

BLOATER FORMATION IN BRINED CUCUMBERS FERMENTED BY *Lactobacillus plantarum*

INTRODUCTION

BLOATER DAMAGE in brine-fermented cucumbers causes serious economic losses to the pickle industry. Thus, attempts to determine the causes of bloating have been made by numerous investigators over the last several decades.

Structural characteristics of cucumbers have been suggested as an important factor relative to bloating susceptibility (Jones et al., 1941; Sneed and Bowers, 1970). It is commonly known that bloater damage is a more serious problem in larger sizes of cucumbers. Thus, structural changes in the fruit as it increases in size, especially increased skin thickness and enlargement and changes in texture of the seed cavity, have been thought to be associated with the occurrence of bloater damage. Piercing the fruit prior to brining is known to prevent bloaters (Etchells and Moore, 1971). Varietal differences in cucumbers (Jones et al., 1954) and growing conditions of the fruit (Wormley, 1939) also have been suggested as factors influencing bloater damage in the brined stock.

Brining conditions influence bloater damage in natural fermentations of cucumbers. Higher brine strengths during the active stage of the fermentation (Jones et al., 1941), the addition of lactic acid or sugar to the fermenting brine (Jones et al., 1940) and fermentations at higher temperatures (Samish et al., 1957), were shown to cause more bloater damage.

Numerous reports have shown a marked relationship between the occurrence of a gaseous fermentation, caused by yeasts and gas-forming bacteria, and the incidence of bloater damage (Jones et al., 1941; Etchells et al., 1952, 1945, 1953, 1968; Etchells and Bell, 1950).

A mechanism for bloater development was proposed by Etchells et al. (1968). They suggested that the fermentation gas, which is produced solely in the cover brine, diffuses into the cucumber in the dissolved state, is released from solution inside the fruit and accumulates in pockets at locations of structural weakness. This theory is consistent with the earlier finding that gas trapped inside bloated cucumbers was essentially of the same

composition as that which evolved from the brine (Veldhuis and Etchells, 1939; Etchells and Jones, 1941).

It was shown recently that both the cucumbers and the bacteria in the fermenting brine contribute to the amount of CO₂ that accumulates in the brine (Fleming et al., 1973). CO₂ diffused from the cucumbers into the brine prior to the onset of bacterial fermentation. The concentration of CO₂ increased further with the onset of bacterial growth in the brine.

No work relating the concentration of dissolved CO₂ in the brine to bloating of cucumbers has been published. Quantitative data on CO₂ production has been based on the CO₂ that evolved from the fermentation (e.g., Veldhuis and Etchells, 1939; Etchells et al., 1945).

In the present work, relationships between brine concentrations of dissolved CO₂ and bloater damage of the fermented cucumbers were studied. Effects of physical treatments of the cucumbers prior to brining, cucumber size and incubation temperature on CO₂ concentrations in the brine, and bloater development, were of particular interest.

MATERIALS & METHODS

Cucumbers

Fresh cucumbers were obtained from near-by commercial pickling plants or from experimental plots of the Dept. of Horticultural Science at North Carolina State University. They were carefully selected for uniformity of size and shape and freedom from mold growth and mechanical damage.

Brining and description of the fermentation vessel

Cucumbers were hand-washed, weighed and packed in 1-gal jars. A semi-rigid plastic netting was placed over the cucumbers to prevent them from rising into the neck of the jar. A cover brine containing 14.5% NaCl, w/v, 0.8% sodium acetate (trihydrate), and sufficient glacial acetic acid to adjust the brine to pH 4.5 was added to the jars. After equilibration with the cucumbers, the brine was pH 4.7 (± 0.2) and contained 6.7% NaCl and 0.4% sodium acetate. The final pack-out ratio averaged about 50:50, grams cucumber:ml brine. The number of cucumbers of a given size packed per jar was held as nearly constant as possible. For size no. 3, 13 cucumbers were packed and, for size nos. 2 and 1, about 35 and 70, respectively.

