Fermentation of Cucumbers Without Sodium Chloride

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ABSTRACT

Cucumbers were successfully fermented and stored in the absence of sodium chloride (salt) under laboratory conditions, provided the fruit were blanched (3 min, 77°C) before brining in a calcium acetate buffer and the brine inoculated with *Lactobacillus plantarum*. Bloater formation was prevented by blanching even when brines were not purged of CO_2. Firmness of cucumbers was similar in salt-free brines or those containing salt after 1 mo, but firmness of salt-free cucumbers was lower after storage for 12 mos. Under pilot-scale, commercial conditions, however, the cucumbers were severely bloated, and the firmness was unacceptable after storage for 7 mo, due apparently to microbial recontamination after blanching.

Key Words: cucumbers, fermentation, sodium chloride, *Lactobacillus plantarum*, blanching

INTRODUCTION

THE U.S. ENVIRONMENTAL PROTECTION AGENCY has proposed a limit of 230 ppm of chloride in freshwater bodies (Fed. Reg., 1987). Many pickle companies throughout the U.S. have difficulty meeting the 230 ppm limit in discharges from their plant operations, mainly because about 40% of the pickling cucumber crop is temporarily preserved in large vessels containing sodium chloride brine. When needed for processing into finished products, salt is leached from the brined cucumbers and, after biodegradation of organic residues, is discharged directly into streams or into municipal waste systems. The industry has made efforts to reduce chloride wastes. They have reduced concentrations of salt used to store brined cucumbers and are replacing wooden tanks which tend to leak with nonleaking polyethylene or fiberglass tanks.

The addition of $CaCl_2$ or calcium acetate to fermentation brines has reduced the concentration of sodium chloride necessary for retaining textural properties of brined cucumbers (Buescher et al., 1979; 1981; Fleming et al., 1978; 1987; McFeeters and Fleming, 1991). However, microbial instability of fermented cucumbers is a problem when the salt concentration is too low. Cucumbers brined at 2.3% NaCl underwent a normal lactic acid fermentation, resulting in >1% lactic acid and pH 3.7 (Fleming et al., 1989). Subsequently, however, the cucumbers spoiled due to production of butyric acid and other products formed by undesirable bacteria while the level of lactic acid was reduced.

Our objectives were to determine the feasibility of fermenting and storing cucumbers in the absence of NaCl by blanching the cucumbers before brining. This method was compared to a procedure developed for fermentation and storage in anaerobic tanks at relatively low NaCl concentration (Fleming et al., 1988). Also, the use of a malate-negative (unable to produce CO_2 from malic acid) culture of *Lactobacillus plantarum* to ferment the cucumbers was evaluated. Predominant growth by such a culture could eliminate the need for purging to prevent bloater formation.

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MATERIALS & METHODS

Cucumber brining

Fresh pickling cucumbers, size 2B (3.5–3.8 cm diameter) or 3A (3.8–4.4 cm) were washed in either a reel washer (laboratory experiments) or brush washer (pilot tank experiment). They were fermented by two different treatments, blanched, no salt (BNS), and salt, not blanched (SNB), (Fig. 1). Cucumber blanching was done in a water-jacketed steam kettle for laboratory experiments and in a heated water flume for the pilot experiment. Unless otherwise specified, blanching was 3 min at 77°C. The cucumbers were packed into 3.8 L jars with expansion reservoirs (Fleming et al., 1973), 19 L pails, or 4,428 L fiberglass tanks to occupy 55 to 60% of the container volume. All laboratory fermentations were duplicated, and data reported are averages of duplicates. Pilot-scale fermentations were not duplicated. The pilot tanks and related cucumber handling methods were as reported (Fleming et al., 1983). The cover brines consisted of either calcium acetate buffer (to equilibrate with cucumbers to 0.053 M acetic acid and 0.018 M calcium), as previously described (Fleming et al., 1988), and/or other components (noted in footnotes to tables). The nitrogen purging rate was 25 mL/min for jars and 400 mL/min for pilot tanks when purging was applied.

