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AIR-PURGING OF SALT-STOCK CUCUMBER FERMENTATIONS TO CONTROL BLOATER (HOLLOW PICKLE) SPOILAGE

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AIR-PURGING OF SALT-STOCK CUCUMBER FERMENTATIONS TO CONTROL BLOATER (HOLLOW PICKLE) SPOILAGE

by

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ABSTRACT

AIR-PURGING OF SALT-STOCK CUCUMBER FERMENTATIONS TO CONTROL BLOATER (HOLLOW PICKLE) SPOILAGE

by

Karen Christine Gates

Comparison of eighteen air-purged salt-stock pickle fermentations with those nitrogen purged showed air-purging as effective in preventing bloater formation. No significant effects of the various treatments were observed. In two tanks, some softening had occurred directly under the purger outlet where disolved oxygen concentrations were highest.

In laboratory studies, cucumber fermentations were purged at high air flow rates to study factors influencing softening in cucumbers and treatment which might prevent softening. These results indicated that softening of air-purged cucumbers is caused by some oxygen-dependent, sorbic acid-sensitive microorganism(s) growing in or on the cucumbers. Pectinolytic enzyme activities were not well correlated with the extent of softening.

Further laboratory studies showed the causative organism(s) to be mold growing in the brined cucumbers.

DEDICATION

To Charlie, Scott and Amy for their support and understanding and to Ralph Costilow for his incredible patience.

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Thanks to Green Bay Foods Co., Eaton Rapids, MI for providing cucumbers, H. J. Heinz Co., Lakeview, MI for use of facilities and the Department of Food Science and Human Nutrition, Michigan State University, for the use of their facilities.

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INTRODUCTION

Etchells et al. (9) and Fleming et al. (12) demonstrated that nitrogen purging of controlled fermentations of brined cucumbers greatly reduced the formation of bloaters (hollow pickles). Subsequently, it was found that equally satisfactory results could be obtained with natural fermentations by continuously purging them with nitrogen at 20 ft^3/h using an apparatus designated as a sidearm purger (5). This system is now widely used in commercial fermentations.

Use of air instead of nitrogen would greatly reduce the cost of purging. Fleming et al. (12) reported that continuous air-purging of fermentations in 5 gal pails at air flow rates of 25 to 100 ml/min resulted in soft and off-colored pickles. However, intermittent air-purging at even 425 ml of air/min for 2 h/day did not result in soft pickles. Purging of pails from the bottom is not comparable to purging large tanks with a sidearm. Even at 25 ml/min, the air flow rate per unit volume of brine in a 5 gal pail is approximately 5X that when 20 ft^3/h air is used in a

10,000 gal tank. In purging from the bottom, the cucumbers are in direct contact with the cucumbers and gas bubbles may be entrapped in the cucumber mass; this does not occur with the sidearm purger.

The softening observed in air-purged laboratory fermentations was believed to have been caused by molds growing on the brine surface along with oxidative yeasts (9). This could not be true with most commercial tanks, however, since their surfaces are exposed to sun and no films of microbes develop on them. In addition, if the softening resulted from pectinolytic or cellulolytic enzymes produced in the brine, essentially all of the cucumbers in the tank would be expected to become soft. This does occur when high levels of these enzymes are present (3,7); however, it has not been reported in air-purged commercial tanks.

The principle objectives of this investigation were:

1. To determine, under properly controlled conditions, if air could be substituted for nitrogen as a purging gas and to measure the amounts of dissolved oxygen in the brine.

2. To study factors which influenced softening in fermentations at high air flow rates, and to investigate possible procedures to prevent such spoilage.
3. Determine what microorganisms are responsible for softening of brined cucumbers during air-purging.

LITERATURE REVIEW

Bloaters (hollow pickles) are believed to be caused by CO_2 diffusion into the cucumber tissues from the brine (11,12,13) and can be prevented by purging of the brines with nitrogen (12). Costilow et al. (5) described a purging system using an apparatus designated as a sidearm purger which circulates the brine and purges it with nitrogen. This purging system is widely used in the pickle industry and has dramatically reduced bloater-type spoilage. A significant cost savings to industry could be achieved if air could be used in place of nitrogen as the purging gas.

Fleming et al. (12) reported continuous air-purging of fermentations in 5 gal pails at air flow rates of 25 to 100 ml/min resulted in soft and off-colored pickles. However, intermittent air-purging at even 425 ml of air/min for 2 h/day did not result in soft pickles. Even at 20 ml/min, the air flow rate per unit volume of brine in a 5 gal pail is approximately 5 times that when 20 ft^3/h air is used in a 10,000 gal commercial tank. The purging units in the pails, unlike those in commercial tanks, are located

in the bottom allowing direct contact of the gas bubbles with the cucumbers and gas bubbles may become entrapped in the cucumber mass.

Costilow et al. (6) reported air-purging in nine commercial tanks. Because of difficulty with an interrupted electrical supply and breakdown problems with air compressors, the air supply was shut off for variable and unknown periods of time during the nights. When the salt-stock was evaluated, there were no significant differences between the air-purged tanks and the nitrogen-purged controls. There was no evidence of softening, bleaching, or off-coloration of the pickles from any of these fermentations. The lactic acid fermentations developed normally in all tanks.

Potential adverse effects on quality factors such as texture and appearance are not the only converns in using air as a purging gas (12,13). The rate of acid development (titratible acidity as lactic acid) has also been demonstrated to develop more slowly resulting from a lower growth rate of lactic acid bacteria (16) in air-purged fermentations. This could indicate an increase in a competitive advantage of yeasts and other nondesirable aerobic microorganisms. Oxygen is supplied for these

organisms from the air being circulated in the brine and from the fresh tissues in which it is dissolved. Large cucumbers were found to contain 45-50 µl gas/gram in the tissues at brining. The gas contained about 23% oxygan (15), however, the amount of oxygen in the tissues at brining would vary with the way the fruit had been handled.

Softening of cucumbers purged with air has been reported to occur directly under the purger outlet (19) where dissolved oxygen would be expected to be highest. Polygalacturonase has been shown to be a major factor in breakdown of pectic substances and high pectinolytic activity in the brine can cause loss of texture during storage (2). Softening due to storage in brines with high pectinolytic activity is retarded by addition of calcium chloride (4).

MATERIALS AND METHODS

Commercial Fermentations

Fermentations Studied

The 20 commercial cucumber fermentations studied were brined at the H. J. Heinz Company station at Lakeview, MI. All tanks were 8 ft. high x 14 ft diameter (∿1,000 bu capacity), and were filled with size 3A (1.5 x 1.75 in. diameter) cucumbers unless noted. They were all brined according to plant procedures. The cucumbers were covered with 45° salometer brine acidified with about 0.05% acetic acid. Sufficient dry salt was added to each tank to equilibrate at 25 to 28° salometer. After the acidity in the brines had attained about 0.6% lactic acid, the brine strength was slowly increased to 45-50° salometer.

The standard sidearm purger equipped with a 2 in. x 12 in. ceramic gas sparger (5) was used for sparging all commercial tanks. Gas flow rates were controlled by use of Brooks gas flow meters, Model 1510B, with a capacity of 5-45 standard cubic ft/h (scfh) (Brooks Instrument Division,

Emerson Electric Co., Hatfield, PA). With all tanks, the tubes to the spargers were equipped with quick disconnects and needle valves. Gas flow rates were checked and adjusted at least daily by insertion of a flow meter into the line. This procedure results in actual gas flow rates somewhat higher than measured with the flow meters in the line. Therefore, the actual gas flow rates in those tanks were probably 2-3 ft^3/h higher than recorded. A high capacity, heavy duty air compressor equipped with an efficent air dryer was used for an air supply. Purging was discontinued when the brine samples were found to have less than 0.05% reducing sugar as determined by the quality control personnel of H. J. Heinz Company. On an average, these tanks were purged for 9 days.

Analysis

Brine samples from the air-purged and from two nitrogen-purged tanks were plated on dextrose agar (Difco) acidified with 2 ml of 5% tartaric per 100 ml in attempts to isolate molds or unusual yeasts. Aliquots (0.1 ml) of serial dilutions of the brines were spread on pre-poured plates within 4 h after removing from the tanks.

Dissolved oxygen concentrations were determined with a dissolved oxygen meter YSI Model 57 (Yellowsprings Instrument Co., Inc., Yellow Springs, OH) equipped with an oxygen probe, YSI 5739, and submersible stirrer, YSI 5791A. The instrument was calibrated daily in air-saturated brine at the same salometer (±2°) and temperature as the fermenting brines which were to be measured. Consistent meter readings of 0.1 to 0.3 ppm oxygen were obtained with brines in nitrogen-purged tanks which should have no dissolved oxygen in them. Therefore, all readings were corrected by -0.2 ppm. Measurements made in brine near the surface of tanks (above the headboards) were made by submerging the probe and stirrer in the brine in the tank. Center of the tank determinations were made by insertion of a stainless steel tube to the approximate geometric center of a tank and siphoning brine into the bottom of a tall 1-liter beaker containing the probe and stirrer. The beaker was allowed to overflow until at least 1 liter of brine had passed through it before the dissolved oxygen reading was taken.

