Reprinted from *J. Food Sci.*
61(4): 832–836

Assuring Microbial and Textural Stability of Fermented Cucumbers by pH Adjustment and Sodium Benzoate Addition

H. P. FLEMING, R. L. THOMPSON, and R. F. McFEETERS

ABSTRACT

Acidification of fermented cucumbers with HCl prevented utilization of lactic acid and resultant rise in brine pH (accompanied by formation of butyric, propionic and acetic acids, and n-propanol by spoilage bacteria) when they were stored at 0 or 4.4% NaCl. Firmness retention of the fermented cucumbers was reduced, however, if the brine pH were less than optimum pH 3.5, which assured microbial stability and acceptable firmness retention with 4.4% NaCl. At 0% salt, pH 3.0 insured microbial stability, but resulted in unacceptable firmness. Addition of 0.1% Na benzoate reduced the need to lower pH to assure microbial stability. Results indicated that pH control could be used to reduce the need for salt to insure stability of fermented cucumbers.

Key Words: cucumbers, fermentation, stability, microbial, texture

INTRODUCTION

BRINED CUCUMBERS traditionally have been held with 5 to 7% NaCl during fermentation (about 1 mo) and then the NaCl concentration increased to 8 to 16% for storage (a few months to a year or longer). Reasons for the NaCl concentrations used included microbial control, prevention of enzymatic softening (Bell and Etchells, 1961) and prevention of freeze damage in frigid climates. The use of calcium chloride increased firmness retention of brined cucumbers at relatively low concentrations of NaCl (Fleming et al., 1987) and provided protection against enzymatic softening (Buescher et al., 1979). Controlled fermentation in anaerobic tanks resulted in acceptably firm cucumbers and proper fermentation at NaCl concentrations of 2.7 and 4.6% NaCl (Fleming et al., 1988). Subsequently, cucumbers fermented and stored in an anaerobic tank at 2.3% NaCl were reported to be microbiologically unstable during storage, after a normal fermentation (Fleming et al., 1989). Lactic acid formed during fermentation was converted to butyric acid and other undesirable end-products during storage. In unreported experiments, we have observed a gradual conversion of lactic to propionic and acetic acids during storage of cucumbers brined at 4 to 5% NaCl.

The pickle industry is increasingly required by regulatory agencies to reduce chloride discharge into freshwater bodies, especially since the U. S. Environmental Protection Agency issued the guideline limit of 230 ppm chloride in freshwater bodies (EPA, 1987). Since most fermented pickle products require 1 to 4% NaCl for desirable flavor, excess salt must be leached from brined cucumbers during processing. Such excess salt has been discharged into private or municipal wastewater streams, and eventually could enter freshwater bodies.

The objective of our study was to determine if brine pH adjustment and sodium benzoate addition after fermentation could serve as alternatives to the addition of high concentrations of NaCl to assure microbial and textural stability of fermented cucumbers. Such alternatives were tested on cucumbers that were either blanched or unblanched before fermentation.

The authors are with the USDA-ARS Food Fermentation Laboratory, and North Carolina Agricultural Research Service, Dept. of Food Science, North Carolina State Univ., Raleigh, NC 27695-7624.

MATERIALS & METHODS

Cucumbers

Fresh pickling cucumbers (size 2B, 3.5 to 3.8 cm diameter) of unidentified cultivar were obtained from a local pickle company. They were washed in either a reel washer (laboratory experiments) or brush washer (pilot tank experiment). The fruit were in good condition, not notably desiccated, diseased, or mechanically damaged.

Brining procedures

The cucumbers were brined and fermented by 2 procedures, as described (Fleming et al., 1995). In a ''blanched, no salt'' (BNS) treatment, fresh cucumbers were blanched in water at 77°C for 3 min; the cover brine contained calcium acetate buffer, but no NaCl. This procedure was used to test the feasibility of fermenting and storing cucumbers in the absence of salt. In a ''salt, not blanched'' (SNB) treatment, the cucumbers were not blanched and the cover brine contained calcium acetate and sufficient NaCl to equalize with the cucumbers at 4.4%. Cover brines for each treatment were supplemented as needed to equalize with cucumbers at 0.053M acetic acid and 0.018 M calcium hydroxide, as described (Fleming et al., 1988). The initial cover brine was pH 4.7.

Fermentation

The fermented cucumbers were described in a previous study (Fleming et al., 1995). The brined cucumbers had been inoculated with the *Lactobacillus plantarum* MOP3-M6 culture, as described (McDonald et al., 1993) and allowed to ferment at 26°C under laboratory conditions or at ambient temperatures (≈23°C) in the pilot tanks.

