

Softening Effects of Monovalent Cations in Acidified Cucumber Mesocarp Tissue

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ABSTRACT

Due to the importance of salt as an ingredient in cucumber pickle products, the effect of salt concentration on first-order softening rates during acid storage was determined. Softening rates of unfermented tissue increased as the NaCl increased from 0 to 1.5M, whether or not the tissue was blanched. For unheated, fermented tissue, softening rates increased between 0 and 0.2M NaCl but did not increase above 0.2M NaCl. Changes in the degree of pectin methylation were not highly correlated with changes in softening rates. The ions Li⁺, K⁺, Rb⁺, and Cs⁺ had softening effects similar to Na⁺. An enthalpy of activation of 145 kJ/mole was determined for softening of blanched tissue stored in 1.5M NaCl. This is the first demonstration of a softening effect by monovalent cations in cucumber tissue.

INTRODUCTION

WITHIN THE PICKLING INDUSTRY, salt has historically been used for directing the fermentation of cucumbers, as a preservative, and as a flavoring agent. The textural effect of NaCl in cucumber pickle products is generally considered to help maintain tissue firmness. Data have been obtained on the effect of NaCl on fruit firmness after fermentation and storage (Fleming et al., 1978; Thompson et al., 1979; Hudson and Buescher, 1985; Fleming et al., 1987). These studies have shown that higher concentrations of NaCl added at the time of brining or after the active fermentation period results in better texture retention during storage. Fleming et al. (1987) found that 2.6% NaCl inhibited softening which occurred without NaCl, but higher NaCl levels did not result in further improvement in texture. This effect of NaCl is most likely due to the fact that it either inhibits degradative enzymes or prevents the growth of microorganisms that produce degradative enzymes since blanching cucumbers prior to fermentation prevented firmness loss during storage even in the absence of NaCl (Fleming et al., 1978). Also, addition of NaCl to cucumbers pasteurized at 74°C and subsequently inoculated with polygalacturonase activity inhibited softening (Bell and Etchells, 1961). Though the effects of acids (Bell et al., 1972), alum (Etchells et al., 1972) and calcium (Etchells et al., 1977; McFeeters et al., 1985) on the retention of firmness in unfermented cucumber pickles have been investigated, the influence of NaCl concentration on texture in unfermented cucumber tissue has not been reported.

Investigations of the effect of NaCl and occasionally other monovalent ions during cooking or retorting have been conducted on several low acid vegetables such as potatoes (Hughes et al., 1975), peas (Blair and Ayers, 1943; Lenz and Weckel, 1967), carrots (Sterling, 1968), and green beans (Van Buren and Peck, 1982; Van Buren, 1986; Van Buren et al., 1988). Van Buren (1986) distinguished two effects of NaCl on snap bean pods. One effect was tissue softening that occurred during heating at 115°C. The second was a salt-soak effect which also

caused firmness loss, presumably due to calcium displacement from the tissue. These studies were conducted using neutral pH conditions, and the tissues were heated so that enzymatic softening reactions would be absent. These different conditions could account for the observed softening effect of salt in low acid vegetables compared to a firming effect for fermented cucumbers where the fruit are not heated and the pH is between 3 and 4.

Despite studies over the years of the effects of chemical treatments such as metal ions, pH, pectin methylation and other factors on the textural properties of fruits and vegetables (Doesburg, 1965; Van Buren, 1979, 1986; Hudson and Buescher, 1985, 1986; McFeeters et al., 1985), determinations of rate constants for textural changes resulting from chemical treatments have not been made. Huang and Bourne (1983) pointed out that kinetic evaluation of texture changes in foods have been done in relatively few cases, even though in situations in which it has been applied, softening reactions have followed apparent first-order kinetics (Nagel and Vaughn, 1954; Nicholas and Pflug, 1962; Paulus and Saguy, 1980). Kinetic analysis in the study of texture, as with chemical reactions in general, is necessary for quantitative evaluation of chemical or physical effects on textural changes. It will allow quantitative comparisons among treatment effects on both the rate and the extent of softening. It can be an important tool in efforts to differentiate among possible mechanisms for tissue softening.

The objective of this study was to determine softening rates for cucumber tissue stored in acid conditions in order to evaluate the effect of monovalent cations on the rate of tissue softening.

