

Effect of Water Stress on Stored Pickling Cucumbers[†]

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Pickling cucumber fruits were stored at 15.5 °C and either 62 or 93% relative humidity (rh) for 28 days. Periodically, fruits were removed from both treatments, and weight loss was measured. Mesocarp tissue was analyzed for osmotic potential, relative water content, membrane leakage, concentration of acids, sugars, and free amino acids, and cell wall composition. Fruit stored at 62% rh lost weight more rapidly, had increased decline in relative water content and osmotic potential, and had increased membrane leakage relative to fruits stored at 93% rh. Fruits stored at 62% rh exhibited more senescence characteristics than fruits stored at 93% rh. Both treatments developed pillowy tissue disorder, but for the fruits stored at 62% rh, the disorder was more severe. Arabinose and galactose levels in cell walls decreased during storage. This research has shown that long-term storage of cucumber fruits prior to pickling would probably result in poor quality products.

INTRODUCTION

Cucumber fruits destined for conversion into pickled food products are not usually stored for long periods of time. However, at times during the processing year, fruits are not available locally and must be transported long distances. Thus, there may be a significant time interval between the harvest and processing dates. This practice can result in a significant amount of stress due to transpirational water loss. Cucumbers are covered with a waxy cuticle and lose water at the rate of 0.17 mg cm^{-2} ($0.1 \text{ Pa H}_2\text{O}$ vapor deficit)⁻¹ h (Burton, 1982). Thus, as the deficit in water vapor pressure between the fruit interior and the exterior environment increases, the rate of transpirational water loss increases. Water loss by a detached plant organ causes stress as the organ water status changes. Depending upon the degree of stress, cellular responses such as osmotic regulation, ultrastructural organization, membrane integrity, RNA and protein synthesis, and respiration are affected (Bewley and Krochko, 1982). These cellular responses are manifested at the plant organ level as a range of effects which impact appearance, color, flavor, texture, storability, and processing characteristics of the commodity.

Ben-Yehosua et al. (1983) and Lurie et al. (1986) showed that for bell pepper and lemon fruit water stress (WS) accelerated senescence-like changes such as loss of firmness, membrane leakage, and changes in cell wall pectins. These workers also showed that prevention of WS by sealing the commodity in plastic film slowed these changes and increased the storage life. Lazan et al. (1987a,b) reported similar results for edible leaves of *Brassica juncea* (leaf mustard) and *Amaranthus caudatus* (Inca wheat), where WS caused declines in ascorbic acid, chlorophyll, and protein content that could be slowed by enclosing the leaves in plastic film. Kailasapathy and Koneshan (1986) reported that leafy green vegetables stressed to the point of wilting lost vitamin C.

Etchells et al. (1973) found that the best conditions for storing pickling cucumbers were low temperature (10 °C) and high humidity [90–95% relative humidity (rh)]. Lee et al. (1982) reported that good quality pickles could be

made from cucumbers stored 6 days or less at 5 °C and 90% rh. The present study was conducted to examine the effect of WS on the water status and composition of stored cucumber fruits. Information of this nature is needed if the mode of action of WS in accelerating senescence is to be understood and used to control this process in stored plant foods.

MATERIALS AND METHODS

Cucumber fruits (44–51 mm in diameter and 175–240 g in weight) were obtained shortly after harvest from Mount Olive Pickle Co., Inc., Mount Olive, NC. After weighing, the fruits were divided into two groups of 80. Each group was put into a separate Hotpack environmental chamber at either 93 or 62% rh and held under these conditions for 28 days. The temperature in both chambers was maintained at 15.5 °C. Periodically, four cucumbers were removed from each chamber and weighed. Each cucumber served as a replicate. Each fruit was peeled, and a 1-cm section was removed from each end and discarded. A tissue core sample removed from each end with a cork borer (13 mm i.d.) was used for water potential (WP) and osmotic potential (OP) determinations. The remaining tissue was cut into 1-cm widths, seeds and placental tissue were discarded, and the mesocarp was cut into eighths and randomized. Samples were selected for determination of dry matter (DM), relative water content (RWC), and conductivity. The remainder of the mesocarp tissue was frozen for determination of cell wall content and composition, organic acids, sugars, and free amino nitrogen.

Water Potential (WP). Cores were removed from the cork borer, and slices 1.0 mm thick were cut, blotted, and placed in the sample compartment of a Wescor Model 5100CRX osmometer. After an equilibration period of 10 min, the WP was recorded.

Osmotic Potential (OP). The remainder of the core was placed in a 3-mL plastic syringe equipped with a piece of glass fiber filter material. The plunger was inserted and pressed against the tissue core, and a plastic cap was used to seal the needle end. The syringe was then placed in dry ice until the core solidified. The frozen cores were held at -10 °C overnight. Each syringe was allowed to thaw 15 min at room temperature and the juice expressed from the tissue by putting pressure on the plunger. The juice was collected and the OP measured on 10-μL portions in a Wescor 5100CRX osmometer.