Brine pH adjustment and brine-stock storage

After primary fermentation (about 30 days), samples of cucumbers and brine (55/45, w/v, cucumbers/brine) were blended to homogeneity and titrated with 6N HCl or NaOH to desired pH. Calculated amounts of HCl or NaOH were added to samples of fermented cucumbers to equalize at the desired pH for storage. Titration curves for the homogenized cucumbers were determined (Fig. 1). Titration curves with 6N acetic and 4.7N lactic acids are reported to illustrate relative amounts compared to HCl for a specified pH.

For storage stability studies, fermented cucumbers (8–10 per jar, 55/45, w/v, cucumbers/brine) and brine were packed into duplicate 1410 mL jars and the appropriate amounts of HCl or NaOH added, based on titration (Fig. 1), to equilibrate at the desired pH between 3.0 and 4.3 (see initial pH values). Sodium benzoate was added to specified samples to equalize at 0.1%. All laboratory-stored products were held at 26°C in hermetically capped jars. Pilot tank-stored brine-stock was held in the original fermentation tanks at ambient temperature.

Chemical analyses

Organic acids, sugars, and alcohols were determined by HPLC, as described (McFeeters et al., 1984). The identity of presumptive peaks for butyric acid, propionic acid, and n-propanol was confirmed by HPLC with a HPX-87H cation column (Bio-Rad Laboratories, Richmond, CA) with 0.01N H_2SO_4 as eluent. Detection was by refractive index detector (model 401, Waters Associates, Milford, MA) and a Varichrom UV detector (Varian Analytical Instruments, Palo Alto, CA) at 210 nm. To confirm the identity of n-propanol, a brine sample found to contain the compound, based upon HPLC, was analyzed by purge and trap gas chromatography-mass spectrometry. The brine sample was diluted fivefold with water. Volatiles were collected from 5 mL of the diluted brine by purging with helium gas and trapping on a Tenax trap (Supelco, Inc.,

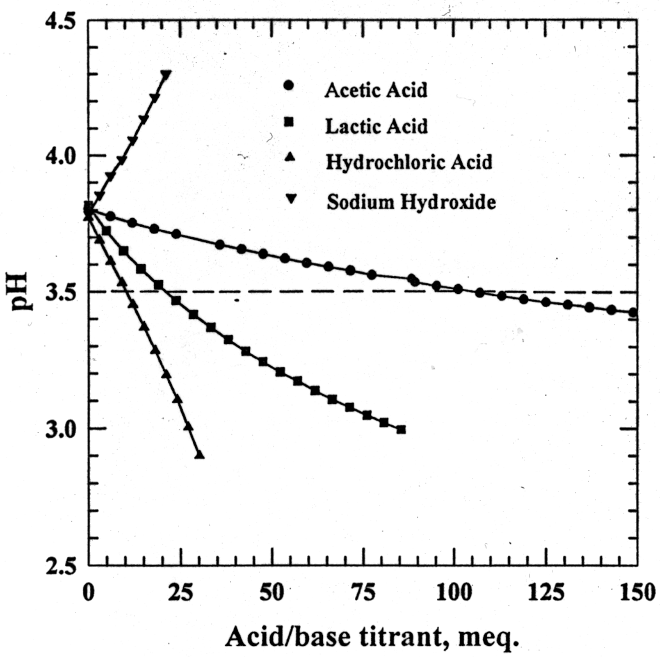

Fig. 1—Titration of fermented cucumber homogenate. Samples (500g each) of fermented cucumbers and brine (55/45, w/w) were blended and titrated to the pH values shown. The horizontal line is drawn to facilitate comparison of relative amounts of acidulant necessary to reduce the brine to pH 3.5.

Table 1—Fermentation substrates and products of cucumbers fermented in pilot and laboratory fermentors

	Concentration of compounds, mM				
		After fermentation			
	Before	Pilot tanks		Lab fermentors	
Compound	fermentation	BNS	SNB	BNS	SNB
Glucose	31.2[a]/28.6[b]	ND[c]	ND	ND	ND
Fructose	34.9/32.0	ND	ND	ND	ND
Malic acid	10.9/10.0	ND	ND	1.4	4.3
Acetic acid	64.2	60.8	70.8	69.9	61.9
Lactic acid	ND	114.2	123.4	114.0	118.8
Succinic acid	ND	2.9	1.1	5.6	1.5
Propionic acid	ND	ND	ND	ND	ND
Ethanol	ND	25.2	30.8	6.9	14.0
pH	4.7	3.7	3.5	3.7	3.3

[a] Equilibrated values (theoretical) for the pilot tanks (60/40, cucumbers/brine pack-out ratio).
[b] Equilibrated values (theoretical) for the laboratory fermentors (55/45, cucumbers/brine).
[c] ND = none detected.