MATERIALS & METHODS

CUCUMBER TISSUES were used either as slices or mesocarp pieces. Size 3A (38 to 44 mm diameter) or size 3B (45 to 51 mm diameter) cucumbers were obtained from local commercial processing plants. When whole slices were used, the fruit were sized, washed and cut into 7 mm thick slices with a food slicer. Approximately 15 mm of tissue from both the stem and blossom ends of the fruit were discarded. All slices within an experiment were randomly mixed prior to blanching and filling jars. Slices were placed in single layers in a wire basket. The layers were separated by a 20 mm wire mesh divider to allow uniform heating. Slices were blanched for 3 min in boiling distilled water and cooled for 2 min in 20°C water. Blanched slices (180 ± 2 g) were packed in 12-oz jars (360 mL), covered with 180 mL of brine and closed. All cover brines contained 1.2% acetic acid and 400 ppm SO₂, added as sodium metabisulfite, which were calculated to equilibrate at 0.6% acetic acid and 200 ppm SO₂ in the jars. Metal ions were added to the cover brines as their chloride salts. After closing, the jars were held at 17°C for 3 to 5 days and inverted occasionally to help assure equilibration of soluble components. The jars were transferred to a 44°C incubator. At the time of transfer and at four subsequent times, duplicate jars were opened and 15 slices were punched with a 3.15 mm diameter flat punch mounted on an Instron Universal Testing Machine (Thompson et al., 1982). Samples were equilibrated at room temperature prior to the firmness measurement. The maximum force required to puncture the cucumber tissue was recorded. First-order rate constants for the loss of tissue firmness were calculated for each experimental treatment (Huang and Bourne,

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1983). The effect of NaCl concentration on the firmness of unblanched cucumber slices was studied using the same brining procedure except that the blanching step was omitted. The effect of temperature on tissue softening rates was determined for blanched cucumber slices in 1.5 M NaCl by using the same procedure described above except that 10 jars were placed at 60°, 52°, 44°, 37°, 30°, and 17°C after the initial equilibration period.

Later experiments were done using mesocarp pieces rather than whole slices. Pieces were prepared by peeling the whole cucumbers with a vegetables peeler. The fruit were cut into 7 mm thick slices, and mesocarp sections from each of the three fruit carpels were removed from the slices. The peel and seed area tissues were discarded. About 400g of tissue were placed in 4 layers in a stainless steel wire mesh basket. Each layer was separated by a stainless steel screen to allow water circulation during blanching. Blanching was done for 3 min in approximately 13L of boiling distilled water and then cooled for 2 min in 20°C distilled water. Mesocarp pieces (30 ± 0.5 g) were packed in 60 mL wide mouth jars (Wheaton Scientific, Millville, NJ), covered with 30 mL of the appropriate cover brine, prepared as described above and closed with a tight fitting snap cap. Equilibration, incubation at 44°C, data collection and analyses were the same as described for whole slices.

Softening of fermented tissue was determined on cucumbers prepared by a modified controlled fermentation procedure (Etchells et al., 1973). Washed whole cucumbers (1892 ± 10 g/jar) were packed into 3.8 L jars. The fruit were covered with 1892 mL of cover brine and closed. Four hours after brining, each jar was inoculated with 2.2 mL of a 16-hr *Lactobacillus plantarum* WSO culture which had been grown in MRS broth (de Man et al., 1960). The cover brines of all treatments contained 107 mM acetic acid and 72 mM NaOH. Salt concentrations added to the cover brines were 0, 0.4, 0.8, 1.2, 2.0 and 3.0M. Duplicate jars were fermented at each NaCl concentration. Fermentations were done at 27°C. After 32 days of fermentation, when all fermentable sugars had been utilized, the cucumbers were sliced (7 mm thickness) and packed into 360 mL (12 oz) jars as described above and covered with the fermentation brines from the respective jars. The fermented slices were then incubated at 44°C and analyzed as described previously.

Pectin methylation analysis. Uronic acid and pectin methylation in the cell wall samples were analyzed as described previously (McFeeters and Armstrong, 1984), except that the Saeman hydrolysates (Blakeney et al., 1983) after ammonium hydroxide addition were used for the colorimetric analysis of the uronic acids. McFeeters and Lovdal (1987) showed that use of these hydrolysates reduced the variability in the analysis compared to that obtained using the hydrolysis procedure of Scott (1979). Methylation analysis was performed on cell wall samples prepared after texture measurements were completed.

pH determinations. Measurement of the pH of brines after equilibration with cucumber tissue was done using an Orion model 901 pH meter with a combination electrode.

