Food Structure

Volume 4 | Number 1 Article 18

1985

Comparison of the Microstructure of Firm and Stem-End Softened Cucumber Pickles Preserved by Brine Fermentation

W. M. Walter Jr.

H. P. Fleming

R. N. Trigiano

FOOD MICROSTRUCTURE, Vol. 4 (1985), pp. 165-172
SEM Inc., AMF O'Hare (Chicago), IL 60666-0507 U.S.A.

COMPARISON OF THE MICROSTRUCTURE OF FIRM AND STEM-END SOFTENED CUCUMBER PICKLES PRESERVED BY BRINE FERMENTATION[1]

W. M. Walter, Jr., H. P. Fleming and R. N. Trigiano*

U. S. Department of Agriculture, Agricultural Research Service,
and North Carolina Agricultural Research Service, Department of Food Science,
North Carolina State University, Raleigh, NC 27695-7624

*Department of Plant and Soil Science,
University of Tennessee, Knoxville, TN

Abstract

Soft and firm stem end tissues of cucumbers fermented at a low concentration of sodium chloride were examined by a combination of light microscopy (LM), transmission electron microscopy (TEM), and scanning electron microscopy (SEM). When compared to firm tissue, softened tissue cell walls were swollen and striated, and the middle lamellae were poorly stained by the uranyl acetate-lead citrate used in TEM. When the tissue was penetrated by a circular punch (1.5 mm diameter), cells in soft tissue tore along the middle lamellae rather than across cell walls, as did cells in firm tissue. Thus, softening was characterized by a weakening of cell-to-cell junctions at the middle lamellae. Ultrastructural changes in the softened tissue were consistent with cellulolytic and/or pectinolytic degradation of cell wall components. Since fungal hyphae were found in both soft and firm tissue, it was not possible to determine if the softening was due to extracellular enzymes produced by invading organisms or to enzymes endogenous to the cucumber tissue.

[1]Paper no. 9772 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, NC 27695-7601.

Initial paper received January 30, 1985.
Final manuscript received April 22, 1985.
Direct inquiries to W.M. Walter.
Telephone number: (919) 737-2990.

KEY WORDS: Cell wall, Middle lamella, Electron microscopy, Light microscopy, Hyphae, Cellulolytic, Pectinolytic, Enzymes, Paraffin, Histochemical

Introduction

Texture is an important quality factor in cucumber pickles. Softening of pickles during brine fermentation and storage can be a source of serious economic losses to the pickle industry. One cause of softening has been demonstrated to be the result of breakdown of cucumber pectic substances by polygalacturonases (PG) of fungal origin present in the fermentation brine (Bell et al., 1950; Etchells et al., 1958; Lampi et al., 1958). Buescher and Hudson (1984) reported that Cx-cellulase in brine can cause delayed but serious softening of cucumber pickles. Softening has been observed in cases where pectinolytic enzyme activity was not detected in the brine (Costilow et al., 1980). In this report molds were found inside softened cucumber pickles fermented with air purging of the brine and were presumed to be the cause of the softening. Softening also may be the result of PG (Pressey and Avants, 1975; McFeeters et al., 1980) and other enzymes endogenous to the cucumbers, particularly at low concentrations of NaCl.

Historically, the pickle industry has used high concentrations of sodium chloride during brine fermentation (5-8%) and storage (10-16%) to prevent enzymatic softening. Bell and Etchells (1961) found that firmness retention of cucumbers in the presence of fungal PG's increased as salt concentration was increased. Addition of Ca^{++} to the fermentation brine results in improved firmness retention at relatively low concentrations of NaCl (Fleming et al., 1978), even in the presence of PG of fungal origin (Buescher et al., 1979; 1981). The use of $CaCl_2$ in combination with use of improved tanking vessels and handling methods, as are being proposed for industry use (Fleming et al., 1983), may offer a practical means for reducing the concentration of NaCl required for firmness retention. Such a reduction is desirable because of salt disposal problems that are created by the need to desalt the cucumbers after brine storage, before they are processed into finished pickles.

The potential for softening at low salt conditions under commercial conditions has not been fully explored. We have observed instances

of softening occurring under low salt conditions in the presence of $CaCl_2$. In such instances, the mechanism of softening has not been obvious. Softening may have occurred by enzymes of microbial origin, or by enzymes endogenous to the cucumber. As low salt brining procedures are introduced into the pickle industry, methods are needed to assess the cause of softening when it occurs, in order to better understand ways to prevent the problem. The purpose of this study was to characterize differences in the microstructure of firm and soft tissue of cucumbers brined at a low concentration of NaCl.

