# Malic Acid Degradation and Brined Cucumber Bloating

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## ABSTRACT

Bloater formation of brined cucumbers increased as more malic acid was degraded to $CO_2$ and lactic acid. $CO_2$ production by the brined cucumber, unrelated to malic acid degradation, was 12.5 mM. This was just sufficient to bring cucumbers to the point of bloating. $CO_2$ from malic acid provided the marginal increase required to cause significant bloating. Fermentation with a strain of *Lactobacillus plantarum*, which did not degrade malic acid, prevented cucumber bloating. Oxygen exchange of cucumbers before brining increased the amount of $CO_2$ required to initiate bloating damage by 8 mM. Nonmalic acid-degrading starter cultures and/or oxygen exchange may be useful alternatives to $CO_2$ purging from brines to prevent bloater damage.

## INTRODUCTION

MALIC ACID degradation to lactic acid and $CO_2$ is the major reaction which results in $CO_2$ production in cucumber juice by lactic acid bacteria (McFeeters et al., 1982). However, when cucumber fruit are brined, a substantial amount of $CO_2$ is produced by the fruit in the absence of microbial fermentation (Fleming et al., 1973). Multiple sources of $CO_2$ evolution in cucumber fermentations may elevate $CO_2$ concentrations enough to cause bloater defects (hollow centers). The relative contribution of $CO_2$ from different sources is important in devising ways to minimize $CO_2$ production in fermentations. Since $CO_2$ production from malic acid is caused by the fermentation bacteria, it is of interest to find organisms which do not degrade malic acid to determine whether bloating could be prevented by eliminating that source of gas production.

The objectives of this study were: (1) to determine the relationship between malate decarboxylation and bloater formation during fermentation of brined cucumbers, and (2) to compare the effects on bloater damage of lactic acid bacteria that decarboxylate malic acid with those that do not. For this purpose, 19 strains of *Lactobacillus plantarum* and 5 strains of *Pediococcus cerevisiae* were surveyed in an attempt to obtain nonmalic acid-degrading lactic cultures.

## MATERIALS & METHODS

FOURTEEN STRAINS OF BACTERIA, designated as *Lactobacillus plantarum* with culture numbers 82, 340, 341, 343, 352, 354, 363, 963, 965, 1193, 1194, 1752, 1939, and 1988 were obtained from the National Institute for Research in Dairying (Reading, England). *Lactobacillus plantarum* YIT-0068 was obtained from Yakult Institute for Microbiological Research (Tokyo, Japan). *Pediococcus cerevisiae* 20 was provided by Dr. J.B. Evans, Dept. of Microbiology, North Carolina State Univ. (Raleigh, NC). *Pediococcus cerevisiae* 23 was from the late Dr. J.O. Mundt, Dept. of Microbiology, Univ. of Tennessee (Knoxville, TN). *Pediococcus cerevisiae* ABC was from A.B.C. Research Corporation (Gainesville, FL). *Lactobacillus plantarum* WSO, 15, 16, 442, and *P. cerevisiae* 61 were from the culture collection of this laboratory. *Lactobacillus cellobiosus* ATCC 11739, a heterofermentive species, was obtained from the USDA-ARS Northern Regional Research Center culture collection.

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A nutritive medium was designed to test for malic acid degradation in the bacteria listed above. The main factors considered for selection of an appropriate medium were that lactic acid bacteria would grow, the medium did not contain major sources of $CO_2$ other than malic acid, and it did not contain components that would interfere with HPLC determination of malic acid and lactic acid. One liter of medium contained 10g Difco peptone, 5g yeast extract, 2g $KH_2PO_4$, 0.2g $MgSO_4 \cdot 7\ H_2O$, 0.05g $MnSO_4 \cdot H_2O$, 10g glucose, and 6.6g L-malic acid. Each organism was grown in MRS medium (De Man et al., 1960) and then inoculated into the test medium. They were incubated at 30°C for 6 days, then fermentation products were analyzed.

