The Modification by Calcium Chloride of the Firmness and Pectic Substances of Highbush Blueberry Fruit

A Thesis

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by

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CHAPTER I

INTRODUCTION

BACKGROUND

Blueberries are native to North America and were consumed by American Indians, who introduced them to early settlers (Eck, 1966). Breeding for desirable characteristics, such as flavor, color and size, has produced many successful cultivars (Eck, 1988). There are three types of commercially important blueberries; highbush (*Vaccinium corymbosum* L.), lowbush (*Vaccinium angustifolium* Ait.) and rabbiteye (*Vaccinium ashei Reade*) varieties. Highbush blueberry cultivars were derived from the two wild highbush species, *V. australe small* and *V. corymbosum*. A few cultivars have been produced by hybridization of highbush and lowbush species.

Highbush and rabbiteye blueberry production in the United States has increased from 73.5 million pounds in 1980 to 137.8 million pounds in 1985 (Eck, 1988). The projected highbush U.S. production for 1990 was 195 million pounds (Hansen, 1990). Acreage has also increased (Hansen, 1990).

Approximately sixty percent of the total American blueberry production is processed (Eck, 1988). Two-thirds of this fruit is frozen and the remainder is used in jams and jellies, dehydrated, canned or crushed for juice (Eck, 1988). Most
frozen fruit is used in yogurt, although its use in baked goods, ice cream and juice is increasing. Overall, the consumption of processed blueberries is growing faster than that of the fresh fruit (Eck, 1988).

Research relating to blueberry chemistry, processing and fruit quality characteristics is limited (Eck, 1988). Blueberry anthocyanins, sugars, titratable acidity and the surface wax structure have been examined (Woodruff et al., 1960; Watt and Merril, 1964; Sapers et al., 1985; Sapers and Phillips, 1985). Pectin degrading enzymes such as pectinmethylesterase (Woodruff et al., 1960) and polygalacturonase (Proctor and Miesle, 1991) have been reported and may be important in berry softening.

**STATEMENT OF PROBLEM**

Thermal processing and freezing result in blueberry softening and loss of berry integrity. Loss of berry integrity limits the use of processed berries and has led to difficulty in producing high quality canned blueberries and blueberry pies.

Studies have shown that mature blueberries contain appreciable quantities of water soluble pectin (WSP) which increases during ripening (Proctor and Peng, 1989). In some fruits and vegetables calcium salts have been used to convert WSP to an insoluble calcium pectate. Insoluble calcium pectate, formed as a result of calcium treatments, has been associated with increased firmness in strawberries (Morris et al. 1986), tomatoes (Buescher and Hobson, 1982) and cucumber pickles (McFeeters et al., 1986).
The objectives of this study were to:

(1) Investigate a possible role for calcium in enhancing the firmness and berry integrity of mature blueberries during blanching.

(2) Observe changes in pectin composition as a result of calcium treatments during blanching.

(3) Investigate the relationships between pectin composition, firmness, and calcium treatment in blueberries.
CHAPTER II

LITERATURE REVIEW

Blueberry cultivation

In the U.S.A. and worldwide, more highbush blueberries are produced than either lowbush or rabbiteye varieties. Highbush blueberries were introduced to the eastern United States during the 1920's (Draper, 1979) and are now grown in New Jersey, Michigan, North Carolina, Washington and Arkansas. Highbush blueberry production totalled 131.4 million pounds in 1985 (Eck, 1988) and is projected to be 195 million pounds for 1990 (Hansen, 1990).

The lowbush varieties are native to the northeastern United States and grown mostly in Maine, Michigan and Canada. The total 1985 lowbush blueberry production was 78.5 million pounds. Rabbiteye blueberries are native to the southeastern United States. The total rabbiteye production was 6.2 million pounds in 1985 and the anticipated production for 1990 was 11.5 million pounds.

Blueberry production is expected to increase with worldwide demand (Eck, 1988). Studies have indicated that varietal differences in firmness, color and resistance to cracking are important in determining quality (Woodruff et al., 1960; Sapers et al., 1985; Sapers and Phillips, 1985). Blueberries will maintain their quality for about four weeks in refrigeration but only about one week at room
temperature (Eck, 1988). Fruit senesce as soon as they are ripe or removed from the plant. Undesirable softening often occurs in fresh blueberries and limits their use in processed foods.

**Blueberry anatomy**

Figure 1 is a representation of the cross-section of a highbush blueberry. The cuticle is the outermost structure of the blueberry, it consists of wax, cutin and the pectin-rich region which would have been the middle lamella had another cell been present. The thick single cell layer, the epicarp, overlays the 4 or 5 cell thick hypocarp. Vascular bundles separate the epicarp and hypocarp from the relatively homogenous mesocarp. Vascular bundles also reach from the core through the mesocarp and surround the endocarp. Stone cells are found within about 1.4mm of the blueberry surface (Gough, 1983; Yarbrough and Morrow, 1947).

The blueberry skin consists of the cuticle, epicarp and hypocarp. This is not a histological definition, but one which is convenient for description. The principal pigment of blueberries are anthocyanins which are concentrated in the epicarp and hypocarp (Sapers and Phillips, 1985).

**The cell wall and middle lamella as structural components.**

The cell wall is important in maintaining fruit firmness. The breakdown of the cell wall is associated with fruit softening. This breakdown occurs during
Figure 1. Blueberry histology. Listed are the major components of blueberry anatomy. Note that "skin" is given the working definition of the cuticle, epicarp and hypocarp (Adapted from Yarbrough and Morrow, 1947; and Gough, 1983).
ripening and senescence. An understanding of fruit cell wall structure is necessary to understand blueberry softening.

The cell wall is a parallel construct composed of a primary cell wall, a secondary cell wall and is associated with the intracellular middle lamella. The primary cell wall is located between the plasma membrane and the middle lamella. Figure 2 illustrates the currently accepted primary cell wall model (Keegstra et al., 1973). The primary cell wall consists of hydroxyproline-rich protein, cellulose, pectin and hemicellulose. The long chain rhamnogalacturonan pectin is connected to the protein by arabinogalactan side chains. In turn the hemicelluloses, xylan and xyloglucan, are covalently bound to cellulose fibers which allow a second side chain, galactan, to provide attachment to rhamnogalacturonan. Any breakdown of rhamnogalacturonan or other cell wall components will weaken the cell wall and result in softening. The primary cell wall is flexible and provides protection for the cell membrane.

The secondary cell wall is located between the primary cell wall and the plasma membrane. It is differentiated, for specialized functions, by modification of polysaccharides and deposition and modification of proteins for selective transport (Darvill, A. et al., 1980). The secondary cell wall is composed primarily of cellulose, but also contains pectin, hemicellulose and lignin. This secondary cell wall provides strength and mechanical support (Eskin, 1979).