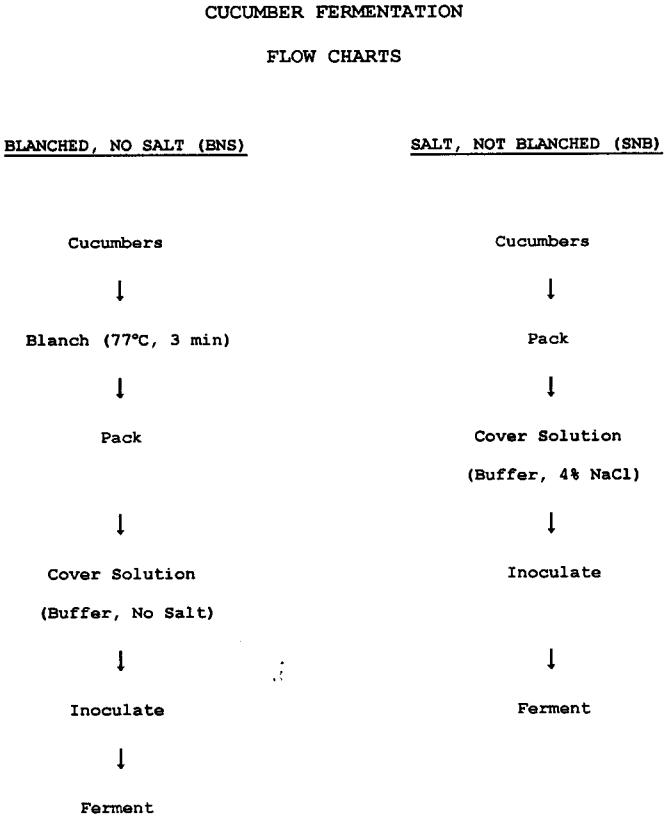

Fig. 1—Flow diagram for two procedures used for fermentation of cucumbers. The calcium acetate buffer (pH 4.7 ± 0.1) indicated in the cover solutions contained acetic acid and calcium hydroxide to attain concentrations, after equilibrium with cucumbers, of 53 and 18 mM, respectively.

Table 1—Effect of BNS and SNB treatments on cucumbers fermented by MDC⁻ and MDC⁺ cultures of *L. plantarum*[z]

Culture	Cucumber treatments		Final brine composition					Cucumber quality	
	Blanched	Salt	pH	Malic acid (mM)	Lactic acid (mM)	Residual sugar (mM)	CO_2 (mg/100 mL)	Bloater index	Firmness FPT (kg)
MDC⁻	−	+	3.5	0.3	120	4	78[c]	22[a]	9.8[a]
	+	−	3.6	4.9	109	1	62[d]	0[b]	9.6[a]
MDC⁻	−	−	3.7	1.2	124	1	114[a]	27[a]	9.5[a]
	+	+	3.5	6.2	103	11	48[ec]	1[b]	10.0[a]
MDC⁺	−	+	3.5	2.4	121	1	76[c]	22[a]	9.1[a]
	+	−	3.7	2.2	129	1	91[b]	4[b]	9.6[a]

[z] Size 3A cucumbers (2081g) were blanched (3 min, 77°C) or unblanched and packed into 3.7L jars and covered with calcium acetate buffer with or without NaCl as noted. Analyses were made after incubation at 26°C for 1 month. Letters within columns designate statistically significant differences ($P \leq 0.05$). BNS = blanched, no salt; SNB = salt, not blanched.

Microbial cultures

Microbial cultures included *L. plantarum* WSO (MDC⁺) and a mutant, M6, from that culture that did not produce CO_2 from lactic acid (MDC⁻) (Daeschel et al., 1984; McDonald et al., 1993). The lactic acid bacteria (LAB) were introduced at a rate of 10^6/mL of brined cucumbers. Brines were inoculated after brine was added to the cucumbers in laboratory fermentations and before brine was added to cucumbers in pilot-scale fermentations. The *L. plantarum* (MDC⁻) culture was prepared as a frozen concentrate by Chr. Hansen's Laboratory (Milwaukee, WI) under a sub-license agreement for a patent (Daeschel et al., 1987). The other culture was grown overnight in cucumber juice broth containing 0.018 M calcium acetate and 2% NaCl (McDonald et al., 1993).

Microbial analyses

General procedures for enumeration of microorganisms were described by Fleming et al. (1992a). Media included standard methods agar (PCA, BBL Microbiology Systems, Cockeysville, MD) for aerobes, violet red bile agar (BBL) + 1% glucose (VRBG) for *Enterobacteriaceae*, MRS broth (Difco Laboratories, Detroit, MI) + 1.5% agar + 0.02% sodium azide (MMRS) for LAB, and standard methods agar (BBL) + 0.1 mg/mL of chlortetracycline HCl + 0.1 mg/mL of chloramphenicol for yeasts (YM). All pour plates were duplicated and incubated at 30°C. Microbial colonies in VRBG plates were enumerated after 24 hr, PCA and MMRS plates after 48 hr, and YM plates after 72 hr.

