Pectinesterase Activity, Pectin Methylation, and Texture Changes During Storage of Blanched Cucumber Slices

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ABSTRACT

Pectin methylation in blanched cucumber slices after 6 months' storage in acid brine (pH 3.7) ranged from 9% (no blanch) to 48% (99°C, 3 min blanch). An 81°C blanch caused complete pectinesterase inactivation, but 15 - 20% reactivation occurred during storage. After a 99°C blanch, only slight reactivation was observed. Pectinesterase was not inactivated at 66°C or less, but up to 85% of the activity was lost during storage. Firmness changes were complex. A clear relationship between pectin methylation and firmness changes was not observed. A 66 or 81°C blanch resulted in best firmness retention. Calcium ion was very effective in prevention of firmness loss regardless of the extent of pectin methylation.

INTRODUCTION

EXTENSIVE DEMETHYLATION of the pectin in cucumbers fermented and stored in 6% NaCl brine was the major change in cell wall components observed by Tang and McFeeters (1983). When brined and fermented, without added calcium ion, cucumber tissue increased in firmness during the period when pectin demethylation occurred. This initial increased firmness was presumed, at least in part, to be a result of formation of a stronger polypectate gel after demethylation.

Since demethylation was associated with substantial texture changes in fermented cucumbers, it was of interest to investigate whether demethylation could occur in heated cucumber tissue. Pectin is demethylated very slowly by acid hydrolysis at pH 3 - 4 (Doesburg, 1965), which occurs in fresh-pack cucumbers. Therefore, pectinesterase, which Bell et al. (1951) found in cucumbers, would be expected to catalyze any significant hydrolysis of the pectin methyl groups.

Plant pectinesterases, including the enzyme in cucumber tissue (Bell et al., 1951), are generally rather heat stable, so it might be possible for active enzyme to survive heat processing and catalyze demethylation during storage. The effect of any changes in pectin methylation on texture of cucumber tissue. Pectin is demethylated very slowly by acid hydrolysis at pH 3 - 4 (Doesburg, 1965), which occurs in fruits and vegetables may result from the action of pectinesterase (Van Buren, 1979). However, in juice products, prevention of enzymatic pectin demethylation can be an important goal of processing (Versteeg et al., 1980).

The objective of this investigation was to evaluate the pattern of changes in pectinesterase activity, pectin methylation, and tissue firmness, which would occur when cucumber tissue was given controlled heat treatments and stored in brine which contained salt and acid at concentrations similar to that used in some types of commercially pasteurized cucumber products, such as kosher dill strips. The relationships among these factors may indicate directions for future efforts to maintain or improve textural characteristics in processed cucumber products.

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MATERIALS & METHODS

THE CUCUMBERS used in these experiments were 'Calypso' cultivar from a local commercial grower. The fruit used to investigate the effect of blanch temperature were held at room temperature and used the day after picking. Fruit for studies of the effect of calcium concentration were held at 10°C for 1 night.

Two major experiments were conducted. First, the effect of blanch temperature on pectinesterase activity, pectin methylation, and tissue firmness was investigated. Unheated cucumber slices and slices blanched for 3 min at 54, 66, 81, and 99°C were packed in brine to equilibrate at 0.6% acetic acid, 2.5% NaCl, 10 mM $CaCl_2$, and 200 ppm SO_2. Samples were analyzed immediately after blanching and at 24 hr, 2 wk, 1 mo, 2 mo, 4 mo, and 6 mo after brining.

For the second experiment, the effect of $CaCl_2$ concentration on tissue firmness was determined by blanching slices at 54, 66 and 81°C for 3 min. The blanched slices were put in brines to equilibrate at 0.6% acetic acid, 2.5% NaCl, 200 ppm SO_2, and 0, 10, 20, and 40 mM $CaCl_2$. Pectinesterase activity and tissue firmness were measured 1 wk, 1 mo, 2 mo, 4 mo, and 6 mo after brining. Pectin methylation was measured only at the 2- and 6-month sampling times.