Cucumber juice was prepared as described previously (McFeeters et al., 1982). NaCl (5% w/v) was added to the nondiluted juice. The juice was sterile-filtered into a Vacutainer tube through a Millipore 0.22 μm Millex filter. *Lactobacillus plantarum* strains WSO, 963, and 965 were grown in MRS medium with 2% NaCl (w/v), washed with sterile saline, resuspended in 4°C cucumber juice, and inoculated into triplicate tubes. After 7 days' incubation at 30°C, fermentation products were analyzed.

Pickling cucumbers (3.8–5.1 cm diameter) from commercial sources were fermented in 1-gal (3.78 liter) jars with a tightly closed lid to prevent $CO_2$ loss during fermentations. Rubber septa were put into the lids to make additions or take samples from jars without opening the lid. Jars were filled with an equal weight of cucumbers and a brine which contained 10% NaCl and 0.32% acetic acid. If malic acid were added, 20, 40 or 60 mL of a 17.6% solution of L-malic acid was added to the brine solution to give equilibrated malic acid concentrations 7, 14, and 21 mM higher than the concentration originating from the cucumbers. The cucumbers were held in this brine for 3 days to allow the salt concentration in the liquid to fall below 6%. The low pH and high salt concentration prevented initiation of a natural fermentation during this time. To raise the pH before inoculation of culture, 24g of sodium acetate trihydrate were dissolved in 30 mL of water. This was injected into the jars through the septum. If malic acid had been added to the cover brine, sufficient 50% NaOH solution was added to neutralize the malic acid. Half of the NaOH was added at the same time as the acetate. The remainder was added 2 days later. Once the pH had been raised to approximately 4.5, each jar was inoculated with 2 mL of a 16-hr culture of the appropriate organism grown in MRS medium with 2% NaCl. The cucumbers were held at 27°C for 3 wk and then analyzed.

Cucumbers were oxygen-exchanged for 30 min at a rate of 300 mL/min according to the procedure of Fleming and Pharr (1980). After brine was added to the exchanged fruit, the expansion tower was removed and replaced with a lid with a single rubber septum, as described above.

Before the closed jars of fermented cucumbers were opened, triplicate 10-mL brine samples were removed through the septum for $CO_2$ analysis. The cucumbers could not bloat in the closed jars because there was no space available for expansion. When lids were removed from the jars, a pressure differential developed, and the cucumbers bloated. Three hours after opening the jars, the fruit were examined for bloater damage. A sample for fermentation product analysis was prepared by blending an equal weight of brine and cucumber tissue. A half cross-sectional piece was cut from 10–12 cucumbers in each jar to obtain a representative tissue sample.

$CO_2$ analysis was done with the method of Fleming et al. (1974). The percentage of bloater damage was expressed as a bloater index value calculated according to Fleming et al. (1977). Concentrations of reducing sugars were determined with the dinitrosalicylic acid reagent (Sumner and Sisler, 1944). Malic acid, lactic acid, and acetic acid were analyzed by HPLC with an 8 x 100 mm, 10 μm $C_{18}$, reversed-phase Radial-Pak column (Waters Associates, Milford, CT) with pH 2.5, 0.05M phosphoric acid as the eluant (McFeeters et al., 1984).

## RESULTS & DISCUSSION

OF THE 19 STRAINS of *L. plantarum* and 5 strains of *P. cerevisiae* that were evaluated for their ability to degrade malic acid in the nutritive medium, only *L. plantarum* 963 and 965 were found to lack the ability to completely degrade malic acid.

These presumptive, nonmalic acid-degrading strains were then compared with *L. plantarum* WSO for their ability to degrade malic acid and ferment cucumber juice containing 5% NaCl. The WSO strain, as has been previously shown (McFeeters et al., 1982), degraded all of the malic acid in cucumber juice (Table 1). However, less than 3% of the initial malic acid disappeared during a 7-day incubation period with strain 965. Strain 963 showed intermediate characteristics in that it degraded 22% of the malic acid. $CO_2$ production by WSO was equivalent on a molar basis to the malic acid metabolized, Both the 965 and 963 strains produced less $CO_2$ than WSO, but more $CO_2$ than could be accounted for by malic acid degradation. The difference between the total lactic acid formed during fermentation and the malic acid degraded by each strain was considered to be the lactic acid production from sugar metabolism. The WSO strain produced nearly twice as much lactic acid as the other two strains.

