Differential Glucose and Fructose Utilization During Cucumber Juice Fermentation

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ABSTRACT: Glucose (32 mM) and fructose (36mM) of cucumber juice were degraded simultaneously by *Lactobacillus plantarum,* but at different rates and extents. Glucose depletion was slightly more rapid than fructose during the exponential growth phase, but slower thereafter and stopped before exhaustion. In contrast, fructose degradation continued until all naturally present fructose was exhausted. When cucumber juice was supplemented with fructose and/or glucose, the starter culture continued to ferment fructose, but not glucose, resulting in an increase in lactic acid production and a decrease in terminal pH. Fructose utilization was not affected by the presence of glucose, but the presence of fructose reduced glucose utilization.

Key Words: lactic acid bacteria, lactic acid, cucumber, fermentable sugars

Introduction

SUGAR CONTENT IN PICKLING CUCUMBERS VARIES WITH CULTIvars and fruit sizes, ranging in total concentration from 0.71 to 5.2% (McCreight and others 1978; McCreight and Lower 1978). Generally, cucumbers contain about 2% fermentable sugars (Handley and others 1983), and rarely higher than 3% (McCombs and others 1976). Nearly all the sugars present in cucumbers are reducing sugars. Glucose and fructose are the 2 predominant sugars, and their molar concentrations are approximately equal (Fleming and others 1988). Sucrose is present only in trace amount (Handley and others 1983). No other fermentable sugars are detectable in cucumbers (McCombs and others 1976). Glucose and fructose are readily fermented by lactic acid bacteria (LAB; Pharr and others 1977). Development of the controlled fermentation process for pickling cucumbers has brought increased interest in the complete sugar utilization by lactic starter cultures during primary fermentation. Any sugars remaining after the lactic fermentation can serve as substrates for fermentative yeasts to grow, resulting in CO_2 production. This may cause bloater formation in the products, resulting in texture defects and serious economic losses to the pickle industry (Fleming and others 1995). Incomplete conversion of fermentable sugars to lactic acid may also result in post-processing microbial growth in nonpasteurized products (Daeschel and Fleming 1987).

Many factors influence the ability of LAB to completely ferment cucumber sugars, including the initial sugar content. Some investigators (Jones and others 1940; Veldhuis and others 1941) showed that addition of sugar to the brine increased the incidence of bloating in cucumber fermentation, suggesting that high sugar content led to more residual sugars for yeasts to use in secondary fermentation, resulting in bloater formation.

Generally, glucose is a preferred energy source over fructose and other carbon sources by many microorganisms (Fisher 1987; Pastan and Adhya 1976; Postma and Lengeler 1985; Chater 1984; Demain and others 1983). However, our previous study showed that residual glucose, but not fructose, was often present at the end of cucumber fermentation, although natural glucose concentration was slightly lower than fructose concentration (Lu 1999). Passos and others (1994) noted that at high concentrations, glucose was de-graded faster than fructose in cucumber juice fermentation by *Lactobacillus plantarum* MOP3, which is the parental strain of the starter culture used in our study. A study of frozen-thawed *L. plantarum* (Raccach and Marshall 1985) reported that *L. plantarum* utilized more fructose than dextrose in Modified All Purpose Tween Broth. The objective of this study was to determine the differences in rate and extent of glucose and fructose utilization during cucumber juice fermentation by a malolactic-deficient strain of *L. plantarum,* MOP3-M6. This strain does not produce CO_2 from malic acid (a natural acid present in cucumbers), and is being investigated for potential commercial use. Knowing the difference in glucose and fructose utilization in cucumber fermentation and understanding the underlying mechanism are practically important in developing a system for complete conversion of cucumber sugars to lactic acid and traces of other end-products during cucumber fermentation.

