Effect of Calcium Ions on the Thermodynamics of Cucumber Tissue Softening

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ABSTRACT

Calcium ion was found to cause inhibition of softening in blanched cucumber tissue under acidic conditions due to a dominant effect of the calcium upon the entropy of activation for the softening reaction. This resulted in slower rates of softening despite a decline in the enthalpy of activation. Enthalpy/entropy compensation was found to occur. The isokinetic temperature for softening was 260 ± 9 K. An assumption was made that the hyperbolic inhibition of softening by calcium was due to saturation of a binding site by calcium. The dissociation constant for calcium from the proposed site was determined to be 1.5 mM at 30°C. This was considerably stronger binding than should occur if calcium was binding to pectin carboxyl groups under similar conditions. The apparent enthalpy and entropy for calcium binding were determined.

INTRODUCTION

IT HAS BEEN SHOWN for many fruits and vegetables that calcium can improve firmness retention during processing and storage (Doesburg, 1965; Van Buren, 1986). However, the effect of calcium on the thermodynamics of texture changes has not been determined. It has recently been found that a linear relationship exists when first-order rate constants for softening of blanched, acidified cucumber tissue over the 17°C to 60°C temperature range are plotted using transition state theory (McFeeters et al., 1989). This indicates that the mechanism of softening does not change over that temperature range. It was also observed that low calcium ion concentrations inhibited the rate of cucumber tissue softening in the presence of high salt levels (McFeeters and Fleming, 1989). Several types of ion binding data for cucumber tissue (McFeeters and Fleming, 1989) indicate that the textural effect of calcium is not due to the "egg box" binding model developed by Grant et al. (1973) to describe calcium binding in pectin solutions. In an effort to better understand this effect of calcium ions on cucumber texture, the thermodynamics of calcium inhibition of the rate of cucumber tissue softening were analyzed.

MATERIALS & METHODS

SIZE 3A CUCUMBERS (38–44 mm diameter) were obtained from a local processor. Cucumber mesocarp tissue pieces were prepared by peeling the fruit and cutting them into 7 mm thick slices. About 15 mm of tissue from both the stem and blossom ends of the fruit were discarded. Mesocarp pieces were cut from the three carpels of each slice. The seed area was discarded. Mesocarp pieces (400g) were blanched for 3 min in 13 L of boiling, distilled water, cooled, and 30 ± 0.5g were packed into 60 mL (2 oz) jars. The cucumber pieces were covered with a volume of brine equal to the volume of cucumber tissue. The brine contained 3.0M NaCl, 0.2 M acetic acid, 400 ppm SO_2 added as sodium metabisulfite, and the appropriate amount of calcium ion added as $CaCl_2 \cdot H_2O$. Samples of the blanched mesocarp tissue were frozen for subsequent analysis of tissue calcium levels and the degree of pectin methylation in isolated cell walls. The sample jars were held at 17°C for 3 days to allow equilibration of brine with the tissue. The equilibrated jars were then placed in 60°C and 52°C water baths and 44°C, 37°C and 30°C incubators. The firmness of the cucumber mesocarp tissue was measured on 15 tissue pieces from duplicate jars at the time samples were transferred from 17°C to the other temperatures. Firmness measurements were made on 15 tissue pieces from single jars at 4 or 5 subsequent times with a 0.315 cm diameter, flat-tipped, stainless steel punch mounted on an Instron Universal Testing Machine (Instron Corporation, Canton, MA; Thompson et al., 1982).

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Calcium analysis

Samples were prepared by blending cucumber tissue with an equal volume of 4% trichloroacetic acid to release calcium from the tissue. Calcium was analyzed by the colorimetric procedure of Gindler and King (1972). An automated version of this procedure has been shown to give results equivalent to atomic absorption analysis for peanut samples (Salazar and Young, 1984).

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.

pH determinations

Measurement of the pH brines after equilibration with cucumber tissue was done at 23°C using an Orion model 901 pH meter with a combination electrode.