Table 2—Effect of pH adjustment on microbial stability of cucumbers fermented in pilot tanks[a]

Brine pH		Brine acidity, %		Secondary fermentation products[e]			
Initial	12 mo	Initial	12 mo	Acetic	Propionic	Butyric	n-Propanol
BNS treatment[b]							
Salt: Fermentation, 0%; storage, 0%							
No sodium benzoate added							
4.3	5.3	0.72	0.47	+	+	+	+
3.7[c]	4.8	1.11	0.82	+	+	+	+
3.6	4.5	1.18	0.95	+	+	+	+
3.5[c]	4.3	1.22	1.10	+	+	–	+
3.4	4.2	1.28	1.12	+	+	–	+
3.3	3.8	1.32	1.24	+	+	–	+
3.0	3.0	1.44	1.46	–	–	–	–
0.1% Sodium benzoate added							
4.3	5.2	0.68	0.51	+	+	+	+
3.7	4.5	1.12	0.88	+	+	–	+
3.6	4.4	1.16	1.02	+	+	–	+
3.5	4.0	1.21	1.19	+	–	–	+
3.4	4.0	1.25	1.17	+	+	–	+
3.3	3.8	1.29	1.24	+	–	–	+
3.0	3.2	1.44	1.46	–	–	–	–
BNS treatment							
Salt: Fermentation, 0%; storage, 4%							
No sodium benzoate added							
4.3	4.6	0.59	0.57	+	+	–	+
3.7	4.0	0.96	0.93	+	+	–	+
3.6	3.8	1.04	1.00	+	+	–	+
3.5	3.7	1.08	1.04	–	–	–	–
3.0	3.2	1.35	1.34	–	–	–	–
0.1% Sodium benzoate added							
4.3	4.6	0.54	0.60	+	+	–	+
3.7	3.8	0.92	0.93	–	–	–	–
3.0	3.3	1.35	1.33	–	–	–	–
SNB treatment[d]							
Salt: Fermentation, 4%; storage, 4%							
No sodium benzoate added							
4.3	4.6	0.54	0.53	+	+	–	+
3.7	4.2	0.90	0.85	+	+	–	+
3.6	3.8	0.98	1.00	+	+	–	+
3.5	3.5	1.06	1.08	–	–	–	–
3.0	3.1	1.34	1.33	–	–	–	–
0.1% Sodium benzoate added							
4.3	4.5	0.53	0.54	+	+	–	+
3.7	3.8	0.90	0.90	–	–	–	–
3.0	3.2	1.32	1.32	–	–	–	–

[a] Size 2B cucumbers were used.
[b] BNS = Cucumbers heated, no salt added for fermentation.
[c] Quantitative changes in these treatments are given in Table 3.
[d] SNB = Cucumbers not heated, 4% salt added for fermentation.
[e] + = a net increase in the concentration of the indicated compound during storage; – = no increase in concentration.

Bellafonte, PA), using a CDS 6000 purge and trap unit (CDS Analytical Inc., Oxford, PA). From the trap, volatiles were chromatographed with a Hewlett-Packard 5890 gas chromatograph equipped with a HP-5, 30 m × 0.25 mm capillary column and detected with a model 5972 mass spectroscopy detector (Hewlett-Packard, Palo Alto, CA) in the EI mode. The mass spectra of volatile components were compared to the mass spectrum of authentic n-propanol. Titratable acidity, pH, and salt concentration were determined, as previously described or referenced (Fleming et al., 1984).

Brine-stock firmness

Fermented cucumbers were cut longitudinally into halves for firmness measurement (skin up) with a USDA Fruit Pressure Tester (FPT) with a 0.79 cm diameter tip and expressed as kg force (Bell et al., 1955). Testing of halves was done to avoid severely bloated fruit, which resulted in about 0.4 kg lower reading than when whole, nonbloated fruit were tested (Fleming et al., 1995).

Statistical analyses

A Randomized Complete Block design was used to assess firmness changes during storage of fermented cucumbers. All statistical inferences were computed with the *General Linear Model Procedure of SAS* (SAS Institute, Inc., Cary, NC).