The jars were fitted with a "Twist-off" cap (White Cap Co., Chicago, Ill.) and assembly as shown in Figure 1. Several items were placed in the cap by means of rubber serum bottle stoppers. These stoppers were described previously (Etchells et al., 1964). One stopper was inserted for sampling. A graduated 250 ml, cylindrical dispensing burette, 37 mm diameter, (Fisher Scientific Co.), hereafter referred to as the expansion reservoir, was inserted through another stopper which had been bored. The orifice of the reservoir, which protruded through

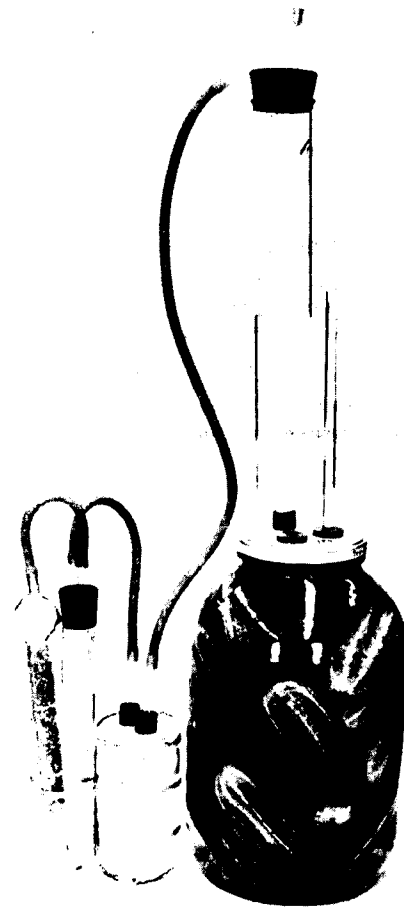


Fig. 1—Fermentation vessel with expansion reservoir and NaOH trap assembly. See text for details.

the stopper, had an internal diameter of 4 mm. This small opening allowed brine to rise in the reservoir during the fermentation, which minimized pressure build-up inside the jar. The small orifice greatly reduced surface exposure of the brine. An 8 mm diameter glass rod was fitted through another bored stopper and served as a plunger as well as support for the expansion reservoir.

When nitrogen purging of the jar contents was desired, a gas-dispersion tube was positioned through a fourth stopper in the jar cap so that the fritted portion rested near the bottom of the jar. Nitrogen was introduced by connection to the portion of the dispersion tube that protruded through the jar cap.

To trap evolved CO_2 , the reservoir was connected to a trap containing standardized NaOH. The NaOH trap was protected from atmospheric CO_2 by means of a second NaOH trap and a tube of Ascarite. For most studies no attempt was made to trap evolved CO_2 , and the reservoirs were loosely covered with a lid, which allowed any evolved gas to escape to the atmosphere.

The jar caps were heated to about 80°C to soften the sealing liner, and the caps with assemblies attached were then screwed onto the jars. It was convenient to first position the jar caps and then the expansion reservoirs. After this, sufficient cover brine was added to reach a level of 50 ml in the reservoir.

Inoculation

The brines and cucumbers were held at 24°C for 1 or 2 days to allow salt to diffuse in and fermentable nutrients to diffuse out of the cucumbers. Then, 5 ml of a culture of *L. plantarum* WSO which had been grown for 16 hr at 30°C in cucumber juice broth (Fleming and Etchells, 1967) was introduced by syringe through the sampling stopper.

