CARBON DIOXIDE PRODUCTION IN THE FERMENTATION OF BRINED CUCUMBERS

INTRODUCTION

BLOATER DAMAGE (hollow stock) in brined cucumbers has been attributed to the production of gases, particularly CO_2, in the fermentation brine (Etchells et al., 1968). Microorganisms that have been associated with gaseous fermentations of commercially brined cucumbers include yeasts (Etchells and Bell, 1950; Etchells et al., 1952; 1953), coliform bacteria of the genus *Aerobacter* (Etchells et al., 1945) and heterofermentative lactic acid bacteria (Etchells et al., 1968).

Previous work in this laboratory with pure and mixed cultures of lactic acid bacteria demonstrated that microbial control in cucumber fermentations can be achieved (Etchells et al., 1964). Recent studies have shown, however, that bloater development can occur, even when cucumbers are fermented by pure cultures of *Lactobacillus plantarum* (Fleming et al., 1973). *L. plantarum*, a homofermentative lactic acid bacterium, is referred to as a non-gas producer, although it is known to produce small amounts of CO_2 (Pederson, 1929). It has not, heretofore, been considered a contributor to bloater damage. It appeared that if CO_2 was responsible for bloater damage, it originated from either activity of *L. plantarum* or the cucumber tissue, or both.

The present work was undertaken to study the contributions of selected homofermentative lactic acid bacteria and the cucumber to the amount of CO_2 produced in brine fermentations.

MATERIALS & METHODS

SOURCES of the cucumbers used in this study were given previously (Fleming et al., 1973), as were the cultures of lactic acid bacteria and preparation of inocula (Etchells et al., 1964; Fleming and Etchells, 1967).

Brining of cucumbers

For fermentation by the natural microflora, cucumbers were packed in 1-gal jars. Brines containing various concentrations of NaCl were poured into the jars together with 3.5 ml of glacial acetic acid. The acid was added to retard growth of coliform bacteria (Etchells et al., 1964) and to favor fermentation by naturally-occurring lactic acid bacteria. The jars were closed with a cap fitted with a 250 ml dispensing burette which allowed the brine level to rise as the fermentation progressed. This system prevented a build-up of pressure in the jar and restricted loss of CO_2 from the brine (Fleming et al., 1973). Incubation was at room temperature (23°C ± 1°).

For fermentation by inoculation with pure cultures of bacteria, cucumbers were handwashed and packed in 1-qt jars. The concentrations of NaCl, sodium acetate and acetic acid in the cover brine were adjusted so that, after equilibration with the cucumbers, the brine was pH 4.7 (±0.2) and contained 6.7% NaCl and 0.5% sodium acetate (Fleming et al., 1973). The final pack-out ratio was 1.3:1, grams cucumbers:ml brine. The jars were capped with "Twist-off" closures (White Cap Co., Chicago, Ill.), which gave a hermetic seal. The headspace volume was 40 ml. A 14-mm diameter rubber serum stopper was placed in each cap for the purpose of sampling (Etchells et al., 1964). Appropriate jars were pasteurized immediately after capping by submerging in a hot water bath as described by Etchells and Jones (1944). After allowing a 24 hr equilibration period at room temperature (23°C ±1°), the jars were inoculated through the sampling stopper with 2 ml of a broth culture of the desired species of bacteria and then incubated at 26.7°C for 3 wk.

Sampling

Headspace gas samples and brine samples when under pressure were taken with a gas-tight needle (22 gauge) and syringe assembly (Hamilton Co.). Otherwise, 10-ml brine samples were taken with 12-ml disposable plastic syringes. Headspace pressures were taken with a pressure gauge equipped with a needle for insertion through the rubber stopper in the jar caps (Hamilton Co.). The jars were equilibrated to 24°C prior to sampling for headspace pressure and gas.

Analyses

Analytical methods for determining titratable acidity, pH, percent NaCl, percent reducing sugar and dissolved CO_2 were described or referred to previously (Fleming et al., 1973).

Sugars in aqueous extracts of fresh cucumbers were identified by thin layer chromatography on cellulose powder MN 300 (Brinkmann Instruments). The plates were developed in a solvent system of formic acid:butanone:t-butyl alcohol:water, 15:30:40:15. Reducing sugars were detected by spraying the plates with aniline phthalate (Stahl, 1969; reagent no. 10). Nonreducing sugars were detected by spraying with silver nitrate-sodium hydroxide (Stahl, 1969; reagent no. 234).

