Relationships Among Cell Wall Constituents, Calcium and Texture During Cucumber Fermentation and Storage

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ABSTRACT

Demethylation of pectin was the major change in cell wall constituents which occurred during controlled fermentation of cucumbers. The average degree of polymerization of the pectin was 402 residues. This did not change until 6 months after brining. Galactose, xylose, glucose, mannose, and arabinose were present in order of decreasing abundance in the mesocarp cell walls. These noncellulosic neutral sugars did not change during cucumber fermentation and storage. Mesocarp firmness increased when pectin was demethylated, but the firmness subsequently decreased. Calcium chloride at 20 and 40 mM prevented firmness loss compared to fresh cucumbers during 11 months of storage.

INTRODUCTION

DESPITE THE IMPORTANCE of firmness to the quality of cucumber products, there have been only a few investigations of the pectin content and structure in cucumbers (Fabian and Johnson, 1938; Lampi et al., 1958; Fukushima and Yamazaki, 1978). The composition of neutral sugars in cucumber cell walls and the effect of brining on these sugars has not been investigated.

Calcium ions help prevent softening in fermented cucumber slices (Fleming et al., 1978). Buescher et al. (1979) found that $CaCl_2$ concentrations of 0.4% could be used to maintain cucumber firmness with low salt levels even when fungal polygalacturonase mixtures were added to brined cucumbers. The calcium firming effects observed in cucumbers are probably similar in nature to those observed during processing of other fruits and vegetables (Hoogzand and Doesburg, 1961; Van Buren, 1968; Collins and Wiley, 1963).

The objectives of this investigation were: (1) to observe changes in cell wall composition during controlled fermentation and storage of cucumbers and (2) to determine the effect of cell wall changes and $CaCl_2$ addition on the firmness of salt-stock cucumbers.

MATERIALS & METHODS

Fermentation and storage

Size no. 3 (3.8–5.1 cm diameter) 'Chipper' cucumbers were grown at the North Carolina State University Horticultural Science Research Farm. The fruit were hand-harvested and stored at 10°C, 95% relative humidity, for 4 days prior to brining. Duplicate 5-gal plastic pails of cucumbers with 0, 20 and 40 mM equilibrated concentrations of $CaCl_2$ were fermented using the controlled fermentation procedure of Etchells et al. (1973). Each pail was filled with 9.5 kg cucumbers and an equal weight of brine which contained 12.1% NaCl, 28 mM acetic acid and the appropriate amount of $CaCl_2 \cdot 2H_2O$. Four days after brining, when the salt concentration was 7.4%, 95g of sodium acetate trihydrate were added to each pail. The pails were inoculated with 10 ml of a 15-hr culture of *Lactobacillus plantarum* WSO grown in MRS broth (DeMan et al., 1960) with 4% NaCl added. The cucumbers were held at 27°C until sampling. The cucumbers were continuously purged with 25 ml/min N_2 for 1 month. One month after brining, the fruit were repacked into 1-gal jars such that the 50/50 cucumber/brine pack-out was maintained. The jars were tightly closed with minimum headspace to prevent subsequent growth of film yeasts or molds. At 1, 3 and 6 months after brining, a 1-gal jar from each of the six original 5-gal pails was analyzed. The firmness of cucumber mesocarp and endocarp was measured. Mesocarp tissue and brine samples from each jar were frozen for subsequent analysis. A texture evaluation of the $CaCl_2$-treated fruit only was done at 11 months.

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Effect of post-fermentation calcium addition

Two experiments were done to determine the effect on cucumber firmness of addition of calcium after fermentation. The first experiment was to add 40 mM $CaCl \cdot 2H_2O$ to duplicate, 1-gal jars of cucumbers from the 0 mM calcium pails 1 month after brining. The texture was analyzed 2 months later.

In the second experiment, cucumbers were fermented without calcium, as described above. Three months after brining, the cucumbers were cut into 0.476 cm thick slices and packed into 8-oz jars with an equal weight of brine. $CaCl_2 \cdot 2H_2O$ was added to the jars to give an equilibrated concentration of 40 mM. The firmness of slices from duplicate jars was analyzed weekly for 8 wk.

Polygalacturonase assay

Brine samples were dialyzed and tested for polygalacturonase activity according to Bell et al. (1955).

