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Softening Rates of Fermented Cucumber Tissue: Effects of pH, Calcium, and Temperature

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ABSTRACT

First-order softening rates for cucumber mesocarp tissue were determined as a function of pH (2.6-3.8), calcium (0–72 mM), and temperature (25–65°C). Fermented tissue, unlike blanched, nonfermented tissue, often showed two softening rates in first-order plots. A five-variable, empirical equation was derived (R^2 = 0.913) which predicted softening rates as a function of pH, calcium concentration, and temperature. Comparison of softening rates in tissue fermented with and without 18 mM added calcium indicated the softening rate depended upon the concentration of calcium present during the period of measurement and not upon previous history of calcium exposure.

Key Words: cucumber, texture, softening, calcium, kinetics

INTRODUCTION

THE NEED TO REDUCE CHLORIDE in waste streams has led to efforts to reduce NaCl concentrations used for storage of fermented cucumbers below the current 5–12%. NaCl helps maintain both the microbial (Fleming et al., 1989) and textural stability (Fleming et al., 1978; Thompson et al., 1979; Hudson and Buescher, 1985; Fleming et al., 1987) of fermented cucumbers. Storage at lower pH can assure microbial stability at lower NaCl levels (Fleming et al., 1992). Based upon previous studies of blanched, nonfermented, cucumber mesocarp tissue, we expected that lower pH would be detrimental to desirable texture (McFeeters and Fleming, 1991), while calcium addition and lower storage temperatures would be beneficial (McFeeters and Fleming, 1989; 1990). While fermentation does not appear to cause major changes in sugar composition of cell walls (Tang and McFeeters, 1983), the pectic substances of the wall undergo extensive demethylation due to the presence of pectin methylesterase (Tang and McFeeters, 1983; Hudson and Buescher, 1986). This change in structure along with other changes in the cell wall during fermentation, could result in softening kinetics of fermented tissue being considerably different from those applicable to nonfermented tissue. We noted in a previous investigation of the effect of NaCl concentration on the rate of cucumber tissue softening that fermented tissue firmness changes were more variable than those for blanched tissue (McFeeters et al., 1989). Larger standard deviations for softening rates occurred in fermented than in blanched tissue.

Our objective was to evaluate the effects of pH, calcium, and temperature on the textural stability of cucumber tissue fermented at 2% NaCl.

MATERIALS & METHODS

DISEASE-FREE, size 3A cucumbers (38–44.5 mM diameter) of an unknown cultivar were obtained from a local processing plant. Eight 19-L plastic pails were filled with 10.4 kg cucumbers and 8.5 kg brine to give 55% cucumbers and 45% brine solution in each pail. The cover brine contained 4.44% NaCl, 118 mM acetic acid, and 80 mM NaOH to give an equilibrated NaCl concentration during fermentation of 2.0%. The brines were inoculated to contain 10^6 CFU/mL of a 24-hr culture of *Lactobacillus plantarum* MOP-3 grown in MRS broth (Difco Laboratories, Detroit, MI). Pails were purged with N_2 gas at 25 mL/min during fermentation to remove dissolved CO_2 and prevent bloater damage. Fermentation was carried out at 25°C. Fermentation was complete after 24 days, as shown by absence of sugars in the brine. The fermented cucumbers were refrigerated until use to minimize loss of tissue firmness.

Five additional 19-L pails of cucumbers were prepared and fermented in an identical manner except 40 mM calcium hydroxide was substituted for 80 mM NaOH in the cover brine to give an equilibrated concentration of 18 mM calcium during fermentation (Fleming et al., 1988).

The fermented cucumbers were cut with a manual slicer to give 7 mM thick cross-sectional slices. Mesocarp carpel sections with peels attached were removed and the seed area discarded. The brines from the eight fermentation pails were mixed to provide a uniform medium for measurement of softening rates. Sodium metabisulfite was added to the fermentation brine to give an equilibrated concentration of 200 ppm SO_2 to prevent microbial growth during softening (McFeeters et al., 1989). Calcium chloride was added to the fermentation brine so that after equilibration with tissue pieces, added calcium levels were 0, 2, 6, 18, 54, and 72 mM. To maintain a constant ionic strength at all calcium levels, NaCl was added to the brines with 54 mM or less added calcium so the ionic strength of all treatments would equal that of the treatment with 72 mM calcium. The pH of the brines was adjusted by addition of 3N HCl or NaOH so that, after equilibration with fermented cucumber tissue, pH values of 2.6, 3.0, 3.4 and 3.8 were attained. The 24 calcium × pH treatments were incubated at 25, 35, 45, 55, and 65°C. Seven 60-mL jars were packed with an equal amount of tissue and brine for each of the 120 calcium × pH × temperature treatments for a total of 840 samples.