To obtain uniform heating of cucumber tissue in a form suitable for subsequent firmness measurements, 0.63 cm (1/4 inch) slices from 3.8 - 4.4 cm diameter fruit were prepared on an accurately calibrated hand slicer. The end pieces from each fruit were discarded. The slices were laid out in a single layer on a coarse wire mesh screen. The layer of slices was covered with a second screen to prevent movement of the slices during heating. Each layer of slices was separated by 2 cm (3/4-inch) spacers to allow circulation of water on both sides of the slices. Approximately 1500g batches of slices were blanched in a 60-L bath of tap water for exactly 3 min. They were constantly agitated during blanching. After heating, the slices were cooled by placing them in an ice water bath for 1 min. Slices were packed 141 ± 1g into 8-oz jars and covered with 94 ± 1g of brine. To preserve the slices without pasteurization of the jars, sodium metabisulfite was added to the cover brine to give an equilibrated concentration of 200 ppm SO_2. The jars were incubated at 27°C until analysis. In all experiments duplicate jars were analyzed for each treatment at each sampling time.

Analysis

Pectinesterase. Enzyme was extracted from the tissue with 0.6% acetic acid, 2.5% NaCl, and 0.05M $CaCl_2$. Previous experiments on the effect of acetic acid, NaCl, and $CaCl_2$ on extraction of pectinesterase activity had shown that this solution gave maximum extraction of activity from fresh cucumber tissue. Since the acetic acid and salt concentrations for maximum enzyme extraction were the same as was used in the experimental jars, 25g of brine, 25g of cucumber slices, and 0 - 1.25 mL of 2.0M $CaCl_2$, to give a 0.05M final concentration of $CaCl_2$ in the solution, were blended with a Tekmar homogenizer.

Five milliliters of the blended slurry were added to 20 mL of a 0.5% solution of rapid-set citrus pectin (Sunkist Growers, Corona, CA) containing 0.15M NaCl. The pH was adjusted to 7.0 with 0.1M NaOH. The pH was maintained at 7.0 by addition of 0.005M NaOH. Measurement of pH was done with an Orion model 901 pH meter equipped with an Orion Ross electrode. A unit of enzyme activity was defined as that amount of enzyme which would require 1.0 μmole of NaOH/min to maintain the pH of the assay mixture at 7.0.

Tissue firmness. Firmness measurements were made using the procedure of Thompson et al. (1982). A single punch with a 0.315 cm diameter, flat-tipped plunger was made in the center of one mesocarp section on each of 15 cucumber slices with the Instron

UTM. A 2-kg compression force transducer was used. The crosshead speed and chart drive speed were 200 and 500 mm/min, respectively. The maximum penetration force was recorded. For firmness measurements on slices after blanching, the slices were held in covered beakers at room temperature until the punch test could be performed. This was 3 hr or less after blanching.

Pectin methylation. Cucumber slices were blended with a Tekmar homogenizer. Fifty grams of 95% ethanol were blended with 10.0g of cucumber slurry. Alcohol insoluble solids were collected on a Whatman no. 1 filter paper, washed with 25g 95% ethanol, then 25g acetone, dried in a vacuum oven at 40°C, and weighed. The degree of pectin methylation in the dried cell walls was measured by the procedure of McFeeters and Armstrong (1984).

RESULTS & DISCUSSION

TABLE 1 SHOWS the initial pectinesterase activity, degree of pectin methylation, and firmness of nonheated cucumber slices and slices blanched for 3 min at four temperatures. Pectinesterase activity did not decrease when slices were heated at 54 or 66°C, but activity was lost at 81 and 99°C. This heat inactivation pattern is similar to that reported by Bell et al. (1951) for pectinesterase extracted from cucumbers. Pectin methylation after blanching was similar for all treatments except for a higher methylation in the 99°C samples, assuming an analytical error similar to that observed for cell walls from unheated cucumbers (McFeeters and Armstrong, 1984). Cucumber slices showed a trend toward increasing firmness as the blanch temperature was increased, except for the 99°C-blanched slices.