Firmness measurements

A single, 0.476 cm thick cucumber slice was cut from the center of each cucumber. A single punch was made with the Instron UMT in the mesocarp and endocarp of 15 slices with a 0.315 cm flat-tipped plunger. A 2-kg compression force transducer was used. The crosshead speed and chart drive speed on the Instron were 200 mm/min and 500 mm/min, respectively. The maximum penetration force expressed in newtons was the only parameter used for firmness measurement (Thompson et al., 1982).

Extraction and purification of pectic substances

The general extraction procedures for pectic substances suggested by Doesburg (1965) were used. The frozen mesocarp tissue, from one of the duplicate gallon jars at each calcium chloride level, was partially thawed and a representative 50g tissue sample was blended for 3 min in 5-fold (v/w) of 95% ethanol with a Tekmar homogenizer. The blended tissue was filtered through a Whatman no. 1 filter paper on a Buchner funnel. The residue was washed twice with 50 ml of 95% ethanol. The volume of the total filtered ethanol was measured. The alcohol insoluble solids (AIS) were collected, dried in a vacuum oven at 50°C to constant weight and stored in a desiccator until further extraction.

Tha AIS from 50g of tissue was extracted for 30 min consecutively with 100 ml of 0.05N HCl at 80°C and 0.5% EDTA solution at pH 6, 50°C. Each extraction step was repeated two to three times until a cooled aliquot of extract did not show pectin flocculation in two volumes of 95% ethanol. At the end of each extraction, the mixture was filtered. Residue from the last EDTA extraction was weighed and refrigerated. Pectic substances, solubilized by the extraction steps, were isolated by adding two volumes of 95% ethanol (v/v) to the extract. The pectic substances precipitated were collected on Whatman no. 1 filter paper. The precipitate was washed with 70% ethanol, 95% ethanol, and acetone. The pectic substances were dried in a vacuum oven at 40°C to a constant weight.

Quantitation and characterization of cucumber pectic substances

The colorimetric technique of Scott (1979) was used to measure

the uronic acid content of each pectin fraction. EDTA was found to interfere with the determination because samples developed a pink color. Therefore, the pectic substances from the EDTA extracts were precipitated with two volumes of 95% ethanol and then redissolved in water before measurement.

The degree of methylation of the pectic substances was measured by titration with poly-NN-dimethyldiallylammonium chloride (Cat-Floc) (Mizote et al., 1975) and the degree of polymerization was estimated viscometrically (Versteeg, 1979). The viscosity at pectin concentrations of 0.2, 0.15, 0.1 and 0.05% in the 0.1M tris-succinate buffer, pH 6.0, with 0.02M NaCl was measured in a no. 1 Ubbelohde viscometer. The intrinsic viscosity $[\eta]$ was determined by plotting the ratio $(\eta_{r-1})/C$ against C and extrapolating to 0 concentration (C = pectin concentration, η_r = viscosity relative to solvent). The formula $[\eta] = 1.4 \times 10^{-6} M^{1.34}$ was used to calculate the average molecular weight (Owens et al., 1946). The acid-soluble fraction was used for the molecular weight estimation of pectin from fresh cucumbers. The amount of EDTA-soluble pectin was too small to be isolated. Since this pectin was 62% methylated, a 184.4 monomer molecular weight was used to calculate the average degree of polymerization. The EDTA-soluble pectic substances were used for size estimation of fermented cucumbers. The amount of the acid-soluble fraction was too small to be isolated after fermentation. A monomer molecular weight of 176 Daltons was used to calculate degree of polyermization since methylation was not detected in the samples.

Cell wall preparation and analysis

Cell walls were isolated from 20 g samples of cucumber mesocarp tissue by the procedure of English et al. (1971). Dried cell walls were hydrolyzed with 2N trifluoroacetic acid (TFA) as described by Albersheim et al. (1967). After hydrolysis, the soluble portion was evaporated to dryness at 50°C under a stream of nitrogen. The dried sample was placed in a desiccator over KOH pellets for 24 hr to remove any residual trifluoroacetic acid. The sample was then dissolved in 0.5 ml of deionized water.

The neutral sugar composition of noncellulosic polysaccharides in the cell wall hydrolyzate was determined by HPLC using a Bio-Rad HPX87 heavy metal column at 73.6°C. Twenty μl of sample was injected and eluted with deionized water at a flow rate of 0.6 ml/min. Glucose, galactose, mannose, xylose, and arabinose were separated and estimated using an external standard calculation. Rhamnose was separated from galactose when standard samples were chromatographed. However, rhamnose, if present, was covered up by a large galactose peak in the cell wall samples.