Materials and Methods

Brined cucumbers

Pickling cucumbers (2.7-3.2 cm diameter, Calypso cv.) were fermented in a 1,200-gal, closed-top, fiberglass tank at a commercial pilot facility, as described by Fleming et al. (1983). The cucumbers were brush-washed and covered with a brine to equalize at 2.7% NaCl, 0.32% acetic acid and 0.018 M calcium hydroxide (initial pH 4.6). The brines were inoculated with a starter culture of Lactobacillus plantarum, purged with nitrogen to remove CO_2 and thereby prevent bloater formation, and allowed to ferment at ambient temperature until completion (all fermentable sugars converted to lactic acid and other end products). The final product contained 1.4% titratable acidity (calculated as lactic acid), pH 3.5, and 2.8% NaCl. The tank was unloaded after 2 months and samples of the fermented cucumbers stored at 3°C until examined. The fermented cucumbers were of good quality, overall, with the exception of slight to moderate softening near the stem end of about 20% of the cucumbers. Pickle firmness was evaluated by grasping the stem end between the thumb and forefinger and exerting a slight pressure. Softened pickles offered much less resistance to compression than did firm pickles.

Preparation of samples for microscopy

Longitudinal slices (0.5 cm in width) were excised from the stem end of softened and firm pickles. Cucumber slices were halved and placed in fixative consisting of 3% glutaraldehyde in 0.1 M acetate buffer (pH 5.5).

Transmission electron microscopy (TEM). Fixed pieces of pericarp were trimmed into 1.0 mm blocks and post fixed in cold, 0.05 M phosphate buffer, 1% OsO_4 (pH 7.2) for 2 hr, dehydrated in a graded ethanol series and embedded in Spurr's low viscosity epoxy medium (Spurr, 1969) as described by Trigiano et al. (1983). Thin sections (700-900 Å) were cut with a diamond knife on an LKB ultratome and mounted on uncoated, 200 mesh copper grids. Sections were stained for 50 min with uranyl acetate and post-stained with basic lead citrate for 7 min. Sections were examined using a JEOL 100S electron microscope operated at 80 kV.

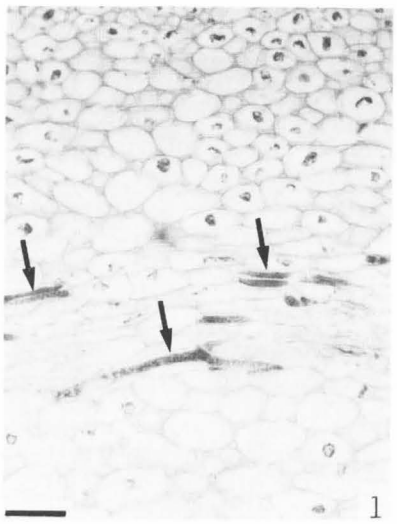
Scanning electron microscopy (SEM). Pericarp tissue was cut into 1 cm cubes. A stainless steel punch (1.5 mm in diameter) was passed downward through the cube beginning in the center of the top face. The punch was removed and the tissue block was dissected with a razor blade such that the blade passed through the center of the hole left by the punch. The tissue blocks were then fixed with 3% glutaraldehyde in 0.1 M acetate buffer, pH 7.0, and dehydrated in a graded series of aqueous ethanol (10, 25, 50, 75, 95, and 100% ethanol). The sections equilibrated in 100% ethanol were then dried in a Ladd Critical Point Dryer using carbon dioxide as the transitional fluid. Sections were gold-coated in a Polaron E 5000 Diode Sputtering System. Samples were observed and photographed at 20 keV with an ETEC Autoscan Microscope.

