

## 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 methyl-esterase (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

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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  $\times$  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  $\times$  pH  $\times$  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.