RESULTS

THE PROCEDURES described for sample preparation and textural analysis allowed relatively rapid measurement of texture changes in large numbers of samples. The firmness measurements have been shown to be highly correlated with sensory firmness ratings (Thompson et al., 1982). An example of firmness changes in cucumber slices at high and low NaCl concentrations is shown in Fig. 1. The slopes of such curves are the first-order rate constants for softening. In all experiments, duplicate jars were analyzed at five different times. Usually, r^2 value for the linear fit of the data was 0.95 or greater. The presence of sodium metabisulfite, added as a preservative to eliminate the need to pasteurize sample jars after packing, did not have a measurable effect on softening rates. This was determined by comparison of softening rates in 1.5 M NaCl at pH 3.2 (data not shown). The lack of development of cloudiness in the jars indicated that under these conditions microbial growth did not occur in the absence of bisulfite.

The initial experiment to determine the effect of NaCl on the firmness changes in cucumber mesocarp tissue was done using blanched slices stored at 44°C. Blanching was done to inactivate enzymes in the cucumber tissue. The pH range of

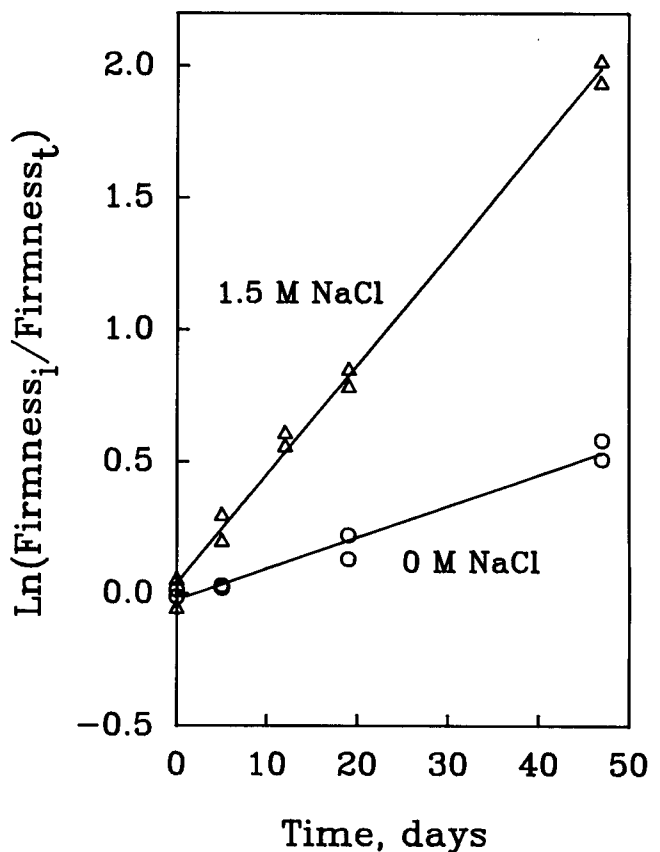


Fig. 1.—First-order plot of cucumber tissue softening at 44°C with 0 and 1.5M added NaCl. The subscript i is the initial firmness, and t is the firmness at time t . The slope with 0M NaCl is 0.0119 ± 0.0008 , and the slope with 1.5M NaCl is 0.0415 ± 0.0011 .

the sample brines after equilibration was 3.5 to 3.7. The relationship between the first-order rate constants for tissue softening and NaCl concentration is shown in Fig. 2. Rather than inhibiting the rate of softening, as has been observed in fermented cucumbers (Fleming et al., 1978; Thompson et al., 1979; Hudson and Buescher, 1985), increasing salt levels increased the rate of tissue softening. This result was confirmed using mesocarp tissue pieces (Fig. 3). When mesocarp tissue was used, the pH of the equilibrated brine samples was in the range of 3.2 to 3.6. The degree of pectin methylation in the mesocarp pieces varied between 57% and 72%. Other alkali metal ions, Li^+ , K^+ , Rb^+ and Cs^+ , increased the rate of mesocarp tissue softening to about the same extent as Na^+ ions (Table 1).

Cucumber slices were stored under the same conditions as described above except that the slices were not blanched. This would allow cucumber enzymes which were not inactivated by the low pH (~3.6) to remain active during the storage period. Salt also increased the rate of softening in unblanched cucumber slices (Fig. 4). Since the cucumber tissue was not heated, pectin methyltransferase would remain active during the experiment (McFeeters et al., 1985). As a result, the degree of pectin methylation was lower and more variable than in the blanched samples.

