

# Complete Heterolactic Acid Fermentation of Green Beans by *Lactobacillus cellobiosus*

K.-H. CHEN, R. F. McFEETERS, and H. P. FLEMING

## ABSTRACT

Only *Lactobacillus cellobiosus*, among eight strains of heterofermentative lactic acid bacteria, removed all fermentable sugars from green beans. Proper blanching of the beans was required to prevent growth by natural lactic acid bacteria. An inoculum of 10 CFU/ml *Lactobacillus plantarum* and 10<sup>6</sup> CFU/ml *L. cellobiosus* resulted in the formation of twice as much lactic acid as inoculation with *L. cellobiosus* alone. A maximum of 3.74% sugar was metabolized by *L. cellobiosus* in bean juice containing 2.5% NaCl and 0.08% acetic acid. Fructose was nearly quantitatively reduced to mannitol with a concomitant accumulation of acetic acid. Ethanol was not observed until most of the fructose was metabolized. The *L. cellobiosus*-fermented beans had a more mild acid flavor than beans fermented with *L. plantarum*.

## INTRODUCTION

PEDERSON (1930) SHOWED the important role of heterolactic acid organisms, particularly *Leuconostoc mesenteroides*, in initiation of sauerkraut fermentations. Heterolactic products, including substantial amounts of mannitol, were formed during the initial stage of sauerkraut fermentations. However, *Lactobacillus plantarum* converted sugars and mannitol to lactic acid in the latter stages of fermentation (Pederson and Albury, 1969). The net result was a high level of acid in the final product. It is now known that many natural food fermentations are initiated by heterofermentative lactic acid bacteria (Pederson, 1979).

Etchells et al. (1964) developed a procedure for pure culture vegetable fermentations by subjecting the vegetables to hot water blanching or gamma radiation prior to fermentation. Both homolactic and heterolactic acid organisms were used. Stamer et al. (1971) fermented sterilized cabbage juice with *L. mesenteroides* and *Lactobacillus brevis* as part of an investigation to determine the source of typical sauerkraut flavor characteristics. In both studies, titratable acidity and pH were the only chemical analyses done to characterize the fermentations. The extent of sugar degradation was not determined. Recently, Kozup and Sistrunk (1982) and Fleming et al. (1983) fermented green beans with the homofermentative organism, *L. plantarum*.

Fleming et al. (1983) found that it is necessary to remove all fermentable sugars from vegetables to prevent undesirable secondary fermentations. The main objective of this investigation was to define conditions which will result in complete fermentation of sugars in green beans by heterolactic acid bacteria. A detailed analysis of the time course of substrate and product changes during fermentation is presented.

## MATERIALS & METHODS

### Fermentation of green beans

Fresh, Blue Lake green beans were obtained from the local produce market. The beans were washed and snapped by hand, then

*Authors Chen, McFeeters, and Fleming are affiliated with the Food Fermentation Laboratory, USDA-ARS, Southern Region, and North Carolina Agricultural Research Service, Dept. of Food Science, North Carolina State Univ., Raleigh, NC 27650.*

refrigerated at 4°C overnight until use. Beans were blanched in boiling water for 3 min and put directly into a tared jar. Eight-ounce to 1-gal jars were used. To minimize any post-blanching contamination, gloves were worn and dipped in 200 ppm chlorine solution before handling the beans. The beans were immediately covered with an equal weight of brine and closed. The brine contained 5.0% NaCl and either 0 or 0.16% glacial acetic acid, depending upon the experiment. The jars and brine were not heated prior to use. After closing, the jars were cooled in tap water. About 8 hr after closing, they were inoculated through a rubber septum in the jar lid with 10<sup>6</sup> CFU/ml of *L. cellobiosus* or other organisms as indicated. Fermentation was carried out at 27°C. In all experiments, duplicate jars were fermented and analyzed.

Two modifications of the usual packing and inoculation procedures were used in the experiments. In one case, to preclude the possibility of contamination of the blanched beans with lactic acid bacteria, the jars were pasteurized for 15 min in boiling water after closing. The second modification was to inoculate beans which were blanched, but not pasteurized, with a mixture of *L. cellobiosus* and *L. plantarum*. All jars were inoculated with 10<sup>6</sup> CFU/ml *L. cellobiosus*, then the same jars were also inoculated with 0, 10<sup>1</sup>, 10<sup>2</sup>, or 10<sup>3</sup> CFU/ml *L. plantarum*.

