Polygalacturonase and Pectinmethylesterase Activities in Developing Highbush Blueberries

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Abstract. Polygalacturonase (PG, E.C. 3.2.1.15) and pectinmethylesterase (PME, E.C. 3.1.1.11) activities, berry size, and texture were measured in fruit of developing highbush blueberries (*Vaccinium corymbosum* L.). Peak PME activity occurred in red berries and preceded peak PG activity, which was observed in blue-red fruit. Extensive softening occurred with the transition in berry color from red to blue-red. Both peak enzyme activities and maximum softening occurred by the time the fruit were 70% of their maximum fresh weight.

Firmness and texture are important quality factors for blueberry fruits; however, blueberry fruit softening is not fully understood. Hamann et al. (1973) observed that firmness was related to storage life and tendency to decay. They separated blueberries into shelf-life classes based on firmness. There is little published information regarding the biochemical basis of softening in blueberries (Ballinger et al., 1973; Proctor and Peng, 1989); therefore, studies of softening may be of value in improving postharvest handling and enhancing fruit processing methods.

Generally, the decrease in firmness in maturing fruits is thought to be due to alterations of the cell wall and middle lamella (Eskin, 1979; Huber, 1983). Pectic substances, cellulose, and hemicellulose are the major cell wall polysaccharides, some of which are depolymerized during ripening. The middle lamella is primarily pectin (Eskin, 1979) and its solubilization is often corre-

lated with a loss of firmness. In blueberries, small green fruit are extremely firm but soften extensively between the green and red stages (Ballinger et al., 1973).

Alcohol-insoluble solids (AIS) and total pectin content decrease during blueberry fruit development until the berries are ripe, after which there is little change in total pectin content (Proctor and Peng, 1989). Furthermore, there is a loss of dilute alkali-soluble pectin (DASP) and a corresponding increase in water-soluble pectin (WSP). The pectin modifications in ripening blueberries suggest

that enzymatic solubilization of pectin may occur. However, in strawberries, pectin solubilization has been reported to occur without pectinase action (Huber, 1984). In many fruits (Huber, 1983), pectin degradation is thought to occur initially by the action of pectinmethylesterase (PME), which de-esterifies the DASP. The de-esterified pectin constitutes a more readily hydrolyzed substrate for polygalacturonase (PG). PME activity has been found in highbush blueberries (Woodruff et al., 1960), but PG activity has not been reported.

The objective of this study was to measure changes in the PG activity of developing highbush blueberry fruit and relate this to PME activity, fruit firmness, and size.

'Collins' highbush blueberries were harvested at various stages of development from a commercial blueberry farm at Mount Vernon, Ohio, during the 1989 season. Care was taken to pick only healthy, undamaged fruit. Blueberries were separated into stages 2 through 8, as described by Ballinger and Kushman (1970). Fruit to be used for enzyme assays were immersed in liquid nitrogen in the field and subsequently stored at -80°C. Physical data were collected from fresh fruit within a few hours of harvest.

The mean weight and diameter of 10 berries from each stage were measured. The firmness of 10 blueberries from each stage was measured using an Instron Universal Testing Machine (Model 1000; Instron Corp., Canton, Mass.) with a flat-ended, 1-cm-diameter cylindrical probe that was moved at

Fig. 1. Changes in the fresh weight of ripening 'Collins' highbush blueberries (LSD_{0.005} = 0.155). Each value is a mean of 10 measurements.

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Fig. 2. Changes in the firmness of ripening 'Collins' highbush blueberries ($LSD_{0.05} = 0.011$). Each value is a mean of 10 measurements. Note: dynes $\times 10^{-2} = N$.