Carbon dioxide in quart jars of cucumbers was determined at the completion of fermentation. An estimate of the total amount in the jars was made by assuming that the concentration of CO_2 dissolved in the brine surrounding the cucumbers was the same as that dissolved inside the tissue. Headspace CO_2 also was included in the total, and was determined by the same method used for dissolved CO_2. To test the validity of the above assumption, the total CO_2 in the jars also was determined by sweeping the entire CO_2 from the jar contents with nitrogen and trapping in standardized NaOH. The values obtained by the latter procedure and

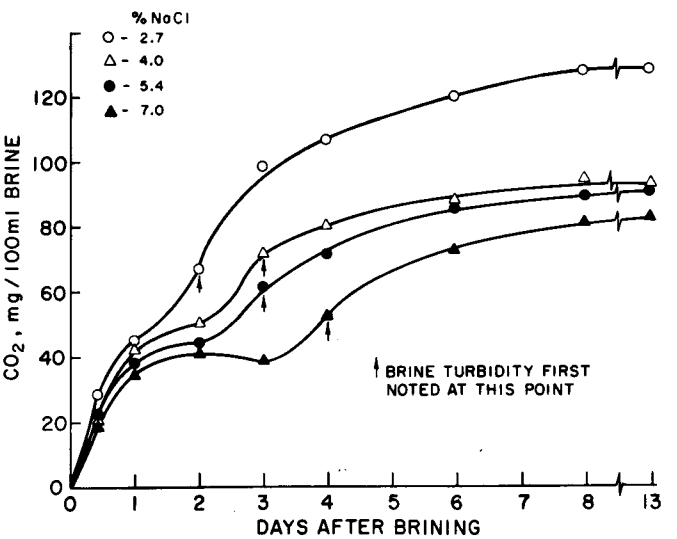

Fig. 1—CO_2 accumulation in cucumber brines undergoing fermentation by natural microflora at four concentrations of NaCl. See footnote "a" of Table 1 for details.

Table 1–Brine analyses of cucumbers undergoing fermentation by natural microflora at four concentrations of sodium chloride[a]

NaCl concentrations in the cover brine			Days after brining								
			2			13			36		
Initial (%)	After 2 days (%)	After equilibration (%)	pH	Acid (%)	Sugar (%)	pH	Acid (%)	Sugar (%)	pH	Acid (%)	Sugar (%)
5.5	2.9	2.7	4.39	0.20	0.37	3.31	0.99	0.00	3.26	1.00	0.00
8.4	4.3	4.0	4.20	0.18	0.38	3.15	1.02	0.00	3.12	1.02	0.00
11.4	5.8	5.4	4.40	0.16[b]	0.38	3.19	0.90	0.07	3.13	1.00	0.00
14.5	7.2	7.0	4.36	0.16[b]	0.42	3.21	0.78	0.18	3.17	0.85	0.12

[a]SMR variety cucumbers, 1-1/16 to 1-1/2 in. diameter, were used. The pack-out ratio was 1g cucumber:0.9 ml brine. Values are averages of duplicate 1-gal jars; CO_2 concentrations are given in Figure 1. Standard deviations for replicate jars were: pH, 0.09; % acid, 0.01; % sugar, 0.05; % salt, 0.1.
[b]This amount of acid was due to the acetic acid added, 3.5 ml/gal, at the time of brining.

those obtained from calculations based on dissolved CO_2 in the cover brine as described above agreed within 6%.

RESULTS

Dissolved CO_2 in brines fermented by natural microflora

CO_2 concentration increased in the 7% NaCl w/v, brine (equilibrated concentration) up to 2 days, and did not increase further until the fourth day, when a slight visual turbidity due to microbial growth was noted (Fig. 1). The concentration of CO_2 after two days was about 40 mg/100 ml brine and was considered to represent the CO_2 that diffused from the cucumbers. Numerous studies since have consistently confirmed that about 30–40 mg of CO_2/100 ml brine is present after equilibration when fresh cucumbers are brined to equilibrate at 7% NaCl. This concentration of CO_2 has been found when the cucumbers were carefully washed and the brine chlorinated, as well as when the cucumbers were brined, unwashed, as in the present experiment.