Materials and Methods

Growth media

Cucumber juice from size 2 fruit (dia = 2.7 to 3.8 cm) was used as a model fermentation system in this study. Fresh pickling cucumbers from the cultivar, Cross Country, were obtained from a local farmer. The cucumbers were carefully sorted for uniformity of size and shape, and freedom from mold growth and mechanical damage and then washed in a reel washer. Cucumber juice was prepared by freezing the fresh cucumbers at −20 °C overnight and then partially thawing and blending to a homogeneous slurry (Daeschel and others 1988). The juice was collected by expressing it from particulate matter in the slurry through cheesecloth, and then stored at −20 °C. When needed, the juice was removed from the freezer and thawed. The thawed juice was then heated in a water bath to 80 °C and rapidly cooled down to room temperature. After centrifugation at 10,000 × g for 20 min, the supernatant juice was collected, and then diluted with cover brine so that the medium contained 55% juice, which simulates the pack-out ratio of 55/45, cucumber/ brine (w/w), used in the pickle industry for bulk fermentation and storage. The cover brine contained 4.4% NaCl, 39

mM $Ca(OH)_2$, and 137 mM acetic acid. After equilibration, the diluted cucumber juice contained 2% NaCl, 17.5 mM $Ca(OH)_2$, and 61.6 mM acetic acid, and the pH was 4.84. The diluted juice was then supplemented with glucose and/or fructose at various ratios (Table 1) to make cucumber juice broths (CJB). Each CJB was then filter-sterilized (0.22 :μm, Costar Bottle Filter, Costar Corp., Cambridge, Mass., U.S.A.).

Culture

MOP3-M6 was used in these studies. This culture is a mutant of *L. plantarum* MOP3 isolated from fermenting cucumbers (Fleming and others 1988). The mutant was obtained by the procedure of Daeschel and others (1984), which involved exposure to N-methyl-N'-nitro-N-nitrosoguanidine and isolation from a differential medium. The culture was stored at -84 °C in MRS broth (Difco Laboratories, Detroit, Mich., U.S.A.) containing 16% glycerol. When needed, the frozen culture was streaked onto an MRS agar plate. After incubation at 30 °C for 2 d, an isolated colony was transferred into 5 mL of MRS broth supplemented with 2% NaCl. After growth at 30 °C for 1 d, 1 mL of the culture was transferred into 100 mL of MRS broth with 2% NaCl and incubated overnight at 30 °C. The culture was harvested at late log phase by centrifugation (Sorvall RC-5B; Du Pont Co., Wilmington, Del., U.S.A.) at 3000 × g for 10 min at 10 to 15 °C and then resuspended in 100 mL of 0.85% sterile saline.

Inoculation and fermentation

The inoculum culture (1%, by volume) was added to each 15-mL sterile plastic tube containing 10 mL CJB to give an initial cell population of approximately 10^6 colony-forming units per mL (CFU/mL). Then each tube was screw-capped tightly and statically incubated at ambient temperature (123 °C). All the treatments were in triplicate. A sample (1 mL) was taken at suitable time intervals until day 60 when acid concentrations and pH remained unchanged (from 45 d). All samples were stored at -20 °C for later analysis.

Analyses

Cell growth was followed during the course of the fermentation by plating on MRS agar with a spiral plater (Autoplate 3000; Spiral Biotech, Inc., Bethesda, Md., U.S.A.). After incubation at 30 °C for 2 d, viable cell count was determined with a colony counter (Protos Plus; Bioscience International, Rockville, Md., U.S.A.).

The NaCl concentration in brine was determined by titration with standard $AgNO_3$ using dichlorofluorescein as an indicator (Fleming and others, 1992). The pH was measured with a pH meter (Model 825 MP, Fisher Scientific Co., Pittsburgh, Pa., U.S.A.). Sugars, organic acids, and ethanol were determined by the HPLC method of McFeeters (1993). Each sample was centrifuged at 12,000 × g for 3 min in an Eppendorf 5415 microcentrifuge (Eastburg, N.Y., U.S.A.). The supernatant was collected and then diluted 25 or 50 times with distilled water and appropriate internal standards (isobutyric acid for acids and *meso*-erythritol for sugars and alcohols). Separation was achieved on a cation-exchange column (Aminex HPX-87H, Bio-Rad Laboratories, Richmond, Calif., U.S.A.) with a 0.8 mL/min flow of 3 mM heptafluorobutyric acid at 65 °C. A conductivity detector (model CDM-2; Dionex Corp., Sunnyvale, Calif., U.S.A.) and a pulse amperometric detector (model PAD-2; Dionex) were connected in series for detection of organic acids and sugars.

