Pectin Methylation Changes and Calcium Ion Effects on the Texture of Fresh, Fermented, and Acidified Cucumbers

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The commercial use of calcium ion in cucumber pickle products has stimulated efforts to better understand the mechanisms by which calcium affects cucumber texture. Recent results suggest that a high level of pectin methylation has little effect upon the ability of calcium to maintain the firmness of acidified cucumber tissue, while in fermented cucumbers maintenance of a minimum degree of methylation may be important to firmness retention. Efforts are being made to measure calcium binding characteristics in cucumber tissue and to obtain estimates of the effectiveness of calcium ion in inhibiting tissue degradation by pectolytic enzymes.

The ability of calcium ions to act as a firming agent in processed fruits and vegetables has been the subject of many studies over the years (1). Among the commodities in which calcium has been observed to cause firming are: snap beans (2), tomatoes (3), apples (4), carrots (5), apricots (6), and jalapeno peppers (7). Cucumbers are commercially preserved by fermentation (processed pickles), acidification and pasteurization (fresh-pack pickles) and refrigeration of mildly acidified fruit (refrigerated dills). Calcium ion has been found to be effective as a firming agent in all three types of products. It is now being used in most commercial cucumber pickle products. Investigations of the structure of cucumber cell walls and the interaction of calcium with the cell walls have followed the practical application of calcium.

There have been major advances in our understanding of the structure of plant cell walls over the past 15 years (8). However, it has not proven to be an easy task to explain specific textural changes which occur during ripening or processing of fruits and vegetables in terms of changes in the structures of cell wall polymers (9). This is perhaps not surprising since the detailed structures of cell wall polymers are proving to be very complex

(8). There also appears to be great variability in the distribution of polysaccharides in different fruits and vegetables (10).

Recently, efforts have been directed toward trying to explain and ultimately control the textural changes that occur in cucumbers during processing and storage. Cucumbers have not been an exception in that it is difficult to see clear relationships between structural changes in the cell wall and texture effects in the tissue. Despite the difficulties which are encountered, the cucumber has several characteristics which make it a good model to investigate texture/cell wall structural relationships. The cucumber mesocarp is a large proportion of the total fruit tissue, and it can be isolated in quantity without great difficulty. The mesocarp tissue is relatively uniform in structure and contains little starch to interfere with the analysis of cell wall polysaccharides (11). A simple texture test has been devised which can be used with small pieces of cucumber tissue and which relates well with human perception of firmness (12). Finally, cucumbers can be obtained for experimental purposes throughout most of the year in a wide range of sizes.

This paper will give a brief background on textural investigations of cucumbers and cucumber products. Recent work on the structure of cucumber cell walls and initial efforts to determine texture/structure relationships will then be reviewed.

Cucumbers can be softened enzymatically by fungal polygalacturonases. They also contain natural pectolytic enzymes, though the conditions in which these enzymes contribute to fruit softening have not been determined. Finally, softening occurs slowly during storage of both pasteurized and fermented cucumbers, where polygalacturonases have been inactivated to nondetectable levels. The mechanism(s) of this post-processing softening has not been determined.

Bell and coworkers in the 1950's investigated the softening of cucumbers in commercial fermentations (13, 14, 15, 16). They found that softening of small size cucumbers was caused primarily by the presence of polygalacturonases in the fermentation brines, which degraded pectic substances in the fruit. These softening enzymes were primarily of fungal origin and were present on the cucumber fruits and flowers when they were put into fermentation tanks. Lampi et al. (17) attempted to measure changes in the pectic substances of cucumbers during fermentation, but were unable to show any consistent pattern of changes with the techniques available to them.

The use of calcium as a firming agent for cucumber products began in the 1960's with commercial trials conducted over several years. It was found that addition of 0.1% $CaCl_2$ to pasteurized cucumber products resulted in a significant improvement in the retention of a firm texture during storage (18). This led to commercial use of $CaCl_2$ in these pickle products. Fleming et al. (19) found that 0.1% $CaCl_2$ also helped prevent firmness losses in fermented cucumber slices and in small whole cucumbers at low NaCl concentrations. Particularly important to the use of calcium in commercial cucumber fermentations was the finding by Buescher and coworkers (20, 21) that at $CaCl_2$ concentrations up to 1%, whole cucumbers remained firm even when fungal polygalacturonases were intentionally added to the fermenting cucumbers (Table 1).