### Cultures

*Lactobacillus plantarum* WSO, *L. brevis* 50, *L. brevis* 70, *L. mesenteroides* 42, and *L. mesenteroides* 43 were obtained from the culture collection of this laboratory. *Leuconostoc mesenteroides* LC 33 was provided by Dr. R.B. Beelman, Dept. of Food Science, Pennsylvania State Univ. *Lactobacillus cellobiosus* ATCC 11739, *Lactobacillus fermentum* ATCC 14936, and *Leuconostoc paramesenteroides* NCDO 803 were obtained from the Northern Regional Research Laboratory, USDA-ARS. Bacteria were grown in 30°C MRS broth (DeMan et al., 1960) with 2% NaCl. Twelve-hour cultures were centrifuged and resuspended in sterile saline.

### Evaluation of fermented green beans

Fermented green beans were evaluated by a taste panel of seven people. The panelists rated firmness, off-flavor, acidity, and overall acceptability using a 1 to 10 rating scale.

### Chemical analysis

The determination of sugars, organic acids, ethanol, and pH was the same as described in the previous paper (Chen et al., 1983a). Reducing sugars were measured with dinitrosalicylic acid reagent (Sumner and Somers, 1944).

### Enumeration of microorganisms

Plate count agar (BBL, Cockeysville, MD) was used for the determination of total counts. Total *Enterobacteriaceae* were grown on violet red bile glucose agar (BBL) (Mossel et al., 1962). The method of Etchells et al. (1961) was used for yeast and mold counts. Lactic acid bacteria were determined on modified MRS containing 0.02 Na<sub>3</sub>N, as described by Mundt et al. (1967). When *L. plantarum* and *L. cellobiosus* were used in the same fermentation, differential colony counts of the individual species present were made by using basal medium (Hansen, 1968) with different carbon sources. Both species could utilize arabinose, but *L. plantarum* was the only one which could ferment mannitol anaerobically. Therefore, it was estimated by plating on a basal medium with mannitol. The *L. cellobiosus* was estimated by the difference of colony counts between the arabinose and mannitol-containing basal media.

Table 1—Acid, ethanol, reducing sugar, and pH of snapped green beans<sup>a</sup> after 2 wk fermentation

Microorganism	Final brine assay						pH
	Malic acid <sup>a</sup> (mM)	Lactic acid (mM)	Acetic acid (mM)	Ethanol (mM)	Reducing sugar (mM)		
<i>L. cellobiosus</i> ATCC 11739	— <sup>b</sup>	43.1	25.0	20.2	—	3.52	
<i>L. mesenteroides</i> 42	8.52	20.3	11.2	22.5	10.2	3.92	
<i>L. mesenteroides</i> 43	8.60	22.4	10.7	25.6	10.4	3.88	
<i>L. paramesenteroides</i> NCDO 803	0.24	37.3	12.1	21.9	4.4	3.95	
<i>L. brevis</i> 50	—	31.3	8.7	26.7	1.1	4.05	
<i>L. brevis</i> 70	—	38.8	12.4	20.5	4.4	3.93	
<i>L. fermentum</i> ATCC 14936	0.40	40.0	14.3	20.9	4.4	3.96	
<i>L. plantarum</i> WSO	—	88.8	6.6	—	8.0	3.38	
<i>L. mesenteroides</i> LC 33	—	72.7	11.0	13.2	6.9	3.56	

<sup>a</sup> Green beans contained 51.2 mM fermentable hexoses and 10.1 mM malic acid prior to fermentation.

<sup>b</sup> Not detectable.

Table 2—Influence of blanching on population of certain microbial groups on green beans

Microbial group	Colony counts <sup>a</sup> (CFU/g)	
	Nonblanched	Blanched
Total	2.15 × 10 <sup>6</sup>	3 × 10 <sup>2</sup>
Lactic acid bacteria	1.58 × 10 <sup>5</sup>	0
<i>Enterobacteriaceae</i>	5.4 × 10 <sup>5</sup>	0
Yeast	1.46 × 10 <sup>2</sup>	0
Mold	4.0 × 10 <sup>2</sup>	0

<sup>a</sup> Counts shown represent the average for two samplings.

## RESULTS & DISCUSSION

### Selection of a fermentation organism

Eight of the 14 heterofermentative strains used in bean juice fermentations (Chen et al., 1983a) were used to ferment blanched green beans in 2.5% NaCl brine without added acetic acid. The jars were refrigerated for 3 days prior to inoculation to prevent growth of microorganisms while sugars diffused from the beans and salt diffused into the beans. The objective was to see whether the fermentations would be similar to that found in juice and whether the fermentations would be taken over by natural organisms.