Dry Matter (DM). Duplicate 10-g samples were dried to constant weight at 100 °C in a forced-draft electric oven.

"Pillow" Disorder. After 6 days, dried mesocarp tissue from some cultivars assumed a white, styrofoam-like appearance described by other workers as pillow disorder (Staub et al., 1988). Dried tissue was rated by the authors on the basis of

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its appearance as follows: 1, dark brown, compact mass; 2, brownish white, slightly styrofoam-like; 3, whitish brown, styrofoam-like; 4, white-slightly brown, very styrofoam-like.

Relative Water Content (RWC). Duplicate 10-g samples were placed in beakers, covered with water, and evacuated to 5 mmHg for 105 min. The vacuum was released, and the tissue was held for 2 h at room temperature, blotted dry, and weighed. $RWC = (W_f - W_d / W_t - W_d) \times 100$, where W_f is fresh weight, W_d is dry weight, and W_t is weight after imbibement (turgid weight).

Membrane Leakage (Conductivity). Duplicate 10-g samples were placed in beakers, covered with 45 mL of water, and held for 2 h at room temperature. The supernatant was decanted and its conductivity measured with an Amber conductivity meter equipped with a Pt electrode system. The supernatant was poured back on the tissue and the tissue homogenized. The slurry was centrifuged and the conductivity again measured on the liquid portion. Percent conductivity = (conductivity of bathing solution/conductivity of homogenate) $\times$ 100.

For the following procedures, the tissue was weighed (while frozen) in a cold room (5 °C).

Cell Walls. Weighed tissue was homogenized and extracted with water, 1% aqueous sodium dodecyl sulfate, phenol/acetic acid (2/1), water, and acetone (Ring and Selvendran, 1978). The walls were held over P_2O_5 for several days and then weighed.

Cell Wall Sugars. Weighed samples of cell walls (ca. 10 mg) were hydrolyzed with sulfuric acid, the sugars transformed into alditol acetates, and the alditol acetates separated on a Hewlett-Packard Model 5980 gas chromatograph equipped with a 30 m $\times$ 0.25 mm capillary column coated with DB-225 (Blakeney et al., 1983; McFeeters and Lovdal, 1987). Oven temperature was 225 °C, injector and detector temperatures were 250 °C, and the flow rate was 25 mL/min. The signal from the gas chromatograph was electronically integrated with a Shimadzu Model C-R3A integrator. Quantitation of the sugars was based on the response factors for authentic alditol acetates.

Uronic Acid. Aliquots from hydrolysis of the cell walls were assayed for galacturonic acid content by using 3,5-dimethylphenol as the chromogen (McFeeters and Lovdal, 1987).

Sugars and Organic Acids. These components were measured by using HPLC. Weighed samples of frozen tissue were thawed, homogenized, and centrifuged. Sugars in the supernatant portion were separated on a Bio-Rad HPX-87P (Pb, 25 cm $\times$ 4 mm) column (65 °C) connected to a Waters 6000A solvent delivery system and detected with a Waters refractive index detector. Flow rate of the water mobile phase was 0.8 mL/min. Organic acids were separated on a Bio-Rad HPX-87H (25 cm $\times$ 4 mm) column (65 °C) connected to a Waters 6000A pump and a Varian Varichrom UV-visible detector operating at 210 nm. The mobile phase was 0.01 N H_2SO_4 , and the flow rate was 0.8 mL/min. Quantitation was performed on the integrated signal with a Shimadzu Model C-R3A integrator.

Free Amino Nitrogen. Supernatant prepared for the sugar determination was also assayed for free amino nitrogen content. The procedure used was essentially that of Church et al. (1983), which is a spectrophotometric assay based on the reaction between α -amino groups and *o*-phthalaldehyde (OPA). We used glycine as the standard and calculated free amino acid content as glycine.

Microscopy. Mesocarp tissue from stem end and midsection of fruit was removed and cut into rectangular blocks of 5–7 mm/surface. The blocks were fixed in 3% glutaraldehyde in 0.05 M KH_2PO_4 . Tissue for light microscopy was dehydrated with a *tert*-butyl alcohol series, embedded in Paraplast-Plus, cut into 12 μ m thick sections, deparaffinized, stained with safranin-fast green, and evaluated on a Wild model light microscope (Walter et al., 1990). For scanning electron microscopy (SEM), fixed tissue sections were dehydrated with ethanol, critical point dried by using CO_2 as the transition fluid, sputter coated, viewed, and photographed with a Phillips Model 505T scanning electron microscope (Walter and Schadel, 1982) at 20 kV.