Brines were analyzed daily for titratable acidity, salometer, and CO_2 concentrations. The CO_2 was measured as described previously (5).

The salt-stock pickles were evaluated 2-3 months after brining. Routinely, samples were taken from near the surface of the brine directly under the purger outlet. Samples of 100 pickles were sliced lengthwise and evaluated for bloater damage, and other defects, and 10 pickles were tested for firmness by puncturing with a USDA fruit pressure tester equipped with a 5/16 in. tip (2). Personnel of the H. J. Heinz Co. conducted the evaluation of the pickles and used the evaluation procedure of Shoup et al. (18).

Laboratory Fermentations

Microbiological

Dilutions of brine samples of cucumber fermentations were plated on dextrose agar (Difco) acidified with 2 ml of 5% tartaric acid per 100 ml to isolate yeasts and molds; on nutrient agar (Difco) to detect aerobic non-fastidious bacteria such as the endospore producers; and on trypticase soy broth (BBL) plus 1% dextrose and 0.2% yeast extract with the pH adjusted to 5.6 to detect more fastidious bacteria.

Thin sections of cucumber tissues (10-30 μm in thickness) were sliced by use of a Hooker microtome (Labline Instruments, Inc., Melrose Park, IL). They were mounted in water on a microscope slide under a coverslip, and examined with a light microscope at 100x magnification. Photomicrographs were taken with a 35 mm camera.

To culture molds from the tissue, cucumbers were surface sterilized by immersion for 10 min in a 10% v/v solution of household bleach (Chlorox, ∿5.5% hypochlorite). Using Chlorox-treated forceps, tweezers, and scalpels, cross-cut slices about 2-3 mm in thickness were cut from near each end and the center of the cucumber. All three slices were pressed onto the surface of agar medium in a petri dish and incubated at 30°C for 5 days. Both 2% agar in water, and potato dextrose agar (Difco) were used as cultural media. Mold cultures isolated from cucumber slices were isolated and maintained on potato dextrose agar.

Cucumber Fermentations

The cucumbers (size 3A, 3.8-4.5 cm diameter) used in this study were obtained fresh from the Green Bay Food Co., Eaton Rapids, MI. They were packed in 1 gal glass jars fitted with a gas sparger (3/4 in. diameter, 2.5 in.

long) made of ultra high molecular weight polyethylene (10-20 μm pore size). A 1 in.-wide strip of semi-rigid polyethylene was placed under the neck of the jars to hold the cucumbers under the brine. Salt brines of the desired equilibrated salometer were poured over the cucumbers. The g cucumber/ml brine ratios varied from 55/45 to 60/40. Dry salt necessary for equilibration at the desired salometer was added in three equal amounts over a period of 1 day. Other additions were as indicated in the results. The controlled fermentation was established according to the procedure of Etchells et al. (9).

The brined cucumbers were incubated under ultraviolet light at room temperature (25-30°C). Unless stated otherwise, they were purged continuously with air at ∿20 ml/min. Air flow rates were controlled by use of gas flow meters (Brooks Instrument Division, Emerson Electric Co., Hatfield, PA) having maximum air flow rates of 146 ml/min.

Non-fermented Brined Cucumbers

Cucumber brines and brined cucumbers were prepared by equilibration of cucumbers in brines in 1-qt jars with enough salt added to achieve the desired final salometer. All were acidified with 0.04% acetic acid. After addition

of toluene, the jars were sealed, and stored in a refrigerator for a minimum of 2 weeks before use.

Equilibrated brines used for shake-flask cultures were pasteurized in a steamer for 15 min. Brined cucumbers to be inoculated with various isolated microorganisms were all equilibrated at 25° salometer and 0.04% acetic acid. For individual tests, 1 brine-equilibrated cucumber was placed in each 1-qt jar and 50-100 ml of the equilibrated brine added. This resulted in partial submersion of the cucumber. The jars were placed in a steamer for 15 min before inoculation.

Pectinase Assays

Brines were assayed for pectinolytic enzyme activities by the procedure of Bell et al. (1), except that viscosity measurements were made with Ostwald pipettes having an upper bulb capacity of ∿3.8 ml. The flow time for water through these pipettes was about 1 min. Four ml of sodium polypectate solution and 1 ml of dialyzed brine sample were used in each pipette. The percentage losses in viscosity were measured after 20-22 h incubation at 30°C. There is considerable variability in these assays, and any value less than 10% is of doubtful significance.

Evaluation of Fermented Cucumbers

All of the pickles were removed from each jar and pressure tested with a USDA fruit pressure tester (5/16 in. tip) (2) 14 days after brining unless stated otherwise. They were then sliced lengthwise, observed for any ususual color changes, and the percent cure estimated. The latter was based on the percentage of the tissue which had lost its white color.

RESULTS

Commercial Fermentations

Effect of Various Schedules for Air-purging on Quality of Salt-Stock

The purging treatments used, the quality of the salt-stock pickles, and the highest and lowest concentrations of CO_2 observed in each tank are recorded in Table 1. Bloater control was excellent in all tanks even through maximal CO_2 concentrations greater than 70 mg/100 ml were observed in three of the tanks (Nos. 9, 17, and 18) during the fermentation.

Some softening was observed in two tanks. In one of these tanks, No. 13, a number of pickles in the first sample taken were found to have soft blossom ends. However, this was obviously restricted to a very small area of the tank since no evidence of softening was found in three additional samples from the same general area. Softening was more extensive in tank No. 19, but was restricted to pickles in the purger outlet side of the tank (Table 2).

TABLE 1

INFLUENCE OF VARIOUS PURGING TREATMENTS ON THE QUALITY OF SALT-STOCK AND THE MAXIMUM AND MINIMUM CO_2 LEVELS IN THE BRINES (1978)[a]

Treatments	Tank No.	% Defect-free	% Recovery	# Pressure Test	mg CO_2/100 ml	
					High	Low
Continuous nitrogen	1	60	80	13	40	15
	2	91	95	15	28	10
Continuous air[b]	3	96	96	--	62	13
	4	91	91	15	43	16
Air turned off	5	93	97	14	42	28
1 hr/day	6	74	82	15	53	12
Air turned off	7	81	92	15	45	25
3 hr/day	8	84	88	14	68	34
Air turned off	9	88	94	16	77	15
6 hr/day	10	98	99	18	52	14
Air replaced with N_2	11	89	94	14	45	18
3 hr/day	12	96	98	15	43	20
Air replaced with N_2	13	75[c]	94	19	48	18
6 hr/day	14	94	97	18	53	14

N_2 first day, then	15	92	98	19	58	13
air off 3 hr/day	16	90	95	17	48	23
25° Salometer, air	17	65(94)[d]	87(94)	15(19)	70	26
off 2 hr/day	18	80	92	12(17)	95	18
Air turned off	19	77	88	9[e]	43	15
3 hr/day[f]	20	98	98	18	53	18

[a]Salt-stock was evaluated by personnel of the H. J. Heinz Company by the procedure of Shoup, et al. (18). The expected percent recovery represents the percentage of all of the salt-stock which was free of gas pockets or other spearations and which would yield good quality cross-cut slices. Samples of 100 pickles were taken from the top of the tank on the purger outlet side.

[b]The cucumbers in these two tanks were 1 1/4-1.5 in diameter.

[c]Several pickles with softening at blossom end were found in first sample, but none in a second sample.

[d]Numbers in parenthesis represent results of a second sample.

[e]Sample from soft area. See Table 3.

[f]Planned to brine at 30° salometer, but neither tank reached that level during first 5 days.

TABLE 2

PRESSURE TESTS OF SALT-STOCK REMOVED FROM DIFFERENT AREAS OF TANK NO. 19 WHICH HAD BEEN PURGED WITH AIR

Area of Tank Sampled[a]	Individual Pressure Tests										Mean Test
	1	2	3	4	5	6	7	8	9	10	
Purger outlet side:											
Top of tank	3	3	4.5	8	11	11	11	13	14	16	9.5
Lower half	5	6.5	7.5	11	12.5	13.5	13.5	15.5	15.5	17	11.6
Purger side:											
Top of tank	16	16	16.5	17	17.5	17.5	18	18.5	19	19	17.5
Lower half	13.5	15	15.5	16	16.5	16.5	17	17	18	21	16.6

[a]Samples taken with a net. Therefore, samples from lower half of tank may have contained some pickles from near the surface.

When this tank was emptied the number of pickles affected was estimated to amount to less than 50 bu (17). Of particular interest is the fact that the softening was not uniform from pickle to pickle even in a very restricted area of the tank. Firm pickles were found adjacent to mushy ones, and a part of a single pickle was frequently soft and the rest firm. There was no evidence that the softening would spread through the tank. No pectinolytic enzyne activity was detectable in the brines (assay conducted by H. J. Heinz Co.). All of these facts suggest that the softening enzyme (s) is produced by an oxygen requiring organism growing on or in the surface tissue of individual cucumbers.

Analysis of all the data failed to reveal any difference between tank No. 19 and other air-purged tanks. The dissolved oxygen levels were not higher than normal, the acid fermentation developed normally, the salt concentration was not unusually low, and no molds or unusual appearing yeasts were detected by plating of five brine samples during the active fermentation period. Unfortunately, pH of the brine was not measured and we cannot be sure that the initial pH was not unusually high.