During ripening cell wall components are degraded, resulting in softening of the fruit, allowing cell elongation and differentiation (Labavitch, 1981). This
Figure 2. Proposed model for the primary cell wall. Rhamnogalacturonan polymers are connected to cellulose fibrils via galactan and to the protein polymer via arabinogalactan (reproduced from Keegstra et al., 1973).
allows cells to function as part of a tissue with specific properties i.e., the epicarp will have thick cell walls while the mesocarp will not (Labavitch, 1981). Softening is achieved by a wide range of enzymes e.g. pectinases, cellulases and hemicellulases using specific cell wall components as substrates.

The middle lamella is a pectin rich region formed between adjacent cells during the course of cell division. It acts as a cement, binding adjacent plant cell walls via the cellulose-rich primary cell walls (McClendon, 1964). Pectin degradation in the middle lamella allows increased movement between cells, resulting in softening.

**The role of pectin in the cell wall and middle lamella.**

Thirty-five percent of the dicot cell wall and almost all of the middle lamella is pectin (Hall, 1976). Pectin is primarily rhamnogalacturonan, polygalacturonic acid (α-1,4-D-galactopyranosyluronic acid units) interrupted with α-1,2 linked L-rhamnopyranosyl units [Figure 3(i)] (BeMiller, 1986). Galactans [Figure 3(ii)], β-1,4 linked D-galactopyranose units, are β-1,4 linked to the rhamnose units. Araban [Figure 3(iii)], α-1,3 and α-1,5 linked L-arabinofuranose residues, are β-1,4 linked to a galactan chain to form arabinogalactan [Figure 3(iv)] (Eskin, 1979). Galactan homopolymers provide bonding of the rhamnogalacturonan to cellulose. Arabinogalactan provides bonding of rhamnogalacturonan to the protein polymer (Figure 2) (Eskin, 1979).
Figure 3. The structure of pectin and related neutral polysaccharides.
(i) Rhamnogalacturonan (reproduced from Eskin, 1979)
(ii) Galactan (reproduced from Hall, 1976)
(iii) Araban (reproduced from Hall, 1976)
(iv) Arabinogalactan (Eskin, 1979)
Many of the carbonyl groups of the galacturonic residues of pectin are methyl esterified. The degree of methylesterification (DE) is indicative of the pectin's physical properties. Low DE indicates greater polarity and increased solubility of the pectin. Pectin DE varies with species and the function and age of cells and in part determines its solubility and gelation characteristics of (BeMiller, 1986).

Changes in pectin chemistry occur during ripening. Typically, pectin in an immature fruit has a high degree of polymerization (DP) and DE. This pectin is relatively water insoluble and contributes structural support and fruit firmness (BeMiller, 1986). This water insoluble pectin is termed dilute alkali soluble pectin (DASP), due to its solubility in a dilute alkali (0.5M NaOH) solution. The DE and DP are diminished as the pectin is degraded. This results in shorter chained water soluble pectin (WSP), which is associated with fruit softening. As pectin is de-esterified, free carboxyl groups are formed which may bind divalent cations, such as calcium. This pectin-bound cation may form bridges across adjacent pectin chains via carboxyl groups, as seen in Figure 4(i) (Grant et al., 1973). A complex matrix of calcium bound pectin may be formed as shown in the "egg box" model proposed by Rees (1982). This agglomerate of calcium pectate is water insoluble and may provide enhanced structural support within the cell wall.

Pectin can be fractionated by differential solubility. Water soluble pectin (WSP) which has a low DP and low DE, may be removed from alcohol insoluble solids (AIS) with water alone. Calcium and other pectates constitute chelator
Figure 4. Pectin and calcium interactions.
i) Calcium and carboxylic acid ionic bonds joining adjacent pectin strands. (reproduced from Eskin, 1979)
ii) The "egg box" model of calcium ion binding by pectin chains. Optimum conditions of calcium binding results in a large aggregate of pectin. (reproduced from Rees, 1982)
soluble pectin (CSP) which may be removed from AIS with a cation chelator such as sodium hexametaphosphate rendering the CSP soluble. Dilute alkali soluble pectin (DASP) possesses both a high DE and a high DP and is removed from the cell wall material with a dilute alkali solution such as 0.5M sodium hydroxide (Hudson and Buescher, 1985).

The preservation of cell wall integrity is important in retaining firmness. An increase in the concentration of WSP, relative to total pectin, often occurs during fruit softening (Huber, 1983). Proctor and Peng (1989) showed an increase in WSP with a concurrent decrease in DASP during blueberry ripening. This trend has also been reported in pears (Dick and Labavitch, 1989), apples (Bartley and Knee, 1982), and tomatoes (Gross and Wallner, 1979).

**Degradation of pectin**

Softening of the cell wall involves degradation of the polysaccharides contributing to fruit firmness (Ben-Arie et al, 1979). Enzymatic breakdown is the most common form of pectin degradation during ripening. Pectinmethylesterase (PME) de-esterifies the methylated galacturonans. The de-esterified pectin is then more susceptible to enzymatic breakdown by polygalacturonase (PG), which requires free carboxyl groups (Brady, 1987). Pectinmethylesterase activity has been reported in blueberries (Woodruff et al., 1960; Proctor and Miesle, 1991), tomatoes (Pressey and Avants, 1982), and necessary for PG activity in snap beans (Van Buren, 1979).
The activity of PG results in DASP being converted to WSP. Polyalacturonase activity is illustrated in Figure 5. In blueberries PME achieves its maximum activity at a physiological stage prior to the maximum activity of PG, and the onset of softening coincides with PME activity (Proctor and Miesle, 1991). Polyalacturonase activity increases during ripening in many fruits: cucumbers (McFeeters et al., 1980), kiwi (Soda et al., 1986), peaches (Pressey and Avants, 1973) and blueberries (Proctor and Miesle, 1991). In contrast, ripening-related softening apparently occurs without PG in strawberry (Barnes and Patchett, 1976).

Pectin can be degraded without PG by $\beta$-elimination in an alkaline environment (Figure 6) with or without enzymatic catalysis (BeMiller, 1986). As the pH rises and acidity drops during processing or ripening, $\beta$-elimination may occur at increasing rates such as in snap beans (Van Buren, 1979).