Statistical analysis of data

Coefficients of variation and significant differences for the treatment means at $P \leq 0.05$ were calculated from analyses of variance for the firmness, pectin fractions and cell wall neutral sugar data.

RESULTS & DISCUSSION

Cucumber characteristics

A normal fermentation occurred in all pails. There was no evidence of bloating or growth of film yeasts or molds in any of the samples. The fruit were normal in odor and appearance. The brine pH was 3.4 and the lactic acid concentration averaged 1.5% during storage. Polygalacturonase activity was not detected in the brine.

Pectin and neutral sugar changes

The acid-soluble pectin decreased while the EDTA-soluble material increased during the first month after brining (Fig. 1). Little or no change occurred after the first month. There was no significant effect of calcium on the distribution of these fractions, so the data presented in Fig. 1 are the averages over the three calcium concentrations.

The acid-soluble fraction would be expected to contain pectin with a high methoxyl content. Analysis of this fraction from fresh cucumbers showed a 62% degree of methylation. This is very close to the 64.6% methylation reported for a Japanese cultivar (Fukushima, 1978). The acid-soluble pectic substances declined to such a low level by the 1-

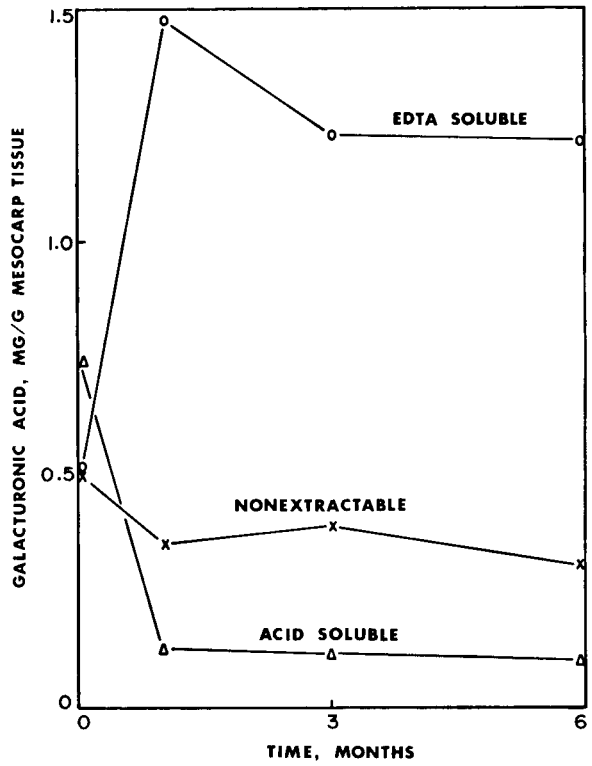

Fig. 1—Changes in pectin fractions during fermentation and storage of cucumbers. Results are the mean of samples over the three calcium levels. The coefficient of variation was 5% for the acid-soluble pectin, 7% for the EDTA-soluble pectin and 10% for the nonextractable pectin in the residue fraction.

month sampling period that they could not be isolated for further characterization. From 1 month onward, the EDTA-soluble fraction was isolated. It was found to have non-detectable levels of methoxyl groups at all three calcium levels, which indicated that complete or nearly complete demethylation of the pectin occurred during the first month of brining. Lampi et al. (1958) reported that conversion of the acid-soluble pectic substances to EDTA-soluble pectic material accompanied the softening of salt-stock cucumbers. However, the measured degree of methylation changed irregularly during softening. A different extraction sequence was used, but we do not know the reason for the apparent large differences in the methylation observed in this study compared to the earlier results. Fukushima and Yamazaki (1978) observed pectin demethylation in chill-injured cucumbers. However, the conversion to low methoxyl fractions did not appear to be as extensive as occurred in the brined fruit.

Neutral sugars in the cucumber cell wall

The neutral sugars from cucumber cell walls had not been previously analyzed, so it was possible that changes in neutral polysaccharides might be responsible for texture changes during brining and storage of the fruit. The amount of cell wall isolated by phosphate buffer extraction did not change as a result of fermentation or storage. The fruit contained 8.3 ± 0.6 mg/g fresh weight of cell walls. After hydrolysis with 2N trifluoroacetic acid, 13–16% of the cell wall was recovered as neutral sugars.