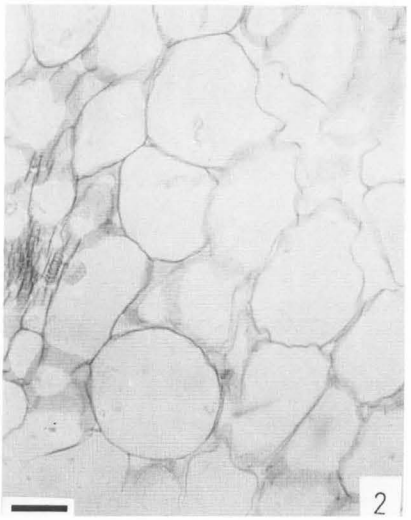
Light microscopy (LM). Fixed tissue blocks were dehydrated in a graded ethanol series, as described previously. Some samples were infiltrated with purified GMA monomer for 1 week at 20°C in gelatin capsules before polymerization for 3 days at 40°C. The GMA-embedded material was cut into 5 µm sections on an ultramicrotome equipped with a glass knife (Walter and Schadel, 1982). Other samples were dehydrated with an ethanol-tertiary butanol series, embedded in paraffin (Jensen, 1962) and sectioned at 12-15 µm on an American Optical Company rotary microtome equipped with a stainless steel knife. Sections were examined and photographed with a Wild M20 microscope.

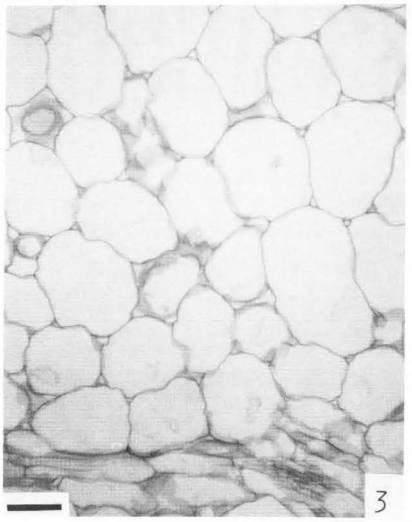
Histochemical and fluorescence studies. Paraffin sections were dewaxed in xylene and either rehydrated with water or equilibrated with a solvent compatible with the histochemical stain to be used. Sections were stained with fast green and counterstained with safranin (fugal hyphae stain; Jensen, 1962), stained for cellulose with Schultze Solution (Gurr, 1965) for carbohydrates with the periodic acid-Schiff series (Jensen, 1962), for pectic substances with ruthenium red (Jensen, 1962), for callose with aniline blue (Jensen, 1962), and with the polychromatic stain, toluidine blue O (O'Brien et al., 1964). GMA sections were stained with the periodic acid-Schiff series (Jensen, 1962). The stained sections were examined with a Wild M20 microscope using bright field illumination. In addition, dewaxed, hydrated sections were stained with 0.1% aqueous calcofluor and examined with a fluorescence microscope (Trigiano et al., 1983).

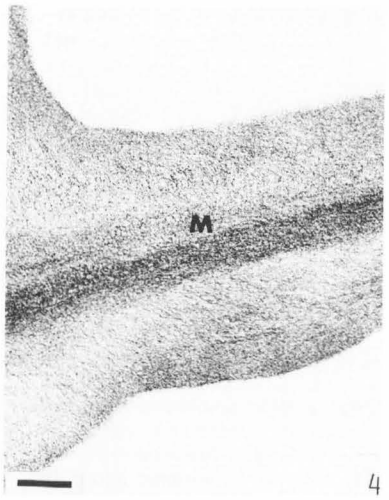
Results and Discussion

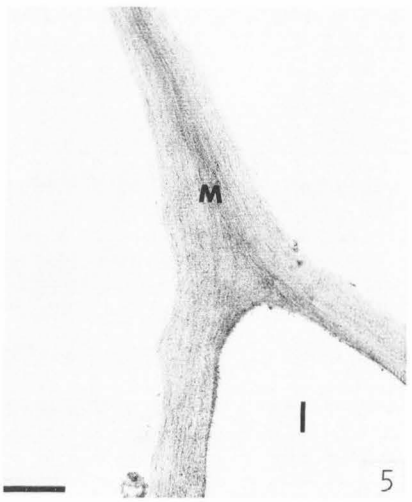
Since it was suspected that the softened stem ends could have been caused by fungal invasion, stem end tissue from eight soft and three firm pickles was examined for infection. Fungal hyphae were found in tissue from both firm and soft pickles. In general, the hyphae were found in or near vascular tissue and adjacent intercellular spaces (Fig. 1). Hyphae stained with fast green and counter stained with safranin (Jensen, 1962) appeared greenish blue, blue or red indicating that more than one type of infecting organism was present. If fungal infection caused the observed softening, the fact that fungi were found in both soft and firm pickles must be rationalized. Since the