RESULTS

Microbial stability when fermented in pilot tanks

Cucumbers were fermented in pilot tanks by the BNS and SNB treatments until all fermentable sugars had been converted to lactic acid and other end-products (Table 1). About 30 days after brining, samples of brine-stock from each treatment were returned to the laboratory, where they were stored at pH 3.0–4.3 in the presence or absence of added sodium benzoate (Table 2). Initial acidities varied due to HCl or NaOH required for initial pH adjustment. Microbial instability of the brine-stock was reflected in increase in brine pH and decrease in acidity after storage for 12 mo. HPLC analysis of the brines after 12 mo storage revealed secondary fermentation products formed as a consequence of microbial instability during storage (Table 2).

BNS cucumbers stored in the absence of salt and sodium benzoate were stable at pH 3.0, but not at pH 3.3 and above. At pH 3.3–4.3, the pH increased, the acidity decreased, and secondary fermentation products were formed. Acetic and propionic acids and n-propanol were formed when storage was at initial pH 3.3–3.5. At pH 3.6–4.3, butyric acid also was formed.

Table 3—Chemical changes in cucumbers fermented in a pilot tank and stored under laboratory conditions without NaCl[a]

Compound	Concentration of compounds, mM: After fermentation	Net change after storage, mo[b]: 3	6	9	12
Storage brine pH 3.7 (unadjusted)					
Acetic acid	51.5	34.2	58.6	57.8	54.6
Lactic acid	114.2	−35.3	−114.2	−114.2	−114.2
Propionic acid	ND[c]	ND	5.5	5.9	6.6
Succinic acid	2.8	0.3	−2.8	−2.8	−2.8
Butyric acid	ND	ND	18.8	20.0	20.1
Ethanol	14.0	15.4	18.4	17.0	16.2
n-Propanol	ND	13.8	36.0	34.6	36.4
Brine pH	3.7	3.9	1.0	4.7	4.8
Storage brine adjusted to pH 3.5					
Acetic acid	63.3	4.1	59.4	62.2	54.5
Lactic acid	114.3	−8.6	−104.2	−104.2	−104.6
Propionic acid	ND	5.5	25.9	26.9	22.4
Succinic acid	3.2	−0.1	0.2	0.3	0.0
Butyric acid	ND	ND	ND	ND	ND
Ethanol	25.4	0.3	0.7	1.6	2.4
n-Propanol	ND	ND	25.5	26.0	26.2
Brine pH	3.5	3.6	4.3	4.2	4.3

[a] See Table 2, footnote b, for the BNS brining treatment represented by these samples.
[b] The net changes reflect the increase or decrease in concentrations of compounds during storage compared to the concentrations immediately after fermentation.
[c] ND = none detected.

Table 4—Firmness and pH stability of cucumbers fermented in pilot tanks and stored under laboratory conditions after NaCl addition to the BNS treatment

Storage time, mo[b]	BNS (0% NaCl storage) Firmness FPT (kg)	pH	BNS+ (4% NaCl storage) Firmness FPT (kg)	pH	SNB (4% NaCl storage) Firmness FPT (kg)	pH
0	6.4[d]	3.7	6.4[d]	3.7	8.5[c]	3.7
3	3.9[gh]	4.1	5.4[ef]	3.6	8.6[c]	3.7
6	3.4[h]	4.4	5.8[de]	3.6	7.9[c]	3.6
9	3.1[i]	4.2	6.0[de]	3.6	8.3[c]	3.7
12	3.2[h]	4.5	5.8[de]	3.7	8.2[c]	3.7

[a] Fermented cucumbers were taken from pilot tanks, 4% NaCl added to a portion of BNS product, and firmness of all three treatments determined after storage at 26°C. Firmness values among all columns and rows with the same superscript letter are not different significantly (P ≥0.05) as determined by the Bonferroni method. Firmness values are means for 18–20 cucumbers.
[b] The zero time sampling was 2½ mo after initial brining.

When sodium benzoate was present, propionic acid was not formed at pH 3.3 or below, and butyric acid was not formed at pH 3.7 or below (Table 2).

When 4% salt was added to BNS cucumbers after fermentation, microbial stability was achieved at higher initial pH values (Table 2). In the absence of sodium benzoate, none of the secondary fermentation compounds were observed with storage at pH 3.5 or below. Butyric acid was not detected with pH as high as 4.3, but acetic and propionic acids and n-propanol were detected at pH 3.6–4.3. When sodium benzoate and salt were added, only storage at pH 4.3 resulted in microbial instability.

