

Chapter 11

Function of Metal Cations in Regulating the Texture of Acidified Vegetables

R. F. McFeeters

Food Fermentation Laboratory, Agricultural Research Service, U.S.
Department of Agriculture, and
Department of Food Science, North Carolina Agricultural Research Service,
North Carolina State University, Raleigh, NC 29695-7624

The use of metal ions to modify the texture of fruit and vegetables has been investigated and used for nearly 50 years. In general, monovalent ions have been found to cause softening while divalent ions, usually calcium, either inhibit softening or in some cases actually increase firmness. However, there have been few instances in which the effect of metal ions on tissue softening rates during processing or storage have been measured. The development of a rapid procedure to measure softening rates in cucumber tissue has made it possible to investigate quantitative effects of metal ions on texture. A salt softening effect in blanched, acidified cucumber tissue was observed when NaCl or other alkali metal chlorides were added. Low concentrations of calcium, strontium, barium, and lanthanide ions inhibited the rate of tissue softening in the presence of a high concentration of NaCl. The effects of multivalent ions on inhibition of tissue softening has led to the conclusion that the egg box model, which was developed to explain gel formation by calcium ions in dilute polypectate solutions, is not a suitable model to explain metal ion effects on cucumber tissue texture. Hopefully, development of this experimental approach will lead both to improved understanding of the mechanisms of plant tissue softening and to better means to retain firmness in cucumber pickle products.

The ability of metal ions to modify the texture of fruit and vegetable tissue has been studied since 1939, when Kertesz (1) found that calcium ions improved the firmness of tomatoes. A very simplified generalization of the effects of metal ions on fruit and vegetable texture is that monovalent cations, usually Na⁺ and K⁺, cause tissue softening. Examples of this softening effect include results with peas (2,3), dried peas (4), carrots (5), potatoes (6) and green beans (7). On the other hand calcium, a divalent cation,

either causes tissue firming or prevents softening of plant tissues. Calcium effects appear to be general in plant tissues. All of the commodities mentioned above respond to calcium addition. In addition, the texture of apples (8) and apricots (9) is affected by calcium.

The USDA Food Fermentation Laboratory has been concerned over a long period of time with the factors which affect the textural quality of acidified vegetables, particularly cucumbers. As is the case with other processed fruit and vegetable products, metal ions have been found to have important textural effects on cucumbers. In contrast to observations with other plant tissues, NaCl has been found to improve firmness retention in fermented cucumbers, in addition to its ability to select for lactic acid bacteria in fermentations and its use as a flavoring (10-13). Table I shows the effect that NaCl has in preventing softening during fermentation and storage of cucumber slices. The probable reason that softening inhibition occurs is that NaCl can inhibit to some degree the action of polygalacturonases which soften tissue. This is suggested in Table I by the fact that NaCl was not required to maintain firmness in blanched cucumber slices during fermentation. In addition, earlier results of Bell and Etchells (14) show that NaCl reduces the rate of softening in pasteurized cucumber tissue to which a fixed amount of polygalacturonase activity is added. Calcium is currently added to most commercial cucumber pickle products due to its ability to prevent texture loss in both fermented (10, 11, 15-17) and non-fermented pickles (18, 19).

Table I. Effect of heat and salt on cucumber slice firmness

Heat Treatment	NaCl, %	pH	Firmness score
None	0.0	3.5	3.1C ¹
	1.4	3.3	4.6B
	3.9	3.2	5.2B
	6.5	3.2	8.3A
77°C, 3.5 min	0.0	3.6	7.9A
	1.4	3.4	8.3A
	3.9	3.3	8.6A
	6.5	3.2	8.9A

¹Means followed by different letters indicate treatments were different at the 0.05 significance level.

Source: Data are from ref. 10.

It is somewhat surprising, considering the amount of work done and the variety of different plant tissues studied, that there have not been multiple mechanisms proposed to explain metal ion effects. There has been nearly complete agreement that these effects occur due to the interaction of metal ions with the demethylated galacturonic acid residues in the pectic substances of the cell wall. Monovalent ions, i.e. NaCl, are thought to act by displacing calcium ions from pectate binding sites and thereby disrupting the

pectin gel structure. This is illustrated by some data of Van Buren (20) on green beans (Fig. 1). A direct effect of NaCl on degradation of cell wall polymers has not been reported. For divalent cations, i.e. calcium, Rees and his group in England proposed an "egg box" model (21) to explain the binding of calcium ions to polypectate in aqueous solution (Fig. 2). The key elements of this model are that calcium cations provide ionic cross-links as negatively charged galacturonic acid polymeric chains line up with each other and provide pockets into which the metal ions can fit. At low calcium levels there will initially be dimerization of pectate molecules. As more ions are bound, the dimers will aggregate into a large gel structure.