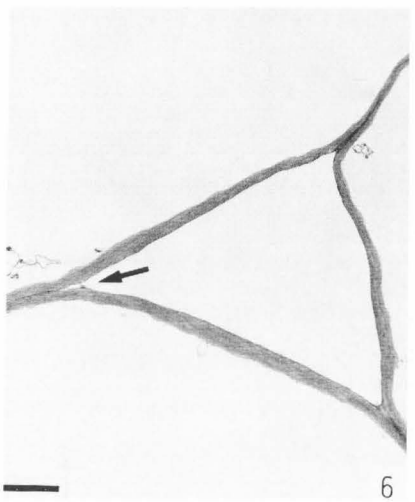


Fig. 1. Light microscopy of a longitudinal section of paraffin-embedded, soft cucumber tissue stained with safranin-fast green. Fungal hyphae are evident in vascular tissue (arrows). Bar equals 40 µm.

Fig. 2. Light microscopy of a cross section of GMA-embedded, soft cucumber tissue stained with PAS series. Cell-to-cell junctions are swollen, and considerable separation is seen. Bar equals 40 µm.

Fig. 3. Light microscopy of a cross section of GMA-embedded, firm cucumber tissue stained with PAS series. Cell-to-cell junctions are regular, and very little separation is seen. Bar equals 40 µm.

Fig. 4. TEM of adjacent cell walls from firm cucumber tissue showing intense staining of microfibrils and well defined middle lamella (M). Bar equals 0.1 µm.

Fig. 5. TEM of adjacent cell walls near intercellular space (I) from firm cucumber tissue. Note well-defined middle lamella (M), highly organized arrangement of microfibrils and strong staining. Bar equals 0.25 µm.

Fig. 6. TEM of intercellular space cell from firm cucumber tissue. Note intensely stained, well organized nature of the walls. Some separation of adjacent cell walls is occurring (arrow). Bar equals 1.0 µm.

histochemical test used in this study does not differentiate fungal species, it is possible that fungal species producing hydrolytic enzymes infected that portion of the cucumbers, which resulted in soft pickles, whereas the remainder of the cucumbers were infected by fungi which did not produce hydrolytic enzymes.

Attempts to differentiate between soft and firm tissue using light microscopy in conjunction with various histochemical tests (for cellulose, carbohydrates, pectic substances, callose) were not successful. Apparently the tests employed are not sufficiently sensitive to detect differences in the chemical composition of the cell walls. However, using GMA sections stained with the PAS reagent, we were able to detect morphological differences between soft and firm tissue. The cell walls in soft tissue appeared to be swollen and torn (Fig. 2). Firm tissue cell walls appeared more organized, and very little cell wall separation was evident (Fig. 3).

TEM of cell walls was more informative. As a rule, cell walls from softened tissue did not adsorb the uranyl acetate-basic lead citrate stain well and appeared less electron dense than firm tissue when viewed on the fluorescent screen of the electron microscope. The TEM photographs do not exhibit this phenomenon because print exposure times were increased to compensate for the decreased staining. Generally, firm tissue (Figs. 4, 5 and 6) cell walls consisted of densely packed fibrillar material with some granular material which may be cell wall matrix. In those sections which contained walls between adjacent cells, the middle lamella was clearly visible as a dark-stained line. However, striated, swollen walls, enlarged intercellular spaces and decreased staining of the middle lamellae were also observed in isolated portions of the firm tissue. Saxton and Jewell (1969) and Jewell (1972) reported that cell wall reorganization occurred during brine fermentation of cauliflower and brown onions. Reorganization involved ultrastructural features like we observed in isolated portions of firm tissue. Likewise, occasional areas of normal cell wall structure were present in softened tissue, but the majority of the walls appeared less dense with the middle lamellae either faintly visible or not visible at all (Figs. 7, 8, and 9). In many cases the cell walls of soft tissue were swollen (Fig. 10) and contained striations in which areas of high and low electron density were observed. Infrequently, separation of two adjacent cell walls caused by dissolution of part of the middle lamella was observed (Fig. 11).

The TEM observations indicated that soft tissue exhibited a loss of material from the cell wall and partial to complete dissolution of middle lamellae. The changes appear to be similar to those which occur during ripening of apples and pears (Ben-Arie et al., 1979), tomatoes (Crookes and Grierson, 1983) and avocado (Pesis et al., 1978). Ben-Arie et al. (1979) applied solutions of PG and/or cellulase (CE) to firm tissue disks from apples and pears and demonstrated that in pears a combination of both enzymes was necessary to cause ultrastructural changes similar to that which occurred during ripening, while PG alone caused ripening-like changes in apple cell walls. In firm tomato fruit PG caused cell wall degradation similar to ripening, which involved dissolution of the middle lamella. Cellulase treatment of firm tomato tissue disks resulted in swelling of the cell wall and the appearance of electron-dense regions (Crookes and Grierson, 1983).