Cucumbers and brines from the pails fermented with 18 mM added calcium were prepared by the same procedure except 0, 18, and 54 mM calcium chloride were added to give equilibrated concentrations of 18, 36, and 72 mM calcium during incubation at the five temperatures. These samples were prepared at pH 3.0. Again, seven jars of cucumber pieces were incubated at each temperature and calcium concentration for a total of 105 samples.

After filling, samples were held overnight at 4°C to allow equilibration. They were then transferred to respective incubation temperatures. All samples were equilibrated at the appropriate temperature in a water bath. The 25, 35, and 45°C samples were then transferred to air incubators. The 55 and 65°C samples were kept in water baths throughout the incubation period. Tissue pieces from two jars were measured for firmness 15 min after the start of incubation. Single jars were sampled at five subsequent times. Time periods over which sampling was done for a treatment varied from 24 hr to 6 mo, depending upon temperature, calcium level, and pH.

Firmness was measured as the maximum force required for a 3.15 mm diameter punch to penetrate the mesocarp tissue (Thompson et al., 1982). Punch tests were done with a Model 1011 Instron Universal Testing Machine on 15 mesocarp tissue pieces from each jar. A first-order plot of the mean firmness vs time was developed. Softening rates were determined from the slopes of the first-order plots (McFeeters et al., 1989).

Cell walls were isolated from fresh and fermented cucumber mesocarp tissue by extraction in 80% ethanol, washing with acetone, and drying. The degree of pectin methylation in the isolated walls was determined using the procedure of McFeeters and Armstrong (1984) except that the methanol analysis was done using a colorimetric analysis as described by Wood and Siddiqui (1971) and modified by Hudson and Buescher (1986). Lactic acid in the fermentation brines was determined by HPLC on a Bio-Rad HPX87-H cation exchange column with 0.01N sulfuric acid as the eluant. Detection was with a Waters model 410 refractive index detector.

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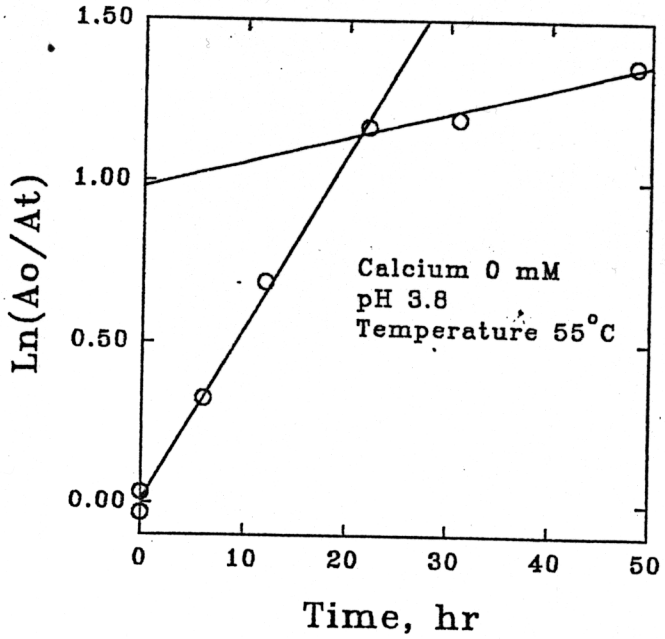

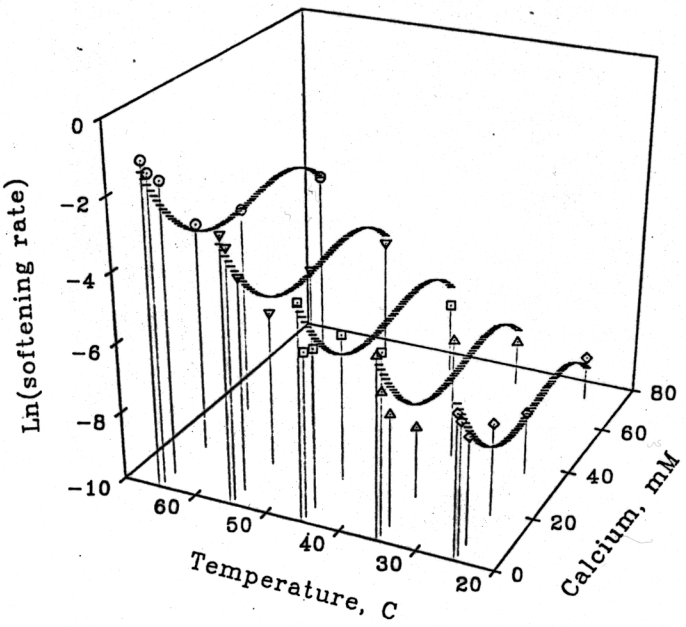

Fig. 2—Measured and predicted (−) softening rates for fermented cucumber tissue as a function of temperature and calcium concentration at pH 3.0. The symbols showing measured softening rates are different for each storage temperature to aid interpretation of the data.