A total of 100 colonies was isolated from MMRS plates of each pilot fermentation at each sampling time for the purpose of determining percentages of LAB that were malate-negative. Isolated colonies were picked and inoculated into individual microtiter wells containing MD broth (Daeschel et al., 1984). Growth in this broth allows differentiation between MDC⁺ and MDC⁻ cultures and has previously been used to determine predominance of the MDC⁻ culture under laboratory conditions (McDonald et al., 1993).

Chemical analyses

Malic, lactic, acetic, propionic, and butyric acids and ethanol, mannitol, glucose, and fructose were measured in fermentation brines using high performance liquid chromatography (HPLC) (McFeeters et al., 1984). An Aminex HPX-87H column (Bio-Rad Laboratories, Richmond, CA) with a cation guard column was used for separation of acids, and an Aminex HPX-87C column (Bio-Rad) with cation and anion guard columns was used for separation of sugars. A refractive index detector was used for quantification of sugars, and a UV detector (210 nm) was used for quantification of acids. In order to detect malic acid using HPLC, reduction of fructose in the sample was necessary. The procedure of McFeeters et al. (1993) was used for fructose reduction. CO_2 was determined according to the method of Fleming et al. (1974). Glucose and fructose concentrations in raw cucumbers were determined by blending four lots of four cucumbers each and determining the average values for the 4 composite blendings.

Product evaluation

Bloater damage (expressed as bloater index, i.e., relative proportion of tissue damaged) and visual cure of the cucumbers were determined as reported (Fleming et al., 1977). Firmness of the cucumbers was determined with a USDA Fruit Pressure Tester (FPT) with a 0.79 cm tip and expressed as kg force (Bell et al., 1955). Since cucumbers were severely bloated in some treatments, firmness of all fruit was determined after they had been longitudinally sliced. Thus, the procedure was to slice the fruit first for bloater evaluation, and then to test firmness of 20 unbloated fruit halves. A comparison of firmness of whole vs half fruit (50 of each) was made on unbloated fruit from the middle section of the SNB pilot tank. The firmness means were 6.8 kg for half and 7.2 kg for whole fruit. This 0.4 kg difference in firmness was not significant ($P > 0.23$).

Statistical analyses

The General Linear Model Procedure of SAS (SAS Institute, Cary, NC) was used to compute all statistical inferences. The experimental design for the pilot tank experiment was a nonreplicated complete block, while the laboratory experiment was a duplicated complete block.

RESULTS

Laboratory fermentation of cucumbers

The effects of the BNS and SNB treatments (Fig. 1) of cucumbers on fermentation by MDC⁻ and MDC⁺ strains of LAB were tested under laboratory conditions. The cucumbers fermented normally by both cultures and under both brining treatments. However, cucumbers fermented faster in the absence of salt and when blanched, as was expected. The fermentations were completed after 1 mo, as evidenced by absence of sugars and cessation of acid production, at which time chemical compositions and product quality were determined (Table 1). The residual sugar in the brine had been reduced to 1–11 mM from a calculated initial concentration of 129 mM in the raw fruit.

The fermentation brines were not purged, thus the CO_2 concentration was permitted to increase to a maximum. In the SNB treatment, the CO_2 concentration by both the MDC⁺ and MDC⁻ strains was similar (76 vs 78 mg/100 mL, respectively). In the BNS treatment, however, the CO_2 concentration was notably higher in cucumbers fermented by the MDC⁺, as compared to the MDC⁻ strain (91 vs 62 mg/100 mL). The higher concentration of lactic acid with the MDC⁺ strain (129 mM) than with the MDC⁻ strain (109 mM) was consistent with expectation, since lactic acid is an end-product of malate decarboxylation. However, difference in CO_2 concentration could not be accounted for on the basis of malate decarboxylation since the difference in residual malate concentrations between MDC⁺ and MDC⁻ strains was only 2.7 mM for the BNS treatments (Table 1).