I emphasize that the egg box model was developed to explain the binding of divalent ions in polypectate solutions, not the effect of calcium ions on the physical properties of plant tissues. A rather large volume of data on the interactions of metal ions with pectin, particularly the work of Rees and his collaborators (21-26) and Kohn and coworkers (27, 29), all seem to be explainable in terms of the egg box model. With plant tissues there is much less quantitative data on ion binding in the tissues or in isolated cell walls. However, the data which have been obtained seem to be generally consistent with the egg box model (30-33). As a result, the egg box model has often been used to attempt to explain calcium ion effects on the physical properties of both living and processed plant tissues (34-40). However, clear demonstrations that physical changes in plant tissues, e.g. firming, inhibition of softening, or inhibition of cell elongation during growth, are caused by egg box binding of metal ions are lacking. In a few instances data have been published which are at least difficult to reconcile with the egg box model. Stoddard et al. (41) were unable to correlate the degree of calcium cross-linking to the rate of cell wall elongation. Tepfer and Taylor (42) found a lack of correlation between the ability of divalent ions to cause pectate gelation and their ability to inhibit the acid-induced elongation of bean hypocotyls. McFeeters et al. (19) found that changing pectin methylation in the presence of calcium ions had little or no effect on the ability of cucumber tissue to maintain firmness during storage. At low methylation there should have been more and stronger calcium cross-linkages with pectin and an inhibition of tissue softening.

Effect of Monovalent Ions on Cucumber Softening Rates

Now let us consider recent results from this laboratory concerning the effects of metal ions on the rates of texture changes in acidified cucumber tissue texture. To investigate the effect of NaCl on cucumber texture in the absence of enzymatic degradation, the firmness changes in blanched tissue under acid conditions, pH 3.2-3.8, were measured (43). The procedure was to blanch cucumber slices or mesocarp tissue pieces in boiling water for 3 min to inactivate pectinesterase (19). After cooling, the tissue samples were covered with a brine which contained acetic acid and SO₂, to equilibrate at 0.6% and 200 ppm, respectively, and the appropriate amount of NaCl. The tissue equilibrated with the brine in the cold and then samples were incubated at 44C so they would soften reasonably rapidly. Firmness of the tissue was evaluated at the

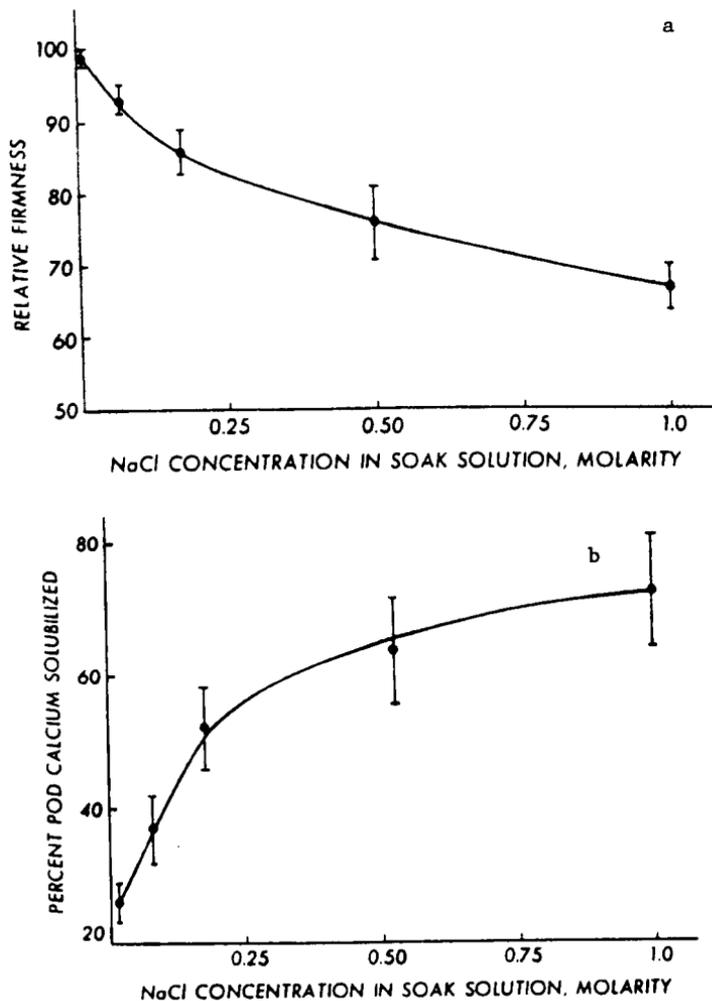


Fig. 1. Effect of NaCl on the texture and solubilization of calcium ions from green bean pods. a) Firmness of pods using a Kramer shear press. b) Solubilization of calcium ions from green bean tissue. Reprinted from reference 20.