**Thermal processing and the use of calcium**

Like many fruit, blueberries tend to soften during thermal processing and this adversely affects fruit quality. Cell membranes are ruptured by heat and organic acids released from vacuoles may chelate pectin-bound calcium (Claypool, 1974). These acids could increase the WSP content of the fruit and positively influence softening (French et al., 1989, Chitarra et al., 1989). This is an important factor in the conversion of CSP to WSP (Grant et al., 1973). The divalent calcium ions provide cross-links between adjacent polyuronide chains via the carboxylic acid groups increasingly formed by PME activity (Van Buren, 1979).
Figure 5. Activity of endo- and exo- polygalacturonases. Exo- cleaves only the terminal galacturonic acid residue as opposed to endopolygalacturonase which cleaves the chain randomly. (reproduced from Eskin, 1971)
Figure 6. Alkaline depolymerization, or β-elimination, of a polygalacturonic acid chain. This is a hydration reaction which only occurs under alkaline conditions. (reproduced from BeMiller, 1986)
With a DE below 50%, the efficiency of calcium binding to pectin is strongly related to the polymer concentration (Thibault and Rinaudo, 1986). The formation of crosslinked pectates often results in fruit firming.

Divalent cations are added to fruits and vegetables to convert WSP to chelated pectate, producing a firming effect. Calcium, strontium and zinc form intramolecular electrostatic bonds between pectic chains. The divalent cadmium, copper and lead ions form complexes with active groups other than only the carbonyl groups; therefore, one pyranosyl residue may bind more than one ion and vice versa (Kohn, 1987). Low molecular weight (MW) fragments of de-esterified pectin bind with cations with varying affinities, as follows: Pb > Cu > Cd > Zn ~ Sr ~ Ca. The difference in binding of Ca, Sr, and Zn to low MW pectins is negligible, and very little of each ion is actually bound. Cadmium, Cu, and Pb are bind according to their solubility. Kohn (1987) assumed other groups, in addition to the carboxylic acids, were involved with chelating heavy metals, as all divalent cations with similar size, solubility and electronegativity should have chelated the same amount of pectin. Further investigation (McFeeters and Fleming, 1989) of cation binding by pectin in cucumber slices showed softening inhibition as follows: Ca ~ Sr > Ba > Cu > Al. Cadmium, Co, Zn, and Mg were ineffective as softening inhibitors on a molar basis. Lanthanum, Ce, Pr, Nd, Sm, and Eu all had greater inhibitory effects on softening than calcium, on a molar basis. This led McFeeters and Fleming to propose that the "egg box" model was incorrect. They predicted that cations with greater binding efficiency will inhibit softening to a greater
degree. They found Ca and Sr had the greatest softening inhibition while possessing the least affinity for pectin (McFeeters and Fleming, 1989). Although calcium inhibited softening effectively, the cross-linking could not explain the effect. It was suggested the textural effects of calcium are not related to binding at the carboxyl groups of pectin alone, and that further research was needed to elucidate the answer.

McFeeters and Fleming (1989) suggested pectin carboxyl group binding is not the method of calcium’s effectiveness in preserving the firmness of cucumbers. The low pH in the cell wall and middle lamella is not conducive to calcium cross-linking of pectin which would limit the effectiveness of forming cross-links (McFeeters and Fleming, 1989; Kohn, 1987).

Strawberries dipped in calcium are significantly firmer than the non-treated berries due to formation of insoluble calcium pectate (Morris et al., 1986). Sliced strawberries were more effectively firmed than whole strawberries. Calcium pectate also inhibits the activity of cucumber PG by reducing the enzyme’s access to the substrate (McFeeters, 1986). Calcium treatment of cucumbers correlated with increased CSP content and firmness (Hudson and Buescher, 1985). Similarly, snap beans soaked in calcium were significantly firmer than those of a water control and twice as firm as those soaked in magnesium (Van Buren, 1984). Calcium chloride effectively firmed strawberries (Main et al., 1986). Tomato products (Buescher and Hobson, 1982) have also been successfully treated.
CHAPTER III
MATERIALS AND METHODS

Materials

Fresh, ripe highbush blueberries (cv. Bluecrop) were obtained from Brady Farms (West Olive, Michigan). The fruit was refrigerated at 4°C and processed within 5 days.

A. ATMOSPHERIC PROCESSING

Calcium Treatments

Sound blueberries were rinsed in distilled water and duplicate 200g samples were immersed in 2L of 0.2M CaCl₂, 0.5M CaCl₂, or 0.2M disodium ethylenediaminetetraacetic acid (EDTA) aqueous solutions and a distilled water control. (EDTA treatments were used to observe the effect of cation chelation.) Berries were blanched at 53°C for three minutes, 83°C for three minutes and soaked for 23°C for eighteen hours (overnight). Each treatment was maintained at the appropriate temperature in a water bath. The blueberries and solution were then carefully poured into a plastic sieve and the fruit rinsed in a...
stream of room temperature distilled water for one minute. The blueberries were placed on white enamel trays for a few minutes to cool.

**Berry integrity evaluation**

The proportion of ruptured blueberries were measured for each treatment. Trays were photographed with a Minolta E7 camera with a 55 mm lens (ASA 100 Fujichrome film) in ambient fluorescent light and an incandescent desk lamp.

**Firmness evaluation**

Immediately after processing, duplicate samples of twenty intact berries were randomly selected from each treatment for firmness measurements. This was performed with an Instron Universal Testing Machine with a 500g crosshead and a 1cm diameter cylindrical probe. Probe speed and chart speed were 10cm/min and 20cm/min, respectively. Berries were oriented perpendicular to their stem/calyx axis and firmness was measured as hardness (Bourne, 1978). Fresh berries at room temperature were used as a control. Mean hardness and LSD$_{0.05}$ were reported for each temperature treatment.
**Total solids**

Duplicate 10g samples of processed intact berries from each treatment were randomly selected and placed in a circulating air oven at 85°C for four hours and weighed. Fresh berries were used as a control.

**Calcium analysis**

Triplicate calcium determinations of each treatment were obtained by atomic absorption (Gaines and Mitchell, 1979).

**Alcohol insoluble solids**

Alcohol insoluble solids (AIS) were prepared according to the method of Proctor and Peng (1989). One hundred gram blueberry samples were ground in a blender for one minute with 100ml of methanol containing 1% HCl. The slurry was filtered on an 18cm Buchner funnel with Whatman #1 filter paper. Insoluble solids were rinsed into the blender with an additional 100ml of acidified methanol and blended for 1 minute. The slurry was again filtered, then 100ml of ethanol was blended into the insolubles, and again blended for 1 minute. The insolubles were then rinsed with 200ml of 80% ethanol/ water (v/v) into a 400ml beaker. This mixture was dispersed and refluxed for 30 minutes. After cooling and filtering, the insolubles were dispersed in 100ml acetone, refiltered and subsequently rinsed in acetone.
The AIS was placed in a vacuum oven at 55°C for 4 hours. The dried AIS was weighed to determine percent total AIS, ground in a cyclone mill twice and stored in glass jars in desiccators. Duplicate determinations of the AIS were made for each treatment.

