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Influence of Temperature and Humidity on Microbial, Enzymatic, and Physical Changes of Stored, Pickling Cucumbers[1]

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Pickling cucumbers stored at five temperatures and four relative humidities were examined for growth of eight microbial groups, the activities of two enzyme systems (pectinolytic and cellulolytic), and weight loss. Twenty-four storage tests for 6 days each were conducted during the 2-year study. In general, microbial populations of the eight groups increased rapidly at the higher temperature (>21 C) and humidity (>70%) treatments. Moisture loss of the cucumbers was rapid with combinations of high temperatures and low humidities. The results suggest that the best environmental conditions for storage or transport of cucumbers would be a combination of low temperature (10 C) with high relative humidity (about 95%). These conditions minimized undesirable microbial, enzymatic, and physical changes of stored, pickling cucumbers.

In recent years, pickle processors have had to extend the holding time of their harvested crop, often for several days, because of the extended times necessary for transit from growing areas to the manufacturing plant. Today, transportation distances by truck equal to that between cucumber production areas in Mexico and processors in the Toronto, Ontario (Canada), area are not uncommon. Also, due to year-round customer demand for pickle products made from raw cucumbers (freshly packed or pasteurized pickles; refrigerated overnight dills), an estimated 3 to 4 million bushels (75,000 to 100,000 tons [about 80,000 metric tons]) of pickling varieties are transported, under refrigerated conditions, from southern growing areas of the country to pickle processors in northern states, prior to their harvest season.

Removal of field heat and respiration heat of freshly harvested cucumbers, either by forced-air cooling (18) or by hydro-cooling (4, 20), for preventing or delaying spoilage losses has been investigated. The effects of cucumber storage temperature and holding time on the quality of pasteurized pickles also have been reported (5, 16). However, physiological or chill damage, characterized by water-soaked spots, pitting, or tissue collapse, was observed (1, 8, 21) when cucumbers were precooled and stored below 10 C. Fellers and Pflug (17), however, recommended a storage temperature as low as 34 F (1.1 C). General recommendations for storage and transit conditions of pickling and "produce" or "slicer-type" cucumber varieties have commonly been 10 ± 2 C, at a relative humidity (RH) of 80 to 85% or higher (5, 19, 23).

We have reported (14) high populations of heterogeneous microflora on small (¾- to ⅞-inch diameter [about 1.91 to 2.23 cm]) cucumber fruit (182 million/U [1 U = 1 cucumber]) and especially on the attached blossom (476 million/U [1 U = 1 blossom]). The average unit weights were: cucumber, 12.4 g; and cucumber blossom, 0.0275 g. Numerical estimates also were reported for eight microbial groups: total aerobes and spores, total anaerobes and spores, coliform bacteria, acid-forming bacteria, yeasts, and molds (higher fungi). The coliform group comprised about 25 to 30% of the bacterial population on the fruit. Mold counts (44 thousand/U) usually were more than double the yeast counts (18 thousand/U) but were much lower than bacterial counts. Species of molds occurring on the pickling cucumber, including

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its retained blossom, and two of their hydrolytic enzyme systems, pectinase and cellulase, have been studied (12, 22). These enzymes, particularly pectinase, have been implicated directly as causing softening of commercially brined cucumbers (12).

Based on this background (12, 14, 22), the present study was designed to follow population changes of the above-named eight microbial groups, and activities of the two enzyme systems, on pickling cucumbers from the time of harvest through 6 days of storage at various temperatures and RHs.

