

EFFECT OF SODIUM CHLORIDE CONCENTRATION ON CALCIUM UPTAKE INTO BRINED CUCUMBERS¹

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ABSTRACT

Calcium from a CaCl_2 brine approximately iso-osmotic with cucumber cell sap penetrated cucumber fruit much more slowly than did calcium ions from a $\text{NaCl} + \text{CaCl}_2$ brine supra-osmotic with cucumber cell sap. After 96 h calcium that penetrated from the iso-osmotic brine was localized mostly in the exocarp and relatively little in the interior tissues, while calcium that penetrated from the supra-osmotic brine reached higher concentrations and was more evenly distributed among the exocarp, mesocarp, and endocarp sections.

INTRODUCTION

Brine fermentation is used to preserve about 40% of the pickling cucumber crop in the U.S. Traditionally, the salt (NaCl) concentration during fermentation is relatively low (5-8%), but is increased after fermentation to 12-16% to maintain firmness and microbial stability (Etchells *et al.* 1951). Environmental concerns with chloride and organic waste disposal over the past 15 years have stimulated an interest in reducing the concentration of salt needed to preserve brined cucumbers.

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It was observed that fermented cucumber firmness could be maintained at relatively low salt concentrations (1.4%) if calcium acetate was added (Fleming *et al.* 1978). Further studies showed that addition of CaCl_2 prevented softening of brined cucumbers, even when softening enzymes were intentionally added (Buescher *et al.* 1979) and at relatively low concentrations of salt (Buescher *et al.* 1981; Fleming *et al.* 1987). These findings have resulted in universal addition of CaCl_2 to fermentation brines to improve firmness retention and to permit the use of reduced salt concentrations.

More recent studies have focused on attempts to understand the mechanism by which calcium increases firmness retention in brined cucumbers. Monovalent cations, including Na^+ , were shown to increase softening of brined cucumbers (McFeeters *et al.* 1989). Salt-induced softening was inversely related to the natural calcium concentration in the tissue (McFeeters and Fleming 1989). Added calcium was shown to reduce the softening rate at 74C, particularly at pH values below 5, a fortuitous effect since brined cucumbers are held under an acidic environment (McFeeters and Fleming 1991). Although the mechanism of calcium's effect on increasing firmness retention in brined cucumbers is not fully understood, evidence has been obtained that the "egg box" model generally applied to explain gelation of polypectate molecules does not provide an adequate explanation (McFeeters and Fleming 1989).

It was suggested that the resistance of plant tissues high in calcium to breakdown by fungal pathogens is due to a calcium-pectin complex which renders the tissues resistant to breakdown (Bateman and Lumsden 1965). Buescher *et al.* (1979) found that CaCl_2 did not inhibit galacturonase activity, but did prevent pickle softening in the presence of such activity and suggested that binding of calcium to pectate in the cucumber could be responsible for resistance to softening.

We anticipated that if the calcium was incorporated into an NaCl brine whose osmotic strength greatly exceeded the osmotic strength of the cell, sap (supra-osmotic) tissue shrinkage and cell plasmolysis would result, thereby compressing all tissue including that adjacent to the intercellular air space. This process would partially block the intercellular network and effectively impede liquid flow through the network. Thus calcium movement would be slowed down. On the other hand, if the cucumbers were initially exposed to calcium containing brine which did not exceed the osmotic pressure of the cell sap (i.e., near iso-osmotic), the intercellular network would remain open and brine flow would be facilitated, resulting in more rapid influx of calcium into the tissue. Thus, the objectives of this research were to determine the effect of NaCl concentration in the brine on calcium uptake into the tissue of the brined fruit.

MATERIALS AND METHODS

This research was conducted in two parts: Experiments 1 and 2. In Experiment 1 cucumber fruit hand-harvested from a nearby NCARS facility was used. Experiment 2 used fruit obtained from a nearby commercial processing facility. For both parts of the study, size #2A cucumbers (25.4-32.0 mm in diameter) were used. The fruit were hand-washed, weighed, and the weight of each cucumber was inscribed on the surface with indelible ink. Three fruits were randomly selected and the osmotic potential of the cell sap determined. To measure osmotic potential, cores of mesocarp tissue 5 mm in diameter were removed and placed in 3-ml plastic syringes. The syringes were placed on solid CO₂ until the core solidified. The syringes were removed, thawed, the cell sap expressed, and the osmotic potential measured with a Wescor Model 5100 CRX Osmometer (Walter *et al.* 1990).