Table 1. Cucumber Firmness 30 Days After the Beginning of Fermentation as Influenced by NaCl, $CaCl_2$ and Polygalacturonase. From Buescher et al. (20)

Treatment	Pickle Firmness (kg)[a]
2% NaCl	
Control	7.9C
0.1 M $CaCl_2$	9.6AB
Polygalacturonase	2.3E
0.1 M $CaCl_2$ + Polygalacturonase	8.9BC
5% NaCl	
Control	8.0C
0.1 M $CaCl_2$	10.0A
Polygalacturonase	5.8D
0.1 M $CaCl_2$ + Polygalacturonase	10.0A

[a]Mean firmness of 18 pickles. Values with the same letters are not significantly different.

High calcium concentrations were also found to prevent breakdown of the locular tissue of large cucumbers during fermentation (22), presumably by preventing the degradation of pectin by cucumber polygalacturonase (23).

These observations have led to efforts to develop a better understanding of the structure of cucumber cell walls and the role that calcium ions play in improving texture. The cucumber cell wall contains about 30% cellulose (10), 15% pectin (24), and noncellulosic neutral sugars (10, 25, 26). Other than glucose, which is present primarily in cellulose, galactose and xylose are the most abundant neutral sugars in the cell wall. The degree of pectin methylation in cucumbers has been reported to be 65% in a Japanese fresh market cultivar (27) and 57% in 4 cm diameter pickling cucumbers (28). Preliminary evidence from this laboratory indicates that pectin methylation may increase during development of the cucumber fruit.

Demethylation of pectin has been the most obvious change observed in cell wall structure, both in the chilling injury of fresh cucumbers (27, 29) and during fermentation (25). Bell et al. (30) showed that cucumber plants contain pectinesterase in all parts of the plant, including the fruit. As part of a series of studies of chilling injury in cucumber fruits, Fukushima and Yamazaki (29) found that a decrease in hot, water-soluble, high methylation pectin and an increase in hot, water-insoluble, low methylation pectin occurred when fruit were stored at 0 or 5°C. They suggested that demethylation of the pectin by pectinmethylesterase resulted in a more rigid cell wall structure, and that this deesterification may be a common characteristic of chilling-sensitive plants (29).

Tang and McFeeters (25) investigated changes in the cell walls of cucumber mesocarp tissue when cucumbers were fermented in 6% NaCl, a procedure similar to commercial fermentations. Figure 1 shows that only small changes in the noncellulosic neutral sugars occurred during the experiment. The total pectic substances showed little change during fermentation and storage. The size of the pectin molecules in the major pectin fractions also showed almost no change. The average degree of polymerization of the major pectin fraction (acid-soluble pectin) isolated from fresh cucumbers was estimated to be 402 residues. After fermentation, the degree of polymerization of the EDTA-soluble fraction, which was the major pectin fraction after fermentation, was 403 after 3 months and declined slightly to 365 after 6 months. The major change observed was a large decrease in the acid-soluble pectin fraction during the fermentation period and an increase in the EDTA-soluble pectin during the same period (Figure 2). The acid-soluble pectin had a degree of esterification of 62%, while the EDTA-soluble material had a degree of esterification too low to measure. These results indicated that pectin deesterification was the major change to occur in the cell wall during fermentation. There were no substantial changes in the cell wall composition from 1 to 6 months after brining, even though there was nearly a 30% decline in tissue firmness when $CaCl_2$ was not added to the fruit. Thus, a situation was observed in which a substantial change in texture occurred in cucumber fruit without an obvious change in the structure of the cell wall.