The highest concentration of CO_2 (114 mg/100 mL) was reached when the cucumbers were not heated and no salt was added (MDC⁻ control treatment). The lowest concentration of CO_2 (48 mg/100 mL) was reached when the cucumbers were blanched and salt was added (MDC⁻ control treatment). The MDC⁻ culture did not appear to predominate the SNB fermentations in our results. Breidt et al. (1992) showed predominance, as evidenced by high residual malate concentration and differential enumeration of MDC⁻ and MDC⁺ LAB. Differences in concentrations of natural MDC⁻ LAB in the two studies were probably responsible for these differences.

Bloater damage in fermented cucumbers was slight when cucumbers were fermented by BNS treatment, though they were fermented by the MDC⁺ culture, and the CO_2 concentration

Table 2—Chemical changes of cucumbers fermented by the BNS and SNB procedures by *L. plantarum* (MDC⁻) in pilot tanks[a]

Fermentation time (days)	Blanched, no salt (BNS)					Salt, not blanched (SNB)				
	CO_2, (mg/100 mL)	Malic acid, (mM)	pH	Titratable acidity (%)	Sugar (%)	CO_2 (mg/100 mL)	Malic acid (mM)	pH	Titratable acidity, (%)	Sugar (%)
0	—	0.0	4.9	0.35	0.00	—	0.0	4.7	0.39	0.00
1	9	1.1	4.8	0.35	0.01	8	0.0	4.7	0.39	0.01
2	67	0.8	4.3	0.39	0.25	43	4.0	4.9	0.25	0.40
3	108	0.0	4.0	0.76	0.15	50	9.4	4.5	0.33	0.46
4	119	0.0	3.9	0.87	0.03	68	3.1	4.6	0.32	0.46
6	150	0.0	3.9	1.04	0.01	122	0.9	3.9	0.73	0.39
8	106	0.0	3.8	0.99	0.01	101	0.0	3.7	0.87	0.29
Purging started[b]										
10	41	0.0	3.8	0.98	0.01	58	0.0	3.7	0.88	0.24
16	34	0.0	3.7	1.06	0.01	30	0.0	3.6	1.01	0.09
30	12	0.0	3.7	1.01	—	10	0.0	3.5	1.07	—

[a] Size 2B cucumbers were fermented in 4,428L pilot tanks. Calcium acetate buffer with or without salt was added as cover liquor at 40% volume of the tank contents. Salt, when added, equalized at 4.4%. Blanching was for 3 min at 77°C.

[b] Purging with nitrogen was started after 8 days at a rate of 400 mL/min. The fermentations occurred at ambient temperature (about 26°C).

reached 91 mg/100 mL (Table 1). No bloater damage was evident in cucumbers fermented by the BNS treatment with the MDC⁻ culture. Bloater damage was severe in cucumbers fermented by SNB treatment, whether fermented by MDC⁺ or MDC⁻ culture (Table 1).

Firmness of cucumbers from all treatments was excellent. There were no statistically significant differences among any of the treatments ($P \geq 0.05$). Since some of cucumbers were bloated, firmness determinations were made on cucumber halves from fruit that had not bloated.

The pH of brines from the various fermentations ranged from 3.5 to 3.7. There was no evidence of propionic and butyric acids or propanol. The products had desirable aromas, with no evidence of fermentation by undesirable microorganisms.

Pilot fermentation of cucumbers

Cucumbers were fermented in 4,428-L fiberglass tanks by the BNS and SNB treatments, using the *L. plantarum* MDC⁻ culture (Table 2). The CO_2 concentrations reached 106 and 101 mg/100 mL for the BNS and SNB treatments, respectively, after 8 days (Table 2). This was considerably higher than that reached in laboratory fermentations for the MDC⁻ culture after 1 mo (Table 1). Since purging of brines by nitrogen was not begun until after 8 days, the CO_2 concentration was an accumulation until that time, and indicated that microorganisms other than the *L. plantarum* MDC⁻ culture were active. After 30 days, no fermentable sugar remained in the brine, and the titratable acidity (calculated as lactic) had reached 1.01 and 1.07%, respectively, for the BNS and SNB treatments. The total fermentable sugars (glucose and fructose) in the raw cucumbers was 117 mM. The brine pH for the SNB treatment was 3.5 and for the BNS was 3.7. There was no evidence of butyric acid in brines of either treatment.

