a marked decline in the soluble pectin content of Jersey blueberries during ripening on a dry weight basis and a simultaneous increase in pectinmethylesterase activity.

As a general observation, unripe fruit contain small amounts of soluble pectin but this increases greatly with ripening (Huber, 1983). Nevertheless, soluble pectin, with free carboxyl groups, can be rendered insoluble by crosslinking to adjacent polymers with divalent cations such as calcium or magnesium (Eskin, 1979). The objective of this study was to measure total pectin, water-soluble pectin, chelator-soluble pectin and insoluble pectin in developing blueberries.

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MATERIALS & METHODS

Fruit sampling and storage

Selected "Bluetta" blueberry bushes at The Ohio State University Experimental Fruit Farm (Overlook, Ohio), were used in this study. Random samples of developing, maturing, and senescing berries were taken from six bushes on five occasions over a period from May 29 to June 26 1987. At each picking the berries were immediately immersed in liquid nitrogen and stored at −23°C prior to pectin analysis. The date of harvest was recorded as days after full bloom.

Alcohol-insoluble-solids preparation

Alcohol-insoluble-solids (AIS) were prepared by a method adapted from Woodward (1972). Fifty grams of fruit were blended with 200 mL 1% HC1 (v/v) in methanol for 1 min to extract anthocyanins. Insoluble solids were then filtered under aspiration with #541 Whatman filter paper, blended once more with 100 mL acidic methanol and filtered again. During the final filtration the residue was washed with 300 mL ethanol.

Alcohol-insoluble-solids were prepared from the pigment free material by refluxing with 200 mL 80% ethanol for 30 min. The solids were then washed with 200 mL acetone before a final filtration. The samples were then dried *in vacuo* at 25°C overnight, weighed and stored in a dessicator at room temperature.

Pectin analysis

Total pectin determinations were made by acid hydrolysis of alcohol-insoluble material, followed by measurement of pectin as uronic acid according to the method of Ahmed and Labavitch (1977). Fractionated pectic substances were obtained as described by Hudson and Buescher (1985). Alcohol-insoluble solids was sequentially extracted using Whatman #541 filter paper with water, removing water-soluble pectin (WSP), 0.5% sodium hexametaphosphate, removing chelator-soluble pectin (CSP) and 0.05% sodium hydroxide, removing dilute alkali-soluble pectin (DASP). Total and fractionated pectin was measured as total uronic acid according to the method of Blumenkrantz and Asboe-Hansen (1973), with the modifications of Kinter and Van Buren (1982), by incubation with sulfuric acid/sodium tetraborate solution and subsequent color development with alkaline m-hydroxyphenyl. Spectrophotometric measurements were made at 520nm on a Spectronic 1201. Each determination was made in triplicate and was expressed as a percentage of AIS and on a fresh weight basis.

RESULTS & DISCUSSION

FULL BLOOM occurred on April 29 and the fruit were ready for harvest on June 16, 48 days after full bloom. At thirty days after full bloom the berries were small and green and developed to larger green berries by day 37. The berries were at various stages of red or blue coloration at day 44 but were dark blue and ready to harvest on day 48. Blueberries collected on day 57 still retained their deep blue color.

Changes in the percent contribution of total cell wall material to fruit fresh weight, as indicated by AIS, are shown in Fig. 1. There was a decline in AIS in fruits up to 43 days after full bloom, which was particularly rapid between day 36 and day 43. Loss of AIS coincided with field observations of fruit

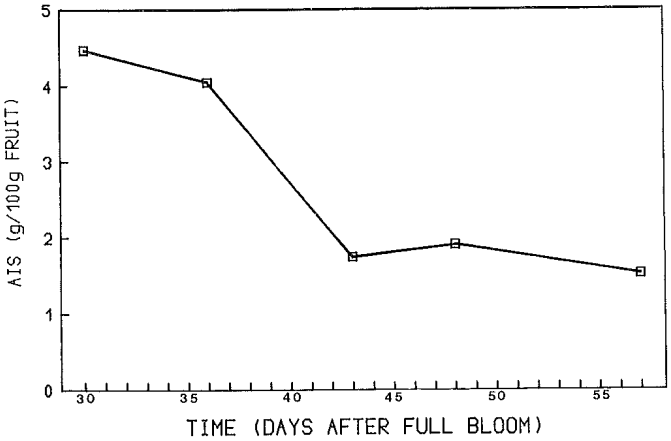

Fig. 1—Changes in alcohol insoluble solids (AIS) derived from ripening "Bluetta" blueberries. LSD = 0.40.