As evident in Table 1, no significant differences in salt-stock quality resulting from the different purging treatments was noted. The salt-stock from the two tanks which were continuously purged with air was excellent, and the soft pickles were from a tank in which the air was turned off 3 h per day. The average quality of the pickles from all 18 air-purged tanks was as good as that for 23 tanks at the same location purged continuously with nitrogen (Table 3).

Dissolved Oxygen in Brines

The air flow rate has a pronounced effect on the dissolved oxygen levels in brines (Table 4). In some instances, the oxygen concentrations were doubled by increasing the flow rate from 10 ft^3/h to 30 or 40 ft^3/h (see data for fermentations 2, 4, and 5 days old). As expected, the highest levels were found in brines directly under the purger outlet. However, significant amounts of oxygen were consistently found on the opposite side (purger side), and the concentrations found were also influenced by air flow rate. In most instances, no dissolved oxygen was detectable in samples taken from the center of the tank.

TABLE 3

COMPARISON ON AVERAGE SALT-STOCK QUALITY FROM TANKS PURGED WITH NITROGEN AND WITH AIR

Purging Gas	No. of Tanks	% With Defects		Expected % Recovery	Pressure Test	
		Severe[a]	Total		Mean	Range
Nitrogen	23	3.0	15	92.0	15.0	13-17
Air	18	3.4	13	93.2	15.5	9-19

[a]These pickles would yield no cross-cut slices.

TABLE 4

EFFECT OF AIR FLOW RATE ON DISSOLVED OXYGEN CONCENTRATIONS IN BRINE FROM DIFFERENT AREAS IN THE TANK[a]

Location in Tank	ft^3/hr Air	Fermentation age (days)									
		1	2	4	5	6	7	8	9	10	Mean
		ppm O_2									
Top, purger outlet[b]	10	1.85	0.95	1.20	0.55	3.0	2.45	1.60	2.00	1.70	1.70
	20	2.25	1.65	--	1.05	3.2	2.55	1.95	2.60	2.30	1.95
	30	--	--	2.70	2.15	--	--	--	2.85	--	2.57
	40	2.50	2.05	--	--	3.45	2.80	2.65	--	2.35	2.63
Top, purger side[b]	10	0.75	0.40	--	--	--	2.05	0.60	--	--	0.96
	20	0.95	0.95	--	--	--	2.15	1.15	--	--	1.30
	30	--	--	--	--	--	--	--	--	--	--
	40	1.65	1.05	--	--	--	2.45	2.20	--	--	1.84

Center of Tank	10	0	0	0	0	0	0	0	0	0.05	--
	20	0	0	--	0	0.05	0	0	0	0.10	--
	30	--	--	0	0	--	--	--	0.05	--	--
	40	0	0	--	--	0	0	0	--	0.25	--

[a]A single tank of a given fermentation age was purged at each air flow rate until the dissolved oxygen concentration remained constant at each location.

[b]The oxygen probe was resting on the headboards, 4 to 6 in. under the brine surface for these readings.

There was a trend for the oxygen concentration to increase as the fermentations progressed in age. This undoubtedly results from differences in the oxygen demand in the brines. The actual demand varied widely from tank to tank, but brines in older tanks (7-10 days) usually had a lower demand than those in young ones (Fig. 1). The cucumbers will take up oxygen rapidly immediately after brining, and the lactic acid bacteria will also use oxygen during their most active fermentation period. Most of the demand in the older fermentations probably results from the development of yeasts. In most instances, the oxygen concentrations in brines from tanks of all ages studied were reduced to essentially zero (less than 0.2 ppm) within 1 h after turning off the air. When nitrogen was used to replace air for purging, the oxygen levels declined very rapidly.

The dissolved oxygen values obtained for bine from one tank (No. 7, Table 1) at 2, 3 and 4 days after brining were very different from those observed in any other tank. The brine did not appear to have any oxygen demand. In fact, readings of samples taken from the center of the tank after 3 days of fermentation appeared to increase in dissolved oxygen from 2.2 ppm when the air was turned off to

FIGURE 1

Rates of depletion of dissolved oxygen from air-purged cucumber brines at various fermentation ages [h or days (d) after brining]. The air was turned off at time zero and measurements made at the points indicated. The dashed line (N_2, 1 d) represents the depletion rate when air was replaced with N_2 for purging.

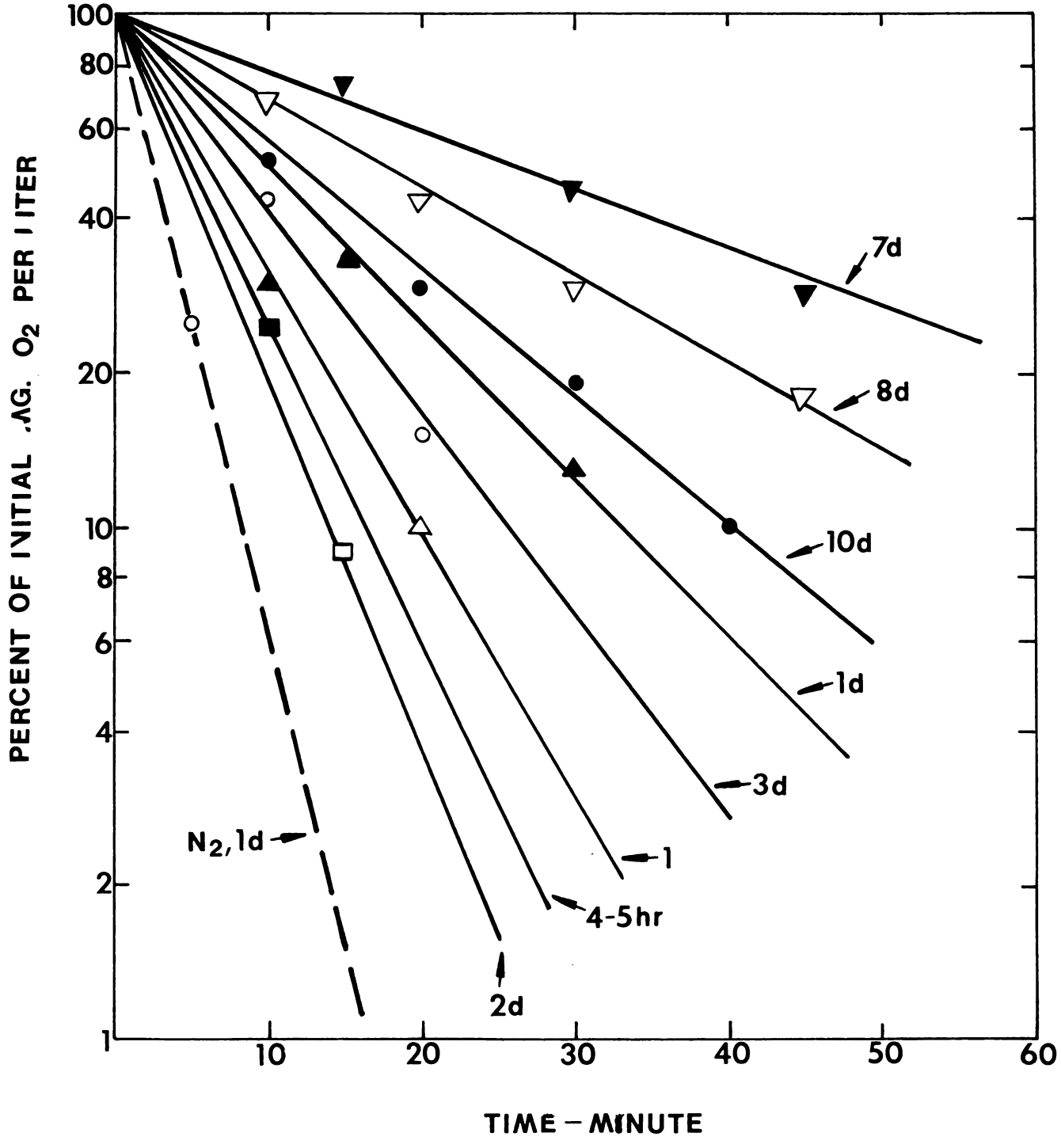
PERCENT OF INITIAL AVG. O_2 PER LITER
100
80
60
40
20
10
8
6
4
2
1
10
20
30
40
50
60
TIME – MINUTE
7d
8d
10d
1d
3d
1
4-5hr
2d
N_2, 1d

FIGURE 1

2.95 ppm 3 h later. Samples taken 1 day and 5-9 days after brining were normal with respect to oxygen demand. The oxygen meter appeared to be functioning normally, since brines in other tanks were found to respond in a normal manner. No reason for this anomalous response was apparent, and it was of much concern. However, as indicated in Table 1 (Tank No. 7), no effect on the salt-stock was evident. It is probable that there was something in that particular brine which was interfering with the meter readings.