Fig. 1 shows the changes in pectinesterase activity during a 6-month storage period after slices were blanched at different temperatures. When pectinesterase was not denatured by blanching, a rapid loss in activity was observed during the first 2 wk of storage in acid conditions (pH 3.7). From that point, only small changes in enzyme activity occurred. At 81°C (Fig. 1B), pectinesterase activity was not detected immediately after blanching. However, a gradual reactivation was observed such that after 1 month the activity was about 20% of that in unheated cucumbers. There have not been previous examples of pectinesterase reactivation. This pattern of partial enzyme inactivation during storage after low temperature blanching and partial reactivation after the 81°C blanch treatment suggests either that multiple pectinesterases with different stability characteristics or a single pectinesterase with multiple binding sites is present in the cucumber tissue. Multiple forms of pectinesterase appear to be rather common in plants. Chromatographically different enzymes have been separated from tomatoes (Delincee, 1976), banana fruit (Brady, 1976), carrots (Markovic, 1978), and oranges (Versteeg et al., 1978). Versteeg et al. (1980) have observed large differences in the thermal stability of pectinesterases from oranges. Pectinesterase from cucumber fruit must be isolated and purified to determine whether multiple forms are present.

Fig. 2 shows that reactivation of pectinesterase occurred in another experiment in which slices were blanched at 81°C and stored in brines with 0 - 40 mM $CaCl_2$. It also shows no apparent effect of calcium concentration on renaturation of pectinesterase. The pattern of pectinesterase changes were also the same at all $CaCl_2$ concentrations after slices were blanched at 54 and 66°C (data not shown). Thus, calcium ion had no effect on pectinesterase stability regardless of blanch treatment.

The possibility that SO_2, used as a preservative for the cucumber slices, may have had a specific effect on pectinesterase changes was checked by packing cucumbers in the same brine as in the experiment shown in Fig. 1, except that in one set of jars 0.2% benzoate was substituted for metabisulfite. Over a 2-wk period there was no apparent difference in pectinesterase activities between the samples with different inhibitors. Harris and Dennis (1979) did not find a specific effect of SO_2 on the stability of fungal polygalacturonases in sulfited strawberries.

Pectin methylation changes from 24 hr after brining blanched slices through 6 months are shown in Fig. 3 for each blanch treatment. At the end of 6 months' storage, the degree of pectin methylation varied from 9% to 48% as the blanch temperature of the cucumber slices was increased. The general course of changes to the final methylation pattern appeared to be influenced, as would be expected, by the pectinesterase activity in the slices. When pectinesterase activity was present after blanching (Table 1), rapid demethylation occurred during the first few weeks of storage. The initial decrease in methylation was particularly rapid in the 66°C blanched slices. After 24 hr in brine, the pectin was only slightly more than 30% methylated,