MATERIALS AND METHODS

Environmental control storage rooms and cucumber samples. The three walk-in rooms, used for the storage tests, were located in the Department of Biological and Agricultural Engineering, and were equipped with temperature (±1 C) and humidity (±3% RH) controls. After calibration and adjustment, the desired environmental conditions were pre-set for each room. Four runs in each of the three rooms were made in each of 2 years for a total of 24 tests (Table 1). Immature fruit, Model cucumber variety, no. 1B size (⅞- to 1 and 1/16 inch diameter [2.23 to 2.70 cm]), was obtained the day of harvest from a receiving and grading station at Zebulon, N.C., about 20 miles from the laboratory. For each storage test 1 and ¼-bushel (about 44.05 liters) lots of cucumbers (about 65 lb [29.5 kg]) were spread out, two to three cucumbers deep, on ¼-inch (about 0.63 cm) mesh, galvanized wire-screen trays fitted with wooden frames. During the second season, the trays were suspended on scales for recording daily changes in cucumber weight. For analysis, each sample consisted of 20 to 25 cucumbers. These were collected aseptically in sterile paper bags and examined promptly for their microbial populations and enzymatic development.

Microbiological procedures. Populations were estimated for the eight groups of microorganisms occurring on the fruit at the start of each storage test and on a 1- or 2-day sampling schedule for 6 days. The procedures for estimating populations for the various microbial groups were described by Etchells et al. (11, 12, 13), but included certain improvements by Borg et al. (3) and Costilow et al. (7), directed primarily to determining the acid-forming bacteria. The eight groups were: (i) total aerobes, (ii) aerobic spores, (iii) total anaerobes, (iv) anaerobic spores, (v) coliform bacteria, (vi) acid-forming bacteria; (vii) yeasts, and (viii) molds (higher fungi).

TABLE 1. *Environmental conditions of cucumber storage*

Temp of storage room (C)	Relative humidity (RH) of storage room (%)				Storage rooms kept at each temperature (no.)
	4[a]	4	7	9	
10.0 (50)[b]	55–60	70–75	80–85[c]	90–95[d]	7
15.6 (60)	55–60	70–75	80–85		3
18.3 (65)			80–85	90–95[d]	4
21.1 (70)	55–60	70–75	80–85		3
26.7 (80)	55–60	70–75	80–85[c]	90–95[d]	7

[a] No. of storage rooms kept at each RH.
[b] Temperature in Fahrenheit.
[c] Two replications, one in each season.
[d] Three replications during season.

Measuring enzyme activity. Pectinolytic and cellulolytic activities were measured on each sample prepared for microbiological examination. The activities of enzymes were determined by viscosity methods as reported by Bell et al. (2). The percentage of loss in viscosity was converted to enzymatic activity units from a standard curve for each enzyme assay.

RESULTS AND DISCUSSION

An experiment, conducted during the latter part of the first year's study, showed that cucumbers that were blended in a saline (0.85% NaCl) diluent had higher microbial plate counts than platings made from cucumber washings in saline. During the second year's study, microbial counts and enzyme determinations were made on both washings and blended extracts to determine the extent of this difference. Based on 53 cucumber samples, blending increased the average counts of various microbial groups from 1.3 to 2.6 times (Table 2). Blending increased enzymatic activity even more: pectinolytic and cellulolytic assay units, on the average, increased by six and three times, respectively.

Initial microbial population estimates on cucumbers. The weights of freshly harvested, pickling cucumber samples examined during the 2-year study and initial populations of the eight groups of microorganisms occurring on these samples are presented in Table 3. The numbers of microorganisms on an individual cucumber can be estimated by multiplying the counts by 20, the average weight for a single fruit. If allowances are made for season, size, and other variables, the populations of the various microbial groups found initially on the fresh fruit were reasonably comparable to those reported by Etchells et al. (14).