Laboratory Fermentations

Effect of Aeration rate and Initial Acidification

As expected, the rate of aeration had pronounced effects on the development of acid fermentation and the rate and extent of softening of air-purged cucumbers (Table 5). Practically all of the cucumbers purged at 100 ml air/min were very soft after 7 days of fermentation, and there was little or no acid development. Those purged at 20 ml air/min all underwent some acid fermentation, and 47-89% of the cucumbers were still reasonably firm after 14 days fermentation.

TABLE 5

EFFECT OF ACIDIFICATION AND OF AERATION RATES ON ACID DEVELOPMENT AND SOFTENING (PRESSURE TEST) OF BRINED CUCUMBERS[a]

Acid Added	Aeration Rate	pH		Maximum Acidity	Pressure Test
		1 day	Final		
% Acetic	ml/min			% Lactic	Lbs
None	20	6.2	3.5	0.50	17.2 (11%)[b]
	100	6.7	4.5	0.13	2.6 (93%)
0.02	20	5.8	3.8	0.26	7.2 (58%)
	100	5.8	4.7	0.06	4.1 (93%)
0.04	20	4.9	3.9	0.36	8.4 (53%)
	100	4.9	5.4	0.04	5.5 (86%)
0.06	20	4.7	3.8	0.35	15.8 (25%)
	100	4.5	3.8	0.23	7.8 (67%)

[a]Cucumbers aerated at ∿20 ml/min were evaluated after 13 days fermentation; those aerated at ∿100 ml/min were evaluated after 7 days. All cucumbers had a completely cured appearance when evaluated.

[b]Numbers in parenthesis represent the percentage of all cucumbers in the fermentation which were severely soft (<11 lbs pressure test).

It is necessary to acidify cover brines to a pH less than 5.0 in order to remove carbon dioxide rapidly during the first day or two of fermentation. This would also be expected to delay development of some aerobic bacteria which could cause softening. However, it would not be expected to influence the growth of most molds and yeasts. Results of these experiments (Table 5) demonstrate that acidification with up to 0.06% acetic acid will not prevent softening. Acid development was highest and the least softening was encountered in the fermentation which was not acidified and was purged at 20 ml/min. However, less extensive softening occurred in fermentations acidified with 0.06% than in those with 0.02 and 0.04% acetic acid.

Measurements of dissolved oxygen levels (mg 0_2/liter brine), microbial activities, and pectinolytic enzyme(s) activities were also made on the fermentations in Table 5. The average dissolved oxygen levels in the four jars purged at 100 ml/min were much higher during the first 2 days of fermentation than the average levels in those purged at 20 ml/min (Fig. 2, top). However, after 3 days, no 0_2 was detectable in the brines purged at 100 ml/min. This was undoubtedly due to the development of very high yeast populations in these fermentations (Fig. 2, bottom).

FIGURE 2

Average dissolved oxygen levels (top) and average yeast counts (bottom) in salt-stock pickle brines. See Table 5 for description of the fermentations. Solid lines represent the averages of values for the four fermentations purged at 100 ml air/min, and dashed lines, the average values for the four purged at 20 ml air/min.

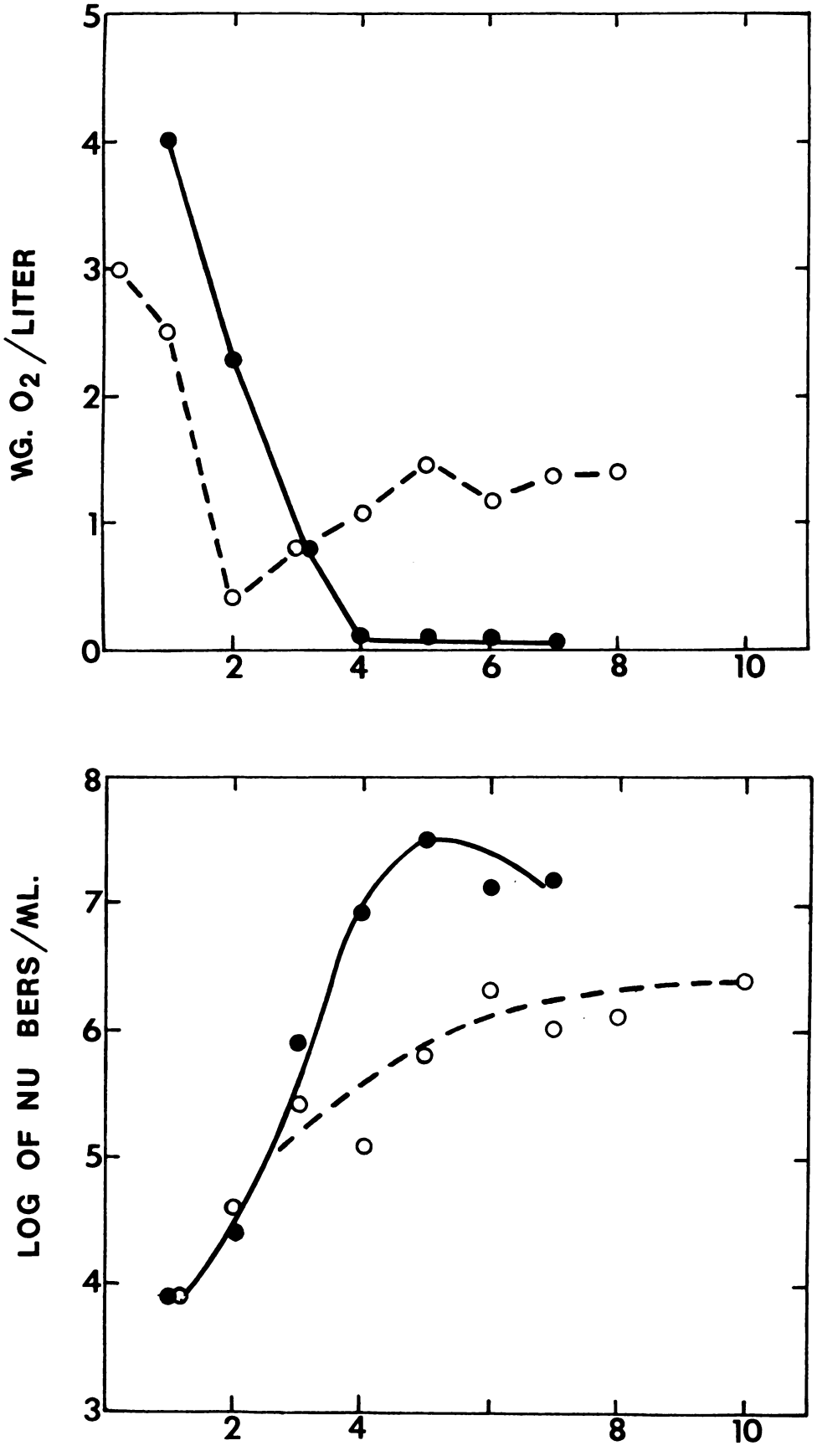

FIGURE 2

The yeast populations were over 10-fold higher in the brines purged at the higher rate. A similar relationship was observed by Potts and Fleming (16).

Pectinolytic activity was very slow to develop in the non-acidified fermentation purged at 20 ml/min (Fig. 3). None was detected until after 8 days of fermentation. In contrast, enzyme activity developed steadily after the third day in the other three fermentations purged at this rate. The activities in the other two brines were very similar to that shown in Fig. 3 for the brine acidified with 0.04% acetic acid. Correlated with the slow development of pectinolytic activity in the non-acidified brine was an initial rapid drop in dissolved oxygen level. After 24 h, no oxygen was detectable in this brine while the other three fermentations purged at 20 ml/min had levels near 3 mg/liter.

Pectinolytic activity developed rapidly in three of the brines purged at 100 ml/min (Fig. 3). However, no significant level of enzyme was detected in the brine acidified at 0.06% acetic acid during 6 days of fermentation even though extensive softening of the cucumbers occurred (Table 5).

FIGURE 3

Effect of aeration rate and of initial acidification on the development of pectinase activities in cucumber brines. Symbols: Dashed lines, 100 ml air/min; solid lines, 20 ml air/min; ○, △, no acid added: ○, ▲, 0.04% acetic acid added: ■ , 0.06% acetic acid added.