beginning of the 44C storage and at several subsequent times by a punch test on 15 tissue pieces from duplicate jars of sample using the Instron Universal Testing Machine (44). The first order rate of the softening reaction was calculated from the slope of the softening curves (Fig. 3).

The rate of softening of cucumber mesocarp tissue increased as the equilibrated salt concentration increased for both whole cucumber slices and cucumber mesocarp tissue pieces (Fig. 4). Therefore, if the enzymatic degradation of cell walls is prevented, cucumber tissue does soften in response to NaCl, as has been observed in other fruits and vegetables. There is a question about the mechanism of the degradative process that leads to softening in acid conditions. The salt softening described for other vegetables has been observed under low acid conditions. It has been proposed that softening in those commodities is caused by β -elimination degradation of pectin, though direct evidence for β -elimination reactions in vegetable tissues has not been obtained (7). However, with the equilibrated pH of the tissue in these experiments in the range of 3.2-3.8, the pH within the cucumber cell wall is almost certainly too low for β -elimination to occur (45). Therefore, there may be a different salt softening mechanism in acidified plant tissues as compared to low acid vegetables. Fig 4b also shows the degree of pectin methylation in the cell walls of the cucumber mesocarp tissue used in this experiment. Two points should be remembered. First, the degree of methylation is relatively constant in blanched tissue and, secondly, it is quite high. Over 60% of the galacturonic acid residues have methyl esters and are, therefore, uncharged. This becomes an important consideration when we consider the effect of calcium ions on the softening rate in light of the requirement of ionized carboxyl groups for cross-linking of pectin molecules according to the egg box model. The salt softening effect in cucumbers was also similar to results in green beans (46) in that sodium and potassium ions had similar softening effects. In fact, at 0.25 M all of the alkali metal ions had a similar effect on the rate of cucumber tissue softening (Table II). Thus, the size of the ions do not appear to affect their ability to increase softening rates.

Table II. Effect of 0.25 M alkali metal ions on the rate of softening of blanched cucumber mesocarp tissue held at 44°C

Metal Ion	Softening rate, day ⁻¹
None	0.055A ¹
Lithium	0.175B
Sodium	0.194B
Potassium	0.159B
Rubidium	0.169B
Cesium	0.166B

¹Means followed by different letters indicate treatments were different at the 0.05 significance level.

Source: Data are from ref. 43.

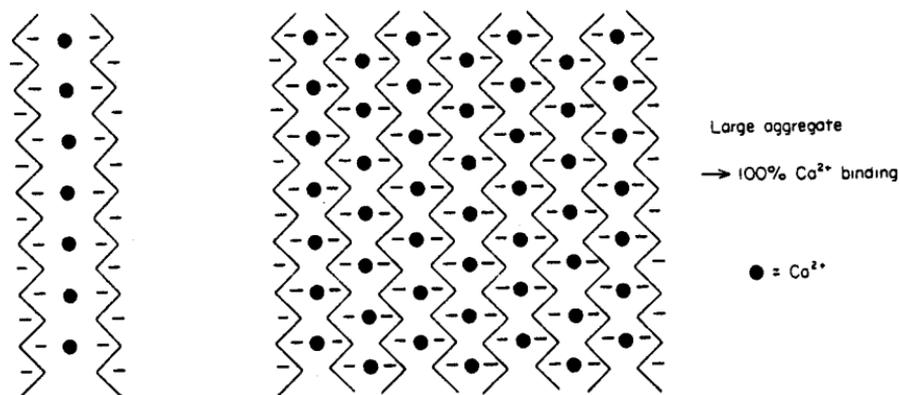


Fig. 2. Egg box model for cross-linking of polypectate chains by calcium ions. Reprinted from reference 25.