However, it is evident from the present study that the time of harvest had a strong influence on initial populations; in most instances the numbers increased as the season progressed from the middle of June to early July. A similar progressive, initial increase during the harvest in the numbers of higher fungi (molds) on cucumber blossoms was noted earlier (12). The initial increase in mold populations as the season progresses probably relates directly to the reduced quality of the late-season fruit often received by pickle plants, especially under the

TABLE 2. *Comparison of washing and blending treatments for preparing cucumber samples for microbial examination and enzyme activity tests*

Treatment of sample[a]	No. of samples tested[b]	Avg colony counts per gram (× 10^3)								Avg enzyme activity (U/g)	
		Bacteria						Fungi			
		Aerobes		Anaerobes		Coliforms	Acid formers	Molds	Yeasts	Pectinolytic	Cellulolytic
		Total	Spores	Total	Spores						
Washed (W)	53	33,000	10.5	700	0.27	2,360	36	36	3.3	7	95
Blended (B)	53	53,330	19.5	980	0.54	6,080	51	46	6.6	44	277
Ratio (B:W)		1.6	1.9	1.4	2.0	2.6	1.4	1.3	2.0	6	3

[a] Washed samples each consisted of 25 no. 1B-size Model variety fruit, which, together with an equal weight of saline in 2-liter flasks, were shaken by hand 100 times; decimal dilutions were prepared from the washings. Blended samples, 20 fruit each, were blended for 2 min in a Waring blender with sufficient saline to make a 1:5 primary dilution.

[b] Duplicate samples were collected at each sampling interval from the storage tests; one sample was prepared for examination by the washing treatment, the other by blending.

TABLE 3. *Initial microbial populations on cucumbers used for storage tests*

Harvest		Avg wt (g)		Avg colony counts per g (× 10^3)							
				Bacteria						Fungi	
Week	Date	Per sample	Per unit	Aerobes		Anaerobes		Coliforms	Acid formers	Molds	Yeasts
				Total	Spores	Total	Spores				
First season (washed samples)[a]											
1st	6/12[b]	618	24.7	570				300		2.5	0.10
2nd	6/19	474	19.0	307	0.23			90	0.12	1.4	0.95
3rd	6/26	493	19.7	3,480	2.50			385	12.9	1.8	0.53
5th	7/8	428	17.1	9,970	5.10			2,130	2.7	6.9	2.30
Avg		481	19.2	3,580	2.6			730	5.3	3.1	0.98
Second season (washed samples)[a]											
1st	6/15	443	17.7	1,063	0.33	25	0.09	177	0.19	0.1	0.08
2nd	6/22	471	18.8	820	0.45	120	0.02	203	2.0	0.2	0.04
3rd	6/29	473	18.9	1,180	0.57	293	0.03	325	0.6	0.6	0.14
4th	7/6	545	21.8	6,170	4.70	311	0.38	847	10.2	2.0	0.20
Avg		483	19.3	2,310	1.5	187	0.13	390	3.2	0.7	0.12
Second season (blended samples)[a]											
1st	6/15[c]	356	17.8	2,670	2.60	42	0.12	116	1.4	0.2	0.80
2nd	6/22[c]	372	18.6	2,630	0.40	335	0.06	934	3.3	1.0	0.17
3rd	6/29	473	18.9	1,870	0.90	135	0.02	570	0.7	1.2	0.03
4th	7/6	545	21.8	5,870	9.10	470	0.30	1,030	34.3	3.9	1.77
Avg		436	19.3	3,260	3.3	246	0.13	660	13.2	1.6	0.69

[a] All values shown based on triplicate samples of 25 no. 1B-size cucumber fruit each, except as indicated otherwise. See Table 2 for details of washing and blending treatments.

[b] Single sample of 25 fruit. Counts not run for aerobic spores and acid formers on cucumbers collected or during 3 storage tests started on this date.

[c] Triplicate samples of 20 fruit each.

weather conditions usually prevailing in the southeast. High humidity, high temperatures, and the absence of refrigeration during this period reduce the quality of cucumber fruit. Such conditions favor rapid production of pectinolytic and cellulolytic enzymes, elaborated by many species of molds known to be present on the pickling cucumber (12, 22).

North Carolina shippers, and those further south, of greenstock pickling cucumbers report that more spoilage claims arise from late- than from early-season shipments.