For both parts of the study, two brines were prepared such that one of the brines contained only CaCl₂ at a concentration which resulted in approximately iso-osmotic with the cucumber cell sap (iso-osmotic). The other brine was at same CaCl₂ concentration as the first brine but contained 9 or 13% NaCl (supra-osmotic). The pack-out ratios, cucumbers/brine, were approximately 30/70 by weight for both experiments. Each of the brines was poured into 3.8 L jars containing the cucumbers.

Experiment 1 [Mean Fruit Cell Sap Osmotic Potential 247 ± 9 Milliosmoles (mOs)]

Twenty-four washed, weighed cucumbers were placed into each of three 3.8 L jars. Brine A (87 mM CaCl₂; 200 mOs) was added to one jar, brine B (87 mM CaCl₂ and 2.09 M NaCl; 3,800 mOs) was added to the second jar, and water was added to the third jar.

Tissue Analysis. Four replicate samples consisting of one cucumber each were removed from each of the jars after 1, 3, 5, and 23 h. The fruit were rinsed in tap water, hand-dried, and weighed. Each fruit was then sliced into a Waring blender, water equal to the fruit weight was added, and the mixture homogenized. A 2-g aliquot was removed and mixed with 2 ml trichloroacetic acid solution (4% weight/volume) and the calcium content measured as described by Gindler and King (1972). Those samples covered in water served as controls for weight change.

Experiment 2 (Mean fruit Cell Sap Osmotic Potential 212 ± 15.3 mOs)

Groups of 24 cucumbers were put into each of four 3.8 L jars. Brine C (75.4 mM CaCl_2 ; 215 mOs) was added to two of the jars, and brine D (75.4 mM CaCl_2 and 1.53 M NaCl; 2,964 mOs) was added to the other two jars as described in Experiment 1. For tissue analysis, three replicate samples (two cucumbers in each replicate) were removed from each brine treatment after 1, 5.5, 21.5, and 96 h. In addition, three replicate samples (two cucumbers in each replicate) of cucumbers was analyzed at the start of the study. These were the 0 time samples.

Tissue Analysis. At appropriate times, cucumbers were removed from the jars, washed, and dried with paper towels. They were weighed and dissected into stem, middle, and blossom sections. The tissues from each section were further subdivided into exocarp (skin), mesocarp, and endocarp types. The separate types were weighed, mixed with an equal weight of water, and homogenized with a Tekmar Tissumizer. An aliquot was removed and mixed with an equal volume of 4% aqueous trichloroacetic acid. This subsample was used for calcium analysis (Gindler and King 1972). The remainder was frozen until analyzed for chloride concentration (Fleming *et al.* 1992). Determination of the chloride concentration allowed us to estimate the sodium concentration because the concentration of chloride contributed by NaCl was 20 times greater than that contributed by calcium chloride and because electro-neutrality must be preserved.

Statistical Analysis

The experimental design was a complete randomized block with nested subsampling. A series of second order polynomial equations was fitted to the data using General Linear Models procedure (version 6.06, SAS Institute, Inc., Cary, NC).

RESULTS AND DISCUSSION

Experiment 1

Calcium penetration into cucumber fruit was much more rapid when the fruit were immersed in the supra-osmotic solution (Fig. 1). After 23 h, fruit immersed in the supra-osmotic brine contained approximately 3.5 times as much

calcium as the iso-osmotic fruit. Statistical analysis of the data showed that calcium uptake from the supra-osmotic brine was significantly greater ($P < 0.001$) than uptake from the iso-osmotic brine.