Data in Table 1 show the product distribution in the green beans and the residual sugar and malic acid after 2 wk of fermentation. With the exception of *L. mesenteroides* LC 33, the distribution of lactic acid, acetic acid, and ethanol were similar to that found in the juice fermentations (Chen et al., 1983a). The results suggested that the fermentations were due to the inoculated organism. Both *L. mesenteroides* LC 33-inoculated jars were apparently taken over by a natural homolactic acid organism, since the lactic acid production was closer to *L. plantarum* than to the heterofermentative organisms.

*Lactobacillus cellobiosus* was the only organism tested which removed all detectable fermentable sugars from the green beans, just as occurred in juice fermentations (Chen et al., 1983a). It also had the lowest final pH among the heterofermentative organisms. *Lactobacillus cellobiosus* produced less than half as much lactic acid as *L. plantarum* (Table 1). Taste panel analysis of the beans indicated that they were significantly less acid in flavor than beans fermented with *L. plantarum*. Kozup and Sistrunk (1982) also found that green beans fermented by *L. plantarum* were very acid in flavor. The acceptability rating of *L. cellobiosus*-fermented beans was also significantly higher. These characteristics led to the selection of *L. cellobiosus* for a more detailed study of the heterolactic acid fermentation of green beans.

### Selection of conditions for *L. cellobiosus* fermentations

Etchells et al. (1964) developed a procedure for "pure culture" fermentation of cucumbers and other vegetables. A similar approach to setting conditions which would result in the dominance of the inoculated organism during fermentation was used in this work. Blanching was used to reduce the natural microbial population on the beans. To protect against the possibility of enzymatic off-flavor development, a blanch of 3 min in boiling water was used. This was based upon blanch treatments used for freezing beans (Luh et al., 1975). A 2.5% level of NaCl was selected because it was similar to that used for sauerkraut fermentation and that level would be suitable for consumption without desalting. Acetic acid at a concentration of 0.08% was added to the jars because it was found that this amount would lower the pH of the beans to near 4.6 at the beginning of fermentation. Etchells et al. (1964) added lactic acid to the cover brine of blanched cucumbers to prevent germination and growth of anaerobic spores.

Data in Table 2 show the microbial population on fresh green beans which had been snapped and refrigerated overnight before plating. A typical heterogeneous population of microflora was present (Etchells et al., 1961). The number of lactic acid bacteria was higher than is usual on fresh produce. This was probably because the lactic acid bacteria began to increase after the beans were snapped. Blanching eliminated lactic acid bacteria, *Enterobacteriaceae*, yeasts, and molds (Table 2). However, a small total count was obtained. When beans were blanched and incubated in 2.5% NaCl without acidification or inoculation with a lactic acid organism, a large population of small cocci was visible microscopically. These organisms were not identified, but demonstrated the fact that the blanched beans were non-sterile. Therefore, spore-forming bacteria, including pathogens, could grow unless proper inoculation and acidification occurred.

### Effect of blanching on fermentation

Since *L. cellobiosus* was inoculated into a nonsterile medium, it was of interest to determine whether the products formed could be attributed to the inoculated organism or whether competition with the remaining natural flora would occur. To evaluate this possibility, control beans were packed in the usual way and then pasteurized. Fermentations in these jars were compared to nonpasteurized jars.

Noninoculated, pasteurized controls showed no growth. However, a nonpasteurized, noninoculated control developed a population of lactic acid bacteria and small cocci after 4 days' incubation. Data in Fig. 1 show the viable counts of lactic acid bacteria in jars inoculated with 10<sup>6</sup> CFU/ml *L. cellobiosus*. The results indicated that similar