SNB cucumbers were microbiologically stable at pH 3.5 and below in the absence of benzoate and 3.7 and below in the presence of benzoate. The addition of 4% salt before fermentation resulted in the same microbial stability profile as addition of salt after fermentation. Apparently, conditions during storage were primarily responsible for microbial stability. Neither blanching of cucumbers before brining, nor presence of salt during fermentation seemed to influence subsequent stability after fermentation when salt and/or sodium benzoate had been added.

The appearance of secondary fermentation compounds and disappearance of lactic acid for cucumbers fermented and stored without salt (BNS) were found (Table 3). At pH 3.7 (unadjusted), lactic acid was reduced by 31% after 3 mo and 100% after 6 mo. Lactic acid depletion during the first 3 mo was accompanied by increases in acetic acid, ethanol, and n-propanol.

Table 5—Effect of brine pH on firmness of fermented cucumbers after extended storage[a]

Brine pH: Initial	Final[b]	Firmness FPT[b] (kg)
4.3	4.6	8.4[c]
3.7	3.8	7.1[d]
3.6	3.7	6.9[d]
3.5	3.6	6.4[e]
3.4	3.5	6.3[e]
3.3	3.4	6.2[e]
3.0	3.2	5.2[f]

[a] Cucumbers were fermented in a pilot tank using the SNB method. After fermentation (30 days), brines were adjusted to the pH indicated with HCl from initial pH 3.7 (the pH after fermentation). The storage brines contained 4% NaCl, 0.2% $CaCl_2$, and 0.1% sodium benzoate.
[b] Firmness and pH were measured after storage at 26°C for 42 mo. Numbers with the same superscript letter are not significantly different (P ≥0.05) as determined by the Waller multiple comparison test. Each firmness value is the mean of 18–20 cucumbers.

Between 3 and 6 mo, butyric and propionic acids began to appear, and n-propanol concentration increased. After 12 mo, no lactic acid remained, the pH had risen from initial 3.7 to 4.8, and secondary fermentation compounds had replaced lactic acid. Overall, about 114 mM lactic acid and 3 mM succinic acid were depleted, and 55 mM acetic acid, 7 mM propionic acid, 20 mM butyric acid, 16 mM ethanol, and 36 mM n-propanol were formed over the 12-mo storage period.

When the brine was adjusted initially to pH 3.5, the initial 114 mM lactic acid was depleted to 10 mM after storage for 12 mo, and the pH increased to 4.3 (Table 3). Acetic acid, propionic acid, and n-propanol were formed. Identity of n-propanol was confirmed by GC-MS, based upon a mass spectrum identical to that of authentic n-propanol. Butyric acid was not formed.

Firmness stability when fermented in pilot tanks

Firmness of cucumbers fermented and stored in pilot tanks by the BNS and SNB treatments has been reported (Fleming et al., 1995). SNB cucumbers fermented and stored in a pilot tank were firm immediately after fermentation (FPT = 8.1 kg) and remained firm after storage for 7 mo in the tank (FPT = 8.4 kg). In contrast, BNS cucumbers were less firm after fermentation (FPT = 6.6 kg) and became considerably less firm after storage in a pilot tank for 7 mo (FPT = 4.0 kg; Fleming et al., 1995).

Samples of brine-stock were removed from the pilot tanks 1 mo after brining for study of firmness stability under laboratory conditions. The SNB cucumbers remained firm over the 12-mo storage (Table 4), similar to the effect when stored in pilot tanks. The firmness of BNS cucumbers declined over the 12-mo storage (Table 4). Addition of 4% NaCl to the BNS cucumbers prior to storage resulted in greater firmness retention during storage for 12 mo than if salt had not been added.

Portions of the pH-adjusted cucumbers used in microbial stability determination were retained for study of pH effect on firmness stability. The effect of pH on firmness retention over 42 mo was determined (Table 5). At pH 4.3, firmness retention was greatest (8.4 kg) and was similar to initial firmness (8.5 kg, Table 4) of the SNB cucumbers. Firmness retention became regressively less as storage pH was reduced to a low of pH 3.0.

Microbial stability when fermented in laboratory fermentors

Fresh cucumbers from the same lot used for pilot studies (Table 1) were returned to the laboratory, where they were brined by the BNS and SNB treatments. The cucumbers, after fermentation, were stored with or without sodium benzoate (Table 6). A portion of the BNS cucumbers was supplemented with 4% salt after fermentation. These treatments were similar to those