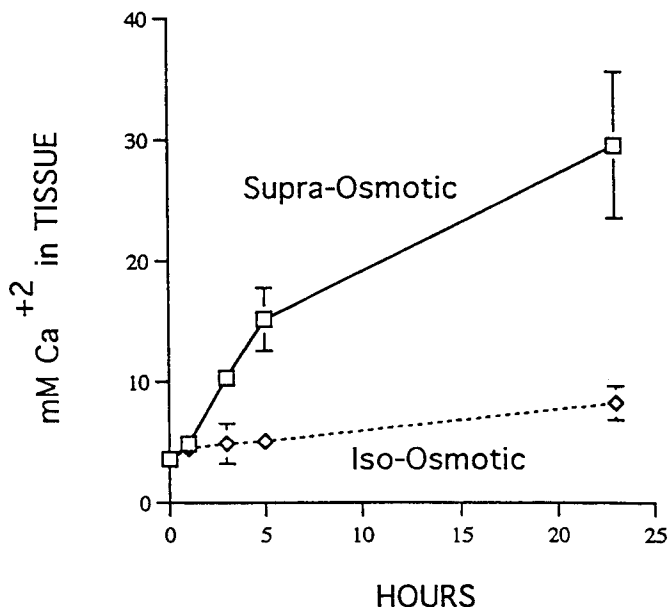


FIG. 1. CALCIUM UPTAKE FOR CUCUMBERS IMMERSSED IN ISO-OSMOTIC (87 MM CALCIUM CHLORIDE) AND SUPRA-OSMOTIC BRINES (87 MM CALCIUM CHLORIDE AND 2.09 M SODIUM CHLORIDE)

Error bars represent standard deviations. The data are from homogenates of whole cucumber fruit (Experiment 1).

With regard to cucumber weight changes, fruit immersed in water showed a slow weight gain throughout the 23 h of the study (Fig. 2), while the weight of fruit immersed in the iso-osmotic solution varied only slightly during the study. Fruit immersed in the supra-osmotic solution had lost about 14% of its weight after 23 h (Fig. 2). Considering the semi-permeable nature of plant cell membranes, these changes in weight were expected. In the case of water, there was a net movement into the cells in response to differences in the osmotic potential (Baker 1984). However, when the solute potential of the brine and the cytoplasm were approximately the same, as was the case for the iso-osmotic

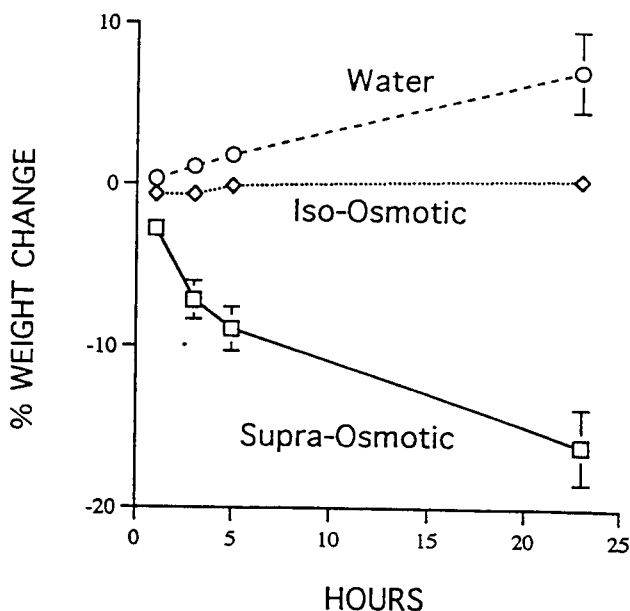


FIG. 2. WEIGHT CHANGES FOR CUCUMBERS IMMERSED IN WATER, ISO-OSMOTIC (87 MM CALCIUM CHLORIDE) AND SUPRA-OSMOTIC BRINES (87 MM CALCIUM CHLORIDE AND 2.09 M SODIUM CHLORIDE)

Error bars represent standard deviations. The data are from Experiment 1 of this study.

brine, no significant uptake occurred. Where the solute potential was much greater than that of the cell solute potential, as it was for the supra-osmotic brine, there was corresponding weight loss.

Experiment 2

The data from Experiment 1 clearly showed that calcium penetration into cucumber fruit was much more rapid when the solute potential of the liquid medium was much greater than that of the cell sap. However, that experiment did not permit us to determine if the calcium had penetrated throughout the fruit and how it was distributed. Since retention of pickle firmness during storage is most likely influenced by the concentration of calcium present in the major regions throughout the fruit, it was important to know the extent of calcium penetration into the exocarp, mesocarp, and endocarp tissues. Experiment 2 of this study was designed to provide information on tissue section calcium concentrations as influenced by brine osmolarity over a 96-h brining period.

Weight change data, although not identical to that in Experiment 1, were similar. That is, weights of cucumbers in the iso-osmotic brine changed only slightly, while cucumbers in the supra-osmotic brine lost significant weight (data not shown). Analysis of the data showed that cucumber section (i.e. stem, middle, and blossom) did not vary significantly ($P < 0.05$) as to calcium and chloride penetration. Consequently data reported herein were pooled over the three sections.