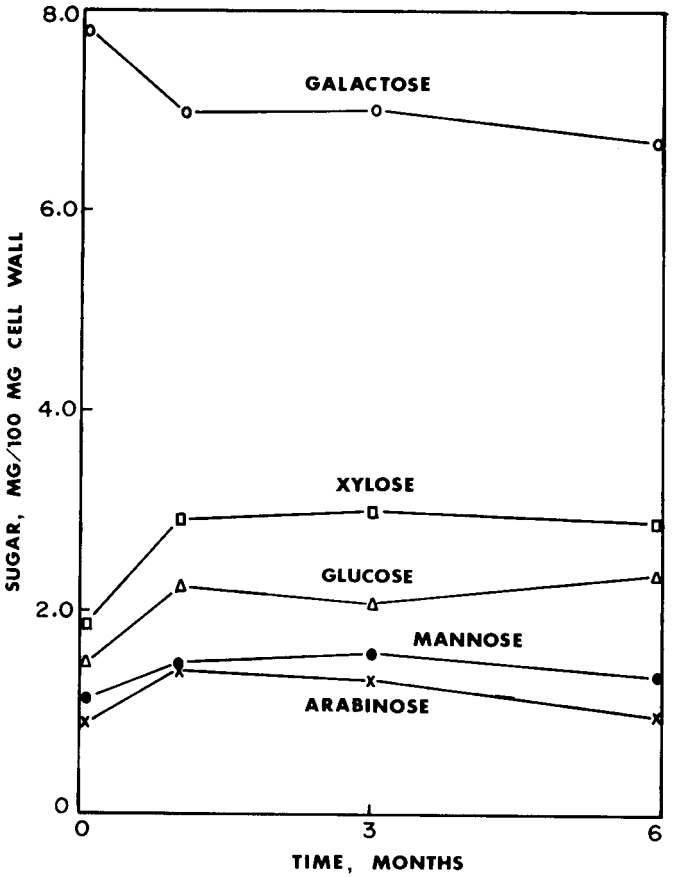

Figure 1. Changes in cell wall neutral sugar content during brining and storage of cucumbers.

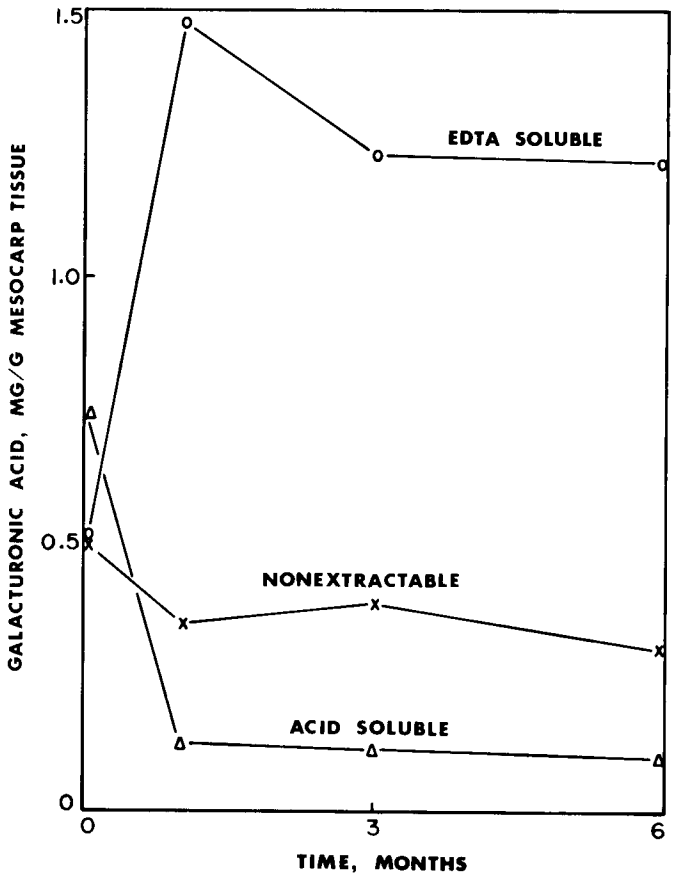

Figure 2. Changes in pectin fractions during fermentation and storage of cucumbers.

