# Butyric Acid Spoilage of Fermented Cucumbers

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## ABSTRACT

Small cucumbers brined to equalize at 2.3% NaCl in an anaerobic tank underwent a normal primary lactic acid fermentation that resulted in 1.09% titratable acidity (as lactic) and pH 3.7. Nine months later the product was observed to have spoiled, as evidenced by an unpleasant odor. Products formed during spoilage in order of concentration were acetic acid > butyric acid > n-propanol > propionic acid. No lactic acid remained. No botulinal toxin was detected. *Clostridium tertium* was identified as contributing to the spoilage, but did not produce propionic acid or n-propanol under test conditions. Evidence indicated that unidentified bacteria, possibly propionibacteria sp., degraded lactic acid causing a rise in pH which allowed *C. tertium* to grow.

## INTRODUCTION

THE PRESERVATION of pickling cucumbers by brine fermentation and storage in bulk containers constitutes approximately 40% of the total crop grown in the U.S. Processors could greatly benefit from brining procedures that minimize salt (NaCl) usage. Much of the salt used for storage of fermented cucumbers must be leached from the brine-stock pickles during further processing. Disposal of salt poses environmental and economic concerns to the pickle industry. The concerns could be increased with current proposals by federal and state agencies for limitation of chloride in waterways into which salt and other processing wastes may be discharged.

Two major functions of salt in the brining of cucumbers include the inhibition of softening enzyme activity (Bell and Etchells, 1961) and control of microbial activity. Recent studies have indicated that the firmness of brined cucumbers can be retained at appreciably lower salt concentrations than traditionally used (Fleming et al., 1987). This optimism is due to recent studies on the use of calcium salts to retard cucumber softening (Fleming et al., 1978; Buescher et al., 1979; Tang and McFeeters, 1983) and the use of anaerobic tanking procedures to preclude entrance of rainwater, foreign matter and aerobic microorganisms (Fleming et al., 1983; 1988).

Higher salt concentrations (10.6–15.8%, w/w) in cucumber brines suppress growth of lactic acid bacteria, but allow growth of bacteria of the genus *Enterobacter* (formerly *Aerobacter*) and yeasts (Etchells and Jones, 1943). The latter two groups of microorganisms produce large amounts of gas, which can result in bloater damage to the brine-stock pickles (Jones and Etchells, 1943). A lower salt concentration (5.3%) was shown to favor growth of lactic acid bacteria and suppress growth of *Enterobacter* and yeasts. Thus, the current commercial use of salt concentrations of 5 to 8% for fermentation and 8 to 16% for storage has evolved as a compromise to achieve a desirable fermentation and maintain a desirably textured product.

In a recent pilot-scale experiment employing anaerobic tank technology (Fleming et al., 1988) at low concentration of salt (2.3%), we observed an unusual spoilage problem of fermented cucumbers. The cucumbers underwent a normal lactic acid fermentation which terminated at pH 3.7. During storage, lactic acid was depleted and acetic acid, butyric acid, propionic acid, and n-propanol were formed. The objective of this study was to characterize chemical changes that occurred during fermentation and spoilage, and to identify spoilage microoganisms.

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## MATERIALS & METHODS

SIZE 1B (2.4 to 2.7 cm diameter) pickling cucumbers were washed with an Osborn U-Brush Washer (model 810–273) and loaded into a dome top fiberglass tank (model CM-CFV-5, Warner Fiberglass Products, Belding, MI) as previously described (Fleming et al., 1983). The tank (4500L total volume) was filled with 60% cucumbers and 40% brine, by volume. The cover brine was added to the tank first and consisted of 0.045M $Ca(OH)_2$, 0.8% acetic acid (added as vinegar) and 6.6% NaCl; the pH was 4.7. The brining procedure was generally that described earlier (Fleming et al., 1988) except for differences in salt concentration and addition of the *Lactobacillus plantarum* culture (isolate MOP7 from fermenting cucumbers) to the brine after addition of cucumbers. The *L. plantarum* culture was grown overnight in MRS broth and added to give about $6 \times 10^5$ cells per mL brined cucumbers. The brined cucumbers were purged with nitrogen at the rate of 15 SCFH (standard cubic feet per hour) during the fermentation; thereafter purging was discontinued and the tank headspace held under a slight nitrogen pressure for the storage period (Fleming et al., 1983).