Finally, whole unblanched cucumbers were fermented at 27°C and then sliced and stored at 44°C to accelerate the rate of tissue softening. The softening rate when 0.2M NaCl was added was greater than when NaCl was not added to the fermentation (Fig. 5). There was no further increase in mesocarp softening rates at salt concentrations above 0.2M NaCl. The degree of pectin methylation in the fermented tissue varied from 7.3%

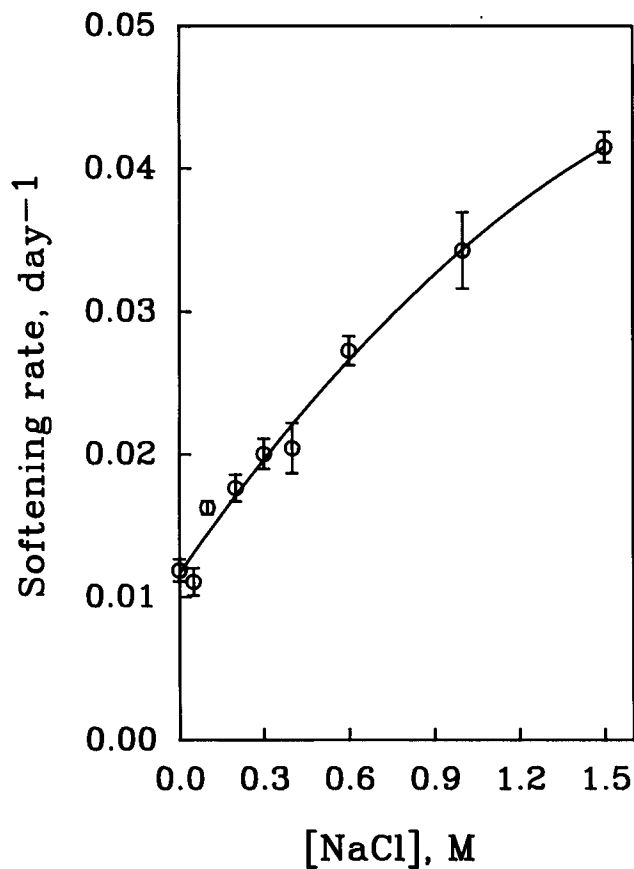


Fig. 2.—Effect of NaCl concentration on mesocarp tissue softening rates during storage of blanched cucumber slices at 44°C. Vertical bars indicate the standard deviation of the softening rates.

to 32%, depending upon the amount of NaCl added at the time of brining.

The studies described above were done at a high storage temperature to reduce the time required for extensive softening to occur. However, it was possible that the mechanism of softening at 44°C might be different from that at lower or higher temperatures. This possibility was tested by measuring the effect of temperature over the range of 17° to 60°C on the rate of softening in blanched cucumber slices stored in 1.5M NaCl. Analysis of the temperature data using transition state theory (Whitaker, 1972) gave a linear relationship $r^2 = -0.995$ (Fig. 6). This suggested that the same softening mechanism was involved over this temperature range. An enthalpy of activation of 145 kJ/mole was calculated for the softening process.

DISCUSSION

THE PROCEDURE used to determine softening rates has several useful features in assessing the effect of chemical treatments on textural changes in acid conditions. Firstly, relatively large numbers of samples can be prepared and evaluated so that it is feasible to determine rate changes under a variety of conditions. Secondly, samples can be equilibrated prior to starting texture measurements, since heating is not required to prevent microbial spoilage of the samples. Thirdly, texture measurements can be made on small pieces of tissue. This has the advantage that it is practical to use compounds which are difficult to prepare or relatively expensive as, for example, CsCl in this study. Also, samples of a single type of tissue can be prepared for chemical analysis after texture measurements have been done. This procedure could easily be adapted for use with a variety of fruit and vegetable tissues.