Pectin fractionation

Water soluble pectin (WSP), chelator soluble pectin (CSP) and dilute alkali soluble pectin (DASP) were obtained by a sequential extraction, in duplicate, by the method of Hudson and Buescher (1985) as follows.

Water soluble pectin:

Fifty mg of AIS was dispersed in 25ml of water. The suspension was filtered with Whatman #1 filter paper in a short stemmed funnel. Tygon tubing was attached to the stem and the end clamped. Two more 25ml additions of water were added to the stoppered funnel which was stirred on an orbital shaker at 110 r.p.m. for ten minutes each. The filtrates were placed in a 100ml volumetric flask with 5ml of 0.5% NaOH added and the total volume was adjusted to 100ml with water.

Chelator soluble pectin:

Similarly, three washes of 0.5% sodium hexametaphosphate (SHMP) were performed on the residue. Five milliliters of 0.5% NaOH was added and the total volume brought to 100ml with 0.5% SHMP.
Dilute alkali soluble pectin:

Finally three extractions were performed on the residue with 0.05M NaOH, and volume brought to 100ml with 0.05M NaOH.

**Uronic acid determination**

Each fraction of pectin was subjected to total uronic acid determination by the method of Ahmed and Labavitch (1977). Triplicate 1ml samples were taken from the well-mixed 100ml volumes of each pectin fraction and pipetted into test tubes in an ice bath. Six milliliters of chilled 0.125M sodium tetraborate in 18M sulfuric acid was added to each sample, which was immediately mixed with a vortexer and held in ice water. Samples were placed in boiling water for 10 minutes. The tubes were cooled in ice water and then brought to room temperature. After reaching ambient temperature (about 5 minutes), 0.1ml of 0.15\% m-hydroxydiphenol in 0.5\% NaOH was added to two of three tubes (the third was the blank and received 0.1ml of 0.5\% NaOH), the mixture was vortexed twice and left to stand for 20 minutes to allow air bubbles to rise. Absorbance was measured at 520nm and a standard curve was prepared using D-galacturonic acid. Means and LSD\textsubscript{0.05} were calculated for each treatment on both an AIS and a fresh weight basis.
B. VACUUM INFILTRATION

Calcium treatments

The same methodology used in atmospheric processing was adopted for vacuum infiltration with the following modifications. Two hundred gram samples were placed in 2L of each solution in a vacuum oven at ambient temperature (20°C). Pressure was decreased with a vacuum pump to 25mm Hg vacuum within two minutes and held for 5 minutes. Pressure was released over two minutes and a 3 minute rest period was allowed. This process was repeated four times. Total time of contact of blueberries with treatment solutions was 50 minutes.

Determinations

The same chemical and physical analyses were performed as were used in atmospheric pressure treatments.
CHAPTER IV
RESULTS AND DISCUSSION

A. ATMOSPHERIC PROCESSING

Blueberry integrity

Table 1 shows the degree of splitting of blueberries processed at the three temperatures (83°C, 53°C, and 23°C) at atmospheric pressure. At 53°C and 23°C there was no fruit damage except for the 18 hours, 23°C EDTA treated blueberries. At 83°C, there was a marked calcium effect in reducing splitting, but 0.5M CaCl₂ was less effective than 0.2M CaCl₂ in this regard. Substantial splitting was observed in the 83°C EDTA treated blueberries. This difference in blueberry splitting between the 83°C and the lower temperature treated blueberries indicates heat is a major source of damage during processing.

The results suggest the possibility that calcium may be incorporated into the blueberry skin (cuticle, epicarp and hypocarp). At 83°C, the calcium treatments have apparently allowed an increased berry integrity despite the elevated temperature. Breakage in the 23°C treated blueberries over an 18 hour period occurred with EDTA despite the lack of added heat. The longer processing time allowed greater diffusion of solutes into the fruit, in contrast to the shorter time
Table 1. Damage to blueberries following thermal processing at atmospheric pressure. Data are reported as the percent split berries and are expressed as a mean of two determinations with the standard deviation.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>83°C 3 min.</th>
<th>53°C 3 min.</th>
<th>23°C 18 hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water Control</td>
<td>8.8 ± 2.1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.2M CaCl₂</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.5M CaCl₂</td>
<td>1.6 ± 0.8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.2M EDTA</td>
<td>31.0 ± 3.7</td>
<td>0</td>
<td>10.4 ± 0.9</td>
</tr>
</tbody>
</table>
of exposure to elevated temperatures. The EDTA may chelate calcium from CSP in the epicarp and hypocarp cell walls. Calcium removal likely weakened the cell wall and middle lamella, this resulting in some berry splitting. Alternatively, the presence of exogenously applied calcium may strengthen the epicarp by chelated pectin formation.

Plates I through IV in Appendix A show the blueberries following treatment under atmospheric pressure for 3 minutes at 83°C with water, 0.2M and 0.5M CaCl₂ and 0.2M EDTA. Damage occurred in the water control and EDTA (Plates I and IV) treated fruit; where a substantial number of blueberries completely ruptured and lost fluid. Many blueberries in the CaCl₂ treated samples (Plates II and III) lost pigments even though relatively few skins ruptured. These photographs compliment the integrity data in Table 1. The greatest degree of splitting occurred with EDTA treatment; and these berries also showed the greatest leakage.

Plates V through VIII in Appendix B show the results of 53°C processing for 3 minutes. At 53°C the EDTA treated berries lost a small amount of pigment but sustained no skin rupturing. The other treated berries sustained little damage from the 53°C heating, with very little pigment loss.

Plates IX through XII in Appendix C show blueberries processed at 23°C for 3 minutes. There is little obvious damage to the blueberries, although the 0.2M EDTA treatment caused some breakage. No pigment loss is visible in the photographs.
Anthocyanin pigment is primarily located within the blueberry epidermal and hypodermal cells. Loss of pigments is also indicative of cell membrane rupture (Sapers and Phillips, 1985; Sanford et al., 1991). Fluid loss is curtailed by calcium treatments as evidenced by the 83°C plates, although this is not obvious in the 53°C or 23°C plates. Anthocyanin presence in the fluid lost by the 0.2M 83°C treated berries appears greater than that in the water treated blueberries. This may indicate a complex of anthocyanins with calcium, increasing the color intensity (Van Teeling et al., 1971). Pectin crosslinking with calcium, however, in the cell wall and middle lamella may form a barrier to pigment diffusion following membrane rupture.