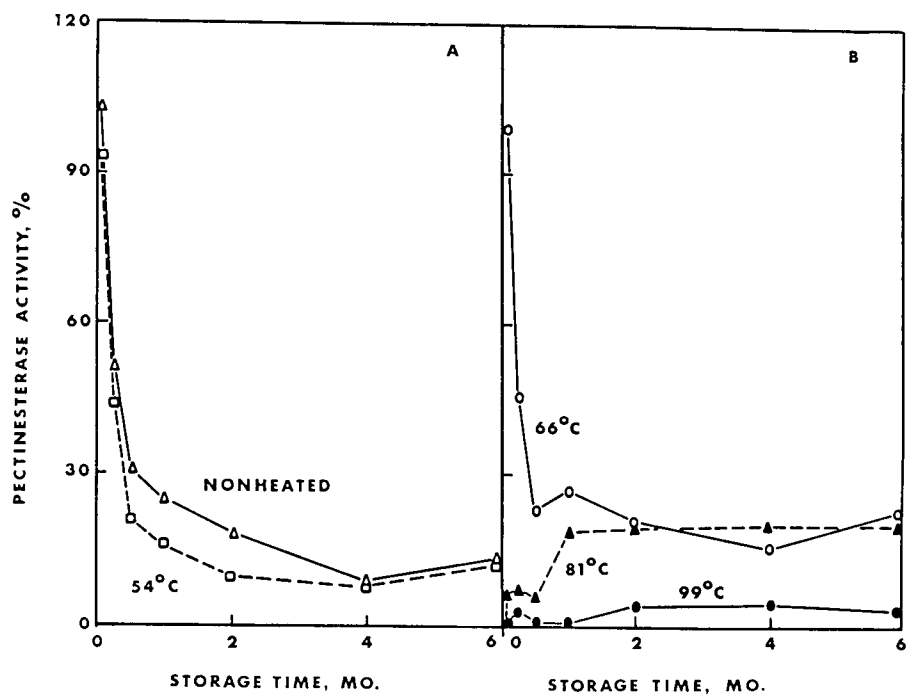

Fig. 1—Changes in pectinesterase activity during storage of cucumber slices blanched at different temperatures for 3 min. Pectinesterase activity was expressed as a percentage of the activity of fresh cucumber slices (0.64 units/g). Coefficients of variation were 5.6% for the nonheated slices, 17.8% for the 54°C blanch, 7.9% for the 66°C blanch, 12.0% for the 81°C blanch, and 62% for the 99°C blanch.

compared to over 45% methylation in the unblanched and 54°C blanched treatments.

Since the pectinesterase activity after the 66°C blanch was similar to that in the nonblanched and 54°C treatments, the lower methylation after only 24 hr in brine suggested some influence on the pattern of changes by undefined factors in the slices. One possibility is that the blanch treatments may have resulted in variable accessibility of methyl groups in the cell wall matrix to pectinesterase. After the first month, a very slow decrease in methylation occurred. There was not a direct relationship at the lower temperature blanch treatments between the final level of pectin methylation, and the pectinesterase activity present during the 1- to 6-month period in that higher methylation was retained after the 66°C blanch even though these samples also maintained slightly higher pectinesterase activity than the unblanched and 54°C blanched samples.

Much higher levels of methylation were maintained when pectinesterase was inactivated by blanching at 81 and 99°C. The 81°C blanched samples showed an initial loss in methylation during the first week even though only 7% of the fresh pectinesterase activity had renatured. During the 1- to 6-month period when about 20% of the initial pectinesterase activity had reactivated, a decrease in methylation from 46% to 27% was observed. At 99°C, where little reactivation of pectinesterase occurred, only a small decline in methylation occurred during the 6-month storage. The relationship between blanch treatment and methylation changes in the cell wall pectin indicates that for experimental purposes the methylation can be varied by blanch treatments. It would be of interest to determine whether substantial pectin demethylation occurs in commercially pasteurized cucumber products since the recommended final internal temperature of 74°C (Monroe et al., 1969) is in a range where pectinesterase may either survive pasteurization or partially reactivate during storage.

The effect of calcium addition to the cover brines on pectin methylation 2 and 6 months after brining is shown in Fig. 4. After 2 months (Fig. 4A), samples with 10 mM $CaCl_2$ maintained a higher level of methylation than samples without added calcium. However, higher $CaCl_2$ concentrations had little additional effect on methylation. Methylation decreased at 6 months (Fig. 4B) compared to 2 months. The slices blanched at 81°C showed no effect of $CaCl_2$ concentration on the degree of methylation after 6 months. Overall, the data indicate that calcium addition had a relatively small effect on pectin demethylation. There was a tendency to maintain a higher degree of methylation when calcium was added to slices blanched at lower temperatures.