Microbial changes during fermentations were followed (Fig. 2). As in the laboratory study, the cucumbers fermented more rapidly in the BNS than in the SNB treatment. The numbers of LAB and total aerobes were nearly identical throughout the first 15 days of SNB fermentation. By comparison, total aerobes were slightly and consistently higher in numbers than LAB during the same period in the BNS fermentation (Fig. 2). We did not determine the reason for this difference, or if the bacteria that grew on the total aerobe plates were actually LAB that failed to grow on the MMRS medium, or were non-LAB. An initial 2-log cycle reduction occurred in *Enterobacteriaceae* count of the BNS, as compared to the SNB-treated cucumbers. Yeast counts were higher in the BNS than the SNB treatment, but never exceeded 1,000/mL and trended lower in both fermentations after about 5 days.

Quality of fermented cucumbers was evaluated after 1 and 7 mo storage in the pilot tanks (Table 3). For comparative purposes, cucumbers taken from the same lot fermented in the pilot tanks were also fermented in 19-L plastic pails under laboratory conditions. The bloater index was relatively high for the BNS treatment compared to the SNB treatment in pilot tanks (Table 3). The index was greater in the top than the middle section, consistent with previous results (Fleming et al., 1977). Also, visual cure was greater in the middle section, as reported previously. Cucumbers were firmer in the SNB than BNS treatment, and the firmness was maintained over a storage period of 7 mo. Firmness was significantly lower ($P \leq 0.05$) in the BNS than the SNB treatment 1 mo after brining, and after 7 mo the firmness had diminished to an unacceptable level.

In contrast, firmness of BNS and SNB cucumbers was similar when fermented under laboratory conditions (Table 3). However, there was a slight reduction in firmness of BNS cucumbers after storage for 12 mo, while there was a slight increase in firmness of SNB cucumbers after 12 mo. The BNS cucumbers had no bloater damage after 1 mo, while SNB cucumbers were slightly bloated.

DISCUSSION

RESULTS OF THIS STUDY indicate that successful fermentation of cucumbers in the absence of added sodium chloride may be possible. Under laboratory conditions, fermented cucumbers of good quality were obtained in the absence of added salt, provided the fruit were blanched prior to fermentation (BNS treatment). Under pilot-scale conditions, however, cucumbers fermented in the absence of salt were notably less firm after fermentation, and the quality deteriorated greatly during storage of 7 mo. Microbial recontamination after blanching was probably responsible for the lesser quality of cucumbers fermented by the BNS procedure under pilot-scale conditions. For the BNS procedure to be successfully applied on a commercial scale, more aseptic procedures may be required. The U.S. pickle industry currently is not structured to implement highly aseptic procedures in the handling of fresh cucumbers for brine-stock storage.

The BNS procedure provides the potential advantage of eliminating the need for purging to prevent bloater damage. Cucumbers blanched before fermentation by the MDC⁻ *L. plantarum* culture did not bloat under laboratory conditions, whether salt was present or absent. Heating may have inactivated microorganisms or enzymatic activity of the cucumber tissue itself. Further studies are needed to establish minimum heating requirements to prevent bloater formation without purging. It is likely that microbial inactivation is important since heated cucumbers bloated under pilot conditions where conditions were less aseptic.

An obvious advantage of BNS treatment was the removal of indigenous microorganisms and the possibility of controlled fermentation by added microorganisms with desirable traits. The addition of and predominance by the *L. plantarum* MDC⁻ culture under laboratory conditions exemplified one type of con-