Chemical analyses of raw cucumbers and of fermentation brines were determined by HPLC as previously described for sugars, organic acids and ethanol (McFeeters et al., 1984). To confirm the identity of presumptive peaks of butyric acid, propionic acid and n-propanol samples were also chromatographed on a Bio-Rad HPX-87H cation exchange column with 0.01N $H_2SO_4$ as the eluent and both refractive index detection with a Waters model 401 detector and UV detection at 210 nm with a Varian Varichrom UV detector. In addition, samples were chromatographed using a Hewlett Packard 5890 GLC equipped with a 1 meter GP 60/80 Carbopack C/0.3% Carbowax 20M/0.1% $H_3PO_4$ column (Supelco no. 1-1825) and an FID detector in order to confirm the identity of these compounds by gas chromatography. Brine samples were injected directly onto the column without derivatization. Helium at a flow rate of 50 mL/min was used as the carrier gas. The column temperature was 120°C. Detector and injector temperatures were 200°C. Titratable acidity, pH, reducing sugar, and salt concentration were determined as described earlier (Fleming et al., 1984). Fermentation balances were calculated by general procedures described by Wood (1961).

Brine samples of spoiled pickles were taken in screw capped tubes which were kept anaerobically (GasPak jar, BBL Microbiology Systems, Cockeysville, MD) overnight at room temperature. The brines were viewed by phase contrast microscopy (500X) for characterization of microbial cell morphology. Samples of brine were heated at 80°C for 15 min. The samples were then streaked onto anaerobic egg agar (Kautter and Lynt, 1978) and incubated anaerobically for 3 days at 30°C. Representative colonies were picked into tubes of thioglycollate broth. The tubes were incubated anaerobically at 30°C for 7 days. Six isolates thus obtained were subjected to identification procedures as described by Holdeman et al. (1977). The procedure of Cato et al. (1982) was followed for determination of the electrophoretic pattern of cell proteins to assist with identification of *Clostridium* isolates.

A sample of brine from the spoiled pickles was sent to the CDC Botulism Laboratory (Atlanta, GA) for determination of the presence of botulinal toxin. The product was then acidified to inhibit further

fermentation and to reduce a potential safety hazard. Concentrated $H_2SO_4$ (13L of 36N) was slowly added to the tank over a 1 hr period through a funnel in the top of the tank. The brine was circulated by nitrogen purging. The pH was 1.6 after acidification. Sodium benzoate (24L of 33%) was added to further stabilize the product. After determination that the product did not contain botulinal toxin, the tank contents were removed and properly discarded.

## RESULTS

THE CUCUMBERS were brined on September 20 and underwent a typical primary fermentation which resulted in 1.09% titratable acidity (as lactic acid), pH 3.7 and no fermentable sugar after 30 days (Table 1). The NaCl concentration equalized at 2.3%. The product was determined to be of excellent quality after fermentation (late October) in terms of firmness, absence of bloater damage, flavor, and general appearance. The brined cucumbers were stored over the winter months in the tank in which they were fermented with no observed changes. On June 3 of the following year (256 days after brining), a sample of cucumbers and brine was removed from the tank for quality evaluation and experimental product formulation and was still of excellent quality. No samples for chemical analyses were taken at this time. The tank was returned to the anaerobic storage mode by flushing the headspace with nitrogen and maintaining a nitrogen blanket under a slight positive pressure. On the following July 10 (293 days after brining), the fermented cucumbers were observed to be spoiled, as evidenced by a strong butyric acid odor and frothing and overflow of the brine. The fermented cucumbers remained firm and intact. A secondary fermentation apparently had occurred as the temperature of the tank contents increased during the summer. At this time, the acidity was only 0.59%, and the pH was 4.8 (Table 1). A sample of the brine was determined not to contain botulinal toxin by the CDC Botulism Laboratory (Atlanta, GA).