Sampling and measurement of "expansion volume"

Brine samples were taken from the center depth of the jar by inserting a long needle through a sample port in the cap and along the inside of the jar. Duplicate 10 ml samples for CO_2 analysis and one 10 ml sample for tur-

bidity and chemical analyses were taken with disposable 12-ml syringes. Trapped gas surrounding the cucumbers was freed by gentle movement of the plunger against the plastic netting placed on the surface of the cucumbers in packing. The cucumbers moved freely and any gas pockets external to the cucumbers rose to the top of the jar and out through the expansion reservoir. Small amounts of gas that remained under the cap were expelled by pressing on the jar cap several times. Then, the brine level in the graduated portion of the expansion reservoir was read. After correcting for the removal of brine due to sampling, net increases in volume were calculated and expressed as the "expansion volume," in milliliters. This rise in brine level that occurred during the bloating stage was attributed to gaseous expansion inside the cucumbers as proposed by Etchells et al., 1968. The volume of bloated cucumbers occupied as trapped gas, in percent, was estimated by dividing the volume which the cucumbers occupied at the time of brining by the expansion volume and multiplication by 100.

Evaluation for bloater damage

Brined cucumbers were cut longitudinally and examined for balloon-, honeycomb-, and lens-type bloaters as described and illustrated earlier (Jones et al., 1941; Etchells et al., 1968).

Subjective evaluations of bloater damage were on the basis of two criteria: (1) The percentage of cucumbers showing each of the three types of bloater defects was determined. When a cucumber had more than one type of defect, the balloon-type bloater was given priority in category placement. Lens and honeycomb categories gained priority over each other depending on the severity of each. (2) The degree or severity of damage was rated as none, slight, moderate or advanced.

Analyses

Analytical methods for determining percent titratable acidity (calculated as lactic acid), pH and percent NaCl in brines were described earlier (Etchells et al., 1964). Total reducing sugar was determined by the method of Sumner and Somers (1944), using glucose as the colorimetric standard.

Determination of CO_2 in brines

Dissolved CO_2 in brines was determined initially by the volumetric method of the AOAC (1965), contained in sections 11.053 through 11.055. Later, an application of the microdiffusion principle (Conway, 1957) was developed, which greatly increased the number of samples that could be analyzed. A 22-ml vial containing 5 ml of standardized NaOH (0.5N) was placed in an 8-oz jar. 10 ml of acid phosphate solution, as described in the AOAC method above, was pipetted into the bottom of the jar. The jar was closed with a "Twist-off" cap. The cap was fitted with a rubber serum stopper, through which 10 ml of brine was introduced by a syringe. The sample was injected so as to enter into the acid solution, care being taken to avoid spillage into the vial of NaOH. After holding the jars at 37°C for 24 hr, the vial was removed, 5 ml of 0.5M BaCl_2 added, and the solution titrated to the phenolphthalein endpoint with standardized 0.5N HCl. The concentration of CO_2 was calculated from the equivalents of base neutralized as described in the AOAC method.

RESULTS

CHEMICAL and volume changes that occurred during a typical fermentation of cucumbers by *L. plantarum* WSO are illustrated in Figure 2. Initial values indicate the conditions after a 2-day equilibration at 24°C . The brine was clear at this time, giving no visual evidence of microbial activity. Chemical and expansion volume changes were dramatic during the first 3 days after inoculation. "Expansion volume" increases were almost linear during this period. After this, the volume increased more gradually over the next several days. The CO_2 concentration increased from the value at equilibration, 39 mg/100 ml brine, to 74 mg/100 ml after 23 days' incubation, for a net increase of 35 mg/100 ml during the fermentation period. Measurement of evolved CO_2 during the 2–3 wk duration of the fermentations indicated that over 90% of the CO_2 produced in the brine was retained.

CO_2 accumulation and bloater development

A series of 1-gal jars of brined cucumbers, with expansion reservoirs attached, was prepared to study the time after brining of bloater development. The incubation temperature was 26.7°C . At specified times, duplicate jars were removed, expansion volumes determined, the brines analyzed and the cucumbers cut for examination of bloater damage (Table 1).