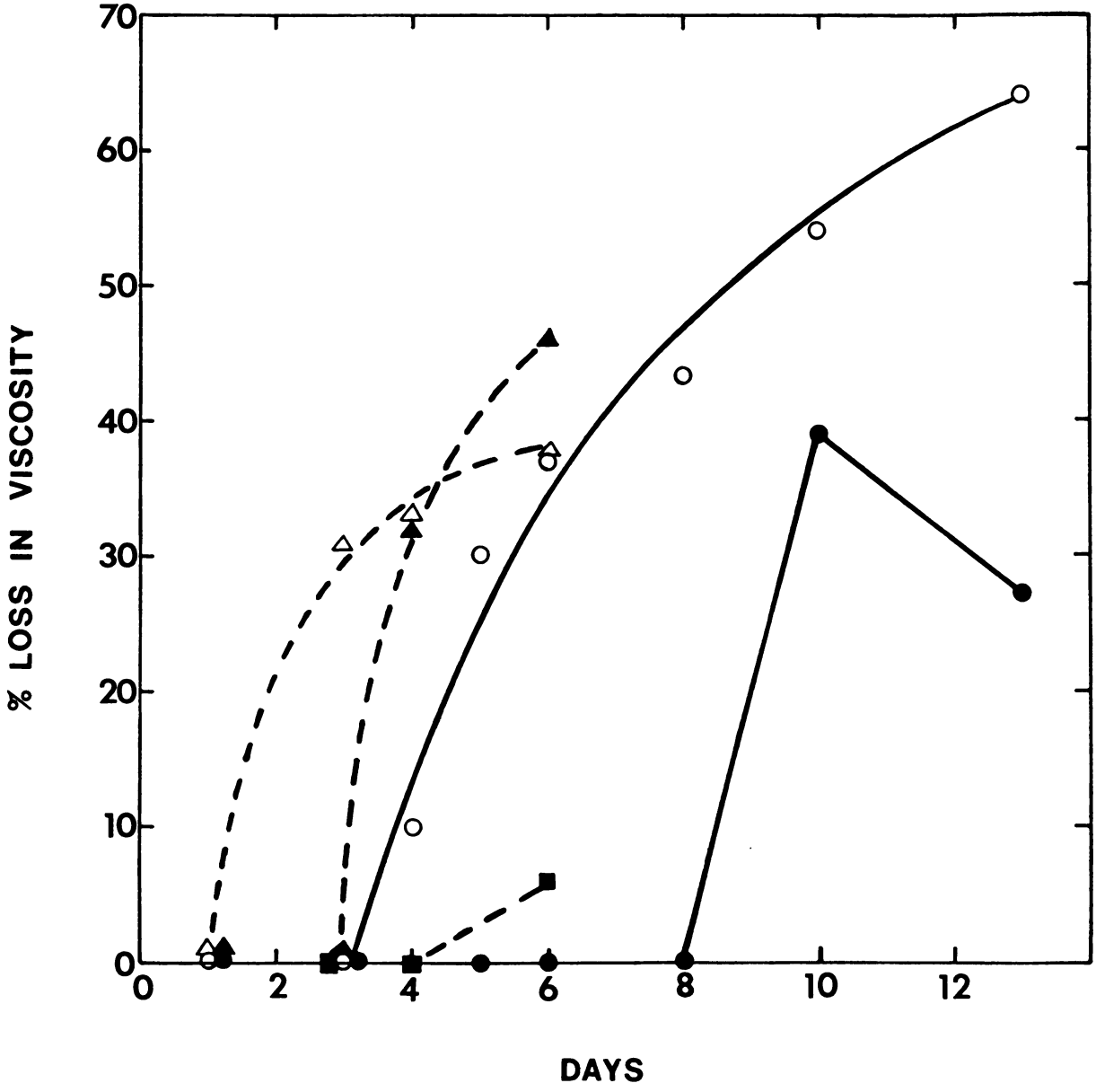

FIGURE 3

Effect of Salt Concentration

Cucumbers brined to equilibrate at salt concentrations between 20° and 35° salometer underwent extensive softening during air-purging for 2 weeks (Table 6). Brines in three of the fermentations developed very high pectinolytic enzyme activities. However the 35° salometer brine had only a marginal level of activity even though many of the cucumbers in the brine were very soft.

Air-purged cucumbers appear to cure (tissue becomes opaque) rapidly. Those in brines aerated at 100 ml/min (Table 5) were cured within 7 days of fermentation, and those in brines up to 25° salometer and aerated at 20 ml/min in Tables 5 and 6 were cured within 14 days. However, high brine strength did delay cure (Table 6).

Effect of Initial Limitation of Oxygen

As noted above in the discussion of the data in Table 5, when the dissolved oxygen level dropped to a low level within 1 day after brining, little or no softening occurred. Many oxygen requiring microbes will die rather quickly in the absence of oxygen. Therefore, experiments

TABLE 6

EFFECT OF SALT CONCENTRATION ON THE DEVELOPMENT OF ACID AND PECTINASE ACTIVITY IN THE BRINE, AND ON SOFTENING AND EXTENT OF CURE OF BRINED CUCUMBERS[a]

Brine-°Salometer	pH		Maximum Acidity	Final Pectinase Activity	Pressure Test	% Cure
	1 day	Final	% Lactic	% Δ Viscosity	Lbs.	
20	4.5	3.4	0.55	69	11 (56%)[b]	100
25	4.5	3.5	0.43	53	11 (44%)	100
30	4.5	4.0	0.17	71	5 (76%)	80
35	4.5	3.6	0.26	17	8 (59%)	20

[a]All fermentations were acidified initially with 0.06% acetic acid, and were purged continuously with air at a flow rate of ∿20 ml/min.

[b]Numbers in parenthesis represent the percentage of all cucumbers in the fermentation which were severely soft (<11 lbs pressure test).

were conducted to determine the effects of limiting oxygen early in the fermentation.

Cucumbers from brines purged with nitrogen the first 2, 3, or 4 days followed by air-purging until 14 days of fermentation were all firm in texture (Table 7). The extent of softening was reduced by purging with nitrogen for 1 day, but 25% of the cucumbers were seriously affected. In another series of fermentations at different brine strengths (Table 8), there was essentially no softening found in fermentations which had been nitrogen-purged for even 1 day before purging with air. No soft pickles were found in brines which had been initially purged with nitrogen for 2 days.

It was not possible to correlate pectinolytic enzyme activities in the brines with the extent of softening observed in these experiments (Table 7 and 8). The 35° salometer brine purged continuously with air (Table 8) had only marginal enzyme activity but 20% of the pickles had pressure tests less than 11 lbs. In contrast, the 30° salometer brines purged with nitrogen for the first 2 days had reasonably high pectinase levels (46-59% but none of the pickles were soft.

TABLE 7

EFFECT OF INITIAL NITROGEN PURGING ON THE DEVELOPMENT OF ACID AND PECTINASE ACTIVITY IN THE BRINE, AND ON SOFTENING AND CURING OF BRINED CUCUMBERS[a]

Treatment	Final pH	Maximum Acidity	Final Pectinase	Pressure Test	% Cure
		% Lactic	% Δ Viscosity	Lbs.	
Non-purged[b]	3.9	0.17	3	16 (6%)[c,d]	15
Continuous air[b]	4.4	0.08	22	5 (86%)	70
N_2 - 1 day	3.6	0.29	16	15 (25%)	99
N_2 - 2 days	3.5	0.27	31	22 (0%)	95
N_2 - 3 days	3.3	0.49	13	18 (0%)	85
N_2 - 4 days	3.4	0.43	19	20 (0%)	85

[a]Cucumbers were brined to equilibrate at 25° salometer and 0.04% acetic acid. All fermentations were aerated continuously at ∿20 ml/min except where indicated.

[b]These two fermentations developed heavy surface scums of yeast because they were not protected by ultraviolet light.

[c]Numbers in parenthesis give the percentages of all cucumbers which were seriously soft (<11 lbs pressure test).

[d]There was one very soft cucumber near the surface of the brine.

TABLE 8

EFFECT OF BRINE STRENGTH AND INITIAL NITROGEN PURGING ON ACID AND PECTINASE ACTIVITY DEVELOPMENT IN THE BRINE, AND ON SOFTENING AND EXTENT OF CURE OF BRINED CUCUMBERS[a]

Brine °Salometer		N_2 Purged	Final pH	Maximum Acidity	Final Pectinase Activity	Pressure Test	% Cure
Initial	Final	Days		% Lactic	% Δ Viscosity	Lbs.	
25	25	0	4.7	0.33	43	18 (10%)[b]	50
	24	1	3.5	0.42	28	21 (0%)	60
	32[c]	1	3.4	0.53	27	21 (0%)	60
	24	2	3.5	0.52	0	21 (0%)	60
	32[c]	2	3.4	0.52	16	22 (0%)	70
30	29	0	4.3	0.39	57	19 (5%)	30
	30	1	3.7	0.42	7	22 (0%)	15
	39[c]	1	3.4	0.50	22	21 (0%)	80
	29	2	3.9	0.43	46	21 (0%)	70
	37[c]	2	3.5	0.50	59	21 (0%)	30

35	35	0	4.0	0.27	7	17 (20%)	50
	34	1	3.9	0.26	4	21 (0%)	50
	39[c]	1	4.0	0.26	21	19 (5%)	40
	33	2	3.5	0.46	12	22 (0%)	20
	40[c]	2	3.7	0.39	18	21 (0%)	10

[a]Size 2B (1 1/4-1 1/2" diameter) cucumbers which had been shipped from Virginia to Michigan (2-3 days post harvest at brining) were brined at the indicated initial brine strengths. All fermentations were acidified with 0.04% acetic acid. All were purged continuously with air (∿20 ml/min) except for the periods indicated for N_2 purging. Heavy scums developed on these brines because they were not protected by ultra-violet light.

[b]Numbers in parenthesis represent the percentage of cucumbers in the fermentation which were severely soft (<11 lbs pressure test).

[c]The brine strength was gradually raised to these values during the second week of fermentation by addition of dry salt.

The effect of air-purging on curing is evident in Table 7 when compared to a non-purged control. Failure of the smaller sized cucumbers used in the experiments reported in Table 8 to cure rapidly is not known.