The yield of noncellulosic neutral sugars from isolated cell walls has been found to vary from 50% in suspension-cultured sycamore cells (Talmadge et al., 1973) to only 12% by weight of the cell walls from mung bean leaves (Nevins et al., 1967). In green tomatoes, the neutral sugar content was 29%. This declined to 20% in ripe tomatoes

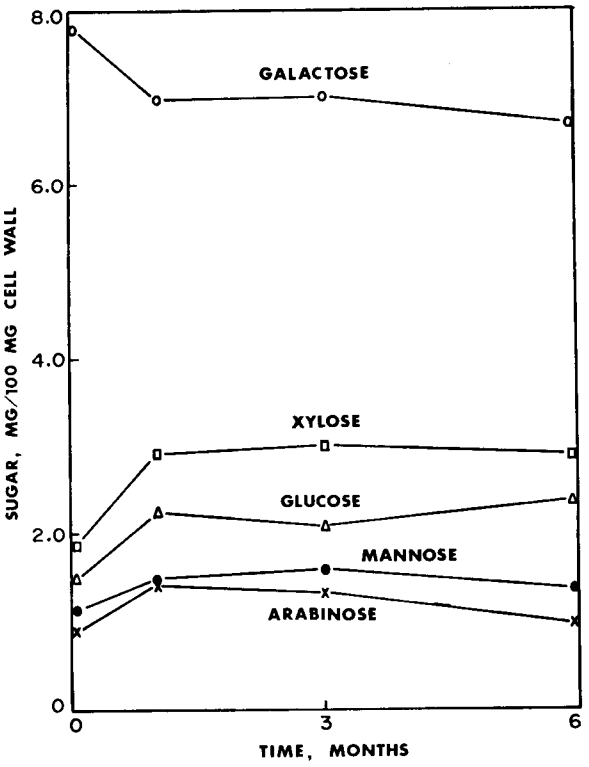

Fig. 2—Changes in cell wall neutral sugar content during brining and storage of cucumbers. Results are the means over the three calcium levels. The coefficients of variation for the individual sugars were galactose, 8%; xylose, 13%; glucose, 16%; mannose, 14%; and arabinose, 25%.

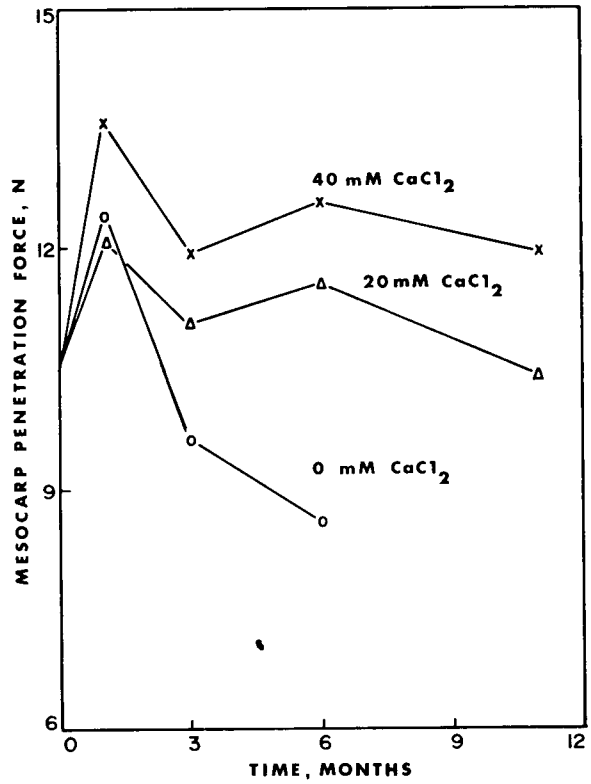

Fig. 3—Effect of $CaCl_2$ on the firmness of cucumber mesocarp tissue during fermentation and storage. The coefficient of variation for the firmness measurements was 16% without added calcium, 15% with 20 mM calcium and 12% with 40 mM calcium.

(Gross and Wallner, 1979). Therefore, cucumber cell walls have a total neutral sugar content in the lower range among the plant tissues which have been analyzed. Fukushima and Yamazaki (1978) found a rather high concentration of cellulose in cucumbers, so cellulose and pectin were probably the major cell wall components not recovered by the TFA hydrolysis.

The presence of calcium ions did not affect the amount of sugars isolated from the wall preparations. Therefore, Fig. 2 shows the mean sugar concentrations over the three calcium concentrations. Galactose, xylose, glucose, mannose, and arabinose were found in order of decreasing abundance. Galactose constituted about 50% of the total hydrolyzable neutral sugars present in the cucumber cell wall. Galactose has also been found to be the major sugar in the walls of apples and tomatoes.