The three strains of *L. plantarum* shown in Table 1, along with a heterofermentative organism, *L. cellobiosus*, were used to ferment cucumbers to observe the relationship between $CO_2$ formation and bloating. Analysis of duplicate jars for malic acid and sugar degradation and lactic acid production is shown in Table 2. Cucumbers did not ferment initially when they were covered with salt and acetic acid. When the pH was raised and no inoculum was added, a natural fermentation occurred. However, when lactic acid bacteria were inoculated immediately after pH adjustment, the inoculated organism apparently dominated the fermentation. This was indicated by the fact that malic acid remained at the end of fermentation, whereas, malic acid is not found in natural fermentations because the natural lactic acid bacteria degrade malic acid. The fact that nearly 80% of the malic acid was degraded in fermentation A with the 963 culture indicated that some competition by natural malic acid-degrading organisms may have occurred.

Even though the 963 and 965 strains were isolated from cheddar cheese (Sherwood, 1939; strains 1.1 and 4.3, respectively), they did carry out an active fermentation of cucumbers under the conditions described. This is indicated by the fact that 85% of the sugars present in the cucumbers were fermented by 965 and 90–92% of the sugars by strain 963. This compares to complete sugar removal by the WSO strain, which is commonly used for cucumber fermentations. The fact that the 965 and 963 strains produced more acid in cucumbers than in juice fermentations was attributed to the fact that the acetate added buffered the cucumber fermentation.

The relationship between bloater index and brine $CO_2$ concentration when cucumbers were fermented with different organisms was linear (Fig. 1). The critical point below which bloating did not occur (Fleming et al., 1978; Fleming, 1979) was 12.6 mM $CO_2$. The 965-fermented cucumbers reduced the $CO_2$ production relative to the control WSO strain to the point that bloating was eliminated by use of the nonmalic acid degrading lactic culture. The $CO_2$ production in the jars 3 days after brining, before inoculation with 965, was 8.2 mM. Therefore, a mean of 4.4 mM $CO_2$ was produced during the fermentation period, compared to a mean of 10.3 mM $CO_2$ production for the WSO fermentations.

An experiment was then conducted to determine the relationship between malic acid degradation and $CO_2$ production in fermented cucumbers. Cucumbers with the natural level of malic acid were fermented with both *L. plantarum* 965 and WSO strains. Cucumbers supplemented with 7, 14, and 21 mM malic acid were fermented only with the WSO strain. A linear relationship between $CO_2$ production and malic acid degradation was observed (Fig.

*Table 1—Malic acid, $CO_2$, and lactic acid in cucumber juice + 5% NaCl after fermentation with strains of L. plantarum for 7 days at 30°C*

| Strain | Malic acid (mM) | $CO_2$ (mM) | Lactic acid | | |
|---|---|---|---|---|---|
| | | | Total (mM) | From malic acid[a] (mM) | From sugar[a] (mM) |
| Noninoculated | 14.7 ± 1.0 | 0.3 ± 0.1 | –[b] | –[b] | –[b] |
| WSO | –[b] | 15.1 ± 0.3 | 134.1 ± 0.4 | 14.7 | 119.4 |
| 965 | 14.3 ± 0.6 | 2.5 ± 0.3 | 62.9 ± 1.6 | 0.4 | 62.5 |
| 963 | 11.5 ± 1.2 | 6.3 ± 1.5 | 64.4 ± 3.8 | 3.2 | 61.2 |

[a] Calculated on the assumption that one mole of lactic acid was formed for each mole of malic acid degraded and that the remainder of the lactic acid was produced by sugar fermentation.
[b] Nondetectable.