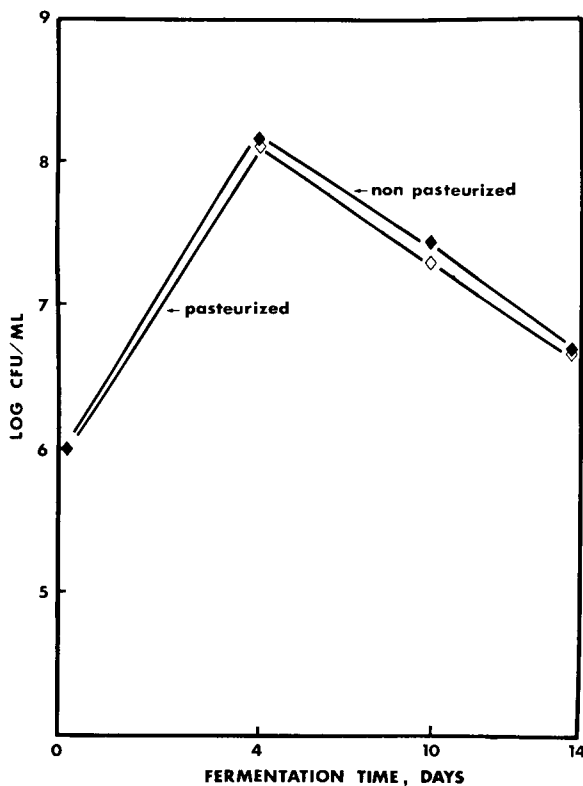


Fig. 1—*Lactobacillus cellobiosus* growth in pasteurized and non-pasteurized, blanched green beans. Viable cell count in pasteurized beans, —◇—; viable cell count in nonpasteurized beans, —◆—.

populations of lactic acid bacteria carried out fermentation in both media throughout the fermentation period.

After 2 wk, analysis of the fermentation products showed almost identical product distributions in both the pasteurized and nonpasteurized beans (Table 3). These results indicated that *L. cellobiosus* could compete with organisms which might survive blanch treatment and that it was the only organism to produce measurable amounts of fermentation products in both pasteurized and nonpasteurized beans. In the course of this work, every bean fermentation inoculated with only *L. cellobiosus* produced a heterofermentative product pattern with calculated carbon recoveries near 100%. There was no evidence which indicated that contaminating organisms had any significant effect on fermentation products.

Natural lactic acid vegetable fermentations normally are dominated by acid-tolerant *L. plantarum* during the final stages. We wanted to determine whether *L. cellobiosus* would dominate fermentations if beans were contaminated with small numbers of *L. plantarum* cells after blanching. Beans were packed in the usual way, but inoculated with a combination of *L. cellobiosus* and *L. plantarum*, as described in the Methods section. Differential cell counts of these two organisms (Fig. 2) showed that within 2 days of inoculation the *L. plantarum* counts had increased to over  $10^8$  CFU/ml even with only a  $10^1$  CFU/ml initial inoculum. Later in the fermentation period, the *L. plantarum* population remained between  $10^8$  and  $10^9$  CFU/ml, while the *L. cellobiosus* counts slowly declined. Data in Fig. 3 show the lactic acid production increased with the initial *L. plantarum* inoculation. With only  $10^1$  CFU/ml *L. plantarum* cells, the final lactic acid concentration more than doubled compared to the *L. cellobiosus* fermentation. Furthermore, more sugar remained after 2 wk when  $10^2$  or  $10^3$  CFU/ml *L. plantarum* was inoculated than when *L. cellobiosus* was inoculated alone (Table 4). These results showed that even small