HPLC analysis of the brine revealed that none of the lactic acid formed during primary fermentation was present in the spoiled pickles and that acetic acid, butyric acid, propionic acid, and n-propanol had been formed. Butyric acid, propionic acid, and n-propanol were identified by their retention times on HPLC and GLC compared to standard compounds. Retention times matched the standards on a Bio-Rad HPX-87H cation exchange column and on a $C_{18}$ reversed phase column using HPLC. On the cation exchange column, the peaks presumptively identified as butyric and propionic acids by refractive index detection also gave peaks with a UV detector at 210 nm, as would be expected for carboxylic acids. Also as expected, the presumptive n-propanol peak gave no response on the UV detector. GLC of the brine samples also showed peaks with the same retention times as standard compounds. The composition of the raw cucumber/cover brine mixture and the brine after primary fermentation (30 days after brining) and after spoilage (293 days after brining) is summarized in Table 2. Carbon recovery after primary fermentation was 109.8%, which is consistent with previously reported carbon recovery of cucumbers fermented by this method (Fleming et al., 1988). The hydrogen/oxygen ratio of substrates before fermentation

*Table 1—Brine chemistry of the cucumber fermentation*[a]

| Days after brining | pH | Acid (%) | NaCl (%) | Sugar (%) |
|---|---|---|---|---|
| **Primary fermentation** | | | | |
| 0 | 4.73 | 0.35 | 5.5 | 0.0 |
| 1 | 4.38 | 0.37 | 2.5 | 0.4 |
| 2 | 3.72 | 0.48 | 2.3 | 0.1 |
| 3 | 3.67 | 0.88 | 2.3 | 0.0 |
| 5 | 3.67 | 1.10 | 2.4 | 0.0 |
| 8 | 3.62 | 1.11 | 2.3 | 0.0 |
| 13 | 3.59 | 1.12 | 2.3 | 0.0 |
| 30 | 3.68 | 1.09 | 2.4 | 0.0 |
| **After spoilage** | | | | |
| 293 | 4.80 | 0.59 | 2.3 | 0.0 |

[a] Concentrations are expressed as %, w/v.

*Table 2—Substrates and products of the cucumber fermentation*

| Compound | Before fermentation | After primary fermentation | After spoilage |
|---|---|---|---|
| | **Concentration of compounds, mM** | | |
| Glucose | 27.1 | ND | ND |
| Fructose | 34.3 | ND | ND |
| Malic acid | 12.6 | ND | ND |
| Acetic acid | 53.2 (0.0)[a] | 63.7 (12.5)[a] | 105.3 (39.6)[a] |
| Lactic acid | ND[b] | 140.1 | ND |
| Ethanol | ND | 7.3 | 1.7 |
| Propionic acid | ND | ND | 8.1 |
| Propanol | ND | ND | 34.5 |
| Butyric acid | ND | ND | 38.7 |
| | **Elemental recoveries, %** | | |
| Carbon | | 109.8[c] | 79.4[d] |
| Hydrogen (H) | | 115.0[c] | 85.9[d] |
| Oxygen (O) | | 104.9[c] | 46.2[d] |
| | **Hydrogen/oxygen ratio of compounds, mM** | | |
| | 1.88 | 2.06 | 3.84 |

[a] Values in parentheses are those used in calculations of elemental recoveries and reflect the assumption that acetic acid was not a substrate for products measured.
[b] ND = none detected.
[c] Recoveries based on composition of compounds before and after fermentation.
[d] Recoveries based on composition of compounds after primary fermentation and after spoilage.

*Table 3—Effect of pH on fermentation of spent cucumber brine by* Clostridium tertium[a]

| Adjusted pH | Visual growth | Brine components after incubation, mM: Lactic acid | Acetic acid | Butyric acid |
|---|---|---|---|---|
| 3.7 (control) | – | 121.8 | 56.1 | ND |
| 3.7 | – | 123.8 | 58.0 | ND |
| 4.1 | – | 120.1 | 55.6 | ND |
| 4.8 | ± | 106.9 | 50.1 | ND |
| 6.0[b] | + + | ND[d] | 6.5 | 86.8 |
| 7.0[c] | + + | 1.5 | 7.4 | 99.7 |

[a] Cucumber brine after primary fermentation was filter-sterilized, pH adjusted and inoculated with *C. tertium* isolates. Observations were made after incubation at 30°C for 6 days.
[b] Carbon recovery, 74.0%, based on pH 3.7 control (uninoculated).
[c] Carbon recovery, 86.1%, based on pH 3.7 control (uninoculated).
[d] ND = none detected.

was 1.88, and this ratio increased slightly to 2.06 for primary fermentation products (Table 2). Carbon recovery after spoilage was only 79.4% (based on products formed during primary fermentation serving as substrates for the spoilage fermentation), and the hydrogen/oxygen ratio of products formed was 3.84.