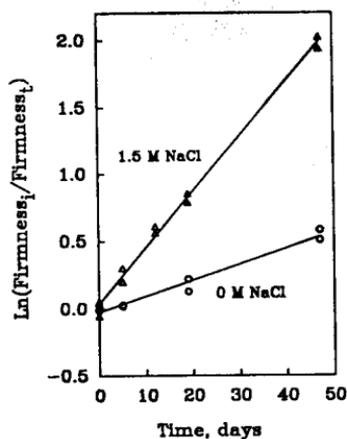


Fig. 3. First order plot of cucumber tissue softening in acid brine with and without added NaCl. The subscript i is the initial firmness, and the subscript t indicates firmness at any subsequent time. From reference 43.

Inhibition of Softening Rates by Calcium

Once it had been demonstrated that NaCl enhanced the rate of cucumber tissue softening, the next question was whether and in what way calcium ions would inhibit the rate of softening. To answer this question a combination of 1.5 M NaCl and 0 to 80 mM calcium ions were added first to blanched cucumber slices and then to cucumber mesocarp tissue (47) (Fig. 5). In both experiments there was an excellent fit of a hyperbolic curve to the softening rates as a function of calcium added, since the hyperbolic model accounted for over 99% of the experimental variation. For the cucumber slices (Fig. 5a), half of the observed inhibition of softening rate occurred at 6.3 mM calcium. For the mesocarp pieces (Fig. 5b), half maximal inhibition occurred at 1.5 mM calcium ion. These results indicated that even in the presence of high NaCl concentrations low calcium ion concentrations could saturate some binding site that resulted in inhibition of texture loss.

A problem with the results from these experiments was that the absolute rates of softening were highly variable from one lot of cucumbers to another even though the pattern of responses to metal ions was very consistent. Notice, for example, that the softening rates without added calcium differ by a factor of about 15 (Fig. 5) even though the rates at high calcium concentrations were similar. In view of the inhibitory effect of calcium on softening rates, the natural calcium levels in lots of cucumbers obtained over a two year period were compared to the softening rates in 1.5 M NaCl, but in the absence of added calcium. Fig. 6 shows a reasonable hyperbolic relationship between calcium level and softening rates with the model accounting for 97% of the experimental variation. This suggested that a major reason for lot to lot variation in softening of cucumber tissue was the natural variation in the calcium content of the fruit.

The "Egg Box" Model as an Explanation for Softening Inhibition

Now let us turn our attention to the question of whether it is reasonable to attribute the inhibition of softening by calcium ions to cross-linking of pectin molecules in the cell wall according to the egg box model(21). The conditions of the calcium inhibition experiment shown in Fig. 5b were such that it would be expected that calcium cross-links would be relatively infrequent. If we consider the calcium concentration which gives half maximal inhibition of softening, 1.5 mM, there was only a small proportion of calcium ions present relative to 1500 mM sodium ions. The pK of galacturonic acid residues in pectate solutions is about 3.6 (48) while the pH in the experiment was 3.2 so that a large proportion of the carboxyl groups would be protonated and, therefore, uncharged. The degree of pectin methylation was 62%, which means that only 38% of the galacturonic acid residues in the cell wall would be non-esterified even if the pH were high enough for the carboxyl groups to be ionized. If it is assumed that the methoxyl groups in the tissue were randomly distributed, as has been reported by Anger and Dongowski (49), the selectivity coefficient between sodium and calcium ions binding to pectin will be relatively small (27). Taking all of these factors into account it was estimated that there would be only one calcium

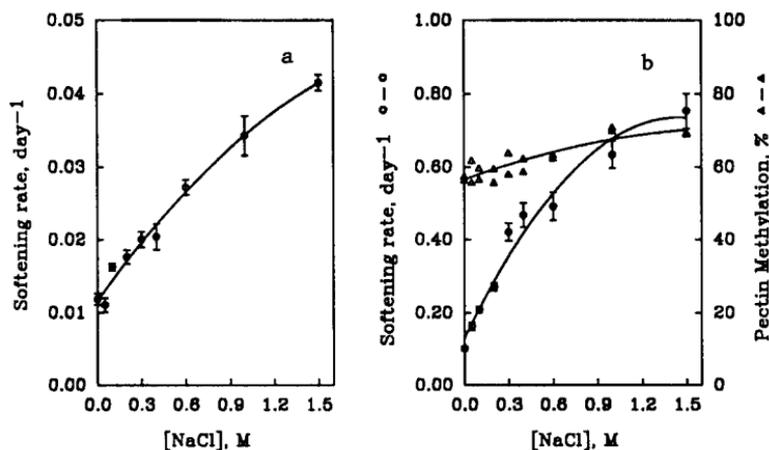


Fig. 4. Effect of NaCl concentration on the rate of cucumber tissue softening in acidic conditions. a) Softening rates of cucumber slices. b) Softening rates and pectin methylation changes in cucumber mesocarp tissue pieces. From reference 43.