Deterioration of the ultrastructure of the cell wall in soft pickles is probably due to enzymatic hydrolysis by PG and/or CE. The origin of these enzymes was thought to be the infecting fungi. Although TEM indicated significant differences in the cell wall ultrastructure between soft and firm pickles, no differences in birefringence (under polarized light) or in the affinity for calcofluor, which stains plant cell wall material (Haigler et al., 1980), were observed, indicating cell wall disorganization was limited.

The difference in the physical characteristics between soft and firm pickles was demonstrated by the punch test. In firm tissue the cells were sheared off by the punch as it passed through the tissue (Figs. 12 and 13), whereas in soft tissue entire cells were dragged along the path of the punch (Figs. 14 and 15). This indicated that in the firm tissue cell-to-cell junctions and cell walls required a similar amount of force to shear them, while in the softened tissue cell-to-cell junctions were severely weakened and tearing occurred at the junction rather than at the stronger cell walls. A similar observation has been reported (Sterling and Bettelheim, 1955) when stress was applied to raw and cooked potatoes. Raw potato tissue ruptured across cell walls, while in cooked potatoes breakage occurred between intact cells. These findings are consistent with the TEM observations which showed that in soft pickle tissue the middle lamellae were separated, faintly stained or not stained at all, indicating a partial or complete dissolution of the cell-to-cell junctions.

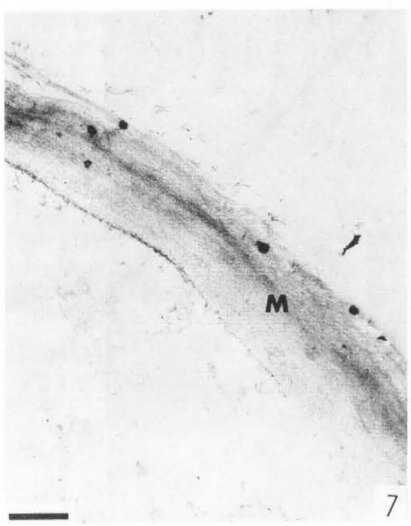
It would be desirable to establish distinctive criteria for delineating the cause of softening when it occurs. Histological and ultrastructural characterization of tissue attacked by various hydrolytic enzymes of microbial or cucumber origin may be useful in this regard and should be further investigated.

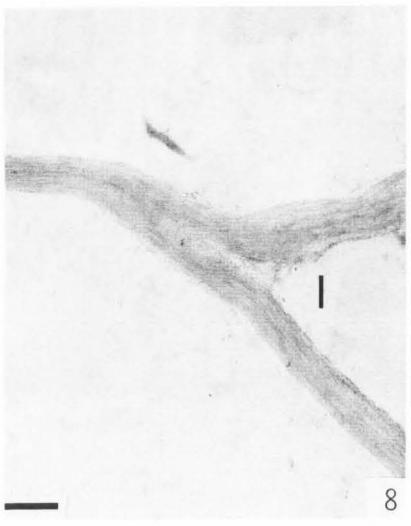
References

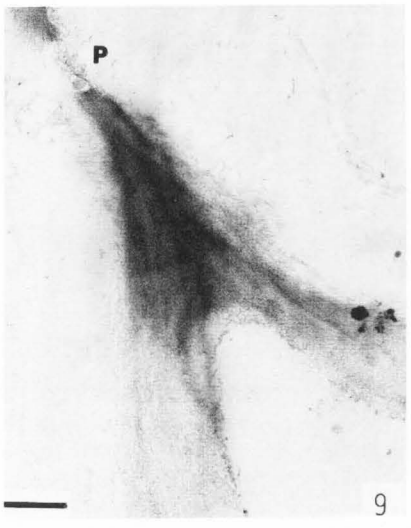
Bell TA, Etchells JL. (1961) Influence of salt (NaCl) on pectinolytic softening of cucumbers. J. Food Sci. **26**, 84-90.