Population changes of microbial groups. Temperature and humidity influenced the development of eight groups of microorganisms on cucumber fruit. The relative rate and extent of development of the two most numerous groups —aerobes and coliforms—and of the molds during six days of storage at five temperatures and at four humidities are illustrated in Fig. 1. To simplify and facilitate comparisons of population growths with initial counts, the estimated population at each sampling interval was divided by the initial population and the log of this ratio was plotted. At the highest temperature tested (27 C), populations of the three microbial groups increased at all four relative humidities.

In most instances, storage at 10, 16, or 18 C prevented any marked increase (less than 10 times) in microbial populations. After 6 days at 90 to 95% RH, however, the numbers aerobic bacteria and molds increased markedly. Molds developed on the stored cucumbers about as rapidly at 21 C as at 27 C. Although there was a definite lag in the growth of the total aerobes and coliforms at 21 C, as compared to 27 C, growth at 80 to 85% RH was comparable at both temperatures.

The four humidity treatments were about equally effective as to control of microbial growth at the lower temperatures. Microbial growth was less at 10 and 16 C than at 18 C and above.

The maximal populations of the eight microbial groups on the cucumbers under some of the different environmental storage conditions are presented graphically in Fig. 2. Aerobes and coliform bacteria predominated; population counts remained high during storage and each increased by 10-fold or more at 27 C. At 10 C and 70 to 75% or lower RH, the populations of these two microbial groups remained fairly constant; at higher humidities counts increased. Populations of anaerobic bacteria and anaerobic spores increased somewhat at 27 C, but changed only slightly at 10 C. Even though cucumbers at the two lower humidity treatments were not examined for these two groups, there was evidence that the higher humidity

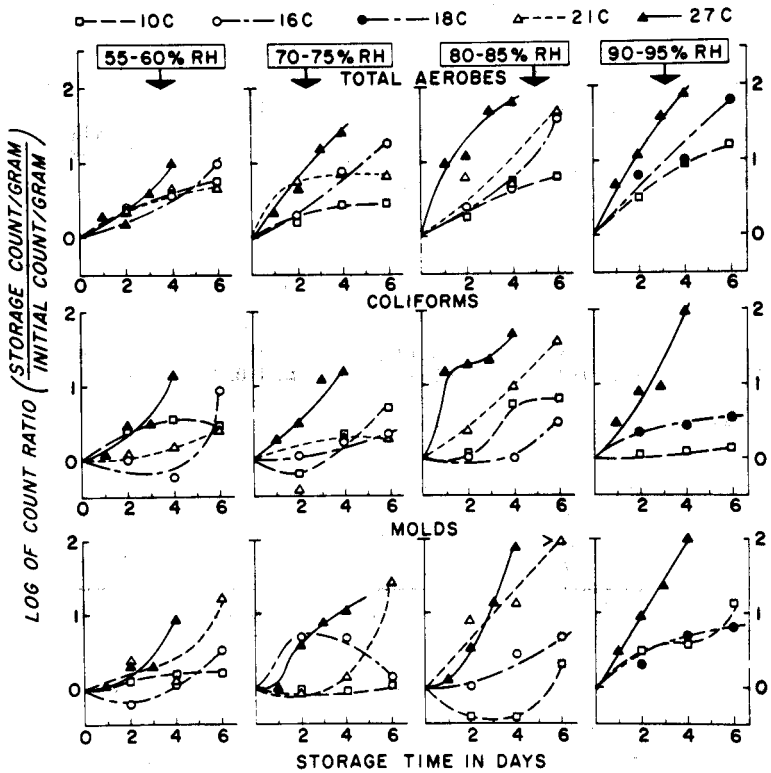

FIG. 1. *Influence of temperature and relative humidity on population changes of certain microbial groups on stored cucumbers.*