When visual turbidity began in the 7% brine, there was an abrupt increase in dissolved CO_2 which subsequently reached a maximal concentration of 82 mg/100 ml (Fig. 1). Lower concentrations of NaCl resulted in higher concentrations of CO_2, but the relative proportions arising from the cucumber and from the bacteria were not as apparent since the plateau in CO_2 concentration prior to microbial growth was less prominent at lower brine strengths. No yeasts were detected by microscopic examination of the brines during the first 2 wk after brining.

Brine assays for samples taken at 2, 13 and 36 days after brining are given (Table 1). The data indicate that acid-forming bacteria were active during the fermentations as about 1% acid was present in the 2.7 and 4% brines after 13 days. The acid was lower in the 5.4 and 7% brines, and residual sugar was present, whereas, no sugar remained in the 2.7 and 4% brines. Sugar remained in the 7% brine after 36 days. The combination of salt and low pH (3.1–3.2) prevented further fermentation by the lactic acid bacteria.

Table 2–CO_2 produced by cucumbers and Lactobacillus plantarum WSO during brine fermentation[a]

Treatment of cucumbers	CO_2 in headspace gas (mg/100 ml)	CO_2 in brine (mg/100 ml)	CO_2, mg/100g cucumber		
			Total	Produced by L. plantarum	Produced by cucumbers
Pasteurized, not inoculated	22.0 (1.3)	15.7 (0.7)	30.1 (1.2)		
Pasteurized, inoculated	81.8 (1.8)	57.9 (1.5)	114.3 (3.4)	84	
Not heated, but chlorinated and inoculated[b]	125.4 (0.0)	90.2 (1.3)	175.4 (2.0)		91
Not heated, not chlorinated, but inoculated[b]	128.7 (1.9)	91.3 (1.9)	178.4 (2.9)		94
Contribution of sources of CO_2 to total, in percent[c]				48%	52%

[a]Model variety cucumbers, 1-1/16 to 1-1/2 inches diameter, were used. The pack-out ratio was 1.3g cucumber:1 ml brine. Data are based on averages of triplicate jars sampled after three weeks of fermentation. Standard errors of the means are given in parentheses.
[b]The cover brine contained 100 ppm available chlorine, added as calcium hypochlorite, before pouring over the cucumbers.
[c]See text for explanation of computations.

CO_2 in pure culture fermented cucumbers

Relative contributions of the cucumber tissue and the fermenting bacteria in the brine to the total amount of CO_2 produced in pure culture fermentations were studied. Four quart-jar lots of cucumbers (three jars per lot) were treated as indicated in Table 2 and examined after the completion of fermentation.

Headspace pressures of the jars, taken at 24°C just prior to sampling, were 5.5 psi for pasteurized, fermented cucumbers and 9 psi for unheated, fermented cucumbers. The pasteurized, uninoculated controls had a slight vacuum.

Jars of pasteurized, unfermented cucumbers contained 30 mg CO_2/100g cucumbers while the pasteurized, fermented product contained 114 mg CO_2/100g (Table 2). The difference, 84 mg CO_2/100g cucumbers, represented the CO_2 produced by *L. plantarum* WSO. Unheated, fermented cucumbers contained 175 mg CO_2/100g. Subtracting the level of CO_2 produced by the culture in the pasteurized, fermented cucumbers from this amount gave 91 mg CO_2/100g cucumbers, which represented the CO_2 contributed by the cucumber tissue.

CO_2 contributed by the cucumbers included that which was present in the fruit at the time of brining plus that formed by respiratory and/or fermentative activity of the tissue subsequent to brining. The initial level of CO_2 present in pastuerized cucumbers (Table 2) may not represent that which was present in the fresh cucumbers. The pasteurizing process may have caused a loss of CO_2 or the heating may have resulted in the formation of CO_2.