Membrane breakage is probably not due to osmotic damage because the water treatment caused less rupturing than the salt solutions. Treatment with EDTA caused breakage or loss of integrity at both 83°C for 3 minutes and 23°C for 18 hours. This may be due to calcium chelation from the CSP within the cell wall, which could allow increased pigment diffusion.

**Firmness evaluation**

Figure 7 shows the firmness of the processed blueberries. At 83°C for 3 minutes, all the processed fruit are significantly softer than the fresh berries. The 0.2M CaCl₂ treated blueberries are significantly firmer than the processed control, but not significantly different from the 0.2M EDTA or 0.5M CaCl₂ treated blueberries. Heat-induced softening exerts a greater effect than that of CaCl₂.
Figure 7. Firmness of blueberries following processing at atmospheric pressure at (a) 83°C for 3 minutes; LSD$_{0.05}$ = 0.276, (b) 53°C for 3 minutes; LSD$_{0.05}$ = 0.375 and (c) 23°C for 18 hours; LSD$_{0.05}$ = 0.395. Data are expressed as the mean of 20 berry samples.
At 53°C for 3 minutes, all the processed fruit except the 0.2M calcium treated blueberries are significantly softer than the fresh blueberries. There are no significant differences between the firmness of the chemically treated blueberries and the water processed control. However, softening was not nearly as severe as in the 83°C treated blueberries.

At 23°C for 18 hours, the 0.2M CaCl₂ treated blueberries were significantly firmer than the fresh and EDTA treated blueberries, but not significantly different from the 0.5M CaCl₂ treated or water control treated blueberries. The 0.5M CaCl₂ treated blueberries were significantly firmer than the fresh berries. No treatment was significantly different from the water processed control. Although EDTA caused berry splitting, it did not cause a significant loss of firmness relative to the fresh fruit.

Illustrated in Figure 8, two sources of anthocyanin and fluid leakage may be identified: first, splitting of the berry skin (cuticle, epicarp and hypopcarp) exposing the mesocarp allowing fluid loss. Second, rupture of epicarp cell without skin breakage which allows anthocyanin loss, indicating cell wall and middle lamella resistance. In the 83°C processed blueberries, skin rupture is observed with moderate skin integrity preservation by calcium treatment although anthocyanins are still lost. Thus while calcium prevents skin breakage, cell rupture still occurs.
Figure 8. Two proposed methods of blueberry anthocyanin or fluid leakage during processing (adapted from Yarbough and Morrow, 1947; and Glough, 1983).
(i) breaking of epicarp cells
(ii) breakage of skin
**Total solids**

Total solids readings ranged from 12.9% to 16.4% (Table 5, Appendix E). This roughly corresponds to a report of 16.8% by Watt and Merrill for highbush blueberries (1964). There were no significant differences at any treatment level. Any juice loss during processing is therefore either small or essentially replaced by water influx. An increase in juice loss would be seen as a greater soluble solids content relative to the controls.

**Calcium content**

Table 2 shows the calcium content of processed blueberries. The 3 minutes, 83°C, 0.5M CaCl₂ treated fruit contains significantly more calcium than the 0.2M CaCl₂ treated blueberries. The 53°C treated blueberries show a similar trend to those treated at 83°C. At 23°C, however, only the calcium content of the 0.5M CaCl₂ treated blueberries was significantly greater than that of the other samples. This data shows the effectiveness of using the calcium chloride solutions to increase the calcium content. However, there was great variation in the calcium content of the samples processed at 23°C, which indicates a variable influx of calcium into the blueberries. There is no significant difference between the calcium contents of the EDTA treated blueberries and the water processed
Table 2. Calcium content of blueberries following processing at atmospheric pressure. Expressed as micrograms of calcium per gram dry weight of blueberry tissue. Data are the mean of three 10g samples. The LSD_{0.05} for the 83°C, 53°C and 23°C treatments are 35.1, 40.2 and 196.9 respectively.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>83°C 3 min.</th>
<th>53°C 3 min.</th>
<th>23°C 18 hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh Berries</td>
<td>122.4</td>
<td>122.4</td>
<td>105.3</td>
</tr>
<tr>
<td>Water Control</td>
<td>73.6</td>
<td>95.1</td>
<td>70.1</td>
</tr>
<tr>
<td>0.2M CaCl₂</td>
<td>206.4</td>
<td>193.2</td>
<td>244.0</td>
</tr>
<tr>
<td>0.5M CaCl₂</td>
<td>246.7</td>
<td>159.0</td>
<td>420.5</td>
</tr>
<tr>
<td>0.2M EDTA</td>
<td>96.4</td>
<td>90.2</td>
<td>71.4</td>
</tr>
</tbody>
</table>
controls. Calcium, although chelated by the EDTA, is apparently not removed from the blueberries.

**Alcohol Insoluble Solids**

There were no significant differences in the AIS content of the treated blueberries at any temperature (Table 6, Appendix E).

**Pectin fractionation**

Figure 9 shows the three pectin fractions of blueberries processed for 3 minutes at 83°C on a fresh weight basis. The WSP content of the water treated blueberries was not significantly different than that of the fresh blueberries. All chemical treatments resulted in a significantly reduced WSP level relative to the fresh blueberries or the water control. However, they were not significantly different from each other. The CSP level in the 0.5M CaCl$_2$ treated berries was significantly greater than that of the water processed control. The CSP content of the other treatments were not significantly different from each other. All four treatments contained significantly less DASP than the fresh blueberries. The 0.2M CaCl$_2$ and 0.2M EDTA treated blueberries contained less DASP than the 0.5M CaCl$_2$ treatment. None of the treatments were significantly different than the water control.

Figure 10 shows the pectin fractionation of the 3 minute, 83°C treatment on an AIS basis. There was no significant difference between the WSP content of the
Figure 9. Pectin fractionation of blueberries processed at 83°C for 3 minutes expressed as a percent of fresh weight. The LSD$_{0.05}$ for the water soluble pectin, the chelator soluble pectin and the dilute alkali soluble pectin are 0.033, 0.015 and 0.015, respectively.
Figure 10. Pectin fractionation of blueberries processed at 83°C for 3 minutes expressed as a percent of alcohol insoluble solids. The LSD_{0.05} for the water soluble pectin, the chelator soluble pectin and the dilute alkali soluble pectin are 1.537, 0.508 and 0.782, respectively.
water processed and fresh controls and the 0.2M CaCl₂ treated fruit. The 0.5M calcium treated blueberries contained less WSP than the 0.2M CaCl₂ treated fruit but was not significantly different from the WSP content of the 0.2M EDTA treated blueberries. In the 0.5M CaCl₂ treated fruit there was a decrease in WSP and a corresponding increase in CSP. The 0.5M CaCl₂ treated blueberries contained a much greater amount of CSP than any other treatment or the controls. The 0.2M CaCl₂ treated blueberries contained more CSP than the water control but the content was not significantly different from the fresh blueberries, or the 0.2M EDTA treated fruit. There were no differences between the DASP content of any treatment and the processed control, but all processed blueberries contained significantly less DASP than the fresh blueberries. There was an apparent conversion of WSP to CSP by processing in 0.5M CaCl₂ at 83°C.