McFeeters et al. (28) recently investigated the effects of pectin methylation in nonfermented cucumber tissue when the degree of methylation was varied between 12 and 50%. Hudson and Buescher (31) have studied the effect of methylation on texture when the degree of esterification was in the range of 6 to 18%. McFeeters et al. (28) were able to vary the degree of methylation by blanching cucumber slices at different temperatures. Heating the slices at 66°C or less caused little or no inactivation of pectinesterase present in the tissue (30). During storage in pH 3.7, 2.0% NaCl brine, the pectin was extensively demethylated in these tissues (Figure 3). When slices were heated at 81°C, pectinesterase was inactivated. However, during storage, partial reactivation of the enzyme occurred. The result was an intermediate level of methylation. Finally, if cucumber tissue was heated in boiling water, only slight reactivation of pectinesterase activity occurred and the degree of methylation remained near 50%.

The effect of changing the degree of pectin methylation on the firmness of cucumber mesocarp tissue was determined over a 6-month storage period. When the slices were stored after blanching in a brine which contained 10 mM calcium ion, there were some texture differences, but the differences observed were not very large. The firmest texture was obtained when the tissue was blanched at intermediate temperatures, i.e. 66 and 81°C. Slices blanched at 99°C were less firm than the other treatments. However, much of the observed difference could be attributed to differences in the tissue firmness immediately after blanching, before the tissue was exposed to brine solutions. The rates of firmness loss during the 6-month storage period were similar, regardless of the degree of pectin methylation.

When the calcium concentration was varied in slices blanched at 54, 66, and 81°C, there was a very clear increase in firmness retention as the calcium concentration increased (Figure 4). However, the pattern of firmness changes was the same, regardless of blanch temperature. Thus, the results of these studies did not show any direct relationship between pectin methylation and firmness changes. Calcium ion was effective in preventing firmness loss during storage, regardless of the degree of pectin methylation. Studies of calcium ion binding by polypectate have shown that blocks of at least 14 consecutive demethylated carboxyl groups on adjacent polygalacturonan molecules are required for cooperative cross-linking to form an "eggbox" type structure (32, 33). The fact that calcium is effective in preventing softening, even at high degrees of pectin methylation, suggests that other types of polysaccharide/calcium interactions may be involved in the cucumber tissue. Calcium ion has been shown to form crystallizable coordination complexes with many mono- and disaccharides (34, 35). Cook and Bugg (35) have speculated upon the possible importance of calcium/galactose interactions in bone tissue. It may be useful to consider whether such interactions occur in plant cell walls.

Hudson and Buescher (31) have found a relationship between cucumber tissue firmness and the degree of methylation of the mesocarp tissue. When the pectin methylation was less than 13%, the firmness of the tissue declined as the degree of methylation

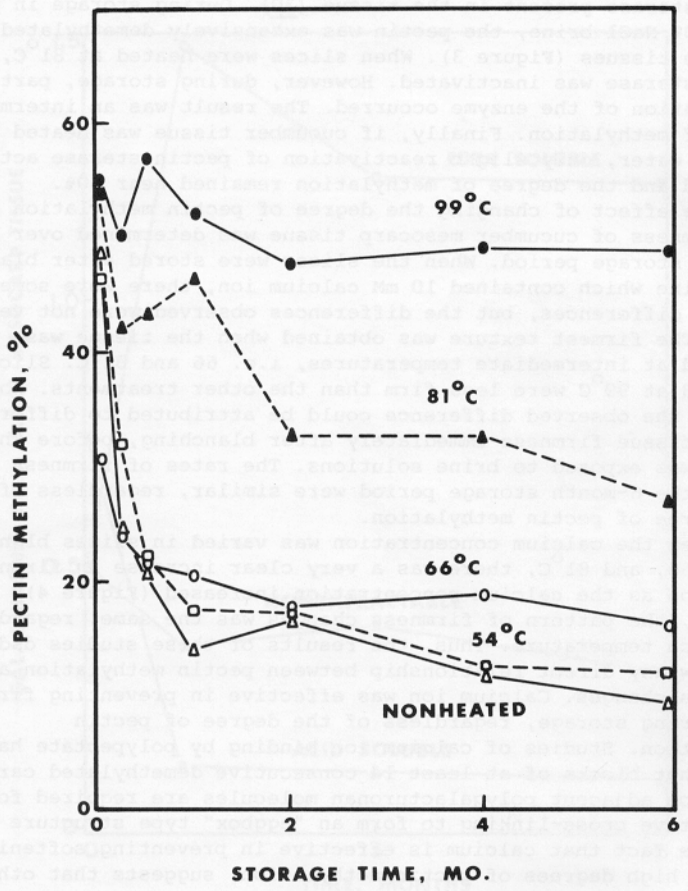

Figure 3. Effect of blanch temperature on changes in pectin methylation of the cucumber cell wall during storage.