Statistical Analysis. Data were analyzed by using the analysis of variance procedure and means separated by Waller-Duncan multiple range test (SAS, 1982).

Table I. Percent Weight Loss and Dry Matter for Cucumbers Stored at 15.5 °C and 93 or 62% Relative Humidity

time	% weight loss ^a		% dry matter	
	93% rh	62% rh	93% rh	62% rh
0	0	0	4.23 $\pm$ 0.53	4.23 $\pm$ 0.53
6	3.44 $\pm$ 0.45	14.30 $\pm$ 2.10	3.80 $\pm$ 0.45	4.00 $\pm$ 0.24
13	7.00 $\pm$ 0.69	25.33 $\pm$ 2.65	3.35 $\pm$ 0.24	3.95 $\pm$ 0.47
21	11.15 $\pm$ 3.45	34.01 $\pm$ 5.60	3.60 $\pm$ 0.22	4.38 $\pm$ 0.51
28	15.02 $\pm$ 3.16	37.81 $\pm$ 2.34	3.10 $\pm$ 0.20	4.60 $\pm$ 0.58

^a Values are for means from four cucumbers ($\pm$ standard deviation).

RESULTS AND DISCUSSION

Water Potential (WP), Osmotic Potential (OP), Relative Water Content (RWC), Weight Loss, Conductivity, and Free Amino Acids. WP, a measure of plant water status, is the difference in free energy between matrix bound, pressurized, or osmotically constrained water and pure water (Baker, 1984). The WP of pure water is 0. When a solute is added, the WP becomes negative as the free energy is decreased. The WP can be defined as

$$WP = (RT/V) \ln a_w \quad (1)$$

and measured with a thermocouple psychrometer, where R is the gas constant, V is the molar volume of water, T is the absolute temperature in degrees Kelvin, and a_w is the water activity (equilibrium rh) of the tissue in a sealed psychrometer chamber. Water flows from areas of low to high WP. Thus, a plant organ with its high water vapor pressure interior tends to lose water via transpiration to the surrounding atmosphere, depending upon the barrier characteristics of the skin. As the plant loses water, WP becomes more negative and stress increases. WP is related as follows

$$WP = OP + TP \quad (2)$$

where OP is osmotic potential and TP is turgor potential. The OP is determined by measuring the osmotic pressure of the cell sap. The TP can be calculated from eq 2 (Tyree and Jarvis, 1982). Another method of evaluating plant water status is the RWC, which is a measure of the actual water content relative to the water content at full turgidity. Since for cucumbers water occupies most of the tissue space, a decline in RWC represents a decrease in cellular volume and thus tissue shrinkage.

Cucumbers stored at the highest rh lost less weight than did those stored at the lowest rh (Table I). DM of fruits stored at 93% rh tended to decline during storage, probably as a result of normal metabolism, while DM of fruits stored at low rh did not change because of the larger water loss which abrogated metabolic DM loss. RWC decreased during storage for fruits from both treatments, with the fruits stored at 93% rh RWC declining least (Figure 1). WP and OP changed in the same manner (Figure 2). For fruits stored at 93% rh, both declined, indicating that solutes dissolved in the cell sap were being metabolized. However, for fruits stored at 62% rh, neither parameter changed, also reflecting the conflicting effect of normal metabolic loss and concentration of the remaining solutes by transpirational water loss. Examination of the sugar content in both groups of fruits (Figure 3) showed that both glucose and fructose levels declined as the storage period increased. No statistically significant trend with respect to storage humidity was observed. Loss of sugars during storage may be of significance with regard to fermentation processing because glucose and fructose comprise most of the substrate for the fermentative microorganisms. Since fruits stored for 13 days lose approximately 25% of their

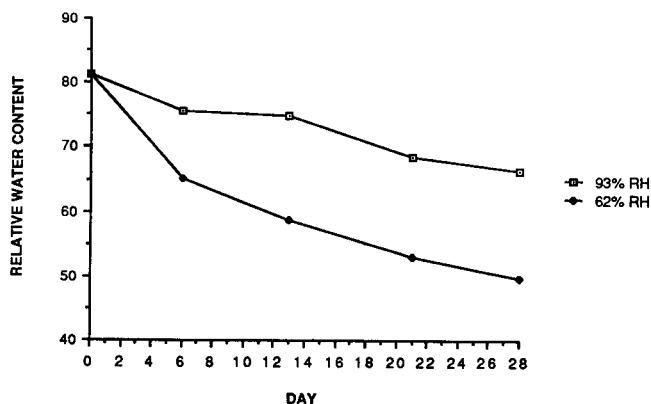


Figure 1. Relative water content (RWC) of cucumbers stored at 15.5 °C and either 93 or 62% rh. Minimum significant difference ($P \leq 0.05$) is 4.80 for 93% rh and 6.11 for 62% rh.