Tomatoes and apples both have arabinose rather than xylose as the second most abundant sugar (Gross and Wallner, 1979; Bartley, 1976). In general, the neutral sugar composition of cucumbers appeared to be similar to that observed in other fruit.

Large decreases of neutral sugars, especially galactose, have been observed during ripening of apples (Knee, 1973; Tavakoli and Wiley, 1968; Bartley, 1976), tomatoes (Gross and Wallner, 1979) and Japanese pears (Yamaki et al., 1979). Wallner (1978) has suggested that removal of neutral sugars that serve as crosslinks could weaken the cell wall structure and contribute to firmness loss. In addition, removal of neutral sugar side chains from pectin could increase its susceptibility to degradation by polygalacturonase. However, Fig. 2 shows that there were not significant changes in neutral sugars during 6 months' storage. Fermentation appears to have little effect on the neutral sugar content of cucumber cell walls.

Effect of calcium on cucumber texture

Fig. 3 shows the firmness changes in the mesocarp tissue of cucumbers at three calcium concentrations during fermentation and storage. Cucumbers with and without added calcium increased in firmness during the first month after brining. This firming effect may be related to crosslinking or gelation of the pectic substances caused by demethylation and the presence of high sodium concentrations and also, calcium ions when $CaCl_2$ was added (Kohn and Sticzay, 1977). An increase in tissue firmness by the interaction of calcium ion and demethylated pectic substances has been observed in the processing of cauliflower (Hoogzand and Doesburg, 1961), snap beans (Van Buren, 1968), and tomatoes (Hsu et al., 1965).

There was a decrease in the firmness of all samples after 1 month. The cucumbers brined without $CaCl_2$ showed a 23% decline in firmness during the 1- to 3-month period and an additional 8% loss from 3 to 6 months (Fig. 3). This decrease could not be readily explained because neither the amount of pectin in the cell walls, the neutral sugars nor the degree of methylation changed significantly during the 1- to 6-month storage period.

Table 1 shows the average molecular weight and degree of polymerization of the major extractable pectin fraction

Table 1—Changes of the average molecular weight and average degree of polymerization of extracted pectic substances during fermentation and storage of cucumbers

Time (months)	Average molecular weight, Daltons	Average degree of polymerization
0	74,200	402
1	71,200	404
3	71,000	403
6	64,400	365

from cucumbers to which calcium was not added. We do not know whether this represents the molecular size of pectin as it exists in the cucumber cell wall. However, the data show that the degree of polymerization of the pectic substances extracted did not change for 3 months after brining. The molecular weight difference between the fresh cucumbers and the cucumbers 1 month after brining was the decrease that should occur if 62% of the galacturonic residues lost a methyl group. There was a 9.4% decrease in the degree of polymerization at 6 months compared to 3 months. This may, perhaps, explain the 8% decrease in mesocarp firmness during this period. However, since the size of pectin did not change during the 1- to 3-month storage period when a large decrease in firmness occurred, this loss of firmness remains to be explained.

The addition of calcium had a significant firming effect on mesocarp tissue. At 3 and 6 months, both 20 and 40 mM calcium chloride showed improved firmness compared to the control fruit without added calcium. Control fruit were not available at 11 months; however, the 20 mM calcium concentration maintained a mesocarp tissue firmness not significantly different from fresh cucumbers. Addition of 40 mM calcium resulted in the mesocarp remaining firmer than the fresh fruit. The endocarp tissue of the cucumbers was less firm and more variable than mesocarp tissue (Fig. 4). Thompson et al. (1982) observed this same result for fresh cucumbers. Due to the variability of the endocarp texture measurements, no significant firming effect of calcium on the endocarp was observed. The endocarp tissue did retain its structure in all treatments so that whole cucumber slices were obtained when the fruit were cut. The data on the firmness changes suggest that properly stored cucumbers could be held in 6% NaCl with 20 mM or more $CaCl_2$ for nearly a year at 27°C without significant loss of firmness compared to fresh fruit. With good tanks and proper control of brining, it may be possible to eliminate the addition of NaCl that is usually carried out after fermentation.

Buescher et al. (1981) found that calcium addition to brined cucumbers after a 2-wk exposure of the fruit to commercial polygalacturonases caused an initial firming response, but the firmness remained much less than that of cucumbers treated at brining with both calcium and polygalacturonase. The breakdown caused by polygalacturonase resulted in some irreversible texture loss.