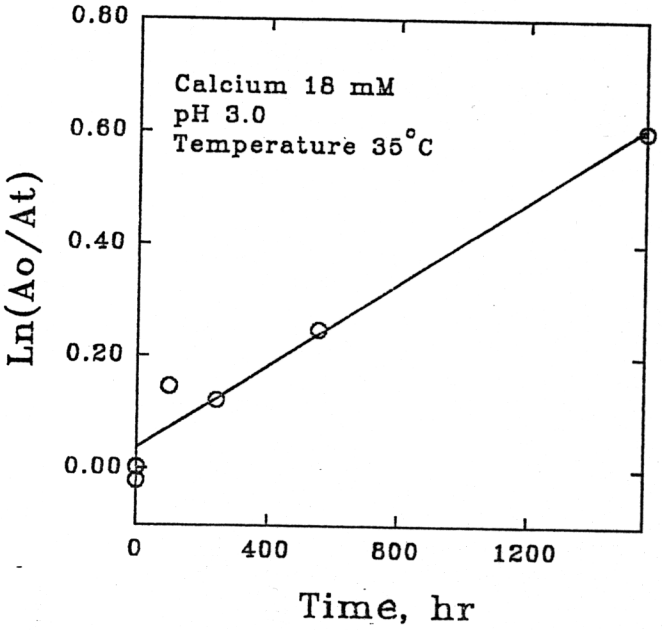

Fig. 1—Examples of broken curve and linear first-order plots of fermented cucumber tissue softening among the 120 calcium, pH, and temperature treatments. A_0 is the initial tissue firmness. A_t is the firmness at subsequent sampling times.

Empirical modeling of softening rates as a function of temperature, calcium concentration, and pH was done using the PC-SAS general linear model (GLM) procedure (SAS Institute, Cary, NC).

RESULTS & DISCUSSION

A NORMAL CUCUMBER FERMENTATION occurred in the eight pails. The final brine pH was 3.5 with a lactic acid concentration of 161.1 mM. There was complete utilization of fermentable sugars. The firmness of the fresh cucumber mesocarp was 9.7N. Mesocarp tissue fermented without added calcium had a firmness of 8.9N, and tissue fermented with 18 mM calcium had a mean firmness of 13.1N prior to the start of the softening experiment. The calcium concentration in the fresh cucumbers was 2.6 mM, so the natural level of calcium in the fermented fruit and brine was 1.4 mM. The degree of pectin methylation in the isolated cell walls was 55.1% in the fresh fruit and 15.1% after fermentation. This was similar to previous results (McFeeters and Armstrong, 1984; Hudson and Buescher, 1986).

This experiment was designed to determine the enthalpy and entropy of activation for softening similar to that which was done for blanched cucumber mesocarp tissue at pH 3.3 (McFeeters and Fleming, 1990). However, we concluded that this type analysis was not appropriate because the softening behavior of fermented tissue was different from that observed for nonfermented tissue in two distinct respects. First, fermented tissue often showed a broken curve in the first-order plot, rather than the straight line relationship that has been reported in a variety of studies with nonfermented tissue (McFeeters et al., 1989; McFeeters and Fleming, 1989, 1990, 1991). Among the 120 treatments, 76 treatments were determined to have a broken curve for the first-order plot (Fig. 1). The point of intersection of the lines for the two softening rates occurred after loss of 25 to 68% of the initial tissue firmness. Average firmness loss at the intersection was 43%.

This was the first case in which this type of broken curve softening has been reported in cucumber tissue softening. The reason for this pattern of softening is not known. In general, broken curves were obtained more often with rapid softening rates and less frequently at low rates of softening. Among the 120 treatments, all of the 20 treatments with the most rapid initial softening rates had a broken curve. Only one of the 20 treatments with the slowest initial rates had a broken softening curve. This could not be specifically related to the calcium, pH, or temperature treatments other than the fact that broken curves became less frequent as temperature and calcium levels declined and as pH increased. The reason a broken curve was not found in all treatments may have been because softening did not proceed far enough to enter the slower part of the softening process, or the difference in rates was not sufficient to differentiate two curves when the data were plotted.

Broken first-order reaction curves suggest at least two different reactions responsible for softening in fermented tissue. The more rapid, associated with the initial softening rate, is the one

responsible for softening within the firmness range that would be acceptable for pickled vegetable products. Softening at low firmness levels, associated with the second softening rate, is not important from the standpoint of sensory textural quality. However, recognition that a second reaction occurs may be of importance in understanding mechanisms for tissue breakdown.

This type of two-reaction kinetic behavior for softening has previously been reported by Huang and Bourne (1983) for retorted vegetables. However, given the differences in conditions of the experiments, the chemical basis for the broken softening curves may not necessarily be similar.