Fig. 5 shows changes in the firmness of slices during the storage period. Over the 6-month experimental period, the unblanched and 54°C blanch treatments gave very similar firmness patterns. Slices blanched at 66 and 81°C were also very similar and consistently firmer than those subjected to lower or higher blanch temperatures. Slices blanched at 99°C were the least firm. Although firmness and pectin

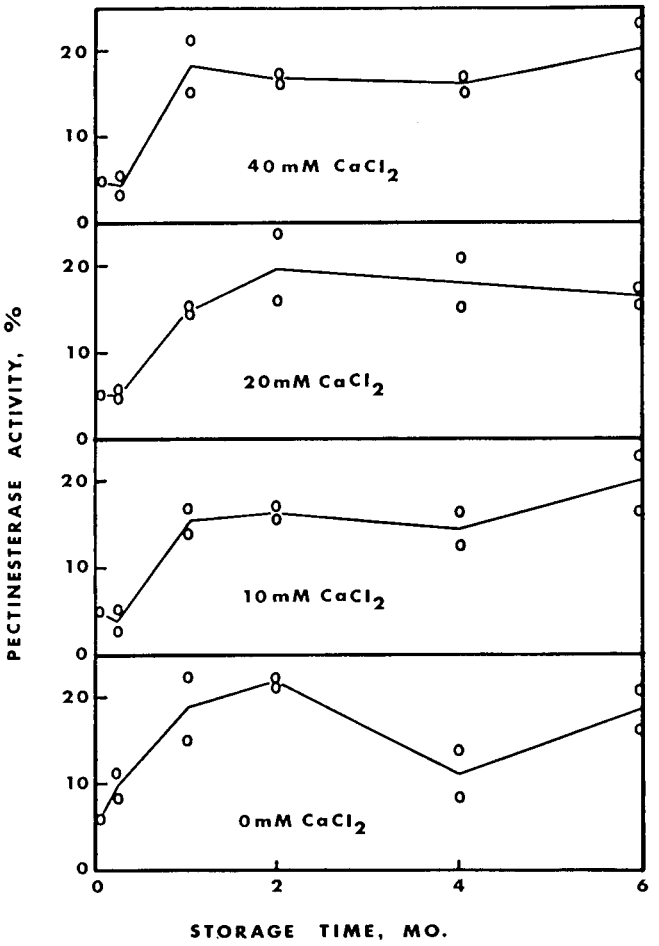

Fig. 2—Recovery of pectinesterase activity in cucumber slices blanched at 81°C and stored in various concentrations of $CaCl_2$. Pectinesterase activity was expressed as a percentage of the activity in the fresh cucumber tissue (0.64 units/g). The coefficient of variation for the pectinesterase measurements over the four $CaCl_2$ concentrations was 20.5%.

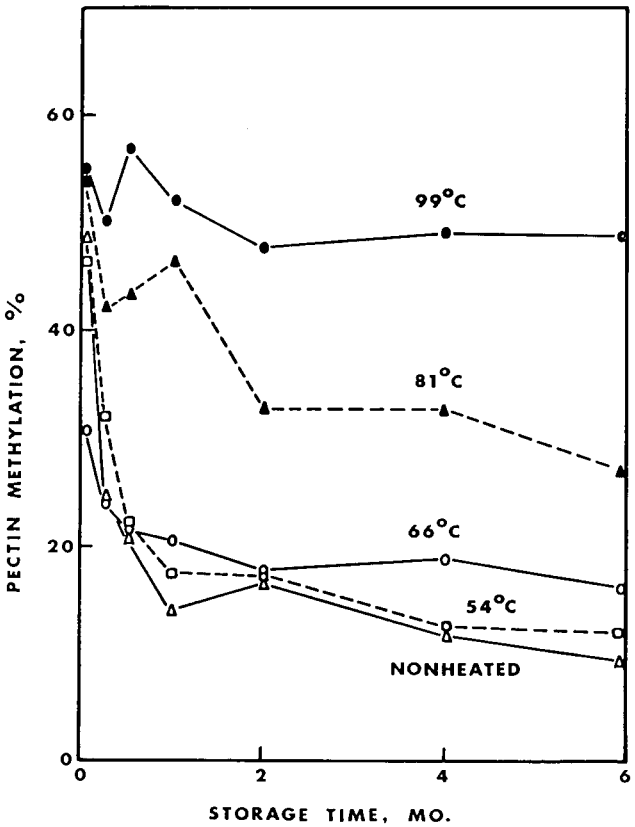

Fig. 3—Effect of blanch temperature on changes in pectin methylation of the cucumber cell wall during storage. The coefficient of variation for the pectin methylation measurements over all treatments was 9.3%.

methylation were retained better at intermediate blanch temperatures, the texture changes were too complex to assign a cause and effect relationship between methylation changes and firmness.