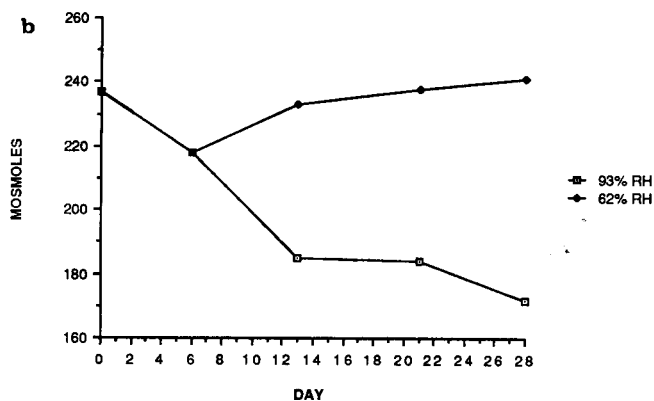
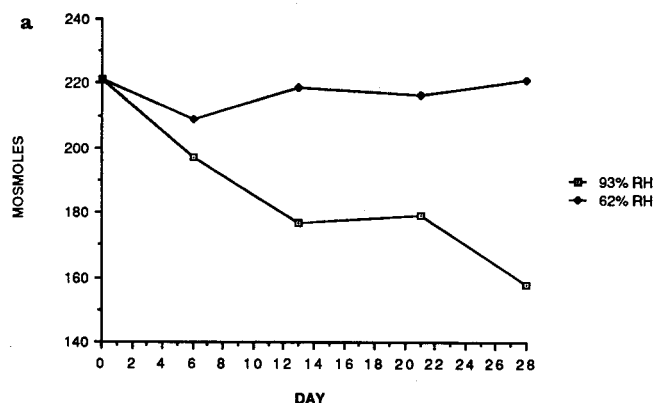


Figure 2. (a) Water potential (WP) in milliosmoles for cucumbers stored at 15.5 °C and either 93 or 62% rh. Minimum significant difference ($P \leq 0.05$) is 42 milliosmoles for 93% rh. There is no significant difference for 62% rh. (b) Osmotic potential (OP) in milliosmoles for cucumbers stored at 15.5 °C and either 93 or 62% rh. Minimum significant difference ($P \leq 0.05$) is 33 milliosmoles for 93% rh. There is no significant difference for 62% rh.

sugar, fermentation characteristics and product quality of stored fruits would be different from those of just-harvested fruits.

Some plants have been shown to adapt to WS by osmotic adjustment in which the osmolality of the cell sap changes in response to WP so that cell turgor and, thus, normal metabolic function are maintained (Oertli and Jenka, 1985; Levitt, 1986). Cucumbers synthesized small amounts of citric acid (Figure 4). Since fruits stored at 62 and 93% rh synthesized similar amounts of both acids, it is unlikely that these compounds represent osmotic adjustment to WS,

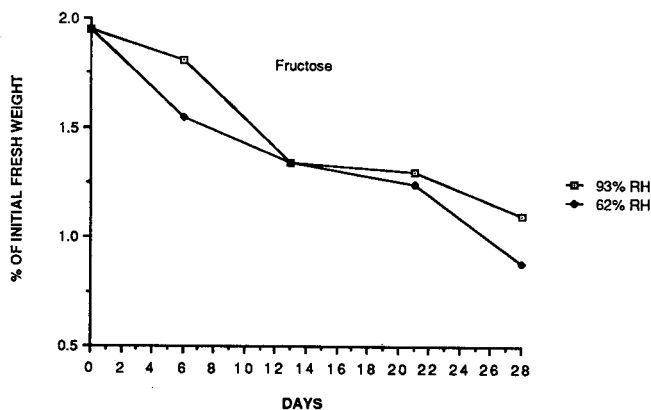
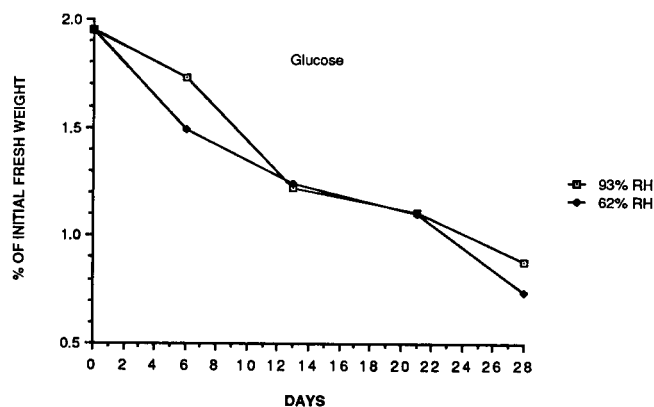