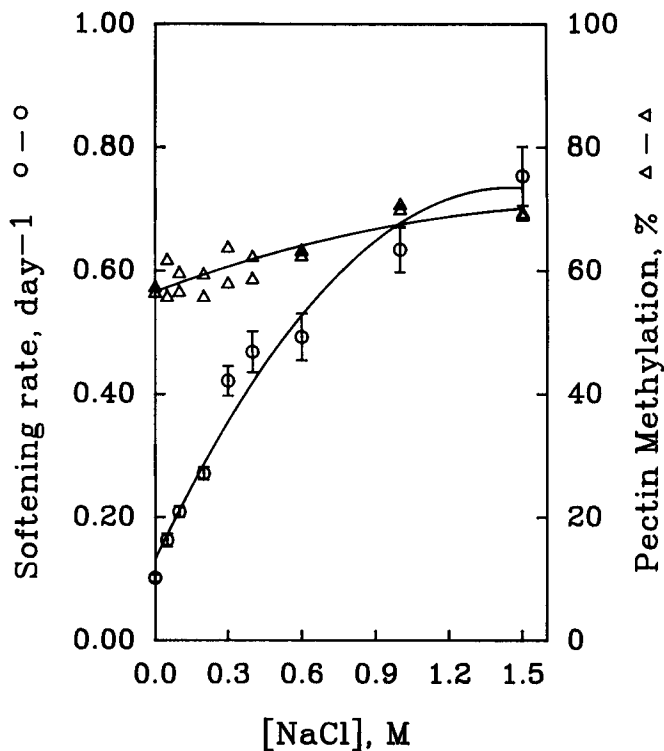


Fig. 3.—Effect of NaCl concentration on blanched mesocarp tissue softening rates and pectin methylation during storage at 44°C. Vertical bars indicate the standard deviation of the softening rates.

Table 1—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	0.0547A
Lithium	0.1753B
Sodium	0.1938B
Potassium	0.1587B
Rubidium	0.1688B
Cesium	0.1656B

* Means followed by different letters indicate treatments were different at the 0.05 significance level using an LSD test.

In contrast to observations on fermented cucumbers that NaCl tends to reduce softening during storage (Fleming et al., 1978; Thompson et al., 1979; Hudson and Buescher, 1985; Fleming et al., 1987), these experiments have shown for the first time a salt softening effect in cucumber tissue. There are likely several reasons for the different salt effects. Firstly, we are probably seeing a direct effect of NaCl on the cell wall polysaccharides, whereas the dominant effect in the fermentation studies was probably an effect of salt on either enzyme activity or microbial activity (Bell and Etchells, 1961; Fleming et al., 1978). Secondly, fermentation may alter the physical or chemical structure of the cell wall to change the direct effect of salt on the wall components. This possibility is suggested by the observation that increasing salt from 0 to 0.2M resulted in an increase in softening rate, but raising the NaCl concentration above 0.2M in fermented tissue had no further effect on the softening rate. In contrast, the unfermented tissues, whether blanched or not heated, showed an increased response to salt up to 1.5M.

A difference was observed in the variability in softening rates between heat-treated and unheated tissues. A comparison of the standard deviations of the softening rates shows that the variation in heated samples (Fig. 2 and 3) was considerably less than the unheated samples (Fig. 4 and 5). Reasons for the

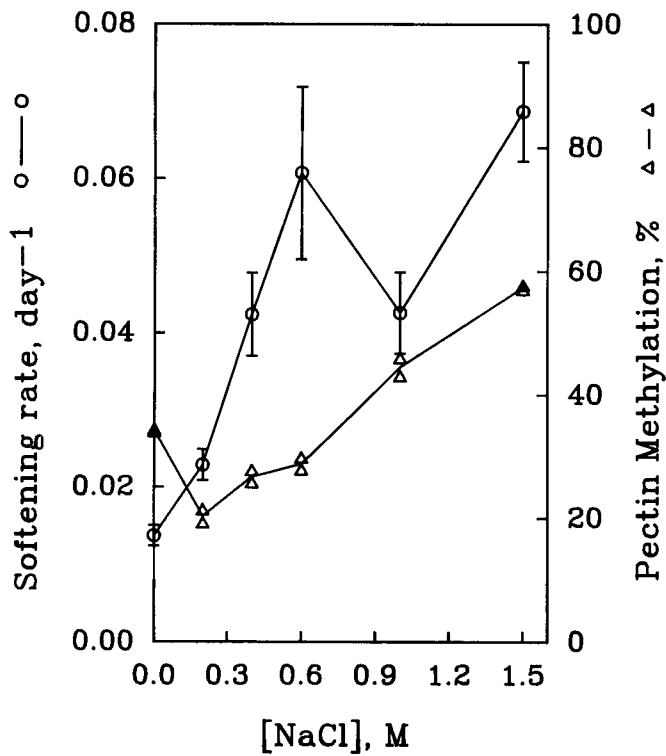


Fig. 4.—Effect of NaCl concentration on mesocarp tissue softening rates and pectin methylation during storage of unheated cucumber slices at 44°C. Vertical bars indicate the standard deviation of the softening rates.