No bloating was evident in the cucumbers prior to inoculating the brine. After 2 days' fermentation, bloater damage was present in all of the cucumbers, 28% being balloon and 72% being of the honeycomb-type. The overall degree of damage for both types of bloaters was rated as only slight. The trapped gas volume inside the cucumbers amounted to 5.6% of the initial volume of the cucum-

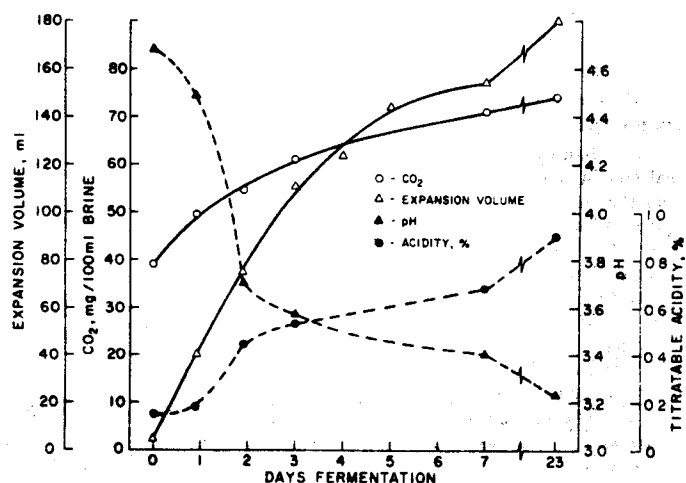


Fig. 2—Chemical changes and expansion volume increases during the fermentation of Explorer variety, size no. 3 cucumbers by *L. plantarum* WSO. Incubation was at 26.7°C .

Table 1—CO₂ accumulation and bloater development at intervals during the fermentation of cucumbers^a

| Fermentation time (Days) | Brine analyses | | | | Expansion volume (ml) | Trapped gas volume inside cucumbers (%) | Bloaters found ^b | |
|--------------------------|----------------|----------|-----------|-----------------------------|-----------------------|---|-----------------------------|---------------|
| | pH | Acid (%) | Sugar (%) | CO ₂ (mg/100 ml) | | | Balloon (%) | Honeycomb (%) |
| 0 | 4.78 | 0.20 | 0.56 | 31 | 25 | 1.7 | 0 | 0 |
| 2 | 3.79 | 0.62 | 0.29 | 61 | 117 | 5.6 | 28 (S) | 72 (S) |
| 4 | 3.54 | 0.86 | 0.19 | 74 | 156 | 7.5 | 32 (S-M) | 68 (S-M) |
| 6 | 3.61 | 0.78 | 0.09 | 71 | 180 | 8.6 | 22 (S-M) | 78 (S-M) |
| 13 | 3.38 | 0.99 | 0.06 | 79 | 245 | 11.6 | 28 (A) | 72 (M) |
| 21 | 3.46 | 0.96 | 0.04 | 78 | 216 | 10.0 | 21 (M-A) | 72 (M-A) |

^aSize no. 3 Model variety cucumbers were fermented at 26.7°C. Duplicate gallon jars were analyzed at the times indicated.

^bValues in parentheses indicate the severity of bloating: S, slight; M, moderate; A, advanced. When two letters are shown, the first indicates the degree of bloating in the majority of the cucumbers.

bers. As the fermentation proceeded to its latter stages, the trapped gas volume amounted to over 10%, and the subjective rating of bloater damage was moderate to advanced. No lens-type bloaters were found in this experiment. CO₂ increased in the brines from 31 mg/100 ml prior to inoculation to 79 mg/100 ml after 13 days' fermentation.