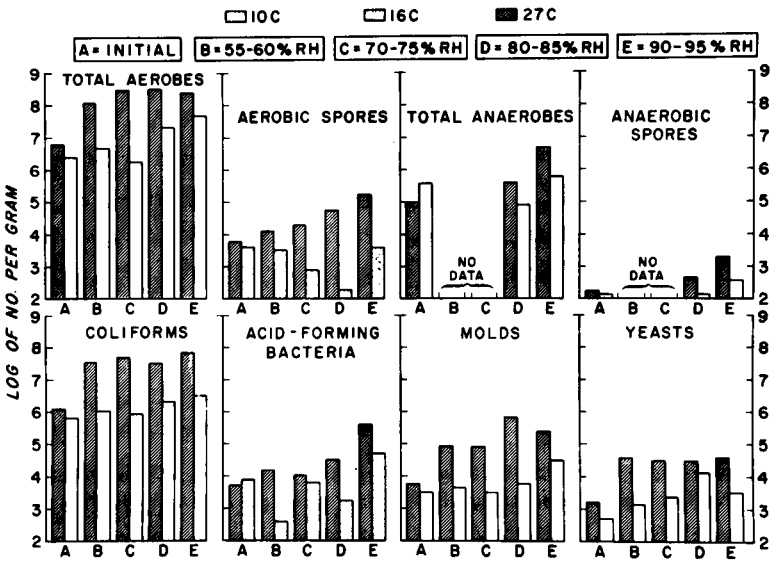

FIG. 2. *Maximal populations of various microbial groups on cucumbers after 6 days of storage at different relative humidities and temperatures.*

treatments encouraged growth.

In the past, acid-forming bacteria have been exceedingly difficult to separate from the very high populations of other microbial groups present on the cucumber. An earlier study (14) reported a few thousand per gram of cucumber in comparison to over a thousand times this number for other bacterial groups. The population ratios of the different microbial groups were very similar in this study, and acid-forming bacteria were present in small but significant numbers. The acid-formers are the most important microbial group present on the cucumbers because certain species of lactobacilli and of pediococci are the predominant acid-forming microorganisms in the natural lactic fermentation of cucumbers for salt-stock pickles (6, 15). The acid they produce has a preserving effect on the cucumber in the brining operation. The acid-forming bacteria were present in populations of nearly 10,000/g on the initial cucumber samples (Fig. 2). In storage at 27 C, this count increased to over 100,000 in the direct order of increasing humidity conditions of storage. At 16 C, there was little change in the population of the acid-forming bacteria, except for a small increase at the 90 to 95% humidity treatment.

Before storage, yeasts and molds (Fig. 2) were present on the cucumbers in substantially lower numbers than most of the bacteria. The initial mold counts were 3,000 or 4,000/g of cucumber, about twice the yeast counts. We have studied these two microbial groups, molds and yeasts, extensively (9–12, 14, 22) because they cause certain spoilage problems, e.g., softening spoilage by molds, bloating (hollow cucumbers) by fermentative yeast species, and surface-growth of oxidative, film-forming yeast species on the brine of salt-stock tanks. Thus, these two groups are highly undesirable organisms in pickle brines and in the pickle plant. The growth and elaboration of enzymes (particularly softening enzymes by molds) on or in the retained blossom of the cucumber under the environmental conditions of this study are most important. At 10 C, the mold population remained fairly constant at the lower humidities tested—70 to 75% and below; population increased slightly at 80 to 85% and still more at 90 to 95%. This indicates that high moisture conditions encourage growth of some mold species even at the lowest temperature used (10 C). However, at 27 C (optimum for most species) the molds increased rapidly from initial counts of about 5×10^3 to nearly 10^6/g, with the highest humidity treatments (80 to 95%) showing the largest populations. Molds are, at best, a most difficult group for which to obtain a true population count; however, coupled with visual observations, cucumbers having high mold populations likewise showed large amounts of fungal growth and decay. Yeast populations (Fig. 2) increased more than 10-fold on the cucumbers stored at 27 C and were not influenced greatly by humidity. At 10 C, the yeasts were somewhat restricted at the lower humidity treatments.