Bell TA, Etchells JL, Jones ID. (1950). Softening of commercial cucumber salt-stock in relation to polygalacturonase activity. Food Technol. **4**, 157-163.

Ben-Arie R, Kisler N, Frenkel C. (1979) Ultrastructural changes in the cell walls of ripening apple and pear fruit. Plant Physiol. **64**, 197-202.







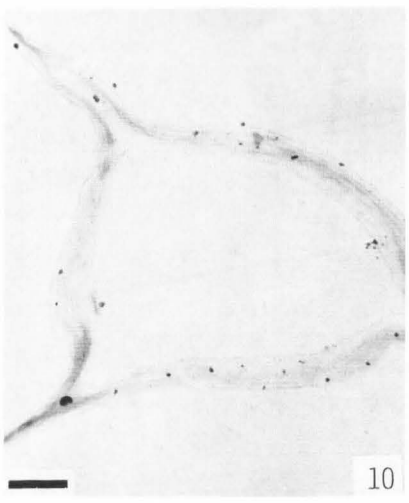


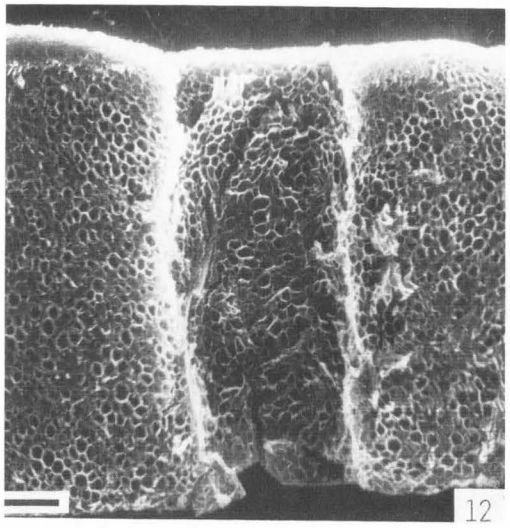
Fig. 7. TEM of adjacent cell walls from soft cucumber tissue showing swelling and decreased microfibril and middle lamella (M) staining. Bar equals 0.33 µm.

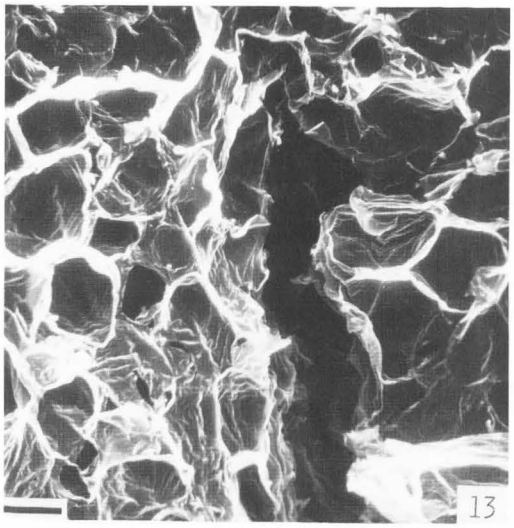
Fig. 8. TEM of adjacent cell walls near intercellular space (I) from soft cucumber tissue. Note decreased intensity of middle lamella staining and striated, swollen areas adjacent to intercellular space. Bar equals 0.33 µm.

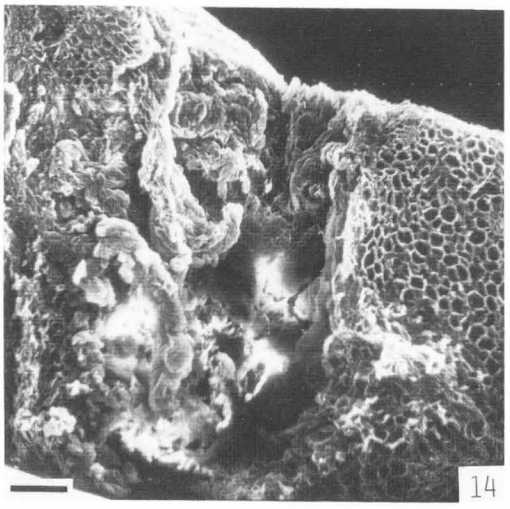
Fig. 9. TEM of adjacent cell walls from soft cucumber tissue showing a primary pit field. Walls are swollen and poorly organized. Very little material is visible in the pit wall (P). Bar equals 0.25 µm.

Fig. 10. TEM of intercellular space from soft cucumber tissue. Note the swollen, striated, poorly organized nature of the walls. Bar equals 1.0 µm.