*Table 2—Malic acid degradation, sugar degradation, and lactic acid production during fermentation of cucumbers with different lactic acid bacteria*[a]

| Organism | Fermentation[b] | Malic acid degradation (%) | Sugar degradation (%) | Lactic acid production (mM) |
|---|---|---|---|---|
| *L. plantarum* WSO | A | 100 | 100 | 140.7 |
| | B | 100 | 100 | 128.4 |
| *L. plantarum* 965 | A | 7 | 85 | 100.4 |
| | B | 26 | 85 | 99.1 |
| *L. plantarum* 963 | A | 79 | 93 | 122.7 |
| | B | 19 | 90 | 102.6 |
| *L. cellobiosus* | A | 93 | 100 | 91.5 |
| | B | 93 | 100 | 51.2 |
| Natural fermentation[c] | A | 100 | 78 | 85.7 |
| | B | 100 | 97 | 63.8 |

[a] Calculated on a brined, equilibrated basis, the fresh cucumbers contained 10.0 mM malic acid and 85.8 mM reducing sugar.
[b] A and B indicate duplicate fermentation jars.
[c] Brine additions and adjustments were the same as the other four treatments, but no starter culture was added.

2). However, only 0.8 moles of $CO_2$ was produced per mole of malic acid degraded. This suggested that a small amount of the malic acid may have been utilized by either cucumber enzymes or the fermentation microorganisms by a reaction other than the malolactic reaction. In cucumber juice fermentations, a 1:1 ratio was observed (McFeeters et al., 1982). The juice used for those experiments had been heated to inactivate cucumber enzymes. The intercept of the curve in Fig. 2 provides an estimate of $CO_2$ produced in the fermentation from sources other than malic acid.

Fig. 3 shows the relationship between $CO_2$ formation and bloating in oxygen-exchanged and nonexchanged cucumbers. The relationship between bloater index and $CO_2$ production in the nonexchanged fruit was not linear, as occurred in the experiment shown in Fig. 1, even though the range of $CO_2$ production was similar. This probably represents differences in the physical strength of the cucumber carpels and the way in which carpel separation occurs in different lots of cucumbers. Further work is needed to characterize quantitative relationships between physical characteristics of cucumbers and bloating in response to $CO_2$. The cucumbers fermented with strain 965 showed slight bloating. Extrapolation to zero bloater index in the nonexchanged condition indicated that the critical $CO_2$ concentration was 12.4 mM. This bloating threshold was very close to that found in the experiment shown in Fig. 1. In both experiments, the $CO_2$ production attributable to the cucumber was sufficient to bring the cucumbers to the point at which bloating could begin. $CO_2$ production from malic acid provided the margin which caused significant bloating of the fruit. Prevention of malic acid degradation would, therefore, prevent most bloating in a controlled, homolactic acid fermentation of cucumbers.

Fleming and Pharr (1980) have shown that oxygen exchange of cucumbers before brining, to replace the air normally present in the gas spaces of the fruit with oxygen, reduces the susceptibility of cucumbers to bloating during fermentation. The results in Fig. 3 show an 8 mM increase in the concentration of $CO_2$ required to initiate bloating (20.8 mM vs 12.4 mM for nonexchanged fruit). This differential was maintained as the $CO_2$ concentration was increased by degradation of larger amounts of malic acid until extensive bloating had occurred. Thus, $O_2$ exchange could provide a margin of protection against bloating, even if some malic acid were degraded. The combination of fermentation with nonmalic acid-degrading bacteria and $O_2$ exchange in cucumber brining tanks could provide a considerable margin for prevention of bloating without the need to remove $CO_2$ by purging. A problem which remains to be solved before $O_2$ exchange can be applied under commercial conditions relates to undesirable bacteria being drawn into the cucumbers (Daeschel, 1982). Daeschel and Fleming (1981) have shown that bacteria enter the fruit through stomatal openings due to the partial vacuum which develops when $O_2$-exchanged fruit are put into brine (Corey et al., 1983).