Overall (Fig. 2) maximal populations of the various groups obtained from the cucumbers

did not indicate competitive or inhibitory properties of one group of organisms for another. The populations of each group remained on the cucumber at about the same or higher populations during exposure to the various environmental treatments, with one or two exceptions. Increase in storage temperature was a greater factor in microbial population increase than was humidity. At all temperatures studied, increased humidity generally brought on increased microbial growth.

Enzyme development. Increased levels of pectinolytic and cellulolytic enzymes on the stored cucumbers (Fig. 3 and 4) correlated closely with the development of mold growth. At 10 C there was little or no enzyme activity at humidities up to 70 to 75%, and development of the two enzyme systems appeared to be delayed more than 2 days even at high humidities. At 27 C, both enzymes increased in activity very rapidly on cucumbers stored at 80% RH and above; there was about a 2-day lag before noticeable increases in enzyme levels were observed in fruit stored at 70% humidity and below.

The pectinolytic and cellulolytic enzyme activities from the first year's study (Fig. 3) were measured on samples of fruit washings and from the second year's on blended samples (Fig. 4). Activities of pectinolytic and cellulolytic enzymes averaged six and three times higher, respectively, in blended samples than in washings (Table 1). Thus, the blending procedure appeared to give a more complete extraction for the two enzyme systems. When the factors of six and three are applied to the first-year's

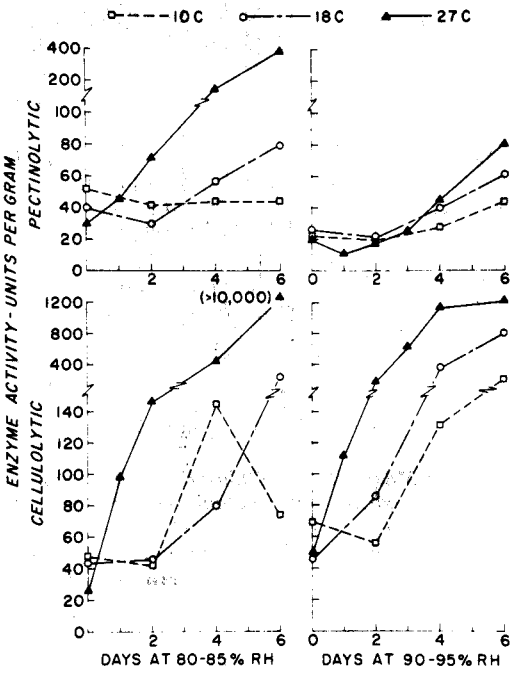

FIG. 4. *Development of pectinolytic and cellulolytic enzyme activities on cucumbers stored at different temperatures and relative humidities—blended samples, second season.*

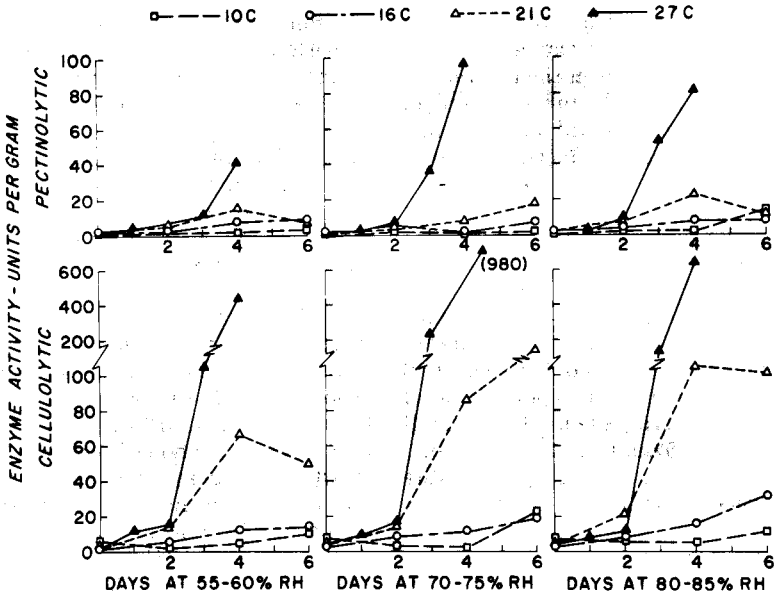

FIG. 3. *Development of pectinolytic and cellulolytic enzyme activities on cucumbers stored at different temperatures and relative humidities—washed samples, first season.*