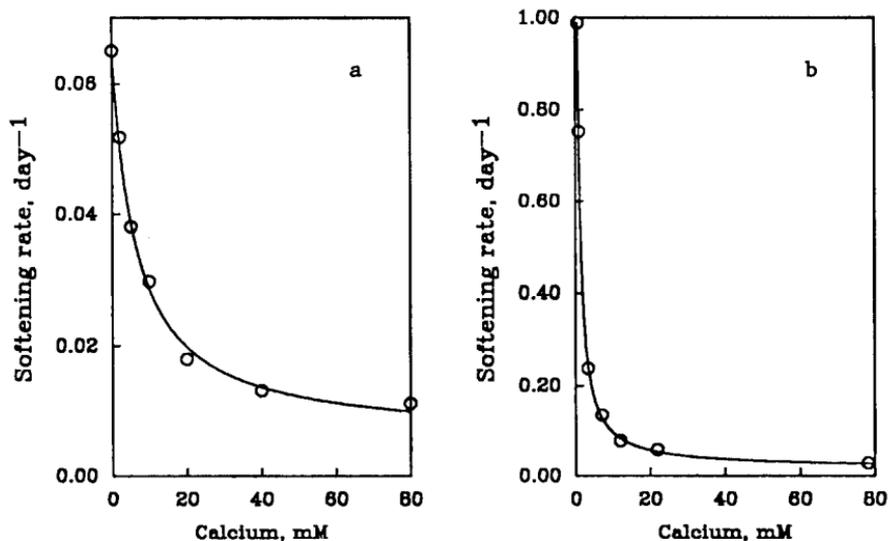


Fig. 5. Effect of calcium chloride concentration on the rate of cucumber tissue softening in 0.6% acetic acid and 1.5 M NaCl. a) Softening rates of cucumber slices. b) Softening rates of cucumber mesocarp tissue pieces. From reference 47.

cross-link per 2700 galacturonic acid residues. It seems difficult to explain such a large calcium effect on texture upon such infrequent cross-linking events in the cell wall. Nevertheless, such an estimate contains many assumptions so that it could easily be off by a considerable magnitude. Also, it is at least possible that in plant cell walls even rare calcium cross-links could have a large effect on texture, though Deuel et al. (50) and Doesburg (45) suggested that isolated calcium cross-linkages would not give a strong pectin gel structure. Therefore, the effects of other ions on softening rates were evaluated to assess in another way whether egg box binding is a reasonable mechanism to explain the effect on calcium on cucumber tissue texture under acid conditions.

The results of the addition of 10 mM levels of various ions in place of calcium are shown in Fig. 7. The ions are shown in the order of increasing binding affinity to pectate based upon the data of Kohn (28, 29), except for barium and aluminum for which binding data are not available. Calcium, strontium and barium were good softening inhibitors. However, zinc, cobalt and cadmium ions, which have affinities equal to or greater than calcium, showed little or no inhibition of softening. Copper, which binds much more strongly to pectin than calcium, has considerably less of an inhibitory effect than calcium. The conclusion from these results was that little or no correlation existed between the affinity of metal ions for pectin and their inhibition of softening. Like the estimate of calcium cross-link frequency, this result also appeared to be inconsistent with the egg box model. In this experiment only the alkaline earth ions, calcium, strontium and barium were good inhibitors of softening so it appeared that the binding sites for calcium, whatever their chemical structure, were relatively specific for calcium. To test this idea another group of ions, the lanthanides, were tested for their ability to inhibit softening. Several lanthanide ions have been found to specifically bind with high affinity to calcium sites in calcium binding proteins such as calmodulin (51). Thus, in their size and electronic structure they seem to be reasonably good analogs of calcium. The lanthanide ions were added to blanched cucumber mesocarp tissue in the presence of 1.5 M NaCl and their ability to inhibit softening was calculated in terms of the equivalent amount of calcium required to give the same degree of inhibition of softening rates. Since all of the lanthanides had a calcium equivalence greater than 0 mM, they all inhibited cucumber tissue softening to some degree (Fig. 8). Since 10 mM concentrations of each ion were used, any calcium equivalence value greater than 10 mM meant that the ion was a better inhibitor of softening than calcium. The six lanthanides with ionic radii greater than gadolinium exceeded the 10 mM equivalence level. The conclusion from these experiments was that the binding sites which affected texture were quite specific for calcium ions or calcium ion analogs rather than for ions which bound to pectic substances with high affinity.