Fig. 11. TEM of adjacent cell walls from soft cucumber tissue showing dissolution of part of the middle lamella (M), increased striations and swelling. Bar equals 0.33 µm.







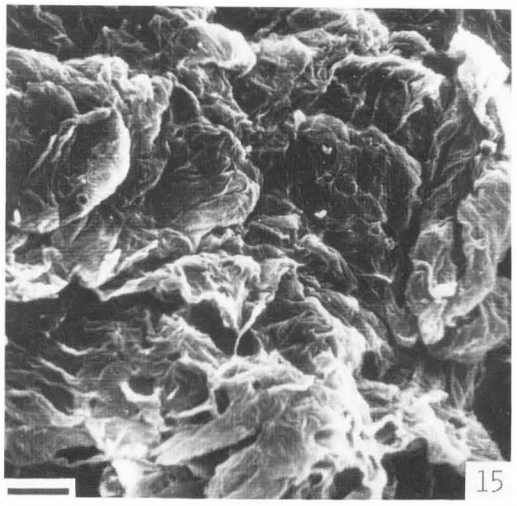


Fig. 12. SEM of a hole in cross section prepared by passing a blunt 1.5 mm punch through a block of firm cucumber pickle tissue, followed by dissection with a razor blade. The hole is flanked by razor-cut faces. Note cells in the hole appear to be sheared off. Bar equals 400 µm.

Fig. 13. SEM of an area on the inside surface of Figure 12 showing shearing across cell walls. Bar equals 100 µm.

Fig. 14. SEM of a hole in cross section prepared by passing a blunt, 1.5 mm punch through a block of soft cucumber tissue. The hole is flanked by razor cut faces. Note clumps of whole, intact cells are visible in the hole where separation has occurred along the middle lamellae. Bar equals 400 µm.

Fig. 15. SEM of an area on the inside surface of Figure 14 showing clumps of cells dragged along the path of the punch rather than sheared across the cell walls. Bar equals 100 µm.

Buescher RW, Hudson JM. (1984). Softening of cucumber pickles by Cx-cellulase and its inhibition of calcium. J. Food Sci. **49**, 954-955.

Buescher RW, Hudson JM, Adams JR. (1979). Inhibition of polygalacturonase softening of cucumber pickles by calcium chloride. J. Food Sci. **44**, 1786-1787.

Buescher RW, Hudson JM, Adams JR. (1981). Utilization of calcium to reduce pectinolytic softening of cucumber pickles in low salt conditions. Lebensm. -Wiss. u. -Technol. **14**, 65-67.

Costilow RN, Gates K, Lacy ML. (1980). Molds in brined cucumbers: cause of softening during air purging of fermentations. Appl. Exper. Microbiol. **40**, 417-423.

Crookes, PR, Grierson D. (1983). Ultrastructure of tomato fruit ripening and the role of polygalacturonase isoenzymes in cell wall degradation. Plant Physiol. **72**, 1088-1093.

Etchells JL, Bell TA, Monroe RJ, Masley PM, Demain AL. (1958). Populations and softening enzyme activity of filamentous fungi on flowers, ovaries, and fruit of pickling cucumbers. Appl. Microbiol. **6**, 427-440.

Fleming HP, Humphries EG, Macon JA. (1983). Progress in development of an anaerobic tank for brining cucumbers. Pickle Pak Sci. **VII**, 3-15.

Fleming HP, Thompson RL, Bell TA, Hontz LH. (1978). Controlled fermentation of sliced cucumbers. J. Food Sci. **43**, 888-891.

Gurr E. (1965). The Rational Use of Dyes in Biology, Williams and Wilkins Company, Baltimore, MD, 311.

Haigler CH, Brown RM, Benziman M. (1980). Calcofluor white ST alters the in vivo assembly of cellulose microfibrils. Science **210**, 903-905.

Jensen WA. (1962). Botanical Histochemistry, W. H. Freeman and Company, San Francisco, CA, 72, 198-204.

Jewell GG. (1972). Structural and textural changes in brown onions during pickling. J. Food Technol. **7**, 387-399.

Lampi RA, Esselen WB, Thomson GL, Anderson EE. (1958). Changes in pectic substances of four varieties of pickling cucumbers during fermentation and softening. Food Res. **23**, 351-363.