Fermentation balances were determined by the calculation of carbon recovery according to the equation:

$$\% \text{ Carbon recovery} = (\text{mM carbon in products}/\text{mM carbon in substrates utilized}) \times 100.$$

It was assumed that glucose and fructose were the only substrates for producing lactic acid and acetic acid. There was no attempt to measure CO_2 since the starter culture is homofermentative and does not carry out a malolactic fermentation.

Results

The natural sugar content in the cucumbers used for making juice was 58 mM (1.04%) for glucose and 63 mM (1.14%) for fructose. After dilution with brine, CJB contained 32 mM natural glucose and 36 mM natural fructose. Figure 1 summarizes changes in the concentrations of substrates and product, viable cell count, and pH during the CJB fermentation without sugar supplementation. Glucose and fructose were utilized simultaneously (Figure 1a) by the starter culture. However, glucose depletion was slightly more rapid than fructose during the exponential growth and stationary phases, but slower thereafter, and finally stopped before complete depletion. In this case, only 23 mM natural glucose was utilized by the starter culture, resulting in 8 mM residual glucose at the end of fermentation. In contrast, fructose degradation continued until all naturally present fructose (36 mM) was exhausted. Most lactic acid was produced during
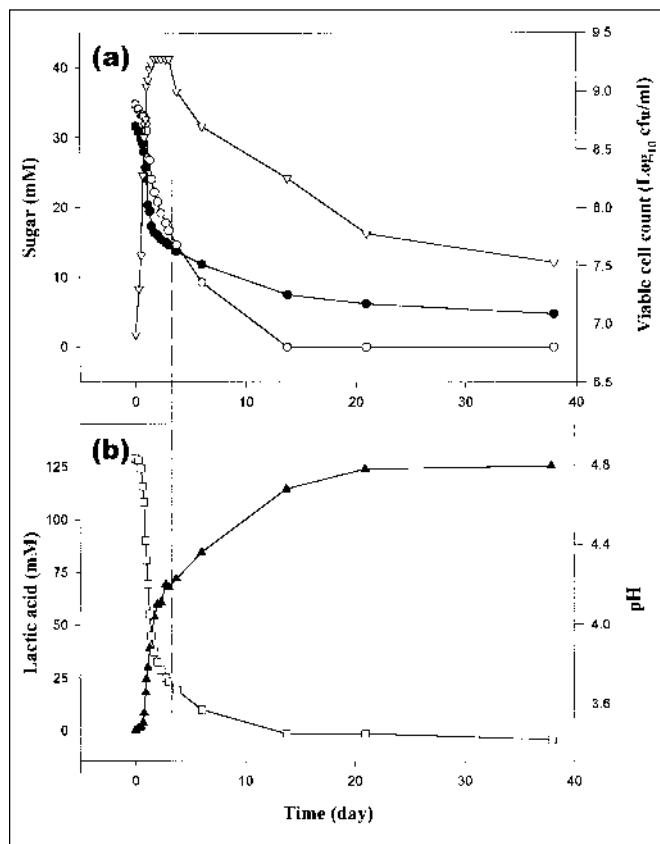

Figure 1—Substrate consumption, product formation, viable cell count, and pH during cucumber juice fermentation by MOP3-M6. ●, Glucose; ○, fructose; ▽, viable cell count; ▲, lactic acid; □, pH. All values represent the means of triplicate samples. The vertical dashed line (−) indicates conditions when cell count starts to decline.

the 1st 3 wk of fermentation. It was noted that lactic acid production continued when viable cell counts started to decline. Totally, 136 mM lactic acid was produced from the naturally present glucose and fructose in CJB, leading to a terminal pH of 3.5 (Figure 1b).