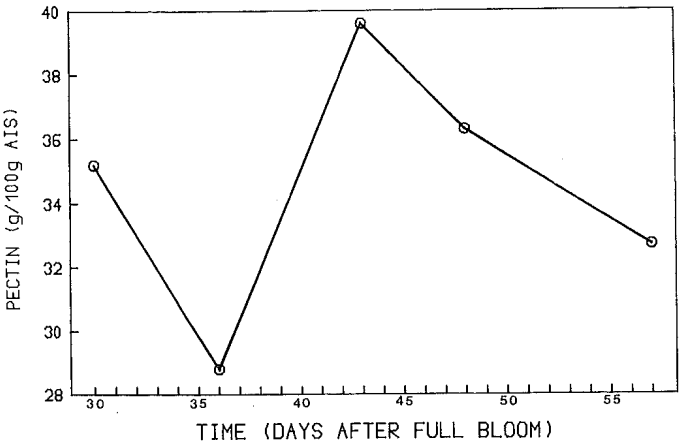

Fig. 2—Changes in the total pectin of alcohol insoluble solids (AIS) derived from ripening "Bluetta" blueberries. LSD = 0.81.

softening. After 44 days AIS levels stabilized and on reaching maturity at 48 days, there was little change in cell wall material, even if the fruit remained on the plant nine days after the maturity date. Decrease in AIS is probably due to an increase in water uptake and subsequent increase in fruit size. Total pectin varied as a component of AIS material (Fig. 2). Between day 30 and 36 there was a reduction in the contribution of total pectin to the cell walls which was followed by a rapid increase in the amount of pectin in the AIS until day 43. Pectin levels declined steadily after day 44, shortly before the harvest date.

When pectin content was expressed on a fruit fresh weight basis (Fig. 3) it declined steadily until day 43, with little change at maturity at day 48. This may reflect the greater increase in water content, relative to pectin, during growth.

Changes in the levels of fractionated pectic substances during fruit development are shown in Fig. 4. The initial rapid increase in DASP corresponded to a large reduction in WSP. After day 36, DASP concentrations fell until day 43, while WSP steadily increased throughout the harvest dates. CSP initially rose with WSP but remained constant after 43 days. Similar trends were observed when the data was expressed on a fresh weight basis (Fig. 5). The sum of the fractions only accounted for part of the reported total pectin. This is due to "nonextractable pectin" which has been described as insoluble, highly methylated pectin that is difficult to quantify by differential solubilization techniques (Hudson and Buescher 1984). However, it is included in total pectin analysis by acid hydrolysis (Ahmed and Labavitch; 1977). The non-extractable pectin constituted 36% of the total pectin on days 30 and 43,

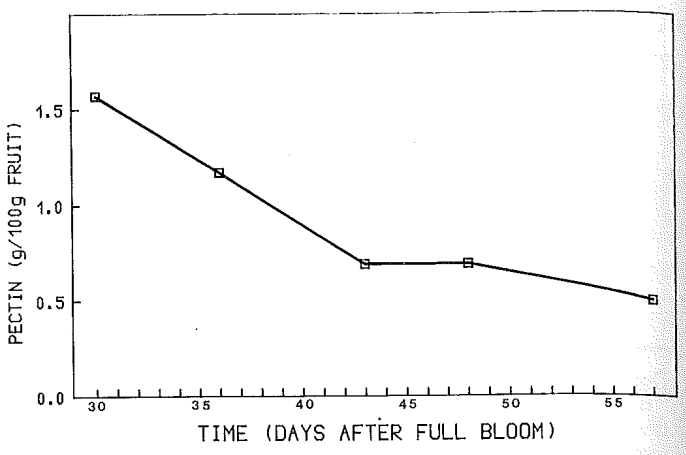

Fig. 3—Changes in the total pectin of "Bluetta" blueberries expressed on a fresh weight basis. LSD = 0.14.