Data analysis

First-order softening rate constants were calculated by linear regression analysis using the mean tissue firmness calculated for each sample jar (Huang and Bourne, 1983; McFeeters et al., 1989). Analysis of activation parameters at each calcium concentration was done using the equation derived from transition state theory (Eyring, 1935):

$$\text{Ln}(k/T) = \text{Ln}(k_B/h) + \Delta S^{\ddagger}/R - \Delta H^{\ddagger}/RT$$

In this equation, k = first-order reaction rate constant for softening expressed in sec^{-1}, T = absolute temperature in K, k_B = Boltzman's constant, h = Planck's constant, R = gas constant, $\Delta S^{\ddagger}$ = entropy of activation, and $\Delta H^{\ddagger}$ = enthalpy of activation. Linear regression analysis of Ln(k/T) vs 1/T allowed the calculation of the enthalpy of activation from the slope and the entropy of activation from the intercept of the line (Whitaker, 1972). The fit of activation parameters to a hyperbolic equation as a function of calcium ion concentration was done using the nonlinear regression analysis of the Asystant+ software package (Macmillan Software Company, New York, NY).

RESULTS & DISCUSSION

THE CUCUMBER mesocarp tissue used in this study had a natural calcium concentration of 3.4 mM after blanching. The degree of pectin methylation of cell walls isolated from the blanched tissue was 59.7%. The mean equilibrated pH of the samples was 3.27 with a range of 3.18 to 3.36. A linear relationship between Ln(k/T) and 1/T was observed at all calcium concentrations (Fig. 1). This result indicated that the rate-limiting step in the softening process did not change with tem-

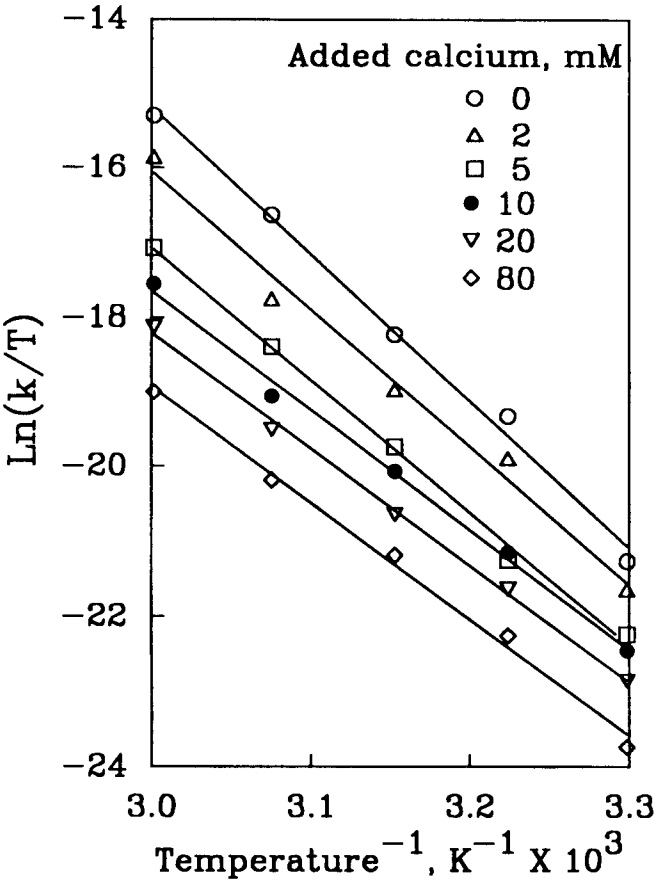

Fig. 1.—Effect of temperature and added calcium ion concentration on the rate of softening of acidified cucumber tissue in the presence of 1.5M NaCl. R^2 values for the fit of the data to a straight lines ranged from 0.987 to 0.997.