Though the results with *L. plantarum* 965 demonstrate the desirability of using nonmalolactic bacteria in cucumber fermentations, it is not suitable as a fermentation organism with current procedures because sugars are not completely fermented. Results of our survey of 24 homofermentative lactic acid bacteria and the results of Caspritz and Radler

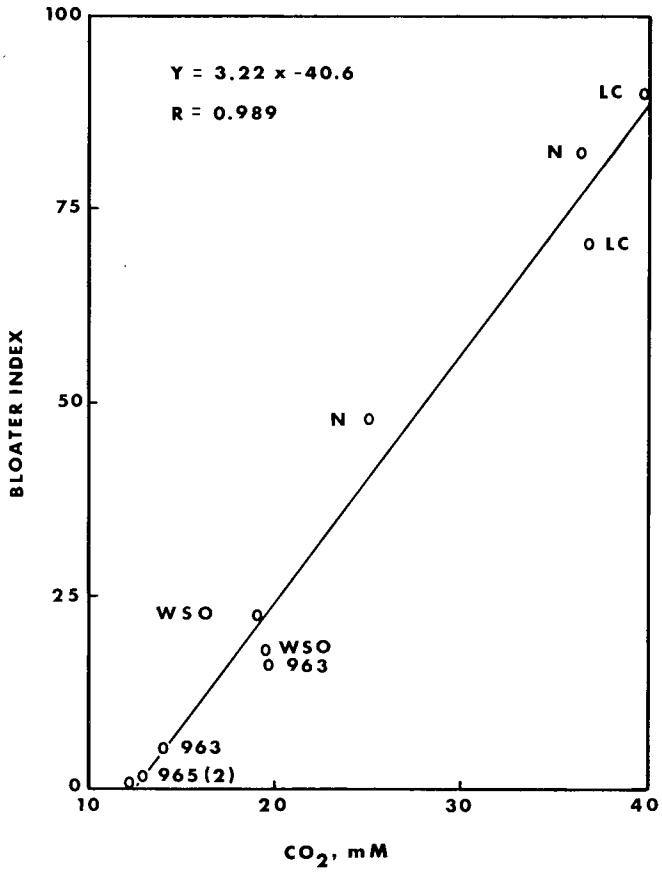


*Fig. 1—Relationship between $CO_2$ production and bloater damage in cucumbers fermented with different microorganisms. The organisms used for each fermentation are indicated as follows: L. plantarum WSO, WSO; L. plantarum 963, 963; L. plantarum 965; 965; L. cellobiosus, LC; and natural fermentation, N. Cover brines were not supplemented with malic acid.*