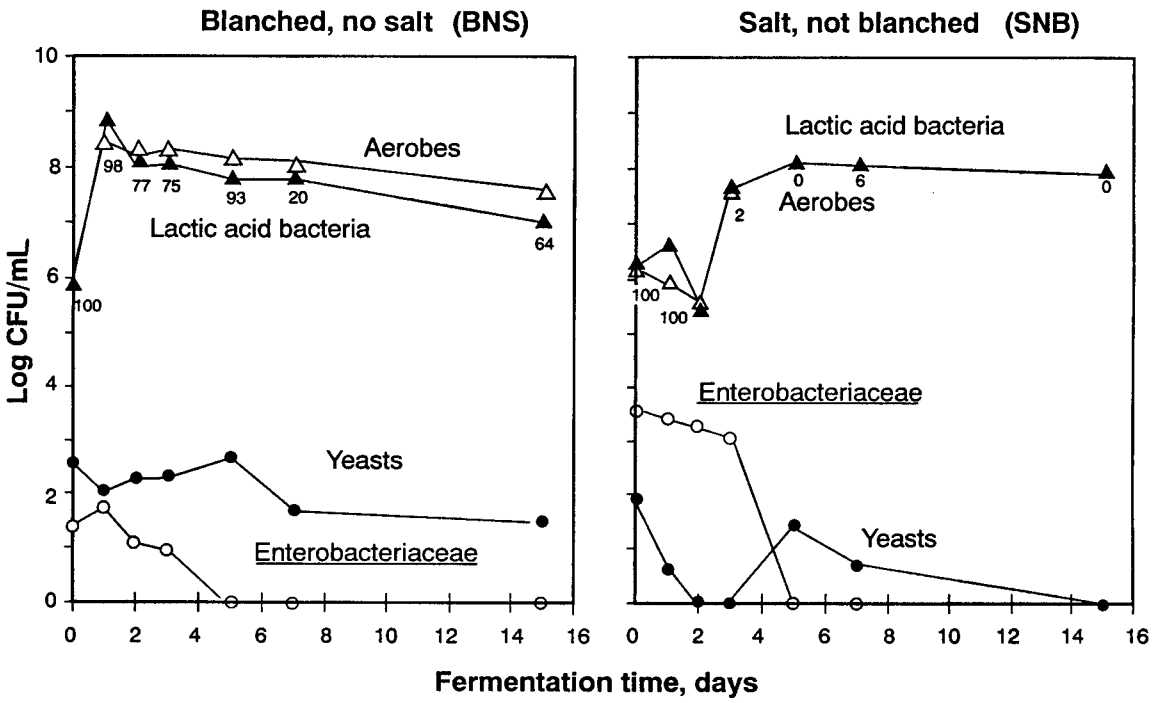

Fig. 2—Microbiological changes in cucumbers fermented in pilot tanks by two different procedures. The numbers that appear under data points for lactic acid bacteria refer to percentages of bacteria determined to be malate-negative. Microbial counts below log CFU/mL of 2 were estimates since the numbers of colonies per plate sometimes were <25.

Table 3—Quality of cucumbers fermented in pilot and laboratory fermentors[x]

		Brining treatment					
		Firmness FPT[y] (kg)		Cure[z] (%)		Bloater index	
Fermentor	Storage time (months)	BNS	SNB	BNS	SNB	BNS	SNB
Pilot							
Top	1	7.1[b]	8.4[a]	60	70	29.0[b]	3.2
Middle	7	TS	8.3[a]	85	75	39.8[a]	10.0[cd]
	1	6.6[b]	8.1[a]	100	98	18.3[c]	1.5[d]
	7	4.0[c]	8.4[a]	100	95	7.8[d]	1.6[d]
Laboratory	1	7.6[b]	7.5[b]	50	55	0.0[d]	13.5[c]
	12	6.7[b]	8.2[a]	58	100	6.6[d]	4.4[d]

[x] Size 2B cucumbers, from the same lot, were fermented in 4,428L fiberglass, pilot tanks at a commercial firm, or in 19L plastic pails under laboratory conditions. Letters within firmness or bloater groupings designate statistically significant differences $P < 0.05$).

[y] BNS = blanched, no salt; SNB = salt, not blanched brining treatments; TS = too soft to determine firmness (<1.4 kg).

[z] Cure % was determined by subjective evaluation of entire lots and was not amenable to statistical analysis.

trolled fermentation. By elimination of purging requirements, economic disadvantages of the BNS system could be at least partially offset.

Clostridia have been shown to cause spoilage in acidified foods (Segmiller and Evancho, 1992). Even in fermented cucumbers, *Clostridium tertium* grew and contributed to the butyric spoilage of fermented cucumbers (Fleming et al., 1989). In that case, the cucumbers had been fermented to pH 3.7, and the acidity was 1% (as lactic acid). However, the salt concentration was only 2.3%, less than half the concentration used in commercial fermentations. Thus, further research is needed to determine the likelihood of spoilage of salt-free, fermented cucumbers by clostridia and other bacteria, and alternatives to salt for assuring microbial stability after fermentation and during extended storage. Addition of HCl to lower the brine pH to 3.3–3.5 after fermentation has been shown to provide the potential for microbial stability of brined cucumbers at 4% NaCl (Fleming et al., 1992b). Textural stability was reduced at lower pH values, but remained acceptable at pH 3.3–3.5

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