The results of processing for 3 minutes at 53°C as expressed on a percent of fresh weight basis are shown in Figure 11. All treatments contained less WSP than the fresh blueberries. The water control contained significantly less WSP than the 0.2M EDTA treated sample. There was no significant difference between WSP content of the water control and the calcium treated blueberries. The CSP content of the water control was much lower than all other treatments, including the fresh blueberries. The CSP content of the 0.2M CaCl₂ and EDTA treated fruit were not statistically different from the fresh blueberries while the water control and the 0.5M CaCl₂ treated blueberries were significantly lower. The fresh blueberries and water processed control were not significantly different
Figure 11. Pectin fractionation of blueberries processed at 53°C for 3 minutes expressed as a percent of fresh weight. The $LSD_{0.05}$ for the water soluble pectin, the chelator soluble pectin and the dilute alkali soluble pectin are 0.036, 0.021 and 0.026, respectively.
but contained greater amounts of DASP than the other treatments. The 0.2M EDTA and 0.5M CaCl₂ treated blueberries contained significantly different amounts in DASP, but neither were different than the 0.2M CaCl₂ treated fruit.

Figure 12 shows pectin fractionation of the 3 minute, 53°C treated blueberries on an AIS basis. The same trends as seen when the data is expressed on a fresh weight basis are evident for the AIS data with respect to both WSP and DASP contents. The CSP data differs slightly. The 0.2M CaCl₂ treated blueberries contained more CSP than the EDTA treated blueberries.

Figure 13 shows the pectin content of fruit held at 23°C for 18 hours as expressed on a fresh weight basis. The WSP content of the 0.5M CaCl₂ and EDTA treated fruit were not significantly different than that of fresh blueberries. There was no significant difference between the water control and 0.2M CaCl₂ treated blueberries but both contained less WSP than the fresh blueberries. EDTA was the only treatment to produce fruit with a greater amount of WSP than the processed control. The CSP content of the 0.2M CaCl₂ treated fruit was significantly less than that of the other treatments and the controls. The CSP content of the water control, 0.5M CaCl₂ and EDTA treatments were not significantly different. The EDTA treated blueberries contained significantly less CSP than the fresh blueberries. The fresh blueberries had a greater CSP content than the processed blueberries. There was no significant difference between the DASP content of the water processed control and the calcium treated blueberries. The EDTA treated blueberries had a greater DASP content than the 0.5M CaCl₂
Figure 12. Pectin fractionation of blueberries processed at 53°C for 3 minutes expressed as a percent of alcohol insoluble solids. The LSD$_{0.05}$ for the water soluble pectin, the chelator soluble pectin and the dilute alkali soluble pectin are 1.724, 1.124 and 1.174, respectively.
Figure 13. Pectin fractionation of blueberries processed at 23°C for 18 hours expressed as a percent of fresh weight. The LSD0.05 for the water soluble pectin, the chelator soluble pectin and the dilute alkali soluble pectin are 0.033, 0.021 and 0.015, respectively.
treatment and water control. The 0.5M calcium chloride treated blueberries were not different from the processed control but contained less DASP than the 0.2M CaCl₂ and EDTA treated blueberries.

Figure 14 shows the pectin content of blueberries processed at 23°C for 3 hours, expressed on an AIS basis. The EDTA treated blueberries are the only sample to contain greater amounts of WSP than the water control. No treatments were statistically different from the fresh blueberries with respect to WSP content. The CSP results were similar to those in the fresh weight determination (Figure 11). The EDTA treated blueberries were the only fruit not lower in DASP content than the fresh blueberries. The DASP content of the 0.2M CaCl₂ treated blueberries was not statistically different from the water processed control but was significantly greater than the 0.5M CaCl₂ treated blueberries. There is no indication of significant CSP production at the 23°C treatment conditions.

Despite an increased calcium content at all treatment conditions 0.5M CaCl₂ processing of blueberries at 83°C was the only treatment to show possible conversion of WSP to CSP. EDTA did not have a significant effect on the WSP or CSP contents. Firmness and berry integrity were positively affected by calcium at both 83°C for 3 minutes and 23°C for 18 hours.

Calcium may penetrate the cuticle first then the epicarp and hypocarp, forming calcium pectate in these areas first. This would serve to reinforce the epidermal cell walls, and subsequently limit diffusion through the skin. Although small relative to total fruit pectin content, CSP formation in the berry skin would
Figure 14. Pectin fractionation of blueberries processed at 23°C for 18 hours expressed as a percent of alcohol insoluble solids. The LSD$_{0.05}$ for the water soluble pectin, the chelator soluble pectin and the dilute alkali soluble pectin are 1.602, 0.965 and 0.604, respectively.
help prevent rupture, increasing the force needed to rupture the blueberries. In addition, the reinforced cell walls would form a barrier to pigment loss, increasing processed blueberry quality. As anthocyanins are primarily located in the berry epicarp and hypocarp (Sapers and Phillips, 1985) they may form large complexes with pectin and calcium, further reducing their diffusion through cell membranes (Van Teeling et al., 1971). However, without diffusion of calcium through the skin, calcium pectate cannot form in the fruit mesocarp. Consequently, the internal blueberry tissue could undergo thermal softening while the skin remains intact. Epicarp firming and integrity retention is a significant result of calcium processing which may be exploited for commercial use.
B. VACUUM INFILTRATION

Blueberry integrity

Table 3 shows the fraction of berries which split during the vacuum infiltration process. Fifty percent of the control fruit ruptured, but processing with 0.5M CaCl₂ reduced this to 23%. More damage, however, was incurred with processing in 0.2M CaCl₂ and EDTA than in the water control. Plates XIII through XVI in Appendix D are photographs of the blueberries following processing. Substantial breakage occurred, although the photographs do not show this effectively, without simultaneous pigment or fluid loss.