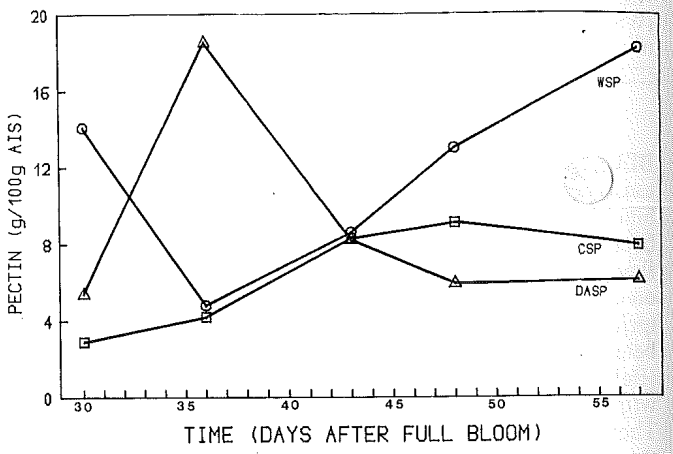

Fig. 4—Changes in the pectin fractions of alcohol insoluble solids (AIS) derived from ripening "Bluetta" blueberries. WSP = Water soluble pectin (LSD = 2.09), CSP = Chelator soluble pectin (LSD = 1.74) and DASP = Dilute alkali soluble pectin (LSD =2.49).

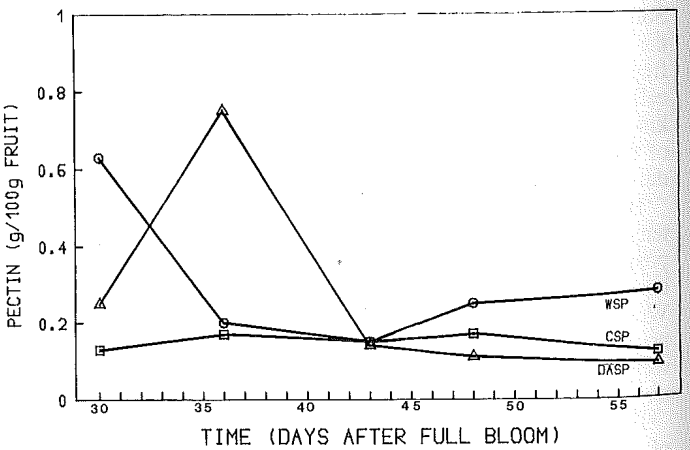

Fig. 5—Changes in the pectin fractions of "Bluetta" blueberries expressed on a fruit fresh weight basis. (WSP = Water soluble pectin (LSD = 0.11), CSP = Chelator soluble pectin (LSD = 0.04) and DASP = Dilute alkali soluble pectin (LSD = 0.11).

declined to 23% on day 47, and was negligible on day 57. The very small reading of 4% nonextractable pectin on day 36 may be an artifact of the experiment.

The data suggest a possible mechanism for pectin metabolism in ripening blueberries. The protopectin, as represented

by the DASP fraction, accumulated up to day 36, after which solubilization was initiated resulting in increased amounts of WSP. In achieving this conversion at least two enzymes may be involved, pectinmethlyesterase (PME) and polygalacturonase (PG). Demethylation by PME would result in greater numbers of carboxyl groups which may facilitate PG activity and binding of cations. Polygalacturonase preferentially degrades de-esterified pectic substances, thus PME may prepare the substrate for PG attack (Huber, 1983). Woodruff et al. (1960) observed PME activity to increase in maturing blueberries. Rising levels of CSP suggest increasing demethylation, probably as a result of PME activity, and subsequent binding of divalent cations. Furthermore, the increase in the gradient of the WSP curve (Fig. 4), as CSP levels stabilized, suggests that the availability of divalent cations may be a limiting factor in CSP formation. The CSP fraction presumably consists of calcium pectate (Hudson and Buescher, 1985). This is supported by a recent finding that the majority of cell wall bound calcium was in the CSP fraction (Howard, 1987). It could be that CSP is formed during AIS preparation when the tonoplast is ruptured. This would allow WSP to bind calcium ions from the cell vacuole. Although pectic enzymes appear to be likely candidates for causing pectin degradation, the possibility of depolymerization by oxidation should not be ruled out (Miller, 1986).

Pectin solubilization of strawberries has been shown to occur without PG (Huber, 1984). Nevertheless, there was an increase in WSP over time and a decline in AIS, when expressed on a fresh weight basis. Monroe and Lee (1987) observed an increase in soluble pectin during boysenberry fruit development, but in contrast to the data obtained from blueberries, there was a reduction in CSP as the fruit approached maturity. A similar trend has been observed in blackcurrants (Green, 1971).

Future research will investigate (1) a possible role of pectic enzymes in cell wall depolymerization, and (2) the effect of calcium infiltration on calcium pectate levels and berry firmness.

In conclusion, this study suggests that there is reduction in the pectin content of developing blueberry fruit, probably due to pectin solubilization.

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