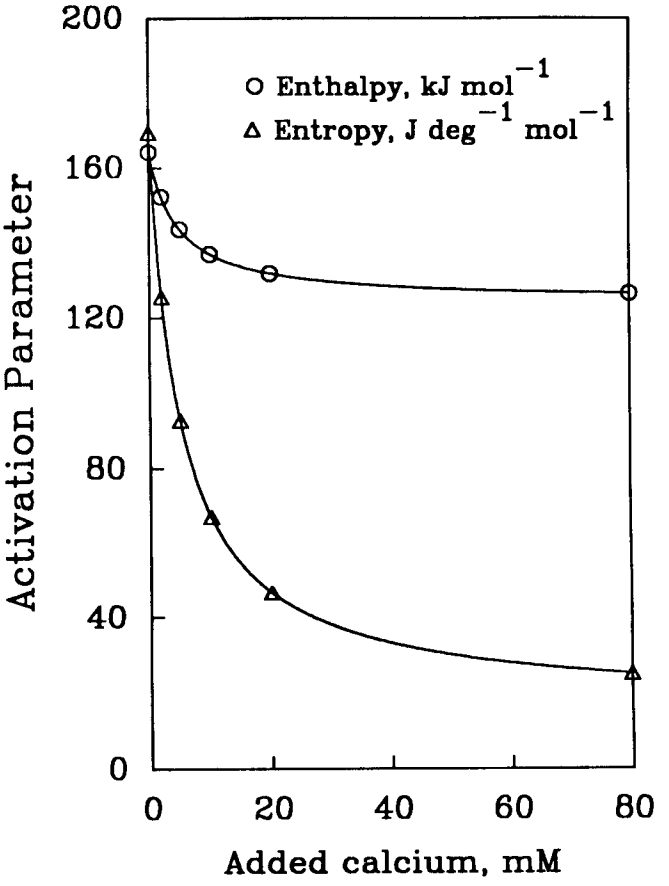

Fig. 2.—Changes in the entropy and enthalpy of activation for cucumber tissue softening as a function of added calcium ion concentration. The equation for the enthalpy of activation was Y = 124.3 +. [(189.7/(4.75 + X)]. The R^2 value was 0.9996. The equation for the entropy of activation was Y = 16.0 + [765.9/(5.00 + X)]. The R^2 value was 0.9939.

perature at any given calcium concentration. The same result was observed for cucumber slices in 1.5 M NaCl without added calcium ions (McFeeters et al., 1989). The apparent enthalpy and entropy of activation for each added calcium concentration were calculated from the slopes and intercepts in Fig. 1. Changes in these quantities as a function of added calcium are shown in Fig. 2. Both the enthalpy and entropy of activation declined as a hyperbolic function as the calcium concentration increased. Consideration of the way changes in these quantities affect the free energy of activation ($\Delta G^{\ddagger}$) and, in turn, the tissue softening rates lead to the conclusion that calcium inhibits softening due to its dominant effect on the entropy of activation. If the entropy of activation was constant, the decline in the enthalpy of activation would decrease $\Delta G^{\ddagger}$ and, as a result, the rate of softening would increase. The fact that softening rates decrease is entirely due to the large decrease in the entropy of activation which results in an increase of $\Delta G^{\ddagger}$ (Fig. 3), despite the decline in the enthalpy of activation as calcium concentration increases.

It has been observed for many chemical reactions that the enthalpy and entropy of activation change in concert as some reaction condition, such as pH, water activity, etc. is changed. This has been called enthalpy/entropy compensation. When there is a linear relationship between the enthalpy and entropy of activation, the slope of the line is the isokinetic temperature. The isokinetic temperature is that temperature at which the reaction rate would remain constant as the reaction condition under study was changed. Labuza (1980) reviewed the concept of enthalpy/entropy compensation, particularly as it related to chemical reactions in foods. The only case cited for which there was adequate data to demonstrate compensation in a degradative reaction in food was that of Kirk (1978) and Kirk and Dennison (1978) for the loss of ascorbic acid as a function of water activity in a dehydrated model food system. The occurrence of compensation in chemical reactions in general has been controversial over the years due to the fact that the plot of the enthalpy of activation vs the entropy of activation, the usual plotting procedure to evaluate compensation, has the unusual statistical property that a linear plot with a high correlation coefficient will nearly always be obtained regardless of the occurrence of chemical compensation (Krug et al., 1976a). In fact, the error structure of the plot is such that a better straight line is obtained as the thermodynamic data become more scattered. It was recommended that compensation be evaluated based upon a plot of the enthalpy of activation vs the free energy of activation at the harmonic mean of the experimental temperatures (T_{hm}) to avoid the problem of statistical compensation between parameter estimates (Krug et al., 1976b). This has been done with the present data (Fig. 4). A linear relationship with a correlation coefficient of 0.906 was obtained, indicating that enthalpy/entropy compensation occurred in the loss of firmness of cucumber mesocarp tissue. To highlight the statistical compensation problem, these data gave a correlation coefficient of 0.997 when the enthalpy vs entropy of activation plot was done. An isokinetic temperature (T_β) of 260 ± 9K was calculated using the slope (γ) of the line in Fig. 4 and the equation from Krug et al. (1976b):

$$T_\beta = T_{hm}/(1 - 1/\gamma)$$

In addition to estimates of the activation parameters for the tissue softening reaction, the data from Fig. 1 can be used to estimate apparent dissociation constants and the entropy and