Two experiments were done to evaluate the effect of calcium added to cucumbers after fermentation under good brining conditions when no detectable polygalacturonase activity was present. Addition of 40 mM $CaCl_2$ to whole cucumbers 1 month after brining prevented the large loss of firmness that occurred in cucumbers without added $CaCl_2$ during the 1- to 3-month storage period. At 1 month the penetration force for cucumbers brined without calcium was 12.4N. By 3 months it had declined to only 9.6N. When calcium was added at 1 month, the firmness decreased to 11.5N compared to 11.9N for fruit brined initially with 40 mM $CaCl_2$.

In the second experiment, fermented cucumbers were held for 3 months without calcium. Calcium was then added to the sliced cucumbers at a 40 mM concentration. Fig. 5 shows that a large increase in mesocarp firmness occurred in the first week after calcium addition, though the slices did not become as firm as when calcium was added at the time of brining. However, the improvement in firmness was temporary. During 8 weeks' storage, the firmness gradually declined until the slices were only slightly firmer than they had been before the addition of calcium. Additional work is required to fully understand the effects of post-brining calcium addition to cucumbers. These limited results indicate that, while later addition of calcium can provide some benefit, the addition of calcium at the time of initial brining is most likely to result in effective control of decreases in cucumber firmness. Buescher et al. (1981) reached the same conclusion based upon their experiments in which polygalacturonases were intentionally added to the brined cucumbers. *—Continued on next page*

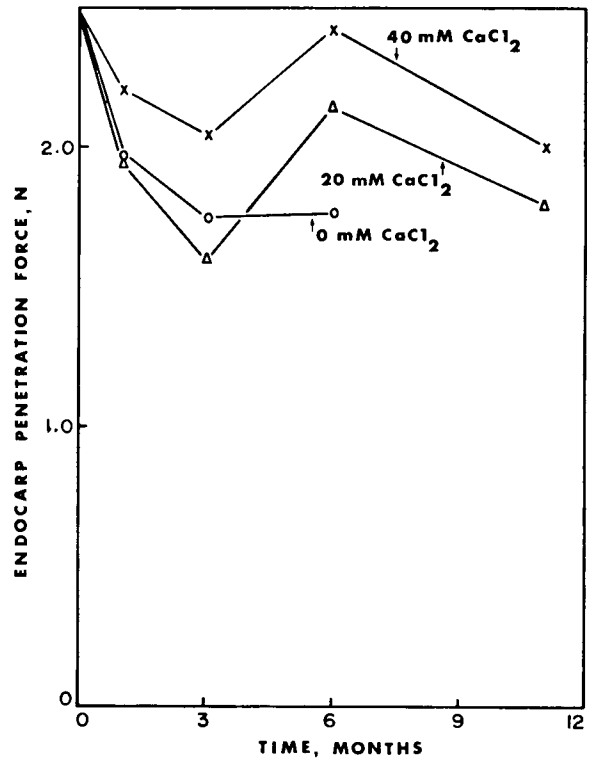

Fig. 4—Effect of $CaCl_2$ on the firmness of cucumber endocarp tissue during fermentation and storage. The coefficient of variation for the firmness measurements was 21% without calcium, 22% with 20 mM calcium and 19% with 40 mM calcium.

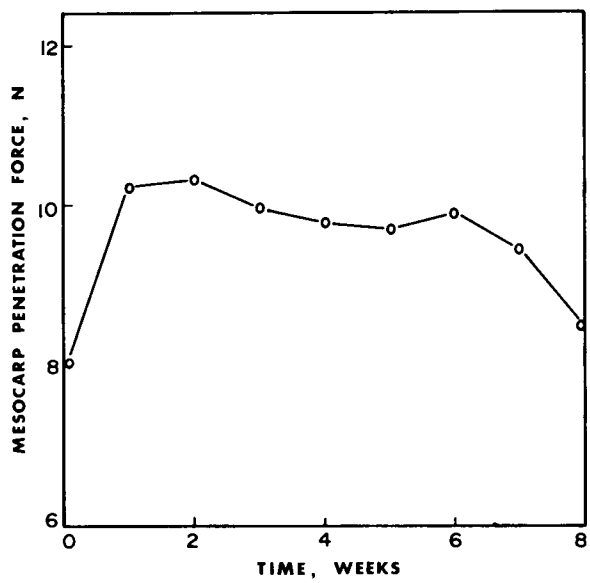

Fig. 5—Effect of 40 mM $CaCl_2$ added to cucumbers after 3 months' storage on the mesocarp firmness of slices. The coefficient of variation for the firmness measurements was 12%.

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