In another experiment, size nos. 1, 2 and 3 cucumbers were brined in separate jars and fermented at 26.7°C. No evidence of bloating was found for size no. 1 cucumbers, whereas, size nos. 2 and 3 were severely bloated after 3 wk of fermentation (Table 2). The CO₂ concentration in the brine of size no. 1 cucumbers was slightly lower at the time of inoculation, but the brines of all three sizes contained 78–80 mg CO₂/100 ml brine after fermentation for 3 wk. Fermentations of the three sizes of cucumbers proceeded at similar rates. The final percent titratable acidity was slightly lower in the brine of size no. 1 cucumbers (0.77%, pH 3.5) than in sizes 2 and 3 (0.88%, pH 3.3). The relationships between CO₂ concentration in the brine and expansion volume, determined at intervals during the fermentation, for the three sizes of cucumbers are illustrated in Figure 3. Although the concentrations of CO₂ in brines during the fermentation were similar for all sizes, the expansion volumes were greatest for the size no. 3 cucumbers at given concentrations of CO₂. Size no. 2 cucumbers had greater expansion volumes than did the no. 1 size.

Size no. 3 cucumbers were fermented at three different temperatures. Bloating occurred in 92% of the cucumbers fermented at 32.2°C, and balloon and honeycomb bloating had reached advanced stages after 3 wk (Table 2). The percentage of cucumbers affected was about the same at 26.7°C, but the severity of those bloated was not as great. At 21.1°C, no balloon bloating was evident and only 11% of the cucumbers had

honeycomb defects. A plot of the relationship between CO₂ concentration in the brine and expansion volume for the fermentations at the three temperatures is given in Figure 4. Greater expansion volumes resulted at higher incubation temperatures for any given concentration of CO₂ in the brine. Although the fermentation rate was higher at the higher incubation temperatures, there were no appreciable differences in pH or titratable acidity after 3 wk.

Effects of physical treatments on bloater formation

Size no. 3 cucumbers were treated in various ways prior to brining. One lot was pierced on two sides to a depth of 1 in. with a bed of 20 gauge needles, 9 needles per square inch. A 1-mm thick layer of peel was removed from the entire surface of a second lot. A third lot was brined without any prebrining treatment. Fritted gas dispersion tubes were positioned in these jars so that gas would diffuse out into the brine near the bottom of the jars. After equilibration and inoculation of the

brines, nitrogen gas was introduced continuously during the fermentation at a rate of about 5 ml per minute. The fourth lot of cucumbers served as a control and received the normal brining treatments.

Piercing of cucumbers eliminated bloaters. The CO₂ concentration was similar to that of the control at equilibration (36 mg/100 ml brine) and at the end of the fermentation (74 mg/100 ml brine). Only slight honeycomb bloating was evident in the peeled cucumbers. The CO₂ concentration was about 15 mg/100 ml less at equilibration and at the end of the fermentation when compared to the control.

Nitrogen-purged cucumbers also were free of bloaters. Upon introducing nitrogen, the CO₂ concentration decreased below the equilibration level and was less than 10 mg/100 ml brine after 1 day. It never increased above this concentration. The expansion volume did not increase greatly in any of the treatments, which was consistent with the absence of bloaters. None of the three treatments appreciably influenced acid development.

Table 2—Effects of cucumber size and incubation temperature on bloater damage of fermented cucumbers^a

| Cucumber size ^c | Incubation temperature °C | CO ₂ in the brine | | Bloaters found ^b | |
|----------------------------|---------------------------|------------------------------|-----------------|-----------------------------|---------------|
| | | At inoculation (mg/100 ml) | Maximum reached | Balloon (%) | Honeycomb (%) |
| 1 | 26.7 | 28 | 80 | 0 | 0 |
| 2 | 26.7 | 34 | 80 | 31 (M-A) | 38 (S) |
| 3 | 26.7 | 39 | 78 | 39 (M-A) | 54 (M-A) |
| 3 | 21.1 | 38 | 77 | 0 | 11 (S-M) |
| 3 | 32.2 | 40 | 68 | 31 (A) | 61 (M-A) |

^aExplorer variety cucumbers were used in this experiment. Examination for bloater damage was made after incubation for 3 wk.

^bValues in parentheses indicate the severity of bloating: S, slight; M, moderate; A, advanced. When two letters are shown, the first indicates the degree of bloating in the majority of the cucumbers.