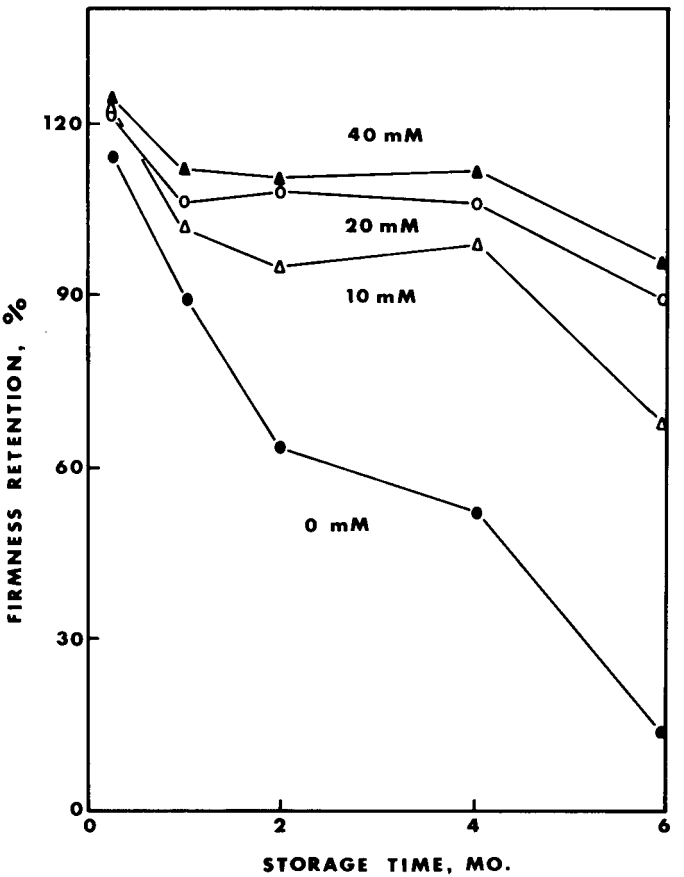

Figure 4. Effect of $CaCl_2$ concentration on the firmness retention of cucumber slices blanched at 81°C for 3 min.

decreased. They suggest that this may be caused by a change in the conformation of the pectin molecules at very low esterification similar to that reported by Leeper and Dull (36) for pectin solutions. However, because it was necessary to compare treatments with and without calcium ions present, the texture differences observed may have been caused by factors other than differences in the degree of methylation.

Since we are interested in trying to better understand the interaction of metal ions with the cucumber cell wall, it was of interest to develop a technique to determine the extent and affinity of ion binding in cucumber tissue. Since it is generally thought that calcium is bound to plant tissues by interacting with the free carboxyl groups present in pectin, an effort has been made to relate calcium bound in cucumber mesocarp tissue to the concentration of the free carboxyl groups of pectin.

Pieces of mesocarp tissue were isolated from cucumbers and placed in a brine to give an equilibrated concentration of 0.6% acetic acid, 200 ppm SO_2 (for preservation), and varying concentrations of calcium ion from 1 to 16 mM. After equilibration, the degree of pectin methylation in the mesocarp tissue was measured (24) and the concentration of free carboxyl groups in the tissue calculated. The concentration of calcium bound by the tissue was calculated as the difference in calcium concentration between the mesocarp tissue and brine solution as determined by a colorimetric procedure (37, 38). These data were used to construct a Scatchard plot to analyze both the moles of calcium bound per carboxyl group in the tissue and the affinity of calcium binding.