Figure 3. Glucose and fructose content (as percent of fresh weight at 0 time) for cucumbers stored at 15.5 °C and either 93 or 62% rh. Minimum significant difference ($P \leq 0.05$) at 93% rh is 0.42 and 0.38% for glucose and fructose, respectively. Minimum significant difference ($P \leq 0.05$) at 62% rh is 0.34 and 0.38% for glucose and fructose, respectively.

but rather that synthesis is the result of normal postharvest metabolic activity as the fruits approach senescence. There was no statistically significant difference in amounts of malic acid at 93% rh (Figure 4). For fruits stored at 62% rh, only day 6 fruits contained lower amounts of malic acid, so there was no increase with increase in storage time. The fact that osmotic adjustment did not occur is not surprising in view of the fact that WP declined for fruits stored at 93% rh and remained constant for fruits stored at 62% rh, even though the former had lost >15% and the latter had lost >38% of their initial weight after 28 days of storage. Because of the high water content of cucumber and the fact that very little reserve carbohydrate is available for metabolic needs after the fruits are detached, metabolism of sugars to provide energy is expected and serves to decrease WP via lowering of the OP. The decrease in RWC (Figure 1) which occurred concurrently with water loss indicated that cellular volume decreased and hence the fruit volume decreased, leading to a shriveled appearance, especially in the fruits stored at 62% rh, which by 28 days had lost >38% of their weight. The degree of shriveling was dependent upon the extent of water loss and will likely adversely affect the appearance and textural properties of fermented cucumbers.

One of the first cellular processes affected by WS is membrane function (Leopold et al., 1981). Damage to membrane integrity can be detected by leakage of cellular electrolytes and amino acids into a bathing medium. We observed a significant increase in conductivity after 13 days of storage of solutions from fruits stored at 62% rh over conductivity of solutions from fruits stored at 93% rh which

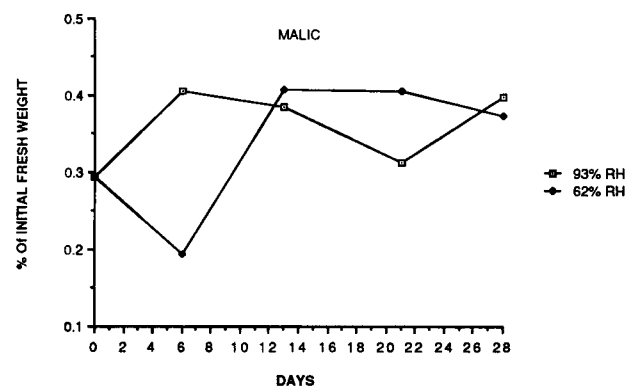
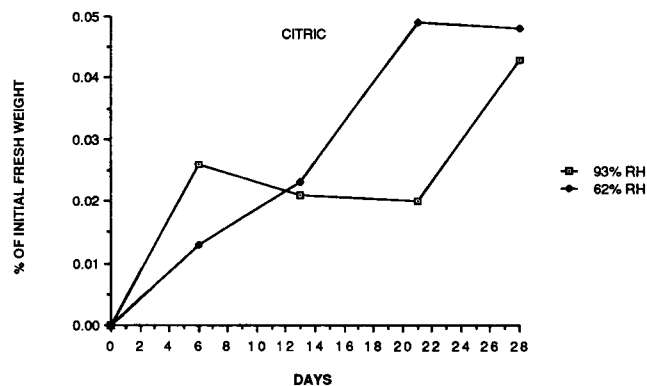


Figure 4. Citric and malic acid content (as percent of fresh weight at 0 time) for cucumbers stored at 15.5 °C and either 93 or 62% rh. Minimum significant difference ($P \leq 0.05$) at 93% rh is 0.016% for citric acid. Malic acid content did not change. For 62% rh the minimum significant difference is 0.014 and 0.139% for citric and malic acids, respectively.

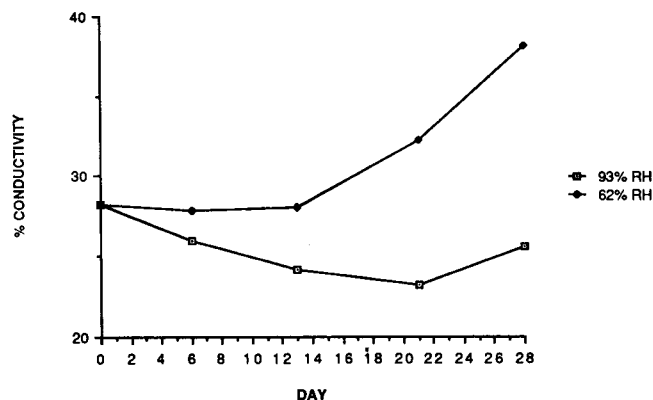


Figure 5. Percent conductivity of the batching liquid from cucumbers relative to conductivity of homogenized cucumber tissue during storage at 15.5 °C and either 93 or 62% rh. Minimum significant difference ($P \leq 0.05$) is 4.5 and 6.3% for 93 and 62% rh fruit, respectively.

did not change during storage (Figure 5). Storage humidity had no effect on free amino acid content, although there was an increase for fruits stored at 62% rh as storage time increased (data not shown). These data indicated that membranes of fruits stored at 62% rh were damaged by WS.