Firmness evaluation

There were no significant differences in the firmness of the fruit processed with vacuum infiltration or the fresh blueberries (Figure 15). Although blueberry integrity is adversely affected by vacuum infiltration, firmness is not. No heat damage occurred as in the atmospheric processing. Likewise, the exposure time of 50 minutes is much shorter than the 23°C, 18 hr treatment. Calcium and EDTA showed little effect at 53°C, because of short exposure time and the low temperature diffusion rate, show little effect on the firmness of the vacuum processed blueberries.
Table 3. Damage to blueberries following processing with vacuum infiltration. Data are reported as the percent split berries and are expressed as a mean of two determinations, with the standard deviation.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percent Breakage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water Control</td>
<td>50.5 ± 0.7</td>
</tr>
<tr>
<td>0.2M CaCl₂</td>
<td>60.0 ± 1.1</td>
</tr>
<tr>
<td>0.5M CaCl₂</td>
<td>23.5 ± 0.4</td>
</tr>
<tr>
<td>0.2M EDTA</td>
<td>71.0 ± 1.0</td>
</tr>
</tbody>
</table>
Figure 15. Blueberry firmness following processing with vacuum infiltration. Data are expressed as the mean of duplicate 20 berry determinations. The LSD$_{0.05}$ is 0.805.
**Total solids**

There were no statistical differences between samples (Table 7, Appendix E). This indicates insignificant fluid loss or uptake.

**Calcium Content**

Table 4 shows the calcium content of the blueberries processed by vacuum infiltration. These data are similar to the readings obtained by atmospheric processing (Table 2) but the variability is much lower, indicating more consistent levels. The calcium content of the fresh blueberries was similar to that of the water processed control and the 0.2M EDTA treated fruit. Both calcium treatments were significantly different, and both contained significantly greater amounts of calcium than the other blueberries. EDTA may enter the berries and chelate the calcium but is not removing that calcium from the fruit. The calcium-EDTA complex may be too large for significant diffusion through the cuticle.

**Alcohol insoluble solids**

No significant differences existed between any samples (Table 8, Appendix E). This suggests that no net large scale cell wall breakdown has occurred.

**Pectin Fractionation**

Figure 16 shows the pectin fractionation from the vacuum infiltration procedure expressed on a percent fresh weight basis. The 0.5M CaCl$_2$ treated
Table 4. Calcium content of blueberries processed by vacuum infiltration. Data are expressed in micrograms of calcium per gram of blueberry tissue. Data are the mean of triplicate 10g samples. The LSD$_{0.05}$ is 6.6.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Calcium Content ($\mu g/g$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh Berries</td>
<td>83.8</td>
</tr>
<tr>
<td>Water Control</td>
<td>86.4</td>
</tr>
<tr>
<td>0.2M CaCl$_2$</td>
<td>169.1</td>
</tr>
<tr>
<td>0.5M CaCl$_2$</td>
<td>200.6</td>
</tr>
<tr>
<td>0.2M EDTA</td>
<td>87.9</td>
</tr>
</tbody>
</table>
Figure 16. Pectin fractionation of blueberries processed at ambient temperature with vacuum infiltration techniques expressed as a percent of fresh weight. The LSD$_{0.05}$ for the water soluble pectin, the chelator soluble pectin and the dilute alkali soluble pectin are 0.037, 0.030 and 0.043, respectively.
fruit contained significantly less WSP than either the fresh blueberries or the 0.2M EDTA treated sample. There were no significant differences between these treatments relative to the processed control. No difference in the CSP content of any samples was found. The 0.5M CaCl₂ treated sample contained significantly greater amounts of DASP than the 0.2M CaCl₂ or EDTA treatments but was not different from the processed or fresh controls.

Figure 17 shows the pectin fractionation from the vacuum infiltration procedure expressed on a percent AIS basis. The CaCl₂ treated blueberries contained significantly less WSP than the controls. The 0.2M EDTA treated blueberries contained significantly more WSP than the calcium treatments or the controls. There were no statistical differences in the CSP content of any samples. The 0.2M CaCl₂ treated blueberries were the only fruit to contain significantly less DASP than the controls. The 0.5M CaCl₂ treated blueberries contained a higher level of DASP than the 0.2M CaCl₂ treated fruit. The chemical treatments have an apparent effect on WSP levels without a corresponding change in CSP content.

Berry integrity was decreased by vacuum processing but the firmness of the surviving fruit was unaffected. The change in pressure may have been too rapid and splitting is probably due to air rapidly expanding and leaving the tissues. Releasing the pressure more slowly may reduce fruit damage.

Blueberry splitting decreases fruit quality (Sapers and Phillips, 1985; Sanford et al., 1991). Therefore methods to maintain intact fruit during processing, even without increased firmness, would provide a marketing advantage.
Figure 17. Pectin fractionation of blueberries processed at ambient temperature with vacuum infiltration techniques expressed as a percent of alcohol insoluble solids. The LSD$_{0.05}$ for the water soluble pectin, the chelator soluble pectin and the dilute alkali soluble pectin are 0.406, 1.4194 and 1.746, respectively.
C. CONSIDERATIONS FOR FUTURE RESEARCH

The effect of calcium processing at atmospheric pressure was to strengthen the blueberry epicarp and reduce the incidents of skin rupture. This may occur by calcium pectate formation in the epidermal cell walls and middle lamellae. Localization of pectate formation in the skin would not significantly increase total calcium pectate content but it would form a barrier to further diffusion of calcium. Therefore, firmness effects due to calcium treatments are probably due to resistance of the berry to rupture, rather than internal tissue firming.

The difference between these findings and those obtained with strawberries (Main et al., 1986; Morris et al., 1986) is probably related to differences in fruit histology. However, even in strawberries, sliced fruit was more effectively firmed than whole berries (Main et al., 1986). Calcium must diffuse through the skin in whole berries in order to reach the mesocarp cells. Mesocarp cells are exposed upon slicing, which may allow increased diffusion, or the vascular system may carry the calcium to more cells.

Calcium may be increasing the resistance of blueberry skin to rupture by means other than calcium pectate formation. McFeeters and Fleming (1989) reported increased firmness in cucumbers without CSP formation. Cell wall proteins may bind calcium, increasing epicarp durability (Labavitch, 1981).

Calcium processing was useful in retaining berry integrity, rather than reducing softening. This is probably related to strengthening of the epicarp,
although the mechanism remains speculative. However, exposure to heat and chelation of calcium by EDTA are important in reducing blueberry skin resistance to mechanical breakage.
CHAPTER V

SUMMARY AND CONCLUSIONS

The objectives of this study were to 1) investigate a possible role of calcium in enhancing the firmness and integrity of mature blueberries during blanching; 2) observe changes in pectin composition as a result of calcium treatments during blanching and 3) investigate the relationship between pectin composition, firmness, and calcium treatment in blueberries.