^cSizes of cucumbers: No. 1, 3/4–1-1/16; No. 2, 1-1/16–1-1/2; No. 3, 1-1/2–2 in. diam.

In another experiment, one-gallon jars of brined cucumbers were purged for 2-hr periods at specified times during the fermentation at a nitrogen flow rate of 80 ml/min. Typical effects of purging intermittently during the fermentation are shown in Figure 5. The expansion volume and CO_2 concentration were greatly lowered after purging on the third day of fermentation. The concentration of CO_2 in the brine was slightly higher the next day as was the expansion volume, but both again were reduced by a second purging. Bloat damage was reduced, but not eliminated, when CO_2 was removed by periodic purging during the first 4 days of fermentation.

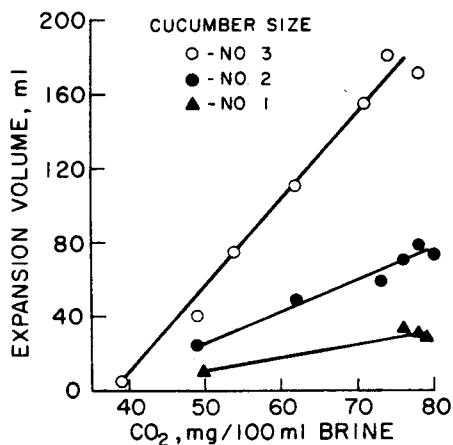


Fig. 3—Relationship between CO_2 concentration and expansion volume during the fermentation of three sizes of Explorer variety cucumbers. Incubation was at 26.7°C .

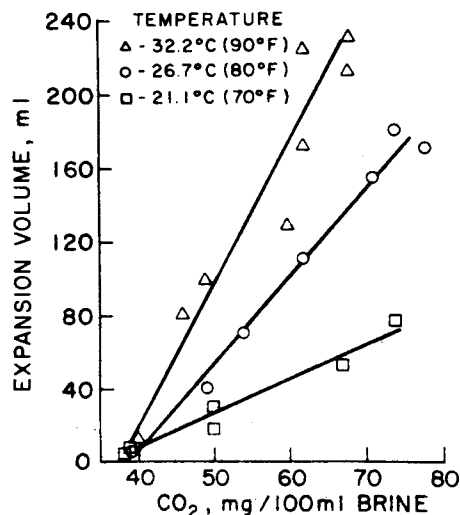


Fig. 4—Relationship between CO_2 concentration and expansion volume during the fermentation of size no. 3 Explorer variety cucumbers at three temperatures.

DISCUSSION

BRINE FERMENTATION of cucumbers with *L. plantarum*, a homofermentative lactic acid bacterium, may result in bloated stock as the foregoing results demonstrated. This finding was surprising as *L. plantarum* is considered to be a non-gas-former, in that it produces relatively little CO_2 in comparison with heterofermentative lactic acid bacteria. The amount of CO_2 which arose from the cucumbers plus that produced by *L. plantarum*, while undoubtedly low in comparison to "gaseous" fermentations caused by yeasts and other microbes that produce large amounts of CO_2 , was largely retained in the brine and was sufficient to cause bloating. High retention of CO_2 in the brine probably was due to the small exposure of the brine surface to the atmosphere as imposed by the fermentation assembly. Also, maximum concentrations of CO_2 reached were 70–80 mg/100 ml brine, which is less than that required for saturation at 7% NaCl and 26.7°C . Under these conditions, CO_2 solubility is about 108 mg/100 ml, based on data tabulated by Quinn and Jones (1936).

Factors influencing the solubility of CO_2 may be related to bloater development in cucumbers. The solubility of CO_2 is reduced at higher temperatures and NaCl concentrations (Quinn and Jones, 1936). Brine pH also is a factor affecting solubility as it regulates the proportion of dissolved CO_2 of the "total CO_2 content" which includes CO_2 , H_2CO_3 , HCO_3^- , and CO_3^{2-} .