Pillow Disorder. By 9 days mesocarp tissue from some of the cucumbers began to assume a white, styrofoam-like appearance. This has been reported to occur in freshly harvested cucumbers and has been designated by the pickling industry as pillow disorder, which renders pickles

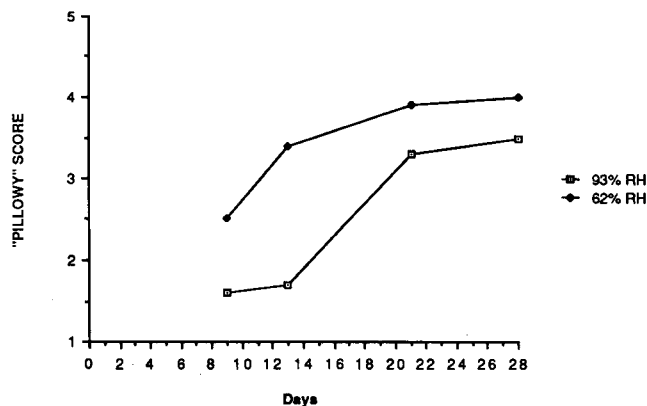


Figure 6. Scores for severity of pillow disorder from oven-dried cucumbers stored at 15.5 °C and 93 or 62% rh. 1, none observable; 4, white, styrofoam-like appearance.

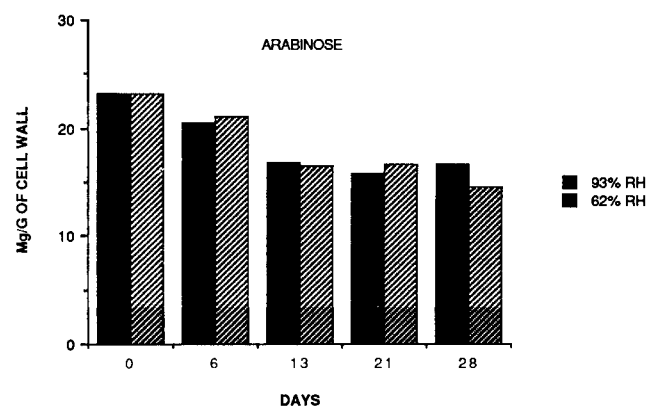
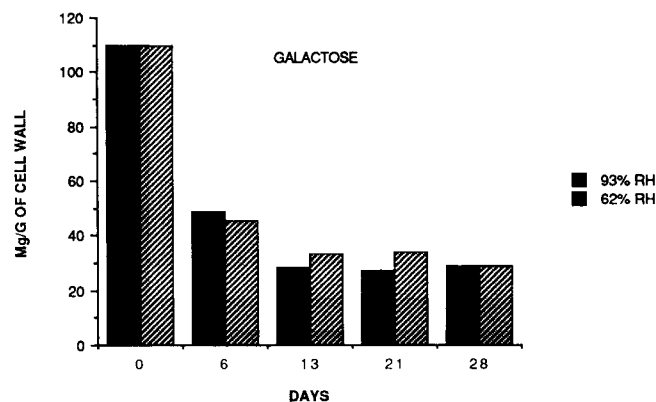


Figure 7. Galactose and arabinose content of cell wall (dry basis) for cucumbers stored at 15.5 °C and 93 or 62% rh. Standard error of the mean is 3.63 and 1.07 mg/g cell wall for galactose and arabinose, respectively.

Table II. Analysis of Variance for Cell Wall Sugars from Cucumbers Stored at 15.5 °C and 93 or 62% Relative Humidity for 28 Days

cell wall sugars	source of variability	
	rh	time stored
galacturonic acid	NS	a
rhamnose	NS	NS ^b
fucose	NS	c
arabinose	NS	a
xylose	NS	a
mannose	NS	a
galactose	NS	a
glucose	NS	a

^a $P \leq 0.01$. ^b NS, not significant. ^c $P \leq 0.05$.