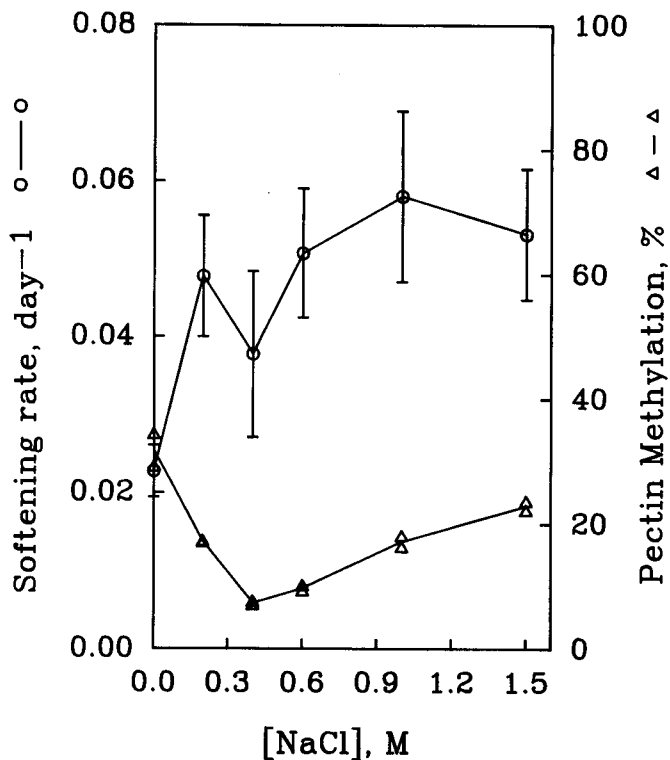


Fig. 5.—Effect of NaCl concentration on mesocarp tissue softening rates and pectin methylation during storage of fermented cucumber slices at 44°C. Vertical bars indicate the standard deviation of the softening rates.

difference in variability are not known. It may be of interest in the future to determine if there is a consistent effect of

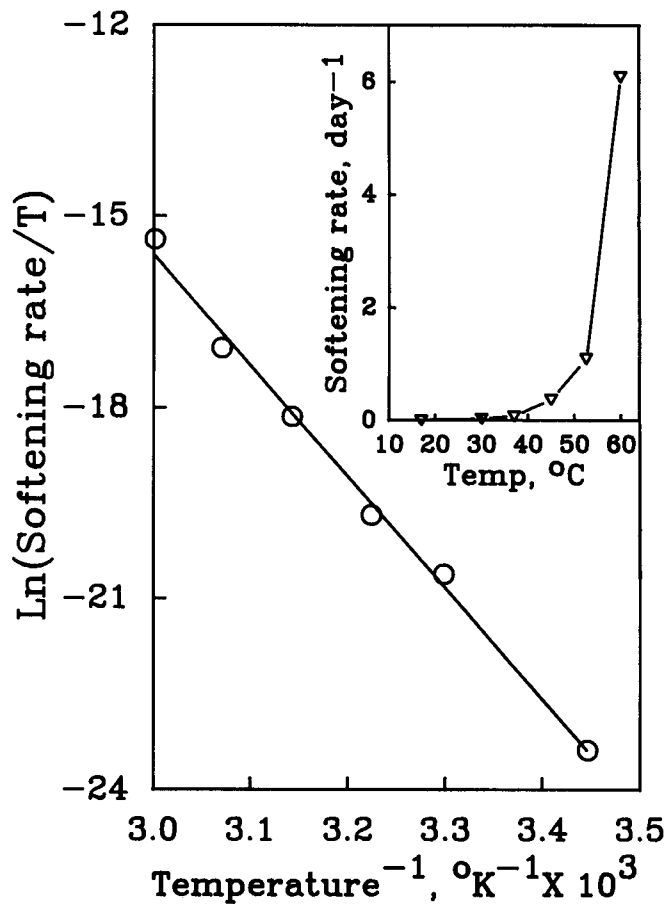


Fig. 6.—Effect of temperature on the rate of mesocarp tissue softening in blanched cucumber slices stored in 1.5M NaCl brine.

heating temperature or duration on the variability of tissue softening.