Effect of Controlled Fermentation of Potassium Sorbate

Pickles from a controlled fermentation which was brined and treated as described by Etchells et al. (9) but purged constantly with air were all firm (Table 9). In addition, no softening enzyme was detectable in this brine after 2 weeks fermentation. The results were exactly the same when 0.035% potassium sorbate was added and a natural fermentation allowed to develop. As the concentration of potassium sorbate was lowered, more extensive softening occurred and higher pectinolytic activities were observed. A fermentation which had not been purged for 1 day (static), and another which had been held static 6 h each day served as controls in this experiment (Table 9). Both of these fermentations contained some soft cucumbers and significant pectinase activities.

TABLE 9

EFFECT OF PARTIAL AIR LIMITATION, CONTROLLED FERMENTATION, AND ADDITION OF POTASSIUM SORBATE ON DEVELOPMENT OF ACID AND PECTINASE IN BRINES AND ON SOFTENING OF THE CUCUMBERS[a]

Treatment	Final pH	Maximum Acidity	Final Pectinase	Pressure Test
		% Lactic	% $\dot{\Delta}$ Viscosity	Lbs.
Static, first day	3.7	0.56	26	14 (33%)[b]
Static, 6 h/day[c]	3.7	0.43	53	17 (7%)
Controlled	3.9	0.69	0	19 (0%)
Plus K sorbate, 0.005%[d]	3.9	--	--	7 (92%)
0.01%	4.0	0.43	--	10 (77%)
0.02%	3.7	0.55	--	15 (42%)
0.035%	3.7	0.68	0	19 (0%)

[a]Cucumbers brined to equilibrate at 25° salometer and 0.04% acetic acid. All fermentations were aerated continuously at ∿20 ml/min except where indicated.

[b]Numbers in parentheses are percentages of all cucumbers which were seriously soft (<11 lbs pressure test).

[c]Aeration was started 6 h after brining, and air was turned off 6 h each day.

[d]Fermentations with added potassium sorbate were not incubated under ultraviolet light.

Effect of Blanching Fresh Cucumbers

Cucumbers which had been blanched at 65°C (149°F) for 5 min before brining did not show significant softening during 2 weeks air-purging (Table 10). This was true even though the cover brine was inoculated by washing unheated cucumbers. Two of three non-blanched cucumbers brined in a jar with blanched cucumbers all became severely soft. Not one of three blanched cucumbers included in a fermentation of non-blanched cucumbers became soft. These data indicate that either the microbe(s) which cause softening of air-purged pickles have infected the cucumber tissue prior to brining and do not readily reinfect after brining, or that blanching the cucumber prevents the development of the microbe(s) in some way.

Microbes Isolated From Brines

A number of yeasts and bacteria were isolated from air-purged cucumber fermentations in which there was extensive softening of the cucumbers. An occasional mold colony was observed on plates, but their frequency of occurrence on platings of various brine dilutions indicated that they were contaminants. None of the yeasts, two mold cultures,

TABLE 10

EFFECT OF BLANCHING CUCUMBERS PRIOR TO BRINING ON SOFTENING DURING FERMENTATIONS WITH AIR-PURGING[a]

Treatment	Pressure tests (lbs)													
	Individual Cucumbers[b]													Average
None - control	0,	0,	0,	5,	7,	0,	13,	0,	0,	4,	0,	5		3
All blanched[c]	16,	16,	19,	16,	18,	17,	19,	17,	19,	19,	20,	17,	19	18
3 non-blanched[c,d]	25,	20,	7,	14,	18,	9,	17,	19,	17,	18,	18,	17		17
3 blanched[e]	18,	14,	18,	20,	17,	18,	13,	9,	11,	0,	18,	5		13

[a]Size No. 3B (1 3/4-2") cucumbers were all brined to equilibrate at 25° salometer and 0.04% acetic acid.

[b]Individual cucumbers were removed and pressure tested in order from top to bottom of each jar.

[c]The cover brine was inoculated by washing fresh non-blanched cucumbers before pouring over the blanched cucumbers.

[d]Three non-blanched cucumbers were identified by tying with a string and packed with blanched fruit. One was packed on the bottom, one in the center, and one at the top of the gallon jar. Pressure tests of these three fruits are underlined.

[e]Three blanched cucumbers were packed with non-blanched ones as described in (d). Pressure tests of these three fruits are underlined.

and only an occasional isolate of an endospore-forming bacterium were found to produce pectinolytic enzyme activity when inoculated into pasteurized brined cucumbers. The spore-forming bacteria were never present at populations over 1,000 per ml in the fermenting brines and the isolates obtained may have arisen from endospores.

Development of Pectinolytic Activities

There was an increase in pectinase activities in brines in most, but not all air-purged fermentations in which extensive cucumber softening occurred as noted above. Therefore, an attempt was made to demonstrate production of such activity in aerated non-fermented cucumber brines inoculated with brine samples from fermentations in which cucumbers did become very soft. No significant pectinase activities were detected in shake-flask brine cultures, while high activities developed during the same period in three of the four air-purged fermentations from which inocula were taken (Table 11).

Extensive softening of cucumbers occurred within 14 days after brining in all four of the air-purged fermentations referred to in Table 11. The average pressure

TABLE 11

PECTINASE ACTIVITIES IN BRINES FOR AIR-PURGED CUCUMBER FERMENTATIONS AT VARIOUS PERIODS AFTER BRINING AND IN SHAKE-FLASK BRINE CULTURES INOCULATED WITH FERMENTING BRINES[a]

Samples	°Salometer Brines			
	20	25	30	35
	% loss in viscosity[b]			
Fermenting brines:				
5 days	6	5	24	2
7 days	24	11	37	5
12 days	64	43	67	0
Shake-flask cultures inoculated with:[c]				
5-day brines	0	3	6	4
7-day brines	3	7	0	7

[a]See Materials and Methods for procedures used for brining and purging of cucumbers, and for determining pectinase activities.

[b]Any values below 10% are of doubtful significance.

[c]Erlenmyer flasks (250 ml) contained 50 ml of cucumber brines at the indicated salometer levels prepared as described in Materials and Methods. Flasks were inoculated with 5 ml samples of corresponding fermenting brines at the fermentation ages indicated. These cultures were incubated on a rotary shaker at 30°C until the original fermentations were 12 days old.

tests of all of the cucumbers were 11, 11, 5, and 8 lbs from fermentations at 20, 25, 30, and 35° salometer respectively. Of particular interest was the failure to detect significant pectinase activity in the brine from the fermentation at 35° salometer. Furthermore, this brine failed to soften non-fermented cucumbers after 40 days storage at 30°C, while the brines from the other three fermentations with high enzyme levels had very significant effects (Table 12).

The failure to find pectinolytic or cucumber softening activities in the 35° salometer brine was not likely the result of either inactivation or inhibition. The enzyme(s) activity in a pooled lot of cucumber brine was stable for four months at 50° salometer, and there was no obvious inhibition of softening until the brine strength exceeded 35° salometer (Table 13). Similar effects of salt on pectinolytic enzyme(s) have been reported (2).

Molds in Brined Cucumber Tissues

The above results indicated that the initial softening of cucumbers in air-purged fermentations resulted from the growth of microorganisms in very close association with the cucumbers, not free in the brines. Microscopic

TABLE 12

EFFECT OF SPENT BRINES FROM AIR-PURGED FERMENTATION OF DIFFERENT BRINE STRENGTHS ON THE PRESSURE TEST OF NON-FERMENTED BRINED CUCUMBERS[a]

Brine °Salometer	Pressure Tests	
	Controls	+ Spent Brine
20	18 (0%)[b]	12 (74%)[b]
25	22 (0%)	14 (63%)
30	19 (0%)	8 (61%)
35	20 (0%)	20 (8%)

[a]Cucumbers in quart jars were brined to equilibrate at the salometers indicated and at 0.04% acetic acid as described in Materials and Methods. At the start of the experiment, the brine in one of two jars at each salometer was replaced with spent brines from an air-purged fermentation conducted at the same brine strength (see Table 11). One jar at which salometer was held as a control. Toluene was added, the jars sealed, and stored for 40 days at 30°C.

[b]Numbers in parenthesis are the final pectinase activities (% loss in viscosity) in the brines after storage.

TABLE 13

AVERAGE PRESSURE TESTS OF CUCUMBERS EQUILIBRATED AND STORED IN BRINES AT VARIOUS SALT CONCENTRATIONS WITH AND WITHOUT HIGH LEVELS OF PECTINASE ENZYME(S) ACTIVITY

°Salometer	Controls	+ Enzyme (s)
	Lbs.	Lbs.
25	13 (0%)	4 (53%)[b]
30	16	8 (60%)
35	15 (0%)	7 (56%)
40	11 (0%)	9 (42%)
45	12	10 (57%)
50	11 (2%)	12 (54%)

[a]Cucumbers in quart jars were equilibrated in brine at the various salometers and at 0.7% lactic acid as described in Materials and Methods. At the start of the experiment, 450 ml equilibrated brine in one of 2 jars at each salometer were replaced with 450 ml of spent brine from air purged fermentations which had been adjusted to 0.7% lactic acid and the appropriate salometer. The spent brine used was from a pooled lot with very high pectinase activity (∿80% loss in viscosity). One jar at each salometer was saved as a control. Toluene was added, the jars sealed and stored at room temperature (∿22°C) for 4 months.