Firmness changes can be divided into three segments. Except for the 99°C blanch, there was an increase in firmness as a result of the blanch treatment (Table 1). Secondly, during the first month of storage, a period of rapid firmness loss occurred (Fig. 5). The rate of firmness loss was 1.12 ± 0.07 N/mo, regardless of the blanch treatment. This suggests some general mechanism of tissue softening which is probably unrelated to enzyme reactions or the degree of methylation of pectin in the cell wall. The third stage from 1 to 6 months was a period in which a slower rate of firmness decline occurred. The 99°C blanched samples were particularly interesting because they underwent the largest firmness decrease during this period, even though these samples were the least likely to retain residual enzyme activities that could catalyze slow degradative reactions.

Fig. 6 shows that 10 mM calcium slowed the loss of tissue firmness in 81°C blanched slices such that after 6 months 68% of the firmness of the unheated slices was maintained compared to only 14% retention when calcium was not added. Higher calcium ion concentrations resulted in further improvement of firmness retention until at 40 mM 95% firmness was retained after 6 months. Very similar texture patterns were also observed in slices blanched at 54 or 66°C (data not shown), which had lower levels of pectin methylation (Fig. 4). These results confirm previous studies on both pasteurized (Etchells et al., 1977) and fermented cucumbers (Buescher et al., 1981; Tang and McFeeters, 1983) that calcium ion is effective in maintaining the firmness of cucumber tissue during storage.

The mechanism by which calcium ion is effective in the maintenance of tissue firmness in the slices under the storage conditions used for Fig. 6 is not clear. The usual concept of calcium firming effects in vegetables is that the calcium ion cross-links pectin molecules by electrostatic interactions between two negatively charged carboxyl groups of the pectin (Van Buren, 1979). 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 calcium ion cross-linking for form an "eggbox" type structure (Kohn, 1975; Powell et al., 1982). Recent titration studies have shown that the apparent pK's of carboxyl groups range from about 3.8 - 4.5 in dilute solutions of pectic acid as it is neutralized with NaOH (Cesaro et al., 1982).

In the present experiments, the pH of the cucumber slices was 3.7. Therefore, well over 50% of the free carboxyl groups would be uncharged due to protonation. Pectin methylation only declined from 57% after blanching at 99°C (Table 1) to about 50% at 2 months and 40% at 6 months. Since the demethylation that did occur was probably enzymatic, a few blocks of free carboxyl groups were probably formed. However, the combination of a high degree of methylation and a high level of protonation would seem to make it very unlikely that 14 or more consecutive, negatively charged carboxyl groups on adjacent molecules could be available for calcium cross-linking. The fact that calcium is so effective in firmness retention under these conditions indicates that other types of polysaccharide/calcium interactions may have an important role. Calcium ion has been shown to form crystallizable coordination complexes with many mono- and disaccharides (Dheu-Andries and Perez, 1983; Cook and Bugg, 1977). Cook and Bugg (1977) have speculated upon the possible importance of calcium/galactose interactions in bone tissue. It may be useful to consider the possibility that such interactions occur in plant cell walls.

Table 1—Pectinesterase activity and pectin methylation after blanching cucumber slices

Blanch temp (°C)	Pectinesterase activity (%)[a]	Pectin methylation (%)	Mesocarp firmness (N)
Nonheated	100	44.0	9.8
54	117	44.3	10.2
66	102	41.1	11.2
81	0	43.3	12.0
99	0	56.8	10.0

[a] Pectinesterase activity calculated as a percentage of the activity in the nonheated cucumbers (0.64 units/g fresh weight).