McFeeters RF, Bell TA, Fleming HP. (1980). An endo-polygalacturonase in cucumber fruit. J. Food Biochem. **4**, 1-16.

O'Brien TP, Feder N, McCulley ME. (1964). Polychromatic staining of plant cell walls by toluidine blue O. Protoplasma **59**, 368-373.

Pesis E, Fuchs Y, Zauberman G. (1978). Cellulase activity and fruit softening in avocado. Plant Physiol. **61**, 416-419.

Pressey R, Avants JK. (1975). Cucumber polygalacturonase. J. Food Sci. **40**, 937-939.

Saxton CA, Jewell GG. (1969). The morphological changes produced in cauliflower stems during pickling and their relationship to texture parameters. J. Food Technol. **4**, 363-375.

Spurr AR. (1969). A low viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruc. Res. **26**, 31-45.

Sterling C, Bettelheim F. (1955). Factors associated with potato texture. III. Physical attributes and general conclusions. Food Res. **20**, 130-137.

Trigiano RN, Van Dyke CG, Spurr HW Jr. (1983). Haustorial development of Peronospora tabacina infecting Nicotiania tabacum. Can. J. Bot. **61**, 3444-3453.

Walter WM Jr, Schadel WE. (1982). Effect of lye peeling conditions on sweet potato tissue. J. Food Sci. **47**, 813-817.

Discussion with Reviewers

W. M. Breene: What is the possible role, if any, of mechanical damage in the inducement of conditions seen in the micrographs?
Authors: It is possible that mechanical damage to the fruit was involved in softening, although no direct evidence was observed. Such damage could activate endogenous enzymes of the fruit or possibly encourage invasion by softening microorganisms. Such questions remain to be answered.

W. M. Breene: My second question is perhaps a reflection of my limited microbiological knowledge and is in reference to the rationalization that the fungi present in firm pickles did not have hydrolytic capacity. How do such fungi manage to eat?
Authors: The fact that fungal hyphae were present without softening was not surprising. Hydrolytic enzymes of fungi may be either constitutive or inducible if they are produced at all. If inducible, they may not be produced by invading fungi as long as simple sugars are available. This could be the explanation in the present case.

R. W. Buescher: Why was the presence of fungi suspected? Could bacteria also be present within the tissue and contribute to softening? Was there any evidence for the presence of bacteria?
Authors: Softening of brined cucumbers in discrete regions is considered to have been caused by microbial invasion, whereas softening of the entire cucumber is thought to result from fungal enzymes that entered the brine via cucumber flowers or some other source. Since polygalacturonases of fungal origin are generally more active at the low pH of fermented cucumbers than are those of bacterial origin (Etchells et al., 1958), we felt that fungi were more likely to have caused the softening if softening occurred after the fruit were brined. We observed no softening of the

fruit before brining, however, it was noted that a few of the fresh cucumbers were infected with fungal mycelium near the stem end.

R. W. Buescher: When would fungi have entered the cucumber: prior to, during, or after brining?
Authors: We think that the fungi had invaded the tissue before brining. It is unlikely that fungi would have grown after brining since the brines were anaerobic, being purged with nitrogen. Costilow et al. (1980) found fungal mycelium to occur in brined cucumbers as a result of air purging. Since fungi are aerobic, the findings of Costilow et al. (1980) are not surprising.

R. W. Buescher: Would the softening of stem end tissue occur in brines with higher NaCl or calcium concentrations? Is it possible that the softening occurred prior to brining?
Authors: Both of these questions will require further research before answering. It is possible that changes in the tissue (which manifested themselves later as softening in the brined cucumbers) occurred before the cucumbers were brined. In this case, higher NaCl or calcium probably would not be effective. If softening occurred after brining, higher NaCl or calcium may be beneficial. We are attempting to answer these questions.

R. W. Buescher: What evidence is there that "The TEM observations indicated that soft tissue exhibited a loss of material from the cell wall"? Could cell wall density (hydration-swelling) be altered without affecting the composition?
Authors: We observed significantly decreased staining by the uranyl acetate-basic lead citrate schedule in the soft tissue whether or not the cell walls were swollen and/or striated. We interpreted this to indicate that those components of the walls which bind the stain were decreased in soft tissue.

M. C. Bourne: Can the authors give mean values for puncture force of soft and firm pickles?
Authors: We regret that puncture force measurements were not made in the softened stem end area. Puncture force measurements made in the dictal (firm) end did not show any difference between firm and stem end softened tissue.