Phase contrast microscopy of the brine revealed the presence of rod-shaped bacteria, some of which were swollen with a terminal, refractile spore. Six anaerobes isolated from the brine were all identified as *Clostridium tertium*, based on identification procedures as described in the Anaerobe Laboratory Manual (Holdeman et al., 1977). The isolates grew both aerobically and anaerobically in PYG broth; however, spores were formed only under anaerobic conditions. The isolates formed large amounts of gas, were Gram-positive and nontoxic by i.p. injection of mice. The isolates produced acids in PYG broth in the following decreasing order of concentration: acetic, butyric, lactic, formic, and succinic. The electrophoretic pattern of proteins (Cato et al., 1982) of cells grown in BHI broth plus Tween and $NaHCO_3$ was similar for all isolates (data not shown). This electrophoretic pattern, when compared to previously described reference strains of *Clostridium* species (Cato et al., 1982), was most similar to *C. tertium*.

Effect of pH on growth and acid metabolism of isolated clostridia (a combination of 8 isolates) in spent cucumber brine is given in Table 3. The spent cucumber brine was taken from the tank before the spoilage occurred. No growth of the isolates or changes in acid composition occurred at pH 3.7 or 4.1 during anaerobic incubation at 30°C for 6 days. At pH 4.8,

slight growth and a decrease in lactic and acetic acids were observed. No butyric acid was detected, although there were slight reductions in concentration of lactic and acetic acids. At pH 6 and 7, heavy growth occurred. Also, lactic and acetic acids were nearly depleted, and butyric acid had been formed. Carbon recovery as butyric acid, based on lactic and acetic acids as substrates, was 74% at pH 6 and 86.1% at pH 7.

## DISCUSSION

REASONS for fermented cucumbers undergoing subsequent spoilage by butyric acid bacteria in the present case are unclear. However, two contributing factors may be the relatively low concentration of NaCl used (2.3%) and the small sized cucumbers to which soil readily adheres. The low NaCl concentration did not adversely affect the primary lactic acid fermentation, but apparently allowed growth of spoilage organisms after primary fermentation. Since clostridia were implicated in the chemical changes, the question of their origin is raised. Although the cucumbers were washed, it is likely that clostridia-bearing soil was present on the fruit.

The addition of acetic acid to the cover brine should be considered as possibly contributing to growth of clostridia in the spoiled pickles. Various species of clostridia produce butyric acid from lactic acid, but the presence of acetate was shown to be necessary for this conversion by *C. lacto-acetophilum* (Bhat and Barker, 1947) and by *C. tyrobutyricum* (Bryant and Burkey, 1956).

While the NaCl concentration was low for commercial cucumber fermentations, the concentration was typical for sauerkraut fermentation (2 to 3% NaCl; Pederson and Albury, 1969). Although butyric acid has been found in sauerkraut (Vorbeck et al., 1961), we are unaware of any instance where it was formed with the concomitant depletion of lactic acid, as in the present case.

Butyric acid formation in brined olives has been attributed to two distinct types of microbial action, both of which result in malodorous products. In one type, *C. butyricum* and a closely related group of four other *Clostridium* species produce butyric acid from sugars during the primary stage of fermentation (Gililand and Vaughn, 1943). From a total of 50 isolates that grew in 1% NaCl (in liver infusion agar), only one (*C. aceto-butylicum*) grew at 6% NaCl. These authors indicated that olive industry experience has shown that a concentration of 7 to 8% salt in olive brines is sufficient to prevent this type of spoilage. All isolates grew in tryptone glucose broth with sodium thioglycollate at pH 7.0, 17 grew at pH 4.5 and none grew at pH 4.3 (Gililand and Vaughn, 1943). The broth pH was adjusted with $Na_2HPO_4$ and citric acid.

In a second type of malodorous olive fermentation, "zapatera" spoilage results from decomposition of organic acids at a time when little or no sugar is present and the lactic acid fermentation stops before the pH has decreased below pH 4.5 (Kawatomari and Vaughn, 1956). Lactic and acetic acids constitute the acids in normal fermentations, but butyric, propionic, formic, valeric, caprylic, and caproic acids have been reported to occur in "zapatera" olives (Delmouzos et al., 1953). Propionic and butyric acids occurred most frequently. From a total of 270 *Clostridium* isolates from "zapatera" olives, 138 were identified as *C. sporogenes* and 77 as *C. bifermentans* (Kawatomari and Vaughn, 1956). Other identified species were *C. butyricum, C. multifermentans, C. beijerinckii, C. pasteurianum,* and *C. sphenoides*. Of 39 isolates tested for salt tolerance, all grew at 1% NaCl, but only 4 grew at 8% NaCl (all were *C. sporogenes*). Of 36 isolates tested for pH tolerance, all grew at pH 6.0 but only 1 grew at pH 4.5 (*C. pasteurianum*. Kawatomari and Vaughn (1956) stated the "zapatera" spoilage can be prevented based on olive industry experience, if the brine pH is 3.8 or below. Interestingly, however, none of the above isolates from olives produced propionic acid under the conditions tested (Plastourgos and Vaughn, 1957).