As we observed in Experiment 1 of this study (Fig. 1), calcium from the supra-osmotic brine penetrated the fruit more rapidly than did calcium from the iso-osmotic brine (Fig. 3). At 96 h for fruit from the iso-osmotic brine, the calcium concentration in both mesocarp and endocarp tissue showed only a slight increase over initial values. On the other hand, for fruit brined in the supra-osmotic solution, calcium content rapidly increased in all three tissue regions the first 21.5 h and approached equilibrium with the brine by 96 h. Since fruit were periodically removed from the jars, mass balance changes made it impractical to calculate equilibrium values.

In addition to calcium concentration we also measured the chloride concentration in order to follow sodium movement into the tissue. The data showed that concentrations of both calcium and chloride increased with time in the brine (Fig. 4). The correlation coefficient for calcium and chloride concentrations was 0.834 ($P < 0.0001$) summed over both brine treatments and three tissue types. This indicated that both calcium and sodium were moving into the tissue together.

A major portion of the driving force for movement of sodium chloride brine into the cucumber tissue appears to be caused by the difference between the osmotic potential of the tissue and the osmotic strength of the NaCl aqueous solution, initially a partial vacuum develops as a result of the dissolution of tissue CO_2 into the aqueous medium, and this acts as the driving force for brine solution migration. Thus, brine moves into the fruit by mass transfer and diffusion. However, in the present study both the supra and iso-osmotic solutions would experience the same internal environment, and both would be affected in the same way.

There are two rationalizations which could account for slower migration of an iso-osmotic solution of CaCl_2 as compared with that of a supra-osmotic brine. In the first place, for an iso-osmotic solution, there is no driving force other than diffusion and the CO_2 driven pressure reduction effect. Moreover, there is a significant amount of discontinuity within the tissue since approximately 5-9% of cucumber volume is intercellular space (Veldhuis and Etchells 1939). This interconnected intercellular space network effectively serves as a barrier and would decrease flow rates because the incoming solution would have to flow around it by movement through capillaries and/or diffusion through cell walls. A second possibility is that calcium ions of the iso-osmotic brine could be

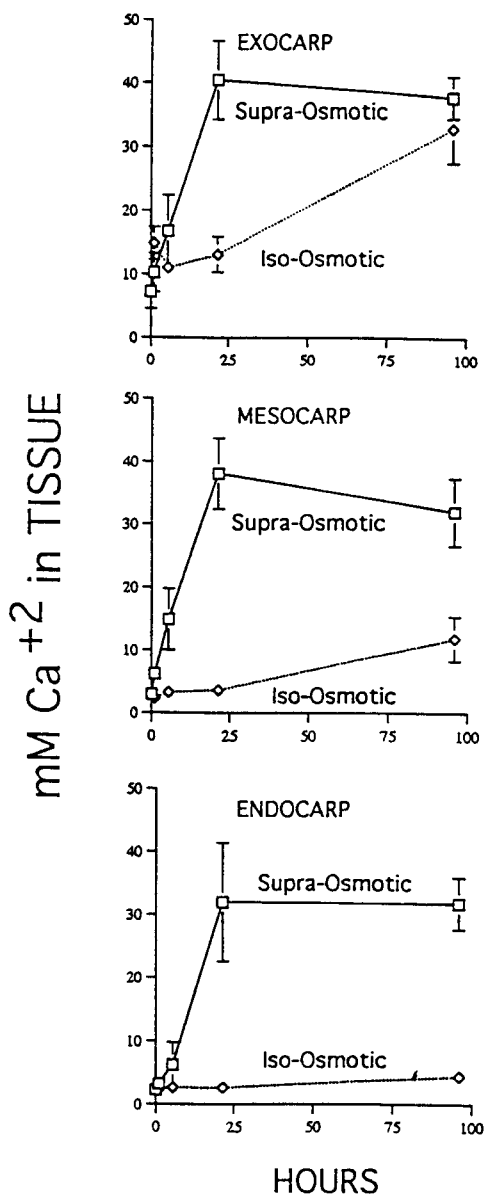


FIG. 3. CALCIUM UPTAKE FOR CUCUMBERS IMMERSSED IN ISO-OSMOTIC (75.4 MM CALCIUM CHLORIDE) AND SUPRA-OSMOTIC BRINES (75.4 MM CALCIUM CHLORIDE AND 1.53 M SODIUM CHLORIDE)

Error bars represent standard deviations. The data are from Experiment 2 of this study.