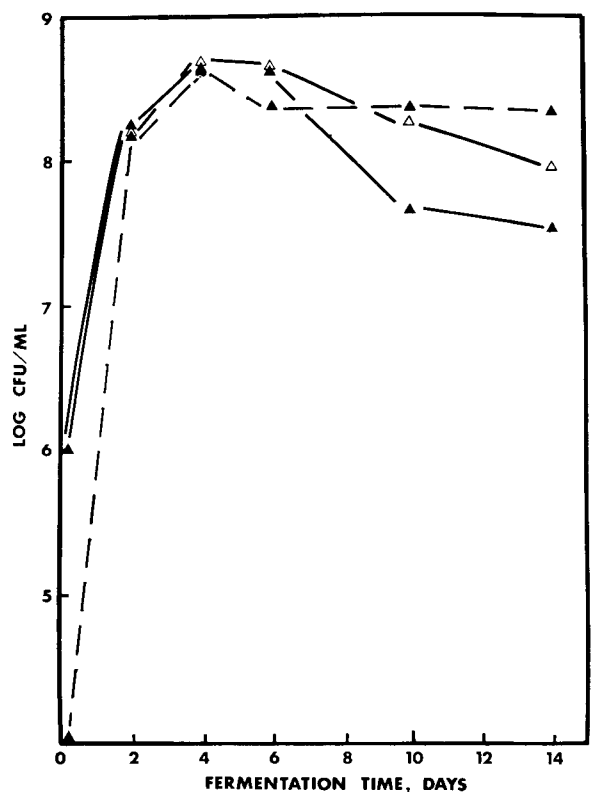


Fig. 2—Competitive growth of *L. plantarum* and *L. cellobiosus* in green bean fermentations. *Lactobacillus cellobiosus* population in beans inoculated with  $10^6$  CFU/ml *L. cellobiosus*, —△—; *L. cellobiosus* population in beans inoculated with  $10^6$  CFU/ml *L. cellobiosus* and  $10^1$  CFU/ml *L. plantarum*, —▲—; *L. plantarum* population in beans inoculated with  $10^6$  CFU/ml *L. cellobiosus* and  $10^1$  CFU/ml *L. plantarum*, —◆—; *L. plantarum* population in beans inoculated with  $10^6$  CFU/ml *L. cellobiosus* and  $10^1$  CFU/ml *L. plantarum*, —◇—. The coefficients of variation were *L. cellobiosus* 77.8% and *L. plantarum* 16.9%.

Table 3—Comparison of end product distribution between pasteurized and nonpasteurized, fermented green beans

End products <sup>a</sup>	Nonpasteurized	Pasteurized
Lactic acid	48.5	46.2
Acetic acid	42.0	41.7
Ethanol	20.9	18.9
Mannitol	39.1	38.4

<sup>a</sup> End products shown represent the average of 2 samples. The green beans contained 6.7 mM sucrose, 28.2 mM glucose, 34.9 mM fructose, and 8.4 mM malic acid. The coefficient of variations were: lactic acid, 2.1%; acetic acid, 0.8%; ethanol, 4.4%; and mannitol, 4.1%.

numbers of *L. plantarum* can be expected to have a major effect on *L. cellobiosus* fermentations. Therefore, it is essential that any post-blanching contamination by this species be prevented to obtain a normal low acid, heterolactic acid fermentation and maximum removal of sugars.

#### Sugar utilization by *L. cellobiosus*

Fleming et al. (1983) found that vegetables were microbologically stable at pH 3.8 and 3.3 after fermentation with *L. plantarum* only if all fermentable sugars were removed. For green beans fermented with *L. cellobiosus*, a secondary fermentation by acid-tolerant yeasts or lactic acid bacteria would also be expected if the beans were exposed to contamination during post-fermentation handling and repacking unless the sugars were removed.

In these experiments, four different lots of beans were fermented. The sugar content of these lots varied from 110 mM (2.0%) to 217 mM (3.9%) hexose on a fresh weight

basis. The maximum amount of hexose removed from green beans was 208 mM (3.74%) on a fresh weight basis or 104

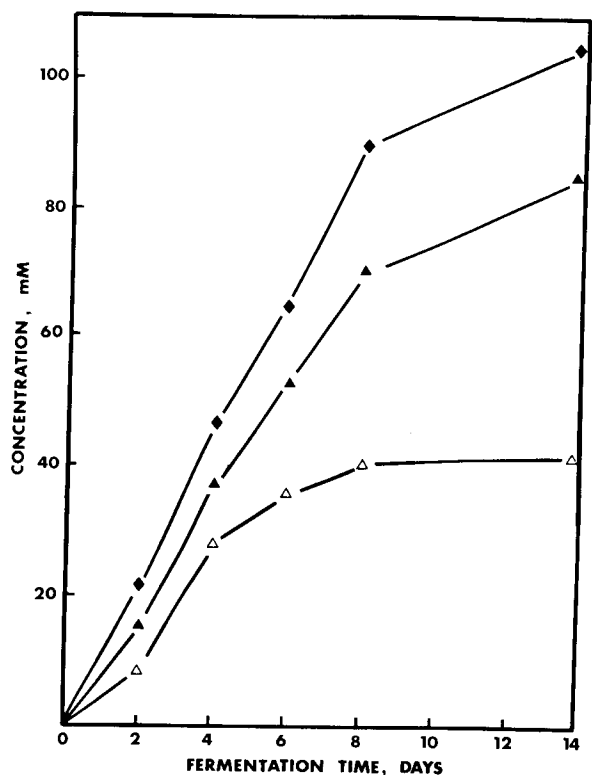


Fig. 3—Effect of competition by *L. plantarum* on lactic acid production in *L. cellobiosus* green bean fermentations. Inoculated with 10<sup>6</sup> CFU/ml *L. cellobiosus*, —△—; inoculated with 10<sup>6</sup> CFU/ml *L. cellobiosus* and 10<sup>1</sup> CFU/ml *L. plantarum*, —▲—; inoculated with 10<sup>6</sup> CFU/ml *L. cellobiosus* and 10<sup>3</sup> CFU/ml *L. plantarum*, —◆—. The coefficient of variation for lactic acid was 6.6%.