Pectin methylation changes at different NaCl concentrations were determined for blanched, unblanched and fermented samples. The degree of methylation was high and showed only a small increase as the salt level increased. This was expected since the blanch treatment was sufficient to inactivate pectin methyltransferase (McFeeters et al., 1985). Both the unblanched and fermented tissues showed a variation of methylation with salt concentration in which the minimum esterification was observed at moderate salt levels. There appeared to be little effect of changing pectin methylation on the softening rates. Hudson and Buescher (1986) found that cucumbers with less than 12.3% methylation were less firm after a 3.5-month storage period at 24°C, regardless of the brining treatment required to achieve the particular degree of methylation. However, in the experiment with fermented cucumbers, softening rates did not change as the degree of methylation changed from 7.3% to 32%. Since the storage temperatures were quite different, it was possible that different softening processes were dominant in the two studies.

The salt softening effect observed in cucumbers would appear to be similar to that found in low acid products such as potatoes (Hughes et al., 1975), peas (Blair and Ayers, 1943; Lenz and Weckel, 1967), carrots (Sterling, 1968) and green beans (Van Buren and Peck, 1982; Van Buren, 1986; Van Buren et al., 1988). However, the mechanism of softening affected by salt in this study may be different. While little direct data has been obtained on this point, it has been suggested that softening at a pH above 5 is due primarily to beta-elimination degradation of pectin molecules, while at lower pH values acid hydrolysis would be dominant (Doesburg, 1965; Keijbets et al., 1976). In the present experiments, the pH was

in the range 3.2 to 3.6. Therefore, the most likely degradation mechanism would be acid hydrolysis, while beta-elimination should be predominant in low-acid vegetables. In the experiments with blanched tissue, enzymatic degradation would be unlikely. The fact that softening rates were similar for all the alkali metal ions studied indicated that this effect was not related to the size of the ion, since the ionic radii vary from 0.76 Å for Li⁺ to 1.67 Å for Cs⁺ (Shannon, 1976), while the hydrated radii vary from 3.40 Å for Li⁺ to 2.28 Å for Cs⁺ (Cotton and Wilkinson, 1988).

The linear relationship obtained from the plot of $\ln(kT)$ vs $1/T$ (Fig. 6) suggested that the mechanism of softening did not change over the temperature range investigated. This in turn strengthens the argument that enzyme reactions are unlikely to be involved in the softening of blanched tissue for two reasons. First, an enzyme would have to be stable not only to a 3 min blanch in boiling water in the fresh tissue, but it must also be stable at pH 3.6 at over the temperature range of 17 to 60°C for periods of hours to months depending upon the storage temperature. Secondly, the enthalpy of activation is very high relative to that expected for an enzyme-catalyzed reaction (Whitaker, 1972). This linear relationship also means that studies of this softening process can be done at high temperatures which will give rapid rates of tissue breakdown. The high enthalpy of activation determined for the softening reaction also suggests, but does not prove, that covalent bond breaking is involved in the softening process. Data have been obtained (McFeeters, 1988) that certain cell wall sugars are lost from cell wall samples prepared from softened tissue, which also indicates that covalent bonds are probably broken.

SUMMARY & CONCLUSIONS

A METHOD for measuring first-order softening rates on relatively large numbers of samples of cucumber tissues was developed. This procedure was used to demonstrate for the first time a salt softening effect in heated, unheated and, to a limited extent, fermented cucumber tissue. A salt softening effect was opposite to the usual observation that salt inhibited softening of fermented cucumbers. On the other hand, these results were consistent with observations of salt softening effects in several low-acid vegetables. Considering the blanch treatment used for the heated tissue, the requirement that a softening enzyme would have to be very stable at low pH and at temperatures up to 60°C and the high enthalpy of activation for softening, heated tissues probably softened by a nonenzymatic mechanism.

All alkali metal ions tested increased tissue softening rates in blanched cucumber tissue to a similar degree. This indicated that the size of these cations had little effect on salt softening under acid conditions. Changes in pectin methylation in the cucumber cell walls occurred in unheated tissues, but methylation changes appeared to have little influence on softening rates. Analysis of the effect of temperature on softening rates indicated that the reaction mechanism that resulted in softening in blanched tissue was the same over the range of 17° to 60°C.

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