data, the activity levels of the two enzymes for the two seasons, on fresh and stored cucumbers at 80 to 85% RH, appear more comparable.

Physical changes. Cucumbers have a moisture content of about 95% and are very susceptible to rapid weight loss accompanied by visible shriveling. Table 4 shows the average weight loss per day at various temperature and humidity treatments. These data may be slightly higher than those from commercial practice because our cucumbers were spread over a wire mesh screen about two to three cucumbers in depth and not stored in bulk containers. Weight loss was most rapid at 55 to 60% RH at 27 C and averaged 25% per day, with severe shriveling. In general, cucumbers stored at 90 to 95% RH lost less than half as much weight daily as those stored at 55 to 60% RH, regardless of temperature. Thus, the lowest rate of cucumber weight loss was at 90 to 95% humidity with the lowest storage temperature used, 10 C (Fig. 5). The rate of weight loss of pickling cucumbers in storage reported herein agrees with a study by Wells (24) who reported that other fruits such as apples, lemons, oranges, peaches, grapefruit, and avocados in storage lost weight as the humidity decreased and temperature increased.

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TABLE 4. *Weight loss of no. 1B-size Model variety cucumbers stored at various temperatures and relative humidities*

Temp (C)	Avg wt loss per day[a] (%)				
	First season[b]			Second season[c]	
	55–60% RH	70–75% RH	80–85% RH	80–85% RH	90–95% RH
10 (50)[d]	11	9	6	8	4
16 (60)	14	12			
18 (65)				12	8
21 (70)	16	13			
27 (80)	25	16	9	18	11

[a] Average weight losses all calculated on a day-to-day basis, i.e., percentage change from previous weighing.

[b] Percentages shown are approximate and based on the weight of individual samples, 20 or 25 cucumber fruit each, collected at each storage interval for bacteriological examination.

[c] Percentages shown for each storage condition are based on actual weight loss of about 65 lb of cucumbers spread out on wire racks mounted on scales.

[d] Temperature in Fahrenheit.

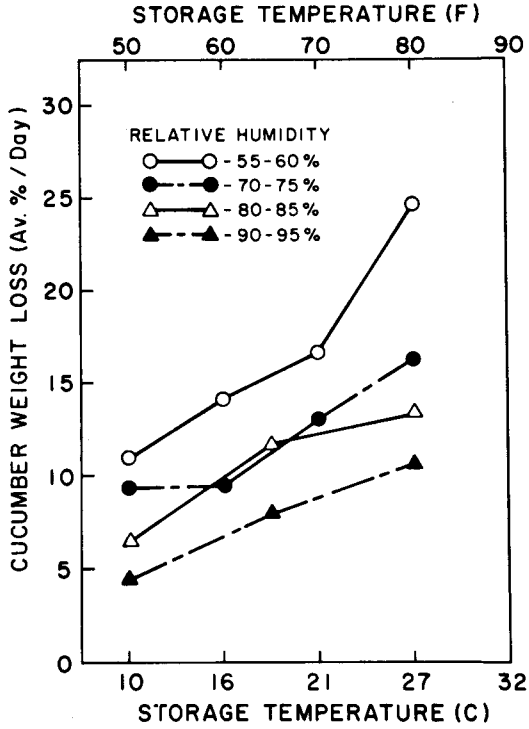

FIG. 5. *Influence of temperature and relative humidity on the rate of cucumber weight loss (%) per day during storage.*

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