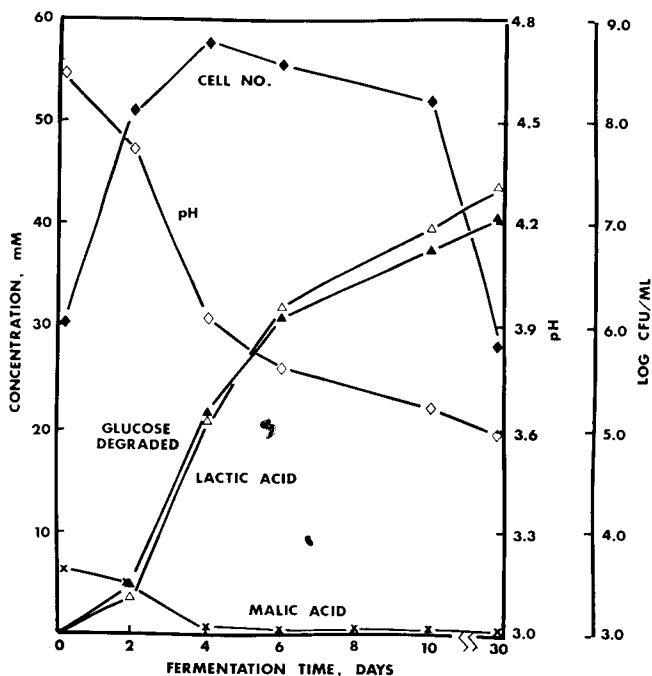


Fig. 4—Time course of changes during *L. cellobiosus* green bean fermentations. The green beans contained 6.2 mM sucrose, 44.8 mM glucose, 52.5 mM fructose, and 5.6 mM malic acid. The coefficients of variation were viable cell count 13.8%, glucose 3.6%, lactic acid 4.1%, and malic acid 6.2%.

mM (1.87%) on a 50:50 brined basis. This will be adequate to remove all sugars from a number of low sugar products. The amount of sugar fermented might be increased for higher sugar products by using pH control during fermentation (Fleming et al., 1983).

**Time course of green bean fermentation**

Fig. 4 and 5 demonstrate the time course of major changes which occurred during the fermentation of green beans in sealed, 8-oz jars. In Fig. 4, viable cell counts increased from 10<sup>6</sup> to over 10<sup>8</sup> CFU/ml in 2 days. It remained above 10<sup>8</sup> CFU/ml for 8 days and then declined to less than 10<sup>6</sup> CFU/ml by 30 days. The pH decreased rapidly during the first 4 days from 4.6 to 3.9. The terminal pH was 3.58. It was an important characteristic of every *L. cellobiosus* fermentation of green beans that the final

Table 4—Effect of initial *L. plantarum* inoculum size on the concentration of sugar remaining at the end of fermentation. The initial *L. cellobiosus* inoculum was 10<sup>6</sup> CFU/ml in each fermentation

Initial <i>L. plantarum</i> inoculum (CFU/ml)	Sugar remaining <sup>a</sup>		
	Glucose (mM)	Fructose (mM)	Total (mM)
0	8.7	—	8.7
10	8.3	—	8.3
10 <sup>2</sup>	12.9	—	12.9
10 <sup>3</sup>	16.4	6.71	23.1

<sup>a</sup> The brined green beans contained 6.2 mM sucrose, 44.8 mM glucose, 52.5 mM fructose, and 5.6 mM malic acid prior to fermentation.