Interestingly, visible bloater damage in size 3 cucumbers began early in the fer-

mentation, after only 2 days, when the concentration of CO_2 was about 60 mg/100 ml brine (Table 1). Although this concentration is considerably less than saturation, the possibility that CO_2 solubility inside the cucumber was different from that in the surrounding brine cannot be disregarded.

The rise and overflow of brine from commercial brining tanks during an active fermentation is common, but the phenomenon has not been fully understood. Briners have attributed such occurrences to a too rapid fermentation. It is clear now, however, that such a rise in the brine level is due to gaseous expansion inside the cucumbers which results in bloater formation (Etchells et al., 1968). During the bloating stage, liquid is expressed from the tissue as is obvious from the desiccated appearance inside the cucumbers upon cutting. Also, the cucumbers become distended due to the internal gas pressure. Upon removal of CO_2 from the brine by nitrogen purging, it was found that the cucumbers lost their distended appearance and actually collapsed when balloon bloating had occurred. The cucumbers were moist inside, but a permanent cavity was present due to the tissue having been pressed toward the skin, and the liquid expressed, when it was under pressure. The rise in brine level, herein termed expansion volume, was used in the present work as a quantitative measure of bloater development. Such measurements proved valuable in that they provided a nondestructive means of monitoring bloater formation during the entire fermentation.

A definite relationship between the expansion volume and the concentration

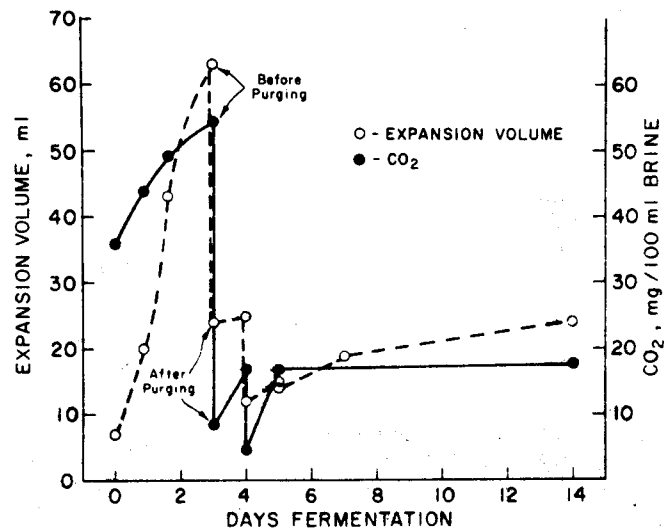


Fig. 5—Effect of nitrogen purging on reducing the CO_2 concentration and expansion volume of brined, Explorer variety cucumbers incubated at 26.7°C . The brine was purged for 2 hr on the third, fourth and fifth days of fermentation.

of CO₂ in the brine was obtained. Removal of CO₂ from the brine by sweeping with nitrogen during the fermentation of cucumbers prevented bloater development. This fact alone provided the most conclusive evidence that the concentration of CO₂ in the brine is related to bloater formation in cucumbers.

It would be desirable to establish the minimum or "critical" concentration of dissolved CO₂ in the fermenting brine which will cause bloating. However, it is clear that this "critical" concentration is variable and depends on the environmental conditions of the fermentation in addition to the variable characteristics of cucumbers.

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Ms received 7/13/72; revised 12/4/72; accepted 12/12/72.

Paper no. 3821, Journal Series, North Carolina State University Agricultural Experiment Station.

The authors express their appreciation to the Mount Olive Pickle Co., Mount Olive, N.C. and Perfect Packed Products Co., Inc., Henderson, N.C., for supplying cucumbers and other materials used in this work. We also thank Drs. C.H. Miller and R.L. Lower for supplying some of the cucumbers.

This investigation was supported in part by a research grant from Pickle Packers International (St. Charles, Illinois).

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