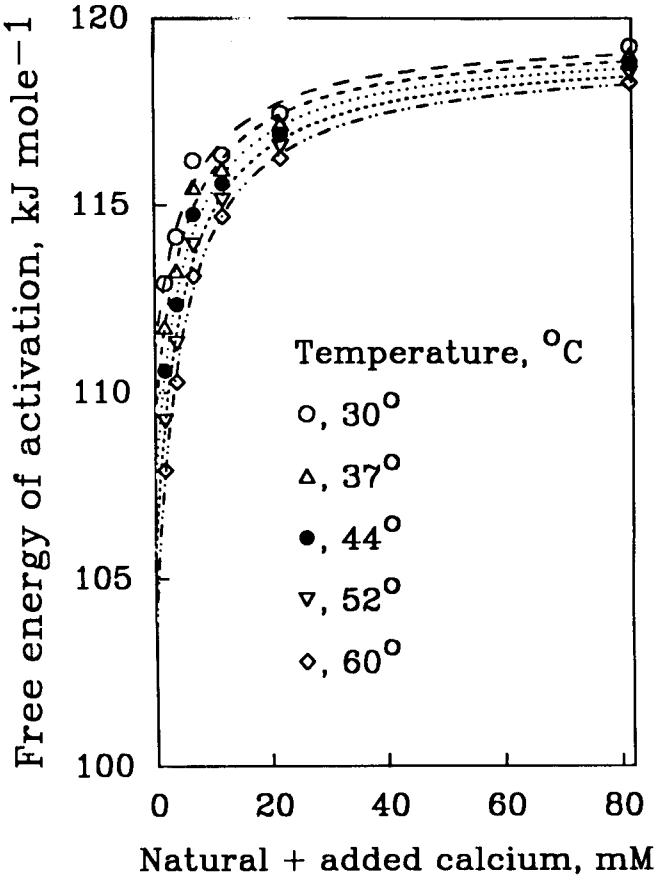

Fig. 3.—Changes in the free energy of activation of cucumber tissue softening as affected by temperature and the natural plus added calcium ion concentration. The R^2 value for the fit of the data to a hyperbola was greater than 0.999 at each temperature.

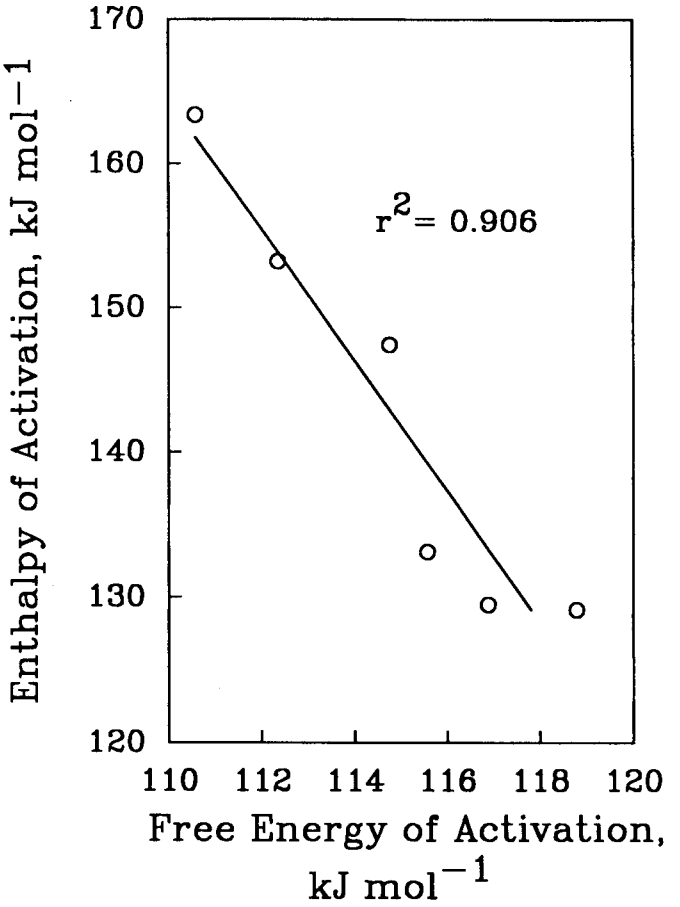

Fig. 4.—Relationship between the enthalpy of activation and the free energy of activation for cucumber tissue softening at 44°C, the harmonic mean of the experimental temperatures.