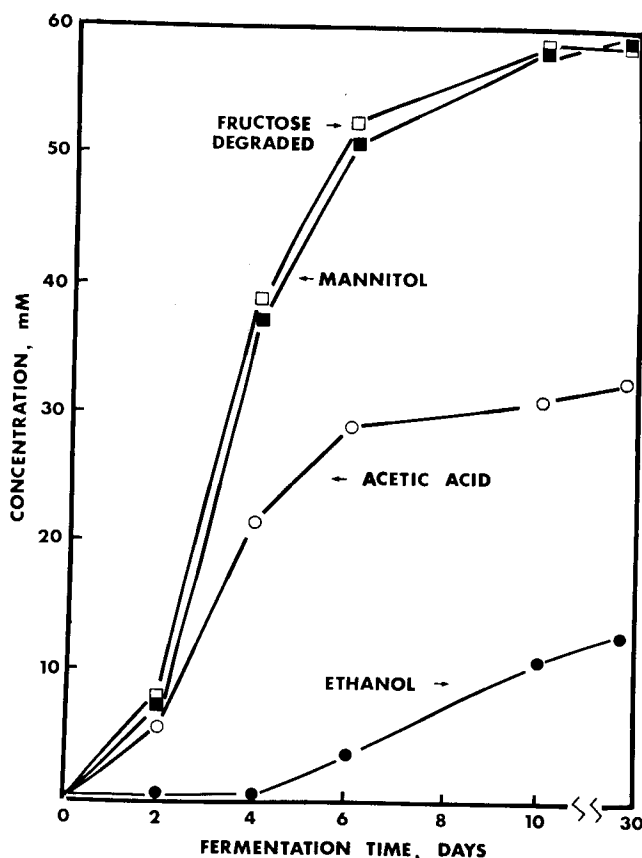


Fig. 5—Time course of changes during *L. cellobiosus* green bean fermentations. The green beans contained 6.2 mM sucrose, 44.8 mM glucose, 52.5 mM fructose, and 5.6 mM malic acid. The coefficients of variation were mannitol 3.8%, fructose 4.0%, acetic acid 3.6%, and ethanol 9.1%.

pH was between 3.50 and 3.60. The malic acid disappeared early in the fermentation, presumably by a malolactic fermentation to lactic acid and CO<sub>2</sub> similar to that which occurs in cucumber juice fermentations (McFeeters et al., 1982). Finally, Fig. 4 shows the formation of 1 mole of lactic acid for each mole of glucose metabolized throughout the fermentation. This is the expected result in a heterolactic acid fermentation. The lactic acid, assumed to have been produced from malic acid, was subtracted from the total lactic acid measured in order to obtain the curve in Fig. 4.

Fig. 5 shows that when glucose was available as an energy source, there was nearly a quantitative conversion of fructose to mannitol. This was a major reason for low acid production in this fermentation relative to the amount of fermentable sugar consumed. Analysis of the time course of fermentation showed a striking relationship between acetic acid and ethanol accumulation. Ethanol was not detected until about two-thirds of the fructose had been converted to mannitol. During this period, it appears that all of the NADH<sub>2</sub> that would have been used to reduce acetate to ethanol in the absence of fructose was used by the cells to produce mannitol. It was observed in the snapped green bean fermentations described above, that about 1 mole of acetic acid was produced for every 2 moles of fructose reduced. This is related to the fact that 2 moles of NADH<sub>2</sub> are required to reduce acetate to ethanol, while it takes only 1 mole of NADH<sub>2</sub> to produce mannitol (Martinez et al., 1963).

These results give a reasonably comprehensive picture of the major changes that occur in this fermentation of green beans. The data are consistent with a simple heterofermentative pathway throughout the fermentation. Fructose was diverted to mannitol because it is a preferred electron acceptor compared to acetic acid.

## CONCLUSIONS

THESE RESULTS SHOWED that it was possible to ferment green beans with the heterofermentative lactic acid bacterium, *L. cellobiosus*, to the point that all detectable sugars were metabolized. This was accomplished by blanching beans in boiling water to kill the natural microbial population, except for spore formers which will not grow in the acidic conditions produced in the product. Blanching is followed by brining with 2.5% NaCl, 0.08% acetic acid, equilibrated concentration, and inoculation with 10<sup>6</sup> CFU/ml *L. cellobiosus*. The beans fermented in this way had a more mild acid flavor than beans fermented with *L. plantarum*.

It was necessary to exclude *L. plantarum* from the green beans during fermentation because only 10 CFU/ml *L. plantarum* WSO cells, when inoculated with 10<sup>6</sup> CFU/ml *L. cellobiosus*, would grow rapidly and equal or exceed the number of *L. cellobiosus* cells within 2 days. This resulted in large increases in the amount of lactic acid.

Investigation of the time course of fermentation showed that fructose was nearly quantitatively converted to mannitol. Acetic acid accumulated until most of the fructose was reduced. From that point ethanol was formed. As expected for the hexose monophosphate pathway, 1 mole of lactic

acid was produced per mole of glucose fermented. The final pH of the heterolactic acid fermentation was between 3.5 and 3.6. *Lactobacillus cellobiosus* could ferment 3.74% sugar on a fresh weight basis from green beans.