McFeeters et al. (1989) observed that fermented cucumber tissue showed considerably greater variability in softening rates than blanched tissue. A similar high level of variability in softening rates was observed for our fermented tissues regardless of addition of calcium or variation of pH and temperature. The reason for this variability in softening of fermented tissue is not clear. Jars of fermented tissue usually contained pieces with a "rubbery" texture in which the tissue would deform before the Instron punch penetrated the surface. The punch would penetrate other pieces within the same jar with little visible deformation. The combination of broken curve, first-order softening rates and variability in softening rates led to the decision to develop an empirical model to relate temperature, pH, and calcium concentration to softening rates.

Statistical analysis of the effects of temperature, calcium concentration and pH upon the initial, more rapid softening rates was performed. The second rate constants calculated from broken curves were not used since there was not a complete set of values. Thompson et al. (1979) previously used empirical modeling to develop a five-variable prediction equation for fruit pressure tester firmness loss in fermented cucumbers as a function of NaCl concentration, temperature, and pH. A nine-component model containing linear, quadratic, and cubic terms for temperature, calcium, and pH as a function of ln(k) was constructed. Then, terms which resulted in a minimal reduction in R^2 were deleted. This process gave the following five-variable prediction equation where C = calcium concentration (mM), T = temperature (°C), pH = pH, and k = first-order softening rate constant (the initial rate constant for broken first-order curves):

$$\text{Ln(k)} = (-1.674) + (-0.2201*\text{C}) + (0.005712*\text{C}^2) + (-0.00004597*\text{C}^3) + (0.001219*\text{T}^2) + (-1.5290*\text{pH})$$

The coefficient of determination for the nine-variable model was 0.924, compared to 0.913 for the five-variable equation. Removal of an additional term gave an $R^2 \leq 0.896$. The relationship between the experimental and predicted first-order reaction rates using this equation were compared (Fig. 2). Since the second rate constants obtained for broken curves were not included in the model, the predictive equation should only be used for the first 40–50% of firmness loss. As was previously found for nonfermented cucumber tissue (McFeeters and Fleming, 1990), calcium decreased softening rates while increasing temperature resulted in large increases in rates of softening. Softening rates declined as the pH was raised from 2.6 to 3.8, but the shape of the response surface was the same as shown in Fig. 2 for pH 3.0, regardless of pH. The pH range was not sufficiently broad to determine whether softening rates would increase as pH approached neutral as was reported for nonfermented tissue (McFeeters and Fleming, 1991). The NaCl concentration in our study was lower than has been used for nonfermented tissue softening studies, and the major acid was lactic instead of acetic, so conditions under which softening occurred were somewhat different. However, we noted that, particularly at low calcium concentrations and higher temperatures, the softening rates for fermented tissue tended to be lower than previously observed for nonfermented tissue.

The salt concentration we used for the fermented cucumbers was considerably lower than those used in the pickling industry. In addition, other factors such as differences in cucumber composition, mineral composition of water used for brining, and the presence of other ingredients may be different from conditions we used. Therefore, the model developed cannot be expected to predict absolute softening rates during commercial storage of fermented cucumbers. However, it may be useful to indicate relative changes in softening rates that would be expected to occur if temperature, calcium levels, or pH used for bulk storage of fermented cucumbers were modified.

Buescher et al. (1981) and Buescher and Burgin (1988) reported calcium was more effective in prevention of firmness loss in fermented cucumbers if added at the time of brining than at a later time. Our cucumber tissues were considerably firmer after fermentation when 18 mM calcium was added in the cover brine. However, the softening rates during subsequent storage at 25 to 65°C were not different based upon a paired t-test when 18 mM calcium was added to the tissue after fermentation, as compared to addition of 18 mM calcium at the time of brining. Likewise, no difference occurred in softening rates in cucumber tissue pieces incubated with 36 and 72 mM calcium, whether the cucumbers for mesocarp pieces had been fermented without added calcium or with 18 mM calcium added. These results suggested that the softening rate depended upon the concentration of calcium during the period when softening was measured and not upon its previous history of exposure to calcium. It is desirable, however, to add calcium as early as possible.

CONCLUSIONS

FERMENTED TISSUE SOFTENING RATES were reduced by higher calcium levels, lower temperature, and increasing pH in the range of pH 2.6 to 3.8. The direction of these effects was similar to that previously observed for blanched, nonfermented tissue. A five-variable empirical equation was derived ($R^2 = 0.913$) to predict softening rates as a function of these variables. An important difference between fermented and nonfermented cucumber tissue is that broken first-order softening curves were observed in fermented tissue. This suggests the involvement of at least two different chemical processes in softening fermented tissue. Statistical comparison of softening rates in cucumbers fermented with and without 18 mM added calcium indicated that softening rate depended upon concentration of calcium during the period of measurement and not upon previous history of calcium exposure.

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