One final experiment addressed to the question of the egg box model and textural effects was to determine the ability of cadmium ions to reverse the effect of calcium ions on softening. Cadmium ion did not inhibit softening (Fig. 7) even though Kohn (29) has demonstrated that it binds to pectate with greater affinity than calcium. It also has an ionic radius nearly the same as calcium

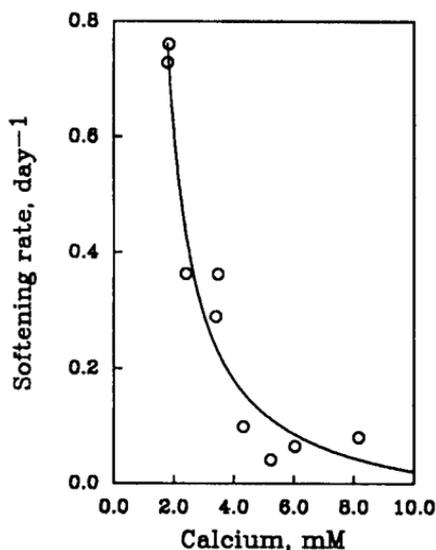


Fig. 6. Relationship between the natural concentration of calcium ions in cucumbers and softening rates of cucumber tissue in 0.6% acetic acid and 1.5 M NaCl. From reference 47.

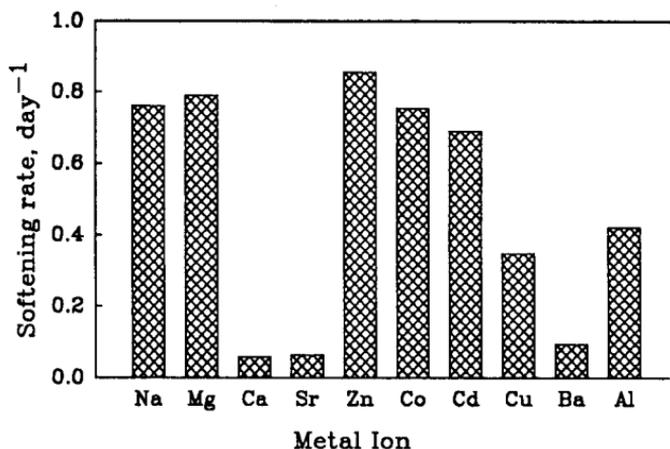


Fig. 7. Effect of 10 mM concentrations of divalent and trivalent cations on the rate of cucumber mesocarp tissue softening in 0.6% acetic acid and 1.5 M NaCl. From reference 47.

(52) and, of course, the same charge as calcium. It was possible that cadmium did not inhibit softening because it did not form cross-linkages with pectin in a way which was effective in inhibiting degradation of the cell wall structure. To test this possibility it was reasoned that if cadmium and calcium ions were added together to cucumber tissue along with 1.5 M NaCl, the cadmium ion should inhibit the calcium effect on softening if the egg box model was operative. Softening rates with 36 combinations of calcium and cadmium ion concentrations were measured. The data were analyzed by doing reciprocal plots similar to those which are used to evaluate enzyme inhibition by competitive, non-competitive and uncompetitive inhibitors. In the reciprocal plot (Fig. 9), high softening rates are toward the x-axis and high cadmium concentrations are toward the y-axis. The line without calcium added to the cucumber tissue shows that the same softening rate occurred with 2 to 80 mM cadmium added. This confirmed the result found for 10 mM cadmium in Fig. 7 over a range of concentrations. The expectation was that if calcium was having an effect due to egg box binding, cadmium ions should competitively displace calcium ions and reverse the calcium effect. This would result in a group of lines at different concentrations of added calcium all intersecting at the y-axis at the zero added calcium intercept. The fact that the reciprocal plots were parallel to the x-axis at all calcium concentrations indicated that cadmium was not competing with the site through which calcium had its textural effect. Since both ions presumably would bind to pectin, the simplest conclusion was that calcium had its effect on softening rates by binding at some site other than pectin carboxyl groups.