[b]Numbers in parenthesis are the final pectinase activities (1% loss in viscosity) in the brines after storage.

observations of thin tissue sections of soft cucumbers from air-purged fermentations demonstrated extensive invasion by mold hyphae (Fig. 4). Molds were consistently observed in sections from soft tissue, while none were found in sections from fresh fruit or from brined cucumbers which had not undergone some softening.

Cultures of cross-cut slices of surface sterilized brined cucumbers which were undergoing softening confirmed the presence of molds in the tissues (Figs. 5 and 6). In both figures, the cucumbers cultured in (A) were from fermentations in which extensive softening had occurred within 14 days after brining; those in (B) from fermentations in which some soft cucumbers were found; and those in (C), and (D) (Fig. 6) from lots in which all of the fruit was still firm after 2 weeks fermentation. Platings of slices of surface sterilized fresh cucumbers were usually free of molds (∿10% of slices with mold colonies). In contrast, after holding fresh cucumbers in the refrigerator for 6 days, molds developed on about 90% of cultured slices. Slices of cucumbers from untreated natural fermentations which had been air-purged for even 1 day usually were found to contain viable molds.

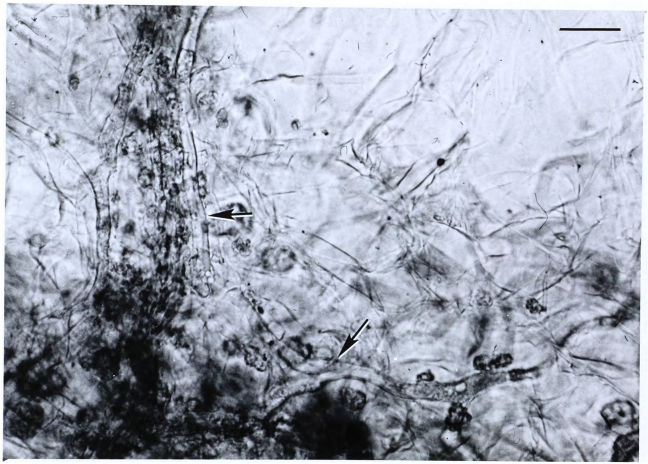

FIG. 4.--Mold hyphae (arrows) in the tissue of soft cucumbers from air-purged fermentations. Bar in lower left corner = 5 µm.

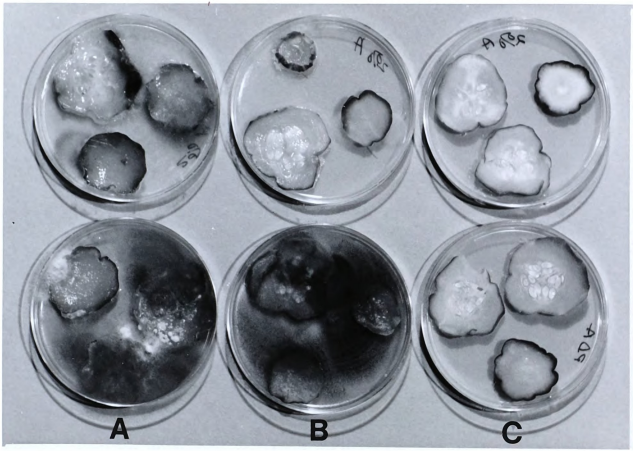

FIG. 5.--Cultures on 2% agar (top row) and potato dextrose agar (bottom row) of slices of surface sterilized cucumbers from fermentations purged for 5 days as follows: (A) continuous air, (B) N_2 for 1 day and then air, and (C) N_2 for 2 days and then air.

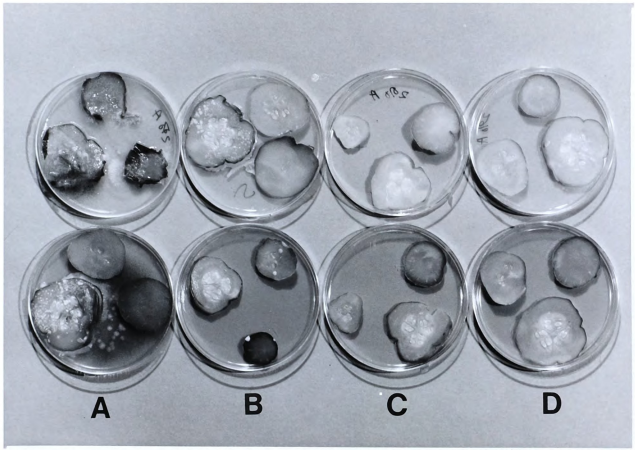

FIG. 6.--Cultures of slices from surface sterilized cucumbers from air-purged fermentation (5 days after brining) on 2% agar (top row) and potato dextrose agar (bottom row). Cucumbers were from fermentations treated as follows: (A) statis for 1 day followed by air, (B) continuously air-purged except for the first 6 h and for 6 h per day, (C) controlled fermentation, and (D) 0.035% potassium sorbate added.

Identity and Softening Activity of Molds Isolated From Cucumber Tissues

Fifteen cultures of molds were isolated from plated slices of cucumbers from air-purged fermentations. These were all identified as members of one of three genera; viz., Alternaria, Fusarium, or Mucor. These all grew when inoculated into pasteurized nonfermented cucumbers equilibrated in brines at 25° salometer and 0.04% acetic acid, and they also softened the cucumbers (Table 14). Most of the cultures produced relatively high activities of pectinolytic enzyme(s) which were found in the brines. However, in a few cases, no significant enzyme activity was detected, but extensive softening of the brined cucumbers occurred.

TABLE 14

PRODUCTION OF PECTINASE ACTIVITY AND SOFTENING OF CUCUMBERS IN BRINES BY MOLDS ISOLATED FROM SURFACE-STERILIZED SLICES OF CUCUMBERS FROM AIR-PURGED CUCUMBER FERMENTATIONS[a]

Culture No.	Genus	Pectinase Activity	Observation of Brined Cucumber
		% Δ Viscosity	
CM-1	Fusarium	12	very soft
-2	Mucor	91	very soft
-3	Fusarium	37	very soft
-4	Alternaria	92	very soft
-5	Alternaria	8	soft area[b]
-6	Fusarium	10	very soft
-7	Alternaria	88	very soft
-8	Fusarium	47	very soft
-9	Alternaria	78	very soft
-10	Fusarium	23	very soft
-11	Mucor	92	very soft
-12	Fusarium	0	very soft
-13	Mucor	0	very soft
-14	Fusarium	0	very soft
-15	Mucor	93	very soft

[a]Individual molds were inoculated by use of a needle into a cucumber equilibrated and partially submerged in brine at 25° salometer and 0.04% acetic acid. See Materials and Methods for procedure for preparation of pasteurized brined cucumbers. The inoculated cucumbers were incubated at room temperature for 9 days.

[b]There was visible mold growth and softening of the tissue in the area where the cucumber had been inoculated.

DISCUSSION

Air-purging, as demonstrated by these results, can and is being used successfully to prevent bloater formation in commercial salt-stock pickle fermentations. During the last 3 years, industry representatives attending meetings at Michigan State University have reported experiences with air-purging about seven million bushels of salt-stock pickles. A few companies are now using air for purging all fermentations of large size cucumbers; one company has air-purged all tanks of cucumbers regardless of cucumber size during the last 3 years (19).

Despite the success of prevention of bloater formation achieved when air is substituted for nitrogen some risk remains. In these experiments, softening was seen in two instances with the soft pickles found directly under the purger outlet. One tank had a few soft pickles (<50 bu) and another had soft ends (<5 bu). Compared to the total number of bushels involved (∿18,000) the loss is negligible. Industry representatives have also reported finding an occasional tank with a few bushels of soft pickles directly

under the purger outlet. However, no one has reported a serious loss.

The relationship of CO_2 levels in the brines and bloater control remains obscure. Originally, Etchells et al. (9) recommended that CO_2 concentrations be maintained below 20 mg/100 ml of brine to control bloater formation in controlled fermentations. Fleming et al. (14) reported that maintenance of CO_2 concentrations below 50% of saturation was necessary to ensure against bloating. However, even with CO_2 concentrations as high as 90 mg/100 ml of brine, essentially no bloaters were found in the commercial tanks studies herein. The temperature of these tanks was $70\pm2°F$ ($\sim21°C$), and most brine strength was near 25° salometer so there would be about 120 mg CO_2/100 ml brine at saturation. Therefore, CO_2 levels in excess of 75% of saturation occurred without any noticeable increase in bloaters.