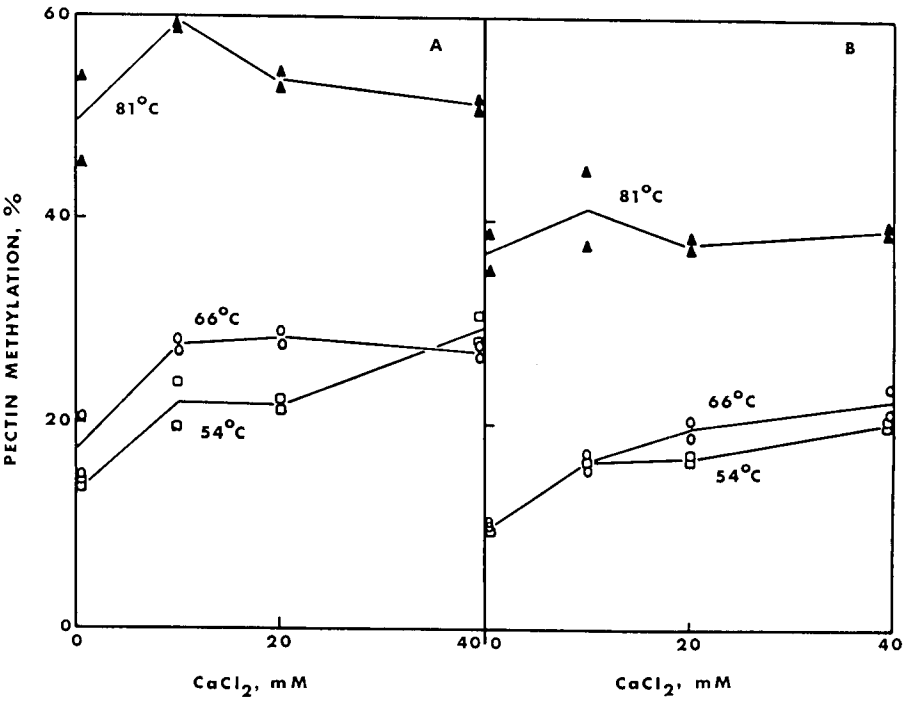

Fig. 4—Effect of blanch temperature and $CaCl_2$ concentration on the degree of pectin methylation in cucumber cell walls: A — pectin methylation after 2 months' storage; B — pectin methylation after 6 months' storage. The coefficient of variation over all treatments for the pectin methylation measurements was 8.9%.

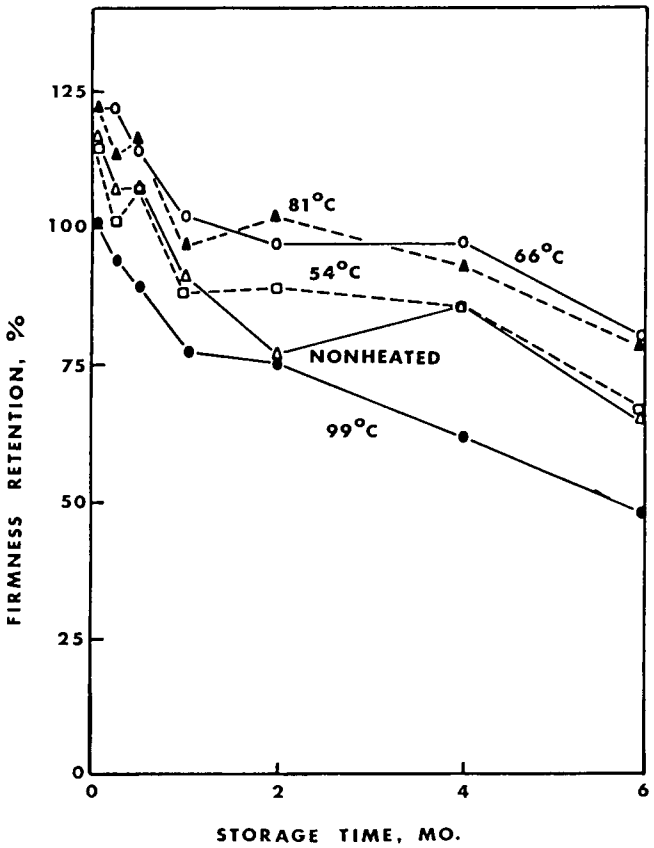

Fig. 5—Effect of blanch temperature on the firmness retention of cucumber slices. Cucumber slices were stored in the standard brine with 10 mM $CaCl_2$ added. Firmness was expressed as a percentage of the firmness of unheated cucumber mesocarp tissue (9.8 Newtons). The coefficient of variation over all treatments for the firmness measurements was 5.8%.