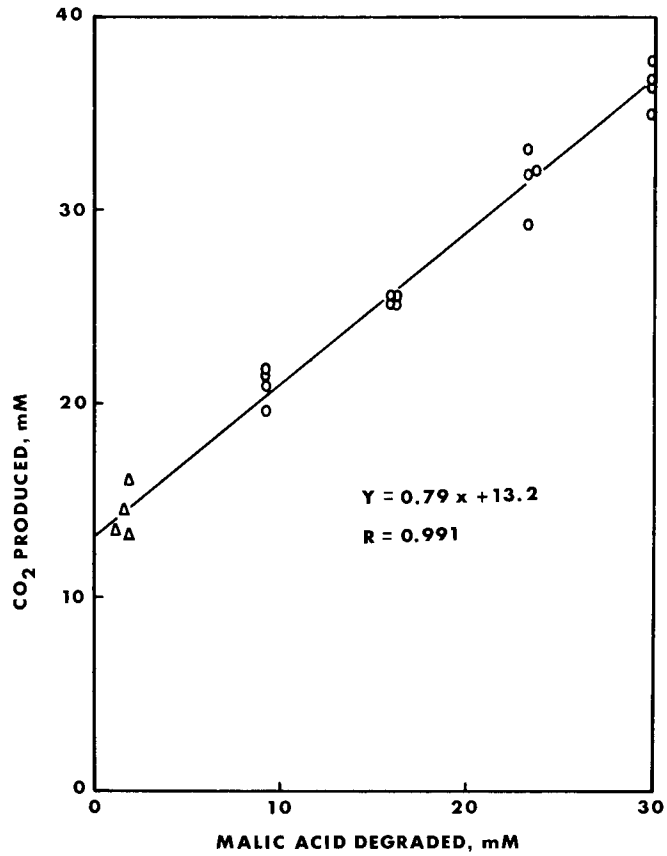


*Fig. 2—Relationship between malic acid degradation and $CO_2$ formation in cucumber fermentations: △ — L. plantarum 965 without malic acid added to the cucumbers; ○ — L. plantarum WSO with 0, 7, 14, and 21 mM malic acid added to the cucumbers.*

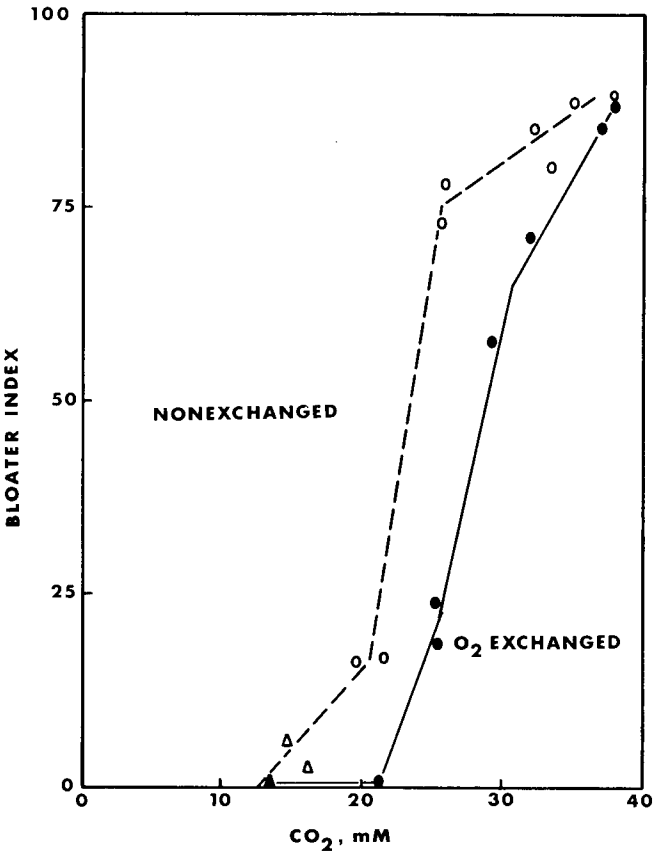


*Fig. 3—Effect of $O_2$ exchange on bloater damage of fermented cucumbers: △, ▲ — L. plantarum 965 without malic acid added to the cucumbers; ○, ● — L. plantarum WSO with 0, 7, 14, and 21 mM malic acid added to the cucumbers.*

(1983) indicate that inability to degrade malic acid is not a common characteristic among this group of bacteria. However, Daeschel et al. (1984) have developed a rapid selection procedure for nonmalic acid-degrading lactic acid bacteria and have demonstrated the isolation of such mutants from *L. plantarum* WSO. Therefore, it may be possible to obtain low $CO_2$-producing organisms with better fermentation characteristics than the *L. plantarum* 965 strain now available.

## SUMMARY

TWO STRAINS of *L. plantarum* (963 and 965) from 24 strains of *L. plantarum* and *P. cerevisiae* tested did not degrade all malic acid from a test medium. *Lactobacillus plantarum* 965, which degraded the least malic acid, prevented significant bloating of cucumbers by reducing the amount of $CO_2$ produced during fermentation in comparison with *L. plantarum* WSO, which completely degraded malic acid.