Summary and Conclusions

To briefly summarize the results as they relate to the egg box model, estimates of a very low frequency of calcium cross-links expected under the experimental conditions used, the lack of correlation between the affinity of metal ions for pectin and the ability of those ions to inhibit softening, the apparent specificity of softening inhibition by calcium or calcium analogs, and the lack of competition between calcium and cadmium ions, all appeared to be inconsistent with the hypothesis that egg box binding was responsible for the inhibition of softening of cucumber tissue by multivalent ions. We need to begin looking for other mechanisms by which cucumber tissue softening can be inhibited by calcium and certain other ions. These results do not in any way suggest that the egg box mechanism is not a valid mechanism to explain metal ion binding in pectin solutions. It appears, however, that the egg box model cannot be extended to explain the physical effects of calcium ion addition to cucumber mesocarp tissue in this situation. It also means that we must be careful in trying to explain the textural effects of metal ions in terms of the egg box model as we study other processing situations. The egg box model may be operative in some situations, but not in all.

There does not exist a known alternative mechanism to the egg box model which can explain the metal ion effects which have been observed. While the experimental results obtained to date argue rather strongly against the egg box idea, they do not suggest an

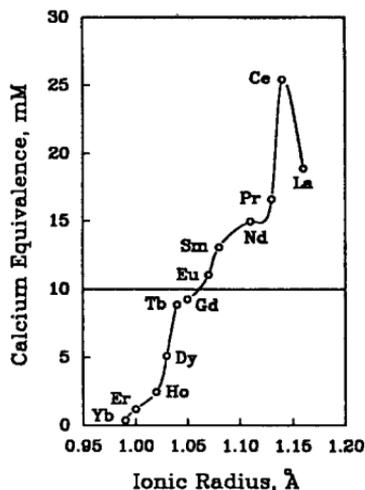


Fig. 8. Comparison of the inhibitory effect of 10 mM lanthanide ions on cucumber tissue softening rates relative to the inhibitory effect of calcium ion. From reference 47.

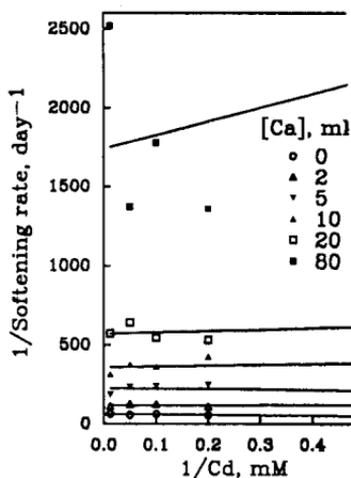


Fig. 9. Evaluation of possible competition between calcium and cadmium ion for inhibition of cucumber mesocarp softening. From reference 47.

obvious alternative. Some possible mechanisms which can be imagined include: 1. Non-enzymatic degradation of one or more cell wall polysaccharides which can be specifically inhibited by calcium and calcium analogs; 2. A heat stable enzyme which degrades a cell wall component and which is inhibited by calcium; or 3. A calcium mediated structural protein, similar to the cadherins found in animal cells (53), which is involved in plant cell interactions. The fact that it is now possible to experimentally affect the rates of softening processes in cucumbers by a variety of metal ions provides us new tools to investigate the chemistry of plant tissue texture. The ability to carry out experiments with gram to kilogram quantities of a single tissue type should aid efforts to isolate and characterize degraded saccharides from softened mesocarp. Several of the lanthanide ions, which proved to be excellent inhibitors of softening, have useful spectroscopic properties, especially their ability to perturb NMR spectra (51). This could be a way to probe the interaction of metal ions with cell wall components. Questions about the generality of these texture effects in other fruits and vegetables and the interaction between metal ions and other processing variables, such as pH, are experimentally accessible with the techniques now available. Hopefully, as these experimental possibilities are exploited new ideas concerning the mechanisms of tissue softening and the effects of metal ions on softening will emerge.

Beyond the interesting scientific questions concerning the chemistry of texture, the purpose for doing research in this area is to have an impact on the processing practices of the industry and ultimately an impact on the quality of products available to people. This sometimes seems to be a distant goal. However, it should be possible to identify processing operations in which changes can be made to improve texture retention as we look at interactions between acidification practices, the addition of sodium and potassium salts, and calcium levels in the tissue, both the natural calcium and that added during processing.

Acknowledgments

This investigation was supported in part by a research grant from Pickle Packers International, Inc., St. Charles, Illinois.

Paper no.12114 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, NC 27695-7643. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U. S. Department of Agriculture or North Carolina Agricultural Research Service, nor does it imply approval of other products that may be suitable.

Literature cited

1. Kertesz, Z. I. Canner 1939, 88(7), 26.
2. Blair, J. S.; Ayres, T. B. Ind. Engr. Chem. 1943, 35, 85.
3. Lenz, R. J.; Weckel, K. G. Research Report N. 27, College of Agriculture, Univ. of Wisconsin, Sept., 1967.
4. Mattson, S. Acta Agric. Suecana 1946, 2, 185.
5. Sterling, C. J. Food Technol. 1968, 3, 367.