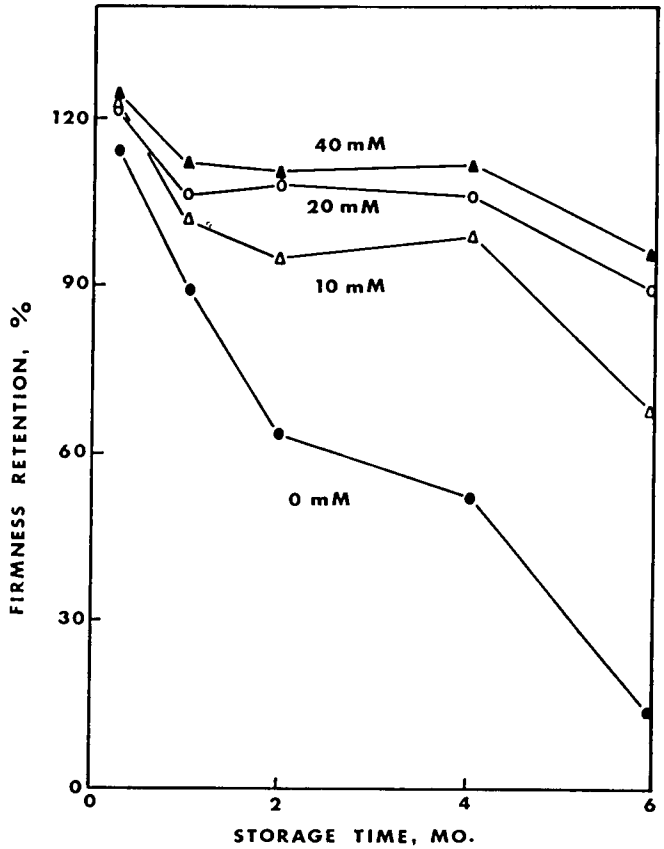

Fig. 6—Effect of $CaCl_2$ concentration on the firmness retention of cucumber slices blanched at 81°C for 3 min. Firmness was expressed as a percentage of the firmness of unheated mesocarp tissue (9.8 Newtons). The coefficient of variation for the firmness measurements over the four $CaCl_2$ concentrations was 5.1%.

CONCLUSIONS

THE EXTENT of pectin demethylation can be controlled by the blanch treatment given to cucumber slices prior to storage in an acid brine. The amount of pectinesterase activity which survived blanching was the primary factor which affected demethylation. However, demethylation stopped at different degrees of methylation even when substantial enzyme activity was present. This indicated that heat treatment also resulted in changes in substrate accessibility, which remain to be analyzed.

The heat stability of pectinesterase in cucumber slices appeared to be similar to that observed by Bell et al. (1951) in cucumber juice with 2% added NaCl. However, after an 81°C blanch, a partial reactivation of pectinesterase was observed in the cucumber slices. Such a reactivation of pectinesterase activity has not been reported previously. The patterns of enzyme inactivation and reactivation during storage suggest the possibility that multiple forms of pectinesterase may be present in cucumbers. Purification and characterization of cucumber pectinesterase needs to be done to determine whether multiple forms exist and whether their stability properties can explain the observed activity changes.

Complex changes in the firmness of cucumber tissue occurred as a result of blanching, acid storage, and the addition of calcium ion to the slices. A direct relationship between pectin methylation and firmness changes was not observed. Calcium was effective in preventing loss of firmness during storage regardless of the extent of pectin methylation. The effectiveness of calcium at high levels of methylation in acid conditions is difficult to explain in terms of current ideas of pectin/calcium interactions.

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