The total $CO_2$ production during a fermentation could be divided into two parts. $CO_2$ from sources other than malic acid, primarily cucumber tissue metabolism, amounted to 12.5 mM. This concentration of $CO_2$ was sufficient to bring the cucumbers to the critical point above which measurable bloating damage would occur. $CO_2$ formation above 12.5 mM was directly related to the amount of malic acid degraded. This additional $CO_2$ provided the marginal increase in gas production required to cause bloater damage. Bloating damage increased with the increase in $CO_2$ formation from malic acid degradation.

Previous work had shown that oxygen exchange of cucumbers prior to brining increased their resistance to bloating during fermentation (Fleming and Pharr, 1980). Comparison of exchanged and nonexchanged cucumbers showed that exchange $O_2$ increased the concentration of $CO_2$ required to initiate bloating by 8 mM.

These results indicate that fermentation of cucumbers with nonmalic acid-degrading starter cultures and/or use of oxygen exchange procedures may make it possible to prevent bloater damage in cucumbers without the need to remove $CO_2$ from the brines by purging (Fleming, 1979).

## REFERENCES

Caspritz, G. and Radler, F. 1983. Malolactic enzyme of Lactobacillus plantarum. Purification, properties and distribution among bacteria. J. Biol. Chem. 258: 4907.
Corey, K.A., Pharr, D.M., and Fleming, H.P. 1983. Pressure changes in oxygen-exchanged, brined cucumbers. J. Am. Soc. Hort. Sci. 108: 61.
Daeschel, M.A. 1982. Entrance of microorganisms into brined cucumbers. Ph.D. Diss. North Carolina State Univ., Raleigh, NC.
Daeschel, M.A. and Fleming, H.P. 1981. Entrance and growth of lactic acid bacteria in gas-exchanged, brined cucumbers. Appl. Environ. Microbiol. 42: 1111.
Daeschel, M.A., McFeeters, R.F., Fleming, H.P., Klaenhammer, T.R., and Sanozky, R.B. 1984. Mutation and selection of Lactobacillus plantarum strains that do not produce carbon dioxide from malate. Appl. Environ. Microbiol. 47: 419.
De Man, J.C., Rogosa, M., and Sharpe, M.E. 1960. A medium for the cultivation of lactobacilli. J. Appl. Bact. 23: 130.
Fleming, H.P. 1979. Purging carbon dioxide from cucumber brines to prevent bloater damage — a review. Pickle Pak Sci. 6: 8.
Fleming, H.P. and Pharr, D.M. 1980. Mechanism for bloater formation in brined cucumbers. J. Food Sci. 45: 1595.
Fleming, H.P., Thompson, R.L., Bell, T.A., and Monroe, R.J. 1977. Effect of brine depth on physical properties of brine-stock cucumbers. J. Food Sci. 42: 1464.
Fleming, H.P., Thompson, R.L., and Etchells, J.L. 1974. Determination of carbon dioxide in cucumber brines. J. AOAC 57: 130.
Fleming, H.P., Thompson, R.L., Etchells, J.L., Kelling, R.E., and Bell, T.A. 1973. Carbon dioxide production in the fermentation of brined cucumbers. J. Food Sci. 38: 504.
Fleming, H.P., Thompson, R.L., and Monroe, R.J. 1978. Susceptibility of pickling cucumbers to bloater damage by carbonation. J. Food Sci. 43: 892.
McFeeters, R.F., Fleming, H.P., and Thompson, R.L. 1982. Malic acid as a source of carbon dioxide in cucumber juice fermentations. J. Food Sci. 47: 1862.
McFeeters, R.F., Thompson, R.L., and Fleming, H.P. 1984. HPLC analysis of sugars, acids, and ethanol in lactic acid vegetable fermentations. J. AOAC. (In press).
Sherwood, I.R. 1939. The bacterial flora of New Zealand cheddar cheese. J. Dairy Res. 10: 426.
Sumner, J.B. and Sisler, E.B. 1944. A simple method for blood sugar. Arch. Biochem. 4: 33.
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