Fig. 3. Changes in the polygalacturonase ($LSD_{0.05} = 26.9$) and pectinmethylesterase ($LSD_{0.05} = 0.051$) activity of ripening 'Collins' blueberries expressed on a fresh-weight basis. Each value is a mean of duplicate determinations of two extracts each each stage.

a speed of 10 $cm \cdot min^{-1}$. The instrument was equipped with a 500-g compression cell. Berries were oriented with the calyx-stem axis horizontal. Firmness was measured as hardness, which is defined as the peak force during the first compression cycle (Bourne, 1978).

Fruit was allowed to thaw for ≈ 1 h at 20C. Blueberries were ground with a pestle, mortar, and a little washed sand in an extraction medium of 0.1 M Na-phosphate buffer (pH 6.5) containing 0.1 M sodium chloride and 1% polyvinylpyrrolidone at 4C and were left for 1 h at this temperature. A 1 fruit :1 medium ratio (w/v) was used. Difficulty was experienced in obtaining extracts from fruit from stage 2 because the tissue was dry and tended to absorb the medium. The extracts were filtered through four layers of cheesecloth and centrifuged at $11,000 \times g$ for 20 min. Each extract was dialyzed for 18 h at 2C against 4 liters of extraction buffer (12-14 kilodaltons exclusion limit). The buffer was changed three times.

PG activity was measured by incubating 1 ml of extract with 5 ml of 0.5% citrus PG

acid in 0.03 M phosphate buffer (pH 6.5) at 30C for 5 min. Reducing sugars were then measured (Nelson, 1944) using galacturonic acid as a standard. The activity of two extracts from stages 3-8 were measured in duplicate. Control experiments were performed with boiled enzyme.

For PME measurement, the spectrophotometric assay of Hagerman and Austin (1984) was used. Twenty microliters of enzyme extract, 2.0 ml of substrate containing 0.5% citrus pectin in 0.2 M phosphate buffer (pH 7.5), 0.15 ml bromothymol blue, and 0.83 ml of water were placed in a cuvette at 25C and the absorbance measured at 620 nm for 1 min. Duplicate determinations of two extracts from each stage were made. Control experiments were performed with boiled enzyme extract from each growth stage.

Blueberry fruit exhibited double-sigmoidal gain in weight, with the initial major growth period occurring between stages 3 and 5 and a second one after stage 7 (Fig. 1). Measurements of berry diameter showed a similar pattern in size increase (data not shown).

Firmness was measured at each stage of development to determine how it changed with development (Fig. 2). Berries softened slightly between stages 2 and 4, but most softening occurred between stages 5 (red fruit) and 6 (blue-red fruit), with no additional softening thereafter. The initial loss of firmness could be due to an increased water content and/or change in berry geometry. Ballinger et al. (1973) reported that most softening in 'Morrow' highbush blueberries occurs as the berries progress from green to red. Our study with 'Collins' blueberries indicated that most softening occurs when the berries progress from stage 5 to stage 6. In 'Bluetta' highbush blueberries, there has been a significant loss of DASP and an increase in WSP at the bluish-red stage of development (Proctor and Peng, 1989).

PG and PME activities of ripening highbush blueberries were expressed on a fresh-weight basis (Fig. 3). PG activity increased until stage 6 (blue-red fruit) and declined significantly by stage 8. PME activity also increased during development, with peak activity occurring at stage 5 (red fruit). Maximum PME and PG activity appeared when the berries were almost totally red and bluish-red, respectively.

The appearance of PG and PME activities in blueberry fruit coincides with pectin solubilization (Proctor and Peng, 1989) and the appearance of anthocyanins (Ballinger and Kushman, 1970). Woodruff et al. (1960) measured PME activity in ripening 'Jersey' blueberries and found increasing activity 6 days after the visibility of red pigment (this would correspond to our stage 3). Enzyme activity increased for the next 14 days, which is approximately in accordance with our findings.

Studies on tomato PG activity have shown that activity in developing fruit may not necessarily be related to softening (Giovannoni et al., 1990; Smith et al., 1988). Therefore, although highbush blueberry PG activity is reported, no causal relationship between fruit softening and enzyme activity should be assumed at this time.

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