enthalpy of calcium binding to the sites that inhibit tissue softening provided certain assumptions are made concerning the calcium interaction with the tissue. These assumptions are: (1) since calcium ions show a hyperbolic inhibition of tissue softening (McFeeters and Fleming, 1989), calcium binds to and saturates sites which inhibit the disruption of cell wall structure; (2) there is only one class of binding sites for calcium which inhibits softening, such that all binding sites have the same binding constant; and (3) extrapolation of $\Delta G^{\ddagger}$ values to zero calcium ion concentration is a valid extrapolation. As mentioned at the beginning of the discussion, the blanched cucumber tissue had a natural calcium concentration of 3.4 mM so that after dilution with brine the calcium contribution from the cucumber would be 1.7 mM. Since the presence of calcium in cucumber tissue does not allow experimental reduction of calcium ion concentration to zero, the free energies of activation were calculated at each concentration of added calcium for each experimental temperature. A least squares hyperbolic fit was calculated at each temperature and extrapolated to zero calcium (Fig. 3). Note that the first experimental point on each curve was at 1.7 mM. Using calculated free energy values from the curves in Fig. 3, reaction rate constants were calculated from 0 to 81.7 mM calcium. By analogy with enzyme kinetics, the calcium ion concentration which gave 50% of the maximal inhibition of the softening rates was calculated and considered to be the dissociation constant for the calcium binding sites at that temperature. The calculated dissociation constants and free energy of binding at each experimental temperature are shown in Table 1. The decline of the apparent dissociation constants with temperature suggested that a rather large increase in entropy might occur with calcium binding. By plotting the $\Delta G^{\ddagger}$ for calcium binding vs temperature, the entropy and enthalpy of calcium binding were calculated to be 30.1 ± 3.3 kJ/mole and 154 ±10 joules/deg/mole, respectively. The large increase in entropy upon calcium binding might be a result of the loss of water from the calcium binding site.

Table 1—Effect of temperature on the dissociation constant and free energy for the calcium ion binding in cucumber tissue which inhibits the softening rate

Temperature, °C	Dissociation constant, mM	Free energy of Ca binding, kJ/mole
30	1.45	−16.5
37	1.07	−17.6
44	0.83	−18.7
52	0.55	−20.3
60	0.52	−20.9

Recent results of ion effects upon cucumber tissue softening (McFeeters and Fleming, 1989) indicate that the calcium effect on softening rate constants cannot be explained in terms of calcium cross-linking of ionized pectin carboxyl groups according to the egg box model of Grant et al. (1973). The results presented here provide additional evidence along this line because the dissociation constant for calcium at 20°C at μ = 1.5, pH 3.27, would be 2 mM, using the calculated enthalpy and entropy for binding. Kohn and Furda (1967) measured a stability constant at 20°C of 49 for 58% esterified apple pectin at μ = 0.15 and neutral pH, which translates to a dissociation constant of 20 mM. Raising the ionic strength and lowering the pH would certainly increase the dissociation constant well above 20 mM. Thus, the inhibition of softening was due to calcium binding to a site with much higher affinity for calcium

than would be expected for pectin at a similar degree of esterification, ionic strength and pH.

In view of these results, there is a need to identify the mechanism by which calcium ions can inhibit softening. Since the cucumber tissue pieces were blanched in boiling water, it is unlikely that softening is due to an enzymatic reaction. The values obtained for the enthalpy and entropy of activation are higher than would be expected for hydrolytic degradation reactions, whether by enzymatic or nonenzymatic mechanisms (Whitaker, 1972). Ginzburg (1961) suggested that a metal ion binding protein in plant cell walls may be important for maintenance of the structure of plant tissues. The large enthalpies and entropies of activation for the calcium effect on tissue softening rates were similar to what was observed for protein denaturation (Eyring and Stern, 1939). Thus, the thermodynamic results were consistent with the possibility of the involvement of a calcium-binding protein in tissue firmness changes. Unfortunately, there are no available thermodynamic data for the unfolding or hydrolysis of suitable cell wall polysaccharides for comparison to determine whether the results obtained here are consistent or not with other possible reactions which might cause tissue softening. A reasonable experimental approach to test the possibility of involvement of a protein in the calcium effect might be to investigate the effect of reagents which disrupt protein structure on the tissue softening reaction.

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