Laboratory scale fermentations using air as the purging gas presents a different perspective from commercial tanks. The purging unit is located in the bottom of the container allowing direct contact of the air with the cucumbers. It is also possible for gas bubbles to become entrapped in the cucumber mass. When very high purging

rates, approximately 500 times that used in commercial tanks (100 ml air/min), were used the fermentations were not of the nature of typical lactic fermentations. Little acid was produced, the pickles were for the most part very soft and an "off" odor was observed. Yeast populations were not well controlled even though the fermentations were continually exposed to ultra-violet light, and high populations of aerobic bacteria were present. Either or both of their microbial groups were probably utilizing any acid produced. This high purging rate produced 100% cure (opaque tissue) in the cucumbers after only 7 days.

When using lower purging rates (20 ml air/min), approximately 100 times the rate used in commercial tanks, extensive softening was still found in many instances. Initial high concentrations of acetic acid (0.06%) and salt 35° salometer tended to retard softening but also retarded the start of lactic fermentation.

Softening was completely eliminated when the cucumbers were prewashed in hypochlorite solution as in the controlled fermentation process, when 0.035% potassium sorbate was added to the fermentation, and when the cucumbers were blanched before brining. These results indicated that the causative organism(s) of softening was located on the skin

or in the tissues of the cucumber. After removal, reinfection did not readily occur since brine made from washings of untreated cucumbers did not produce any softening in cucumbers which had been previously blanched. Softening was also severely curtailed or totally eliminated if nitrogen was used as a purging gas for the first 24-48 h. It is likely that the causative organisms could not survive the anaerobic environment for this long of a period.

Using pectinolytic activity as an indicator, attempts to culture an organism(s) capable of causing softening from the brines were unsuccessful. Inoculation of fresh brines with samples of brine which contained high pectinase activity was also unsuccessful. Organisms isolated from liquid withdrawn from the inside of a soft cucumber with an intact skin using a sterile hypodermic syringe produced no significant pectinase activity. Neither did they cause any softening of blanched prebrined cucumbers.

The results of these experiments are consistent with the findings that molds growing in brined cucumber tissues are primarily responsible for softening of pickles from air-purged fermentations. When cucumbers were surface-treated with hypochlorite, sliced and plated on agar, mold

growth was consistently seen on slices of brined cucumbers which were air-purged and conversely not seen in nitrogen-purged cucumbers. When thin sections of pickles were examined microscopically, mold hyphae was visible in every instance when the section was taken from a soft pickle or soft spot in the pickle. No hyphae was observed in firm tissues. Molds which were cultured from sections of hypochlorite treated cucumbers and inoculated into pasteurized brined cucumbers resulted in softening of the cucumber tissues. All three genera of molds isolated from cucumber slices have been previously found to be associated with pickling cucumbers (10).

The softening of pickles in these experiments was not correlated with pectinolytic enzyme activities in brines. Thus, extensive softening was observed in some fermentations in which negligible enzyme activity was found and vice versa. Therefore, the softening observed after 2 weeks of air-purging was primarily the result of enzymatic activities produced by the molds in pickle tissues.

Further softening would occur if pickles were stored in brines with significant pectinase activities (3,7). It is possible that some of the enzyme(s) in the brine was

produced by microorganisms growing in the brine, on the surface of the cucumbers or in the pores of the polyethylene sparger. This may have been the source of enzyme in those fermentations in which no soft pickles were found. Mold growth on the spargers was observed and, in some cases, resulted in partial plugging of the pores. However, no pectinase activities developed in pasteurized aerated cucumber brines inoculated with brines from fermentations in which enzymatic activity was increasing.

No comprehensive studies have been reported of softening enzyme(s) levels in commercial fermentations which have been air-purged. No activity was found in the brine from the one tank which contained a few bushels of soft pickles in a localized area under the sidearm outlet. The soft pickles observed in air-purged commercial tanks have remained localized in a small area of the tank during several months storage (19). In addition, not all pickles in a given area are soft and some pickles have only soft spots.

Data from these experiments indicates that the first 24-48 h after brining is a critical period when air is used as the purging gas. Molds growing in tissue would be protected to some extent from inhibitory effects of the brine during this period. Also, there may be considerable

air dissolved in fresh tissues. Large cucumbers were found to contain 45-50 µl gas/gram in the tissues at brining. The gas contained about 23% oxygen (15). However, the amount of oxygen in the tissues at brining would vary with the way the fruit had been handled. Those from the central areas of large masses of cucumbers would probably have used all the oxygen in respiration, while those near the surface would have high amounts. This variable could be important in commercial fermentations. Fresh cucumbers in the top few feet of large tanks are frequently not covered with brine for several hours before the brining operation is completed.

No soft pickles were found when nitrogen was used for purging for 2 or more days after brining before starting continuous aeration. It is possible that careful limitation of the air flow rate and purging on an intermittent schedule would eliminate the sporatic softening problems. These results and those of Potts and Fleming (16) show that dissolved oxygen usually disappears from brines rapidly when the air supply is shut off. Although the oxygen demand is variable, it is usually maximal during the first 3 days of fermentation. Fleming et al. (12) found no softening of pickles fermented in five 1 gal pails and

air-purged on an intermittent schedule (2 h/day). In experiments designed to purge commercial fermentations at varying rates with air, difficulties with the air compressors and electrical supply, caused frequent shut-off of the air supply for variable and unknown periods of time during the night (6). In these nine tanks, although the CO_2 levels were not well controlled, there were no significant differences found in the number of bloaters compared to control tanks of nitrogen-purged pickles and no softening was observed.

CONCLUSIONS

1. Air can be used successfully to replace nitrogen as a purging gas in commercial fermentations.

2. When air is used as a purging gas, a limited amount of softening of the salt-stock may occur.

3. Softening may be prevented by keeping the fermentation anaerobic for the first 48 h or by pretreatment of the cucumbers to destroy surface microbes.

4. Molds growing in the cucumbers are the cause of softening.

BIBLIOGRAPHY

1. Bell, T. A., and J. L. Etchells. 1955. A method for testing cucumber salt-stock brine for softening activity. USDA, ARS-72-5.

2. Bell, T. A., and J. L. Etchells. 1961. Influence of salt (NaCl) on pectinolytic softening of cucumbers. J. Food Sci. 26:84-90.

3. Bell, T. A., J. L. Etchells, and I. D. Jones. 1950. Softening of commercial cucumber salt-stock in relation to polygalacturonase activity. Food Technol. 4:157-163.

4. Buescher, R. W., J. M. Hudson, and J. R. Adams. 1979. Inhibition of polygalacturonase softening of cucumber pickles by calcium chloride. J. Food Sci. 44(6):0000.

5. Costilow, R. N., C. L. Bedford, D. Mingus, and D. Black 1977. Purging of natural salt-stock pickle fermentations to reduce bloater damage. J. Food Sci. 42:234-240.

6. Costilow, R. N., K. Gates, and C. L. Bedford. 1980 Air-purging of commercial salt-stock pickle fermentations. (Submitted)

7. Demain, A. L., and H. J. Phaff. 1957. Softening of cucumbers during curing. Agr. Food Chem. 5:60-64.

8. Etchells, J. L., T. A. Bell, R. N. Costilow, C. E. Hood, and T. E. Anderson. 1973. Influence of temperature and humidity on microbial, enzymatic and physical changes of stored, pickling cucumbers. Appl. Microbiol. 26:943-950.

9. Etchells, J. L., T. A. Bell, H. P. Fleming, R. E. Kelling, and R. L. Thompson. 1973. Suggested

procedures for the controlled fermentation of commercially brined pickling cucumbers - The use of starter cultures and reduction of carbon dioxide accumulation. Pickle Pack. Sci. 3:4-14.

10. Etchells, J. L., T. A. Bell, R. J. Monroe, P. M. Masley, and A. L. Demain. 1958. Population and softening enzyme activity of filamentous fungi on flowers, ovaries, and fruit of pickling cucumbers. Appl. Microbiol. 6:103-110.

11. Etchells, J. L., A. F. Borg, and T. A. Bell. 1968. Bloater formation by gas forming lactic acid bacteria in cucumber fermentations. Appl. Microbiol. 16:1029.

12. Fleming, H. P., J. L. Etchells, R. L. Thompson, and T. A. Bell. 1975. Purging of CO_2 from cucumber brines to reduce bloater damage. J. Food Sci. 40:1304.

13. Fleming, H. P., R. L. Thompson, J. L. Etchells, R. E. Kelling, and T. A. Bell. 1973. Bloater formation in brined cucumbers fermented by Lactobacillus plantarum. J. Food Sci. 38:499.

14. Fleming, H. P., R. L. Thompson, and R. J. Monroe. 1978. Susceptibility of pickling cucumbers to bloater damage by carbonation. J. Food Sci. 43:892.

15. Jorge, F. 1978. Gas diffusion from cucumber fruits. North Carolina State University, Raleigh, NC (M.S. Thesis).

16. Potts, E. A., and H. P. Fleming. 1979. Changes in dissolved oxygen and microflora during fermentation of aerated, brined cucumbers. J. Food Sci. 44:429.

17. Rogers, P. 1979. Private communication. The H. J. Heinz Co., Lakeview, MI.

18. Shoup, J. L., J. J. Cuniglio, and B. F. George. 1976. Relationship between fermentation time and bloater-type damage of brined cucumbers. J. Food Sci. 41:1335.

19. Switzer, R. G. 1980. Private communication. Western Food Products Co., Inc., La Junta, CO.