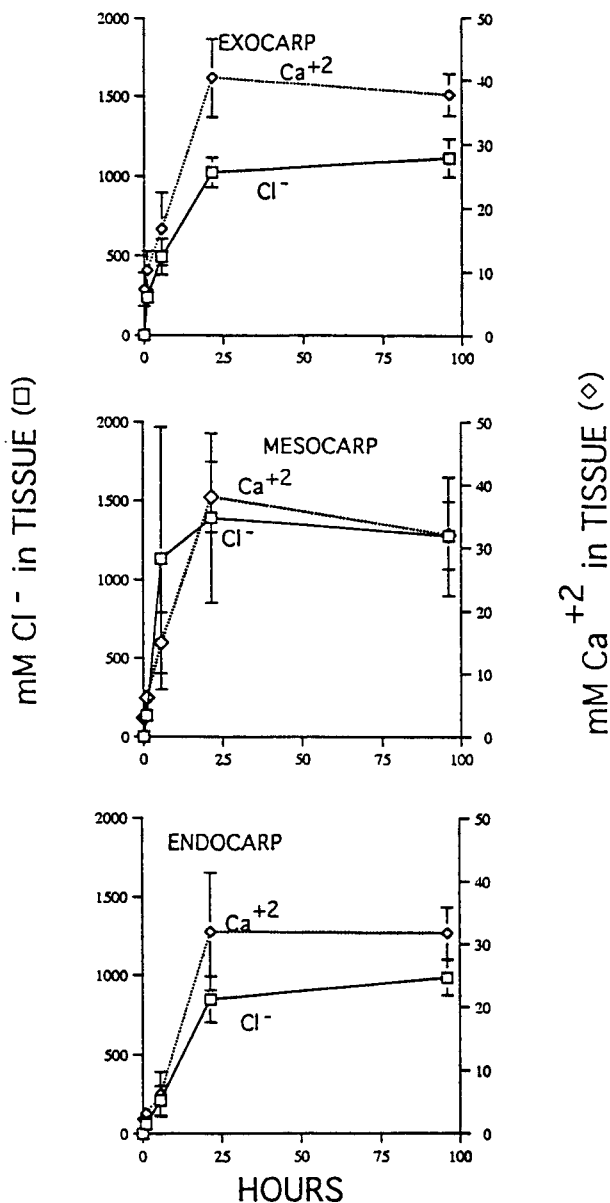


FIG. 4. CHLORIDE ($\square$) AND CALCIUM ($\diamond$) UPTAKE FOR CUCUMBERS IMMERSSED IN SUPRA-OSMOTIC SODIUM CHLORIDE BRINE CONTAINING CALCIUM (75.4 mM CALCIUM CHLORIDE AND 1.53 M SODIUM CHLORIDE) Error bars represent standard deviations. The data are from Experiment 2 of this study.

partially immobilized by the tissue, thereby slowing their rate of movement. This would be less likely to occur in the case of the supra-osmotic brine since the large number of sodium ions would have a tendency to saturate binding sites. This second possibility does indeed seem to be the case since at 96 h the calcium concentration in the exocarp tissue from the iso-osmotic brine is the same as that in the exocarp exposed to supra-osmotic brine, and yet very little calcium had moved beyond the exocarp (Fig. 3).

Although we have no direct evidence to rule out active transport of calcium into the cells as being a factor in the accumulation in the exocarp tissue, plant cells have a very low capacity for calcium in the cytosol (Poovaiah 1988). If calcium concentrations accumulate to millimolar levels which are present in the cell wall/apoplast regions, phosphate-based energy metabolism would be disrupted because calcium (ions) react with inorganic phosphate to form an insoluble precipitate. To avoid this problem, plant cells have an active pumping mechanism which maintains the proper calcium concentration by removing excess calcium from the cell. Enhanced movement is observed for calcium in the supra-osmotic brine because in addition to the CO₂-driven pressure reduction, when the NaCl solution crosses the epidermis and begins to penetrate the tissue, water is osmotically drawn from the cells, causing membrane plasmolysis (Jewell 1972) and thereby filling the cells, the intercellular spaces and saturating the cell walls, thus providing a pathway for calcium movement.

This research has shown that calcium penetration into whole cucumber fruit was facilitated when the brine solution was supra-osmotic to the cell sap of the fruit, and that calcium movement into brined cucumbers occurred simultaneously with chloride movement.

ACKNOWLEDGMENT

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