The principal results drawn from this study are:

1) Blueberry integrity decreased with increasing temperature.
2) Blueberry splitting was decreased by calcium and increased by EDTA.
3) Calcium treatments were effective in increasing the calcium content of blueberries.
4) Blueberry firmness was enhanced with 0.2M calcium chloride treatment at 83°C for 3 minutes and both 0.2M and 0.5M CaCl₂ treatments at 23°C for 18 hours.
5) Blueberry total solids content and percent alcohol insoluble solids were independent of treatment.
6) There was significant conversion of water soluble pectin to chelator soluble pectin in the 0.5M CaCl₂ treated blueberries at 83°C for 3 minutes.
7) Vacuum infiltration processing caused an increased incidence of splitting without a corresponding increase in softening.

The principal conclusions drawn from this study are:

1) Calcium may be incorporated into the epicarp and hypocarp, making it more resilient.

2) The skin may be acting as a barrier to prevent calcium diffusion into the berry interior.

3) The reason for no significant increase in CSP following calcium treatment may be localization of CSP in the blueberry epidermis.

4) Blueberry firming following calcium treatment may be a result of epicarp toughening rather than interior firming.

5) Vacuum release may have been too rapid, allowing the expansion of entrapped air and rupture the blueberries.

6) Fruit integrity retention by calcium treatment (reduced incidence of splitting and pigment leakage) is of potential commercial value.
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Huber, D.J. 1983. The role of cell wall hydrolases in fruit softening. Hort. Rev. 5:169-219


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Appendix A

Photographs of Blueberries Processed Under
Atmospheric Pressure at 83°C for 3 Minutes
Plate I. Water Control.
Plate II. 0.2M Calcium Chloride Treatment.
Plate III. 0.5M Calcium Chloride Treatment.
Plate IV. 0.2M EDTA Treatment.
APPENDIX B

Photographs of Blueberries Processed Under

Atmospheric Pressure at 53°C for 3 minutes
Plate V. Water Control

53° Bleach, 3 Minutes
Distilled Water Control
Plate VI. 0.2M Calcium Chloride Treatment.
Plate VII. 0.5M Calcium Chloride Treatment.
Plate VIII. 0.2M EDTA Treatment.
APPENDIX C

Photographs of Blueberries Processed Under

Atmospheric Pressure at 23°C for 18 Hours
Plate IX. Water Control.
Plate X. 0.2M Calcium Chloride Treatment.
Plate XI. 0.5M Calcium Chloride Treatment.
Plate XII. 0.2M EDTA Treatment.
APPENDIX D.

Photographs of Blueberries Processed With Vacuum Infiltration
Plate XIII. Water Control.
Plate XIV. 0.2M Calcium Chloride Treatment.
Plate XV. 0.5M Calcium Chloride Treatment.
Plate XVI. 0.2M EDTA Treatment.
APPENDIX E

Tables not Included In Thesis Text
Table 5. Total solids (%) of blueberries processed under atmospheric pressure. Data are expressed as the mean of triplicate 10g samples dried at 80°C for 4 hours. The LSD$_{0.05}$ for the 83°C, 53°C and 23°C treatments are 5.27, 4.98 and 5.42, respectively.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>83°C 3 min.</th>
<th>53°C 3 min.</th>
<th>23°C 18 hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh Berries</td>
<td>14.8</td>
<td>14.8</td>
<td>16.4</td>
</tr>
<tr>
<td>Water Control</td>
<td>13.5</td>
<td>12.9</td>
<td>12.9</td>
</tr>
<tr>
<td>0.2M CaCl$_2$</td>
<td>13.7</td>
<td>14.4</td>
<td>15.9</td>
</tr>
<tr>
<td>0.5M CaCl$_2$</td>
<td>13.8</td>
<td>13.4</td>
<td>16.0</td>
</tr>
<tr>
<td>0.2M EDTA</td>
<td>14.5</td>
<td>13.7</td>
<td>12.9</td>
</tr>
</tbody>
</table>
Table 6. Alcohol insoluble solids of blueberries processed under atmospheric pressure. Data are expressed as means of duplicate 50g samples. The LSD_{0.05} for the 83°C, 53°C and 23°C treatments are 0.98, 0.98 and 0.06, respectively.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>83°C 3 min.</th>
<th>53°C 3 min.</th>
<th>23°C 18 hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh Berries</td>
<td>2.00</td>
<td>2.00</td>
<td>1.95</td>
</tr>
<tr>
<td>Water Control</td>
<td>2.05</td>
<td>1.99</td>
<td>1.97</td>
</tr>
<tr>
<td>0.2M CaCl₂</td>
<td>1.94</td>
<td>2.01</td>
<td>1.91</td>
</tr>
<tr>
<td>0.5M CaCl₂</td>
<td>2.00</td>
<td>1.99</td>
<td>1.96</td>
</tr>
<tr>
<td>0.2M EDTA</td>
<td>2.06</td>
<td>1.96</td>
<td>1.95</td>
</tr>
</tbody>
</table>
Table 7. Total solids of blueberries processed with vacuum infiltration. Data are reported as the mean of triplicate 10 gram samples. The LSD$_{0.05}$ is 6.88.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percent Total Solids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh Berries</td>
<td>12.5</td>
</tr>
<tr>
<td>Water Control</td>
<td>12.6</td>
</tr>
<tr>
<td>0.2M CaCl$_2$</td>
<td>14.5</td>
</tr>
<tr>
<td>0.5M CaCl$_2$</td>
<td>15.0</td>
</tr>
<tr>
<td>0.2M EDTA</td>
<td>13.6</td>
</tr>
</tbody>
</table>
Table 8. Alcohol insoluble solids of blueberries processed with vacuum infiltration. Data reported are the mean percentage of two 50g blueberry samples. The LSD$_{0.05}$ is 0.37.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percent AIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh Berries</td>
<td>2.2</td>
</tr>
<tr>
<td>Water Control</td>
<td>2.3</td>
</tr>
<tr>
<td>0.2M CaCl$_2$</td>
<td>2.3</td>
</tr>
<tr>
<td>0.5M CaCl$_2$</td>
<td>2.1</td>
</tr>
<tr>
<td>0.2M EDTA</td>
<td>2.3</td>
</tr>
</tbody>
</table>
APPENDIX F

Glossary of Abbreviations and Terms
AIS. Alcohol insoluble solids.

CSP. Chelator soluble pectin.

DASP. Dilute alkali soluble pectin.

DE. Degree of methylesterification of pectin.

DP. Degree of polymerization of pectin.

Dyne. The force needed to move one gram one centimeter in one second squared.

EDTA. Disodium ethylenediaminetetraacetic acid.

PG. Polygalacturonase.

PME. Pectinmethyltransferase.

SHMP. Sodium Hexametaphosphate.

WSP. Water soluble pectin.