Figure 5 shows an example of the analysis of such a binding experiment. The intercept on the X-axis gave a ratio of 0.43 calcium ions bound per free carboxyl group in the tissue. If each divalent calcium ion were binding to two pectin carboxyl groups, a ratio of 0.5 would be expected. Analysis of the slope of the Scatchard curve, gave an affinity constant of 888 for calcium binding. Kohn (32) determined calcium binding constants as a function of the degree of pectin esterification for pectin solutions at neutral pH. Stability constants varied from <100 for highly methylated pectin to nearly 10,000 with very low degrees of methylation. The degree of esterification expected for pectin with a binding constant of 888 is 38% based upon his data. Table 2 shows that the esterification of the mesocarp samples varied with calcium concentration, but the predicted degree of esterification was within the observed range. These results suggest that this may be a useful approach to the analysis of ion binding in intact tissues under conditions similar to those found in fermented and acidified vegetable products. Additional data need to be obtained to determine whether a detailed analysis of binding of calcium and other ions in cucumber mesocarp tissue can help provide an understanding of the textural changes that occur in processed cucumbers.

The development of commercial applications of calcium addition to improve the textural qualities of fermented and acidified cucumber products has stimulated efforts to understand the mechanisms by which this ion affects the texture of cucumber

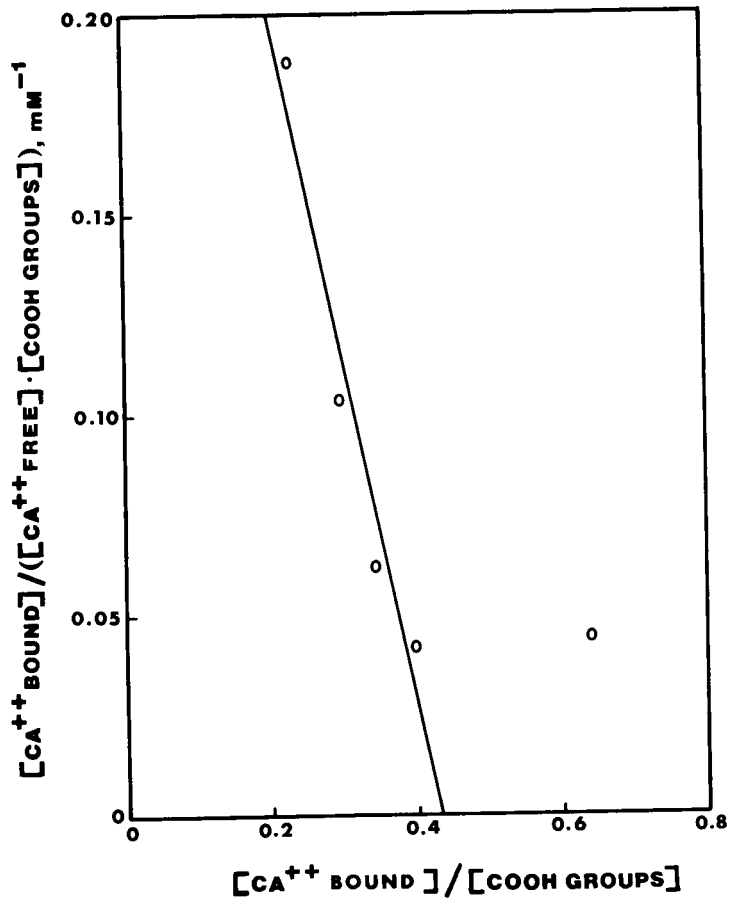

Figure 5. Scatchard plot of calcium binding to the free carboxyl groups of pectin in acidified cucumber mesocarp tissue.

Table 2. Degree of Pectin Methylation in Cucumber Mesocarp Tissue After Equilibration With Calcium Ions

Added $[Ca^{++}]$ (mM)	Degree of Pectin Methylation (%)
0	42.3
1.8	33.1
4.6	27.4
8.8	27.5
16.1	25.4

tissue. Procedures have been developed to measure the firmness of mesocarp tissue (12) and to measure pectin methylation in small samples of cell walls (24). Methods to quantitatively analyze ion binding by cucumber tissue are being developed. Techniques to analyze the neutral polysaccharides to plant cell walls are being used to determine changes in the neutral sugars of the wall during processing procedures (39). A number of interesting observations have been made concerning the effects of calcium ion and pectin methylation on the texture of cucumber tissue, but the structural basis for the textural effects remain to be explained.

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