6. Hughes, J. C.; Grant, A.; Faulks, R. M. J. Sci. Food Agr. 1975, 26, 739.
7. Van Buren, J. P. In Chemistry and Function of Pectins; Fishman, M. L.; Jen, J. J., Ed.; American Chemical Society: Washington, DC, 1986; p. 190.
8. Archer, R. W. Canner-Packer Sept., 1962, 131, 28.
9. Mohammadzadeh-Khayat, A.; Luh, B. S. J. Food Sci. 1968, 33, 493.
10. Fleming, H. P.; Thompson, R. L.; Bell, T. A.; Hontz, L.H. J. Food Sci. 1978, 43, 888.
11. Thompson, R. L.; Fleming, H. P.; Monroe, R. L. J. Food Sci. 1979, 44, 843.
12. Hudson, J. M.; Buescher, R. W. J. Food Biochem. 1985, 9, 211.
13. Fleming, H. P.; McFeeters, R. F.; Thompson, R. L. J. Food Sci. 1987, 52, 653.
14. Bell, T. A.; Etchells, J. L. J. Food Sci. 1961, 26, 84.
15. Buescher, R. W.; Hudson, J. M.; Adams, J.R. J. Food Sci. 1979, 44, 1786.
16. Buescher, R. W.; Hudson, J. M.; Adams, J. R. Lebensm.-Wiss. u. -Technol. 1981, 14, 65.
17. Tang, H. C. L.; McFeeters, R. F. J. Food Sci. 1983, 48, 66.
18. Etchells; J. L., Bell, T. A.; Fleming, H. P. Advisory Statement: Use of Calcium Chloride to Improve the Texture of Pickles; Pickle Packers International, Inc.: St. Charles, IL.
19. McFeeters, R. F.; Fleming, H. P.; Thompson, R. L. J. Food Sci. 1985, 50, 201.
20. Van Buren, J. P. J. Food Sci. 1984, 49, 910.
21. Grant, G. T.; Morris, E. R.; Rees, D. A.; Smith, P. J. C.; Thom, D. FEBS Letters 1973, 32, 195.
22. Gidley, M. J.; Morris, E. R.; Murray, E. J.; Powell, D. A.; Rees, D. A. J.C.S. Chem. Comm. 1979, 1979, 990.
23. Gidley, M. J.; Morris, E. R.; Murray, E. J.; Powell, D. A.; Rees, D. A. Int. J. Biol. Macromol. 1980, 2, 332.
24. Thom, D.; Grant, G. T.; Morris, E. R.; Rees, D. A. Carbohy. Res. 1982, 100, 29.
25. Morris, E. R.; Powell, D. A.; Gidley, J. J.; Rees, D. A. J. Mol. Biol. 1982, 155, 507.
26. Powell, D. A.; Morris, E. R.; Gidley, J. J.; Rees, D. A. J. Mol. Biol. 1982, 155, 517.
27. Kohn, R.; Furda, I. Coll. Czech. Chem. Commun. 1967, 32, 4470.
28. Kohn, R. Pure Appl. Chem. 1975, 42, 371.
29. Kohn, R. Carbohy. Res. 1987 160, 343.
30. Knee, M. Phytochem. 1978, 17, 1257.
31. Irwin, P.; Sevilla, M.; Shieh, J. Biochim. Biophys. Acta 1984, 805, 186.
32. Irwin, P.; Sevilla, M.; Stoudt, C. Biochim. Biophys. Acta 1985, 842, 76.
33. Bush, D. S.; McColl, J. G. Plant Physiol. 1987, 85, 247.
34. Van Buren, J. R. J. Texture Studies 1979, 10, 1.
35. Darvill, A.; McNeil, M; Albersheim, P.; Delmer, D. P. In The Biochemistry of Plants: A Comprehensive Treatise; Stumpf, P. K.; Conn, E. E.; Tolbert, N. E., Eds.; Academic Press: New York, NY, 1980; Vol. 1, Chapter 3.
36. Knef, M.; Bartley, I. M. In Recent Advances in the Biochemistry of Fruits and Vegetables; Friend, J.; Rhodes, M. J. C., Eds.; Academic Press: New York, NY, 1981; p. 133.

Reprinted from ACS Symposium Series No. 405

Quality Factors of Fruits and Vegetables: Chemistry and Technology

Joseph J. Jen, Editor