Plastourgos and Vaughn (1957) isolated 68 cultures of propionic acid bacteria from commercial olives with indications of "zapatera" spoilage. Of these isolates, 46 were indentified as *Propionibacterium zeal* and 22 as *P. pentosaceum*. All isolates grew at 7% salt in lactate medium. The minimum pH tolerated by the isolates was pH 4.8 to 5.2. The maximum pH for growth was 7.2 to 8.0. These authors conjectured that propionic acid bacteria appeared first in the sequence of microorganisms responsible for the development of "zapatera," caused a rise in pH and thus permitted *Clostridium* species to grow. They cautioned against complete acceptance of this hypothesis, however, since Kawatomari and Vaughn (1956) had previously shown that many species of *Clostridium* are as tolerant to low pH as the two species of *Propionibacterium* tested.

More recently, Borbolla y Alcala et al. (1975) and Rejano Navarro et al. (1978) reported the formation of propionic acid in Sevillian olives and suggested that acidity, salt concentration and pH can be controlled to prevent its formation. Gonzalez Cancho et al. (1980) identified 26 isolates of propionic acid-producing bacteria from Spanish-style green olives as belonging to the species *Propionibacterium acnes*. At pH 7.0, 11% NaCl was required to prevent growth of these isolates; at pH 5.1, 9% NaCl was required; and at pH 3.5, no NaCl was required. Borbolla y Alcala and Rejano Navarro (1981) characterized the fermentation of Sevillian olives into four stages, with the formation of acetic and propionic acids occurring at the expense of lactic acid in the fourth phase. They suggested that the resultant rise from this conversion could encourage the spoilage problem, "zapatera." They concluded that this fourth phase can be prevented by sufficiently high salt concentration and sufficiently low pH.

In an attempt to reconstruct the sequence of microbial changes that resulted in the spoilage of fermented cucumbers, we propose the following: The cucumbers underwent a normal lactic acid fermentation with no evidence of acids other than acetic (including that added) and lactic being present after primary fermentation. Upon subsequent storage, the lactic acid was slowly degraded to propionic and acetic acids (by unidentified bacteria, possibly propionic acid bacteria) with resultant rise in pH. This rise in pH eventually allowed growth of *C. tertium* (and perhaps other clostridia).

We do not believe that clostridia directly converted lactic acid to butyric acid for two reasons. First, the isolated *C. tertium* did not grow or produce butyric acid in the spent cucumber brine at pH 4.1 and below, whereas, the pickles were originally pH 3.7. Secondly, *C. tertium* did not produce propionic and acetic acids in the spent brine when allowed to ferment at pH 4.8 and above, whereas, the spoiled pickles contained large amounts of each acid. Plastourgos and Vaughn (1957) also found that *Clostridium* isolates from "zapatera" olives did not produce propionic acid. They concluded that an intermediate propionic acid fermentation occurred. Propanol also was present in the spoiled pickles, but we did not determine whether its formation occurred during propionic acid fermentation or butyric acid fermentation.

We have been unable to duplicate the spoilage problem. The isolated *C. tertium* does not grow in the spent cucumber brine without pH adjustment. Currently, we are attempting to isolate and characterize propionic acid bacteria from fermented pickles held under low salt conditions.

Limiting conditions for microbial stability of fermented cucumbers during anaerobic storage are yet to be established. It is likely that a combination of factors are involved including salt concentration, pH, acidity, and perhaps others. We have not previously observed butyric acid spoilage of larger sized cucumbers (3.5 to 5.1 cm) that were fermented and stored at 2.7 to 4.4% salt by the procedure described herein. Our observations represent more than 20 pilot-scale and 14 commer-

cial-scale fermentations over the past 5 years. However, we have observed a rise in pH accompanied by formation of propionic acid and n-propanol during storage under laboratory conditions of a few samples from the tanks.

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