Table III. Cell Wall Sugars^a from Cucumbers Held at 15.5 °C and 93 or 62% Relative Humidity

sugars ^b	0 time	93% rh				62% rh			
		6 days	13 days	21 days	28 days	6 days	13 days	21 days	28 days
galacturonic acid	207.5	235.0	245.0	230.3	225.0	245.0	245.0	202.5	226.7
rhamnose	10.4	11.2	10.9	11.0	12.1	11.4	12.4	11.3	10.8
fucose	3.9	4.6	4.5	4.2	5.0	4.3	4.7	4.8	4.5
xylose	60.3	60.8	62.4	63.2	71.2	64.2	65.4	69.8	69.3
mannose	42.6	40.9	42.2	44.3	46.0	42.8	45.2	47.5	46.0
glucose	413.1	444.6	444.0	457.8	482.8	442.3	478.1	511.3	485.5

^a Milligrams per gram of dry cell wall. ^b Each value mean of sugar content from four cucumbers.

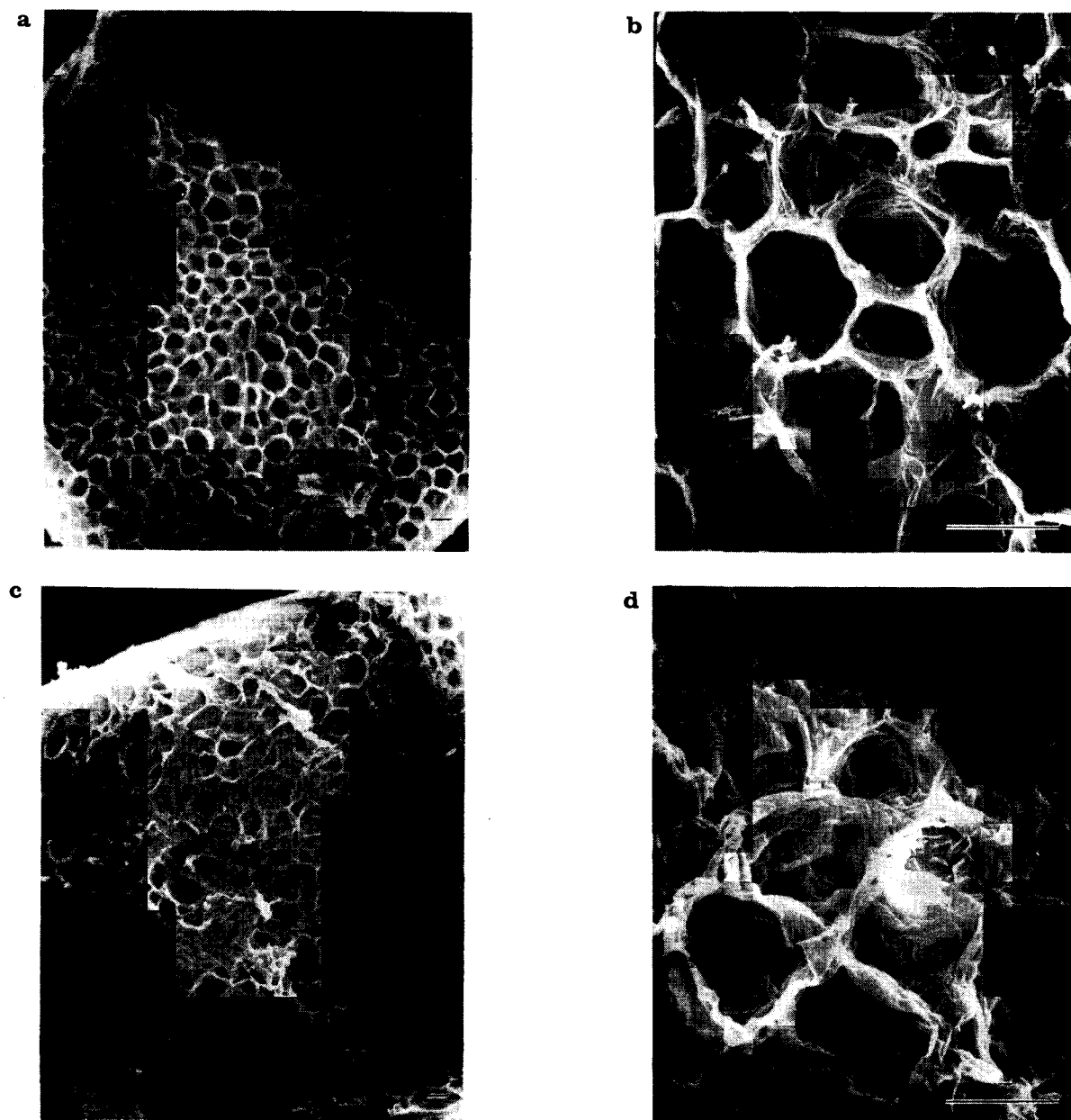


Figure 8. Scanning electron photomicrographs of mesocarp from normal and pillowy disorder cucumber fruit. Note regular shape (a) and slightly wrinkled cell walls of normal fruit (b). Pillow tissue has crushed, severely wrinkled cell walls (c, d). Each bar is 100 μ m.