Table 3—Production of CO_2 by three cultures of lactic acid bacteria during the pure culture fermentation of pasteurized, brined cucumbers[a]

Bacteria	Brine analyses				CO_2 produced by bacteria per g sugar fermented[b] (mg)
	pH	Acid (%)	Residual sugar (%)	CO_2 (mg/100 ml)	
Uninoculated control	4.67 (0.01)	0.28 (0.00)	1.46 (0.01)	15.7 (0.7)	
L. plantarum WSO	3.39 (0.02)	1.23 (0.03)	0.12 (0.03)	57.9 (1.5)	34.6 (1.0)
L. plantarum 442	3.58 (0.03)	0.98 (0.03)	0.48 (0.05)	56.2 (0.8)	45.9 (2.1)
P. cerevisiae 39	3.91 (0.01)	0.70 (0.01)	0.75 (0.02)	63.8 (2.4)	75.2 (5.0)

[a]Standard errors of means are given in parentheses. See footnote "a" of Table 2 for other details.
[b]Values are based on total CO_2 in the jars which included dissolved CO_2 and that present in the 40 ml headspace. Amount of sugar fermented was calculated as the difference between residual sugar in inoculated and uninoculated controls.

Since the unheated cucumbers undoubtedly contained a small number of contaminating microorganisms, their contribution to the CO_2 produced in unheated cucumbers cannot be wholly disregarded, but CO_2 from this source was thought to be very small. Very little difference was noted in CO_2 concentration between the unheated cucumbers that received chlorination and those that did not (Table 2). Microscopic examination revealed a homogenous population of short rods characteristic of *L. plantarum* WSO. No yeasts were detected.

Overall, the fermentation of unheated cucumbers resulted in 48% of the CO_2 being derived from metabolic activity of *L. plantarum*WSO and 52% from the cucumber tissue.

Three cultures of homofermentative lactic acid bacteria were compared regarding amounts of CO_2 produced in pure culture fermentations of pasteurized cucumbers (Table 3). *L. plantarum* WSO and *L plantarum* 442 produced similar amounts of CO_2, while *P. cerevisiae* 39 produced slightly more. Expressed on the basis of mg of CO_2 produced per gram of sugar fermented, however, *P. cerevisiae* 39 produced over twice as much and *L. plantarum* 442 about 30% more CO_2 when compared to *L. plantarum* WSO. Nearly all of the sugar was fermented by *L. plantarum* WSO during the 3 wk of fermentation while 0.48 and 0.75% sugar remained in brines fermented by *L. plantarum* 442 and *P. cerevisiae* 39.

Since sugar content in the cucumbers was determined as reducing sugar, it was essential to establish the identity of the sugars present. Sucrose is fermentable by the lactic acid bacteria, but would not have been detected by the reducing sugar assay.

Thin layer chromatography of extracts from fresh cucumbers indicated that glucose and fructose constituted the major portion of sugars present. Only traces of other unidentified sugars were found. Sucrose was not detected. These findings are consistent with unpublished data collected by C. L. McCombs for numerous varieties of pickling cucumbers (personal communication).

DISCUSSION

THE CUCUMBER TISSUE and homofermentative lactic acid bacteria have not been seriously considered as sources of CO_2 in the brine of fermenting cucumbers. Present results show that the concentration of CO_2 resulting from these combined sources is as high as that reached when bloater damage has been shown to occur, 60—80 mg/100 ml brine (Fleming et al., 1973). While this level of CO_2 is undoubtedly low compared to fermentations characterized by yeasts, coliforms and heterofermentative lactic acid bacteria, a further reduction in the amount present would appear desirable.

There was variation among the homofermentative lactic acid bacteria regarding the amount of CO_2 produced. Strains of *L. plantarum* produced lower levels of CO_2 than did *P. cerevisiae*. Production of CO_2 by homofermentative lactic acid bacteria has not been studied extensively, but amounts reported herein appear to be consistent with those reported for *L. plantarum* (Pederson, 1929). Apparently small amounts of CO_2 are inevitable, although CO_2 is not an end product of the major metabolic pathway in *L. plantarum* (Wood, 1961).

Amounts of CO_2 in cucumbers prior to brining probably vary, depending on the physiological state of the fruit and the storage conditions prior to brining. Temperature and duration of storage have an important influence on the respiratory rate of cucumbers (Eaks and Morris, 1956). The respiratory activity of cucumbers submerged in brine has not been reported. Such activity probably would be limited by the amount of oxygen present and the acid and NaCl which diffuse into the tissue from the surrounding brine. Therefore, prebrining as well as brining treatments undoubtedly influence the amount of CO_2 originating from cucumbers after they are brined.

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