The fact that complete heterolactic fermentations can be done with green beans and the products of fermentation do not undergo a secondary fermentation when properly stored (Chen et al., 1983b) may provide a basis for use of heterolactic acid fermentations for vegetable preservation.

## REFERENCES

- Chen, K.H., McFeeters, R.F., and Fleming, H.P. 1983a. Fermentation characteristics of heterolactic acid bacteria in green bean juice. *J. Food Sci.* 48: 962.
- Chen, K.H., McFeeters, R.F., and Fleming, H.P. 1983b. Stability of mannitol to *Lactobacillus plantarum* degradation in green beans fermented with *Lactobacillus cellobiosus*. *J. Food Sci.* 48: 972.
- DeMan, J.C., Rogosa, M., and Sharpe, M.E. 1960. A medium for the cultivation of lactobacilli. *J. Appl. Bacteriol.* 23: 130.
- Etchells, J.L., Costilow, R.N., Anderson, T.E., and Bell, T.A. 1964. Pure culture fermentation of brined cucumbers. *Appl. Microbiol.* 12: 523.
- Etchells, J.L., Costilow, R.N., Bell, T.A., and Rutherford, H.A. 1961. Influence of gamma radiation on the microflora of cucumber fruit and blossoms. *Appl. Microbiol.* 9: 145.
- Fleming, H.P., McFeeters, R.F., Thompson, R.L., and Sanders, D.C. 1983. Storage stability of vegetables fermented with pH control. *J. Food Sci.* 48: 975.
- Hansen, P.A. 1968. The strains of *Lactobacillus* species: a report by the taxonomic subcommittee on lactobacilli and closely related organisms. American Type Culture Collection, Rockville, MD.
- Kozup, J. and Sistrunk, W.A. 1982. Quality attributes of fermented and acidified green beans. *J. Food Sci.* 47: 1001.
- Luh, B.S., Feinberg, B., and Meehan, J.J. 1975. Freezing preservation of vegetables. In "Commercial Vegetable Processing," (Ed.) B.S. Luh and J.G. Woodroof, p. 334. Avi Publishing Co., Westport, CT.
- Martinez, G., Barker, H.A., and Horecker, B.L. 1963. A specific mannitol dehydrogenase from *Lactobacillus brevis*. *J. Biol. Chem.* 238: 1598.
- McFeeters, R.F., Fleming, H.P., and Thompson, R.L. 1982. Malic acid as a source of carbon dioxide in cucumber juice. *J. Food Sci.* 47: 1862.
- Mossel, D.A.A., Mengerink, W.H.J., and Scholts, H.H. 1962. Use of a modified MacConkey agar medium for the selective growth and enumeration of Enterobacteriaceae. *J. Bacteriol.* 84: 381.
- Mundt, J.O., Graham, W.F., and McCarty, I.E. 1967. Spherical lactic acid-producing bacteria of southern-grown raw and processed vegetables. *Appl. Microbiol.* 15: 1303.
- Pederson, C.S. 1930. Floral changes in the fermentation of sauerkraut. N.Y. State Agr. Exp. Sta. Bull. 168.
- Pederson, C.S. 1979. Fermented vegetable products. In "Microbiology of Food Fermentations," 2nd ed., p. 153. Avi Publishing Co., Westport, CT.
- Pederson, C.S. and Albury, M.N. 1969. The sauerkraut fermentation. N.Y. State Agr. Exp. Sta. Bull. 824.
- Stamer, J.R., Stoyla, B.O., and Dunckel, B.A. 1971. Growth rates and fermentation patterns of lactic acid bacteria associated with sauerkraut fermentation. *J. Milk Food Technol.* 34: 521.
- Sumner, J.B. and Somers, G.F. 1944. "Laboratory Experiments in Biological Chemistry." Academic Press, New York.
- Ms received 11/10/82; revised 1/21/83; accepted 1/26/83.

Presented at the 42nd Annual Meeting of the Institute of Food Technologists, Las Vegas, NV, June 22-25, 1982.

Paper no. 8585 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, NC.

Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Dept. of Agriculture or North Carolina Agricultural Research Service, nor does it imply approval to the exclusion of other products that may be suitable.

This investigation was supported in part by a research grant from Pickle Packers International, Inc., St. Charles, IL.

The technical assistance of Mrs. Suzanne Armstrong and the cooperation of Mr. R.L. Thompson in doing statistical analysis of the data are greatly appreciated.