unacceptable for sale (Staub et al., 1988). These workers reported that pillowy disorder severity increased from stem to blossom end. In our experience, the disorder began in the stem end and was most severe in that area. None of our fruits analyzed prior to day 9 had the disorder. At day 9 pillowy disorder was observed in some of the tissue and by 28 days was severe and evident in all. It was especially noticeable in the tissue dried for determination of the moisture content. Initially, the cucumber tissue after drying had a compact, dark brown appearance which, as

stored time increased, changed to a swollen, white, styrofoam-like material. Scores for pillowy disorder on the dried tissue showed that fruits stored at 62% rh had a more severe case of the disorder than did the fruits stored at 93% rh (Figure 6), indicating that WS can increase the severity. Moreover, since pillowy disorder was not observed in the fresh fruits, it seems that WS can cause the disorder to appear. At present, it is not possible to establish whether storage time had any part in development of the disorder since even the fruits stored at 93% rh had a decrease in

RWC of 14.9 (Figure 1) by 28 days and, thus, were themselves stressed.

Cell Wall Composition. The amount of cell wall per unit dry weight increased from 17.3% initially to ca. 26% after 28 days, probably because metabolic utilization of sugars lowered the absolute amount of non-cell-wall solids. No significant differences were noted between the two groups of fruits held at different humidities. Although differences were noted in the amounts of individual cell wall sugars from the two humidity treatments for some time periods, there was no indication that treatment had any effect on cell wall sugar composition (Table II). This was not the case for storage time (Table II). In fact, rhamnose was the only sugar that did not show a statistically significant change over storage time. Fucose, mannose, xylose, and glucose concentrations increased slightly with increasing storage time, although these changes may be apparent rather than real due to losses in galactose and arabinose (Table III). Uronic acid content increased slightly up to day 13 and then declined. The largest change occurred for galactose, which declined from 109.9 to ca. 28 mg/g of cell wall, and the next largest change was for arabinose, which fell from 23.1 to ca. 15 mg/g of cell wall after 28 days (Figure 7). Since the appearance of pillowy disorder coincided with significant loss of galactose and arabinose from the cell wall, it was tempting to postulate a cause and effect relationship. However, Gross and Wang (1984) found that cucumbers stored at 12.5 °C lost galactose at a rate similar to that we observed and that arabinose content did not change during storage. Their data for arabinose showed a larger degree of variability than did ours, and, consequently, a decrease of the magnitude we observed may have occurred and been obscured. Thus, it appears that pillowy disorder does not involve significant changes in cell wall composition, although we cannot rule out the possibilities either that the fruit in the study of Gross and Wang (1984) developed the disorder and it was not noted or that arabinose loss from the cell wall which we observed was caused by pillowy disorder.

The loss of galactose and arabinose without accompanying loss in uronic acid may indicate that the cell wall changes observed in this study were for nonpectic substances. McFeeters and Lovdal (1987) reported a positive correlation between the concentration of total cell wall sugars and mesocarp tissue firmness. Thus, loss in cell wall material during refrigerated storage would probably have a deleterious effect on textural properties of pickled products made from stored cucumbers.

Microscopy. Staub et al. (1988) reported that pillowy disorder caused hypertrophy and rounding of parenchyma cells, leading to a significant decrease in intercellular space. We observed parenchyma cells in pillowy tissue to be larger and rounder, but to have more intercellular space and be more resistant to staining than normal tissue. SEM of normal and pillowy tissues clearly showed that the diseased tissue was compressed and torn when cut with a sharp razor blade during preparation for microscopy, whereas normal tissue was cleanly cut (Figure 8a,b). At higher magnification, cell walls from the pillowy tissue are seen to be crumpled and compacted, while cell walls from normal tissue were undulated but rigid (Figure 8c,d). That this disorder affected the structural integrity of the cell walls is obvious, but since changes in composition of cell wall sugars could not be shown unequivocally to have been caused by the disorder, protein and/or other components must be involved. Alternatively, it is possible that the disorder triggered a rearrangement of cell wall components

in such a way that wall properties were changed, leaving the gross composition unchanged.

This research has shown that storage of pickling cucumbers greater than 9 days could result in poor quality products due to loss of fermentable sugars and cell wall material. If the fruits are water stressed during storage, product quality would be further degraded due to the above factors as well as shriveling and wrinkling, which occur as a result of transpirational water loss. Long-term storage and/or WS may also cause development of pillowy disorder, resulting in unacceptability of pickled products.

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Registry No. Glucose, 50-99-7; fructose, 57-48-7; citric acid, 77-92-9; malic acid, 6915-15-7; water, 7732-18-5.