The extent of sugar utilization possible during the fermentation was studied by supplementation of each sugar to increase the initial sugar content up to 75 mM for glucose and 78 mM for fructose in CJB. The highest initial glucose and fructose concentrations were over 100% greater than the concentrations usually present in CJB with the pack-out ratio of 55/45, cucumbers/brine. It was found that the starter culture always utilized the same amount of glucose (23 mM), but all natural fructose was used whether or not the media were supplemented with glucose and/or fructose (Figure 2). Addition of glucose into the media resulted in more residual glucose in CJB. Surprisingly, when fructose or both fructose and glucose were added into CJB, fructose degradation continued until an additional 14 mM fructose was utilized; no additional glucose was utilized (Figure 2). The utilization of additional fructose resulted in a significant increase (30 mM more) in lactic acid production and 0.12 unit decrease in pH (Figure 3). Maximally, 23 mM glucose and 50 mM fructose were utilized by the starter culture, resulting in 165 mM lactic acid produced and terminal pH 3.37.

Approximately 10 mM acetic acid was produced during fermentation, but no ethanol was detected (data not shown). The concentration (9 mM) of natural malic acid remained unchanged during the fermentation (data not shown). The carbon recoveries ranged from 108 to 118% (Table 1).

Discussion

THE RESULTS CLEARLY DEMONSTRATED THAT GLUCOSE AND fructose were utilized at different rates and to different extents during cucumber juice fermentation by the starter culture (*L. plantarum* MOP3-M6). Much more fructose than glucose was utilized. Similar observations were made in the fermentation of whole cucumbers from different fruit sizes (Lu 1999). Moreover, the presence of glucose did not affect

Table 1—Sugar content effect on sugar utilization in cucumber juice fermentation.

Added		Initial		Utilized		
Glucose (mM)	Fructose (mM)	Glucose (mM)	Fructose (mM)	Glucose (mM)	Fructose (mM)	Carbon recovery (%)
0.00	0.00	31.73	36.44	23.51	36.44	117.58
14.24	0.00	45.97	36.37	22.95	36.37	116.10
28.62	0.00	60.35	36.37	22.14	36.37	114.40
43.26	0.00	74.99	36.97	21.37	36.97	115.52
0.00	14.69	31.74	51.13	23.67	48.02	110.97
0.00	28.07	31.47	64.51	22.69	48.88	112.41
0.00	41.36	31.62	77.80	22.88	48.40	113.84
7.67	6.51	39.37	42.95	24.03	42.95	112.37
14.78	14.00	46.51	50.44	23.74	50.44	108.36
29.27	28.02	61.00	64.46	23.01	48.53	109.20

fructose utilization. In contrast, more glucose was utilized when fructose was not present in cucumber juice (Lu 1999) or in modified MRS-based media containing the combination of glucose and fructose by the same starter culture used in this study (unpublished data). These results suggest that different mechanisms regulate the utilization of the two sugars by the starter culture.

Why did the starter culture ferment much less glucose than fructose in the fermentation? What factor(s) limited the ability of the starter culture to utilize more glucose? In homofermentative LAB, such as *L. plantarum*, glucose and fructose are transported into cells via the phosphoenolpyruvate (PEP)-dependent sugar phosphotransferase system (PTS) (Romano and others 1979; Thompson 1987) and metabolized via glycolysis (Embden-Meyerhof pathway) to produce primarily lactic acid (Gottschalk 1986; Thompson 1987). The metabolism of the two sugars differs only in the sugar transport step and the subsequent 1 or two steps of glycolysis (Figure 4). Glucose is transported into cells via the glucose-PTS (EII[glc]) and mannose-PTS (EII[man]) (Thompson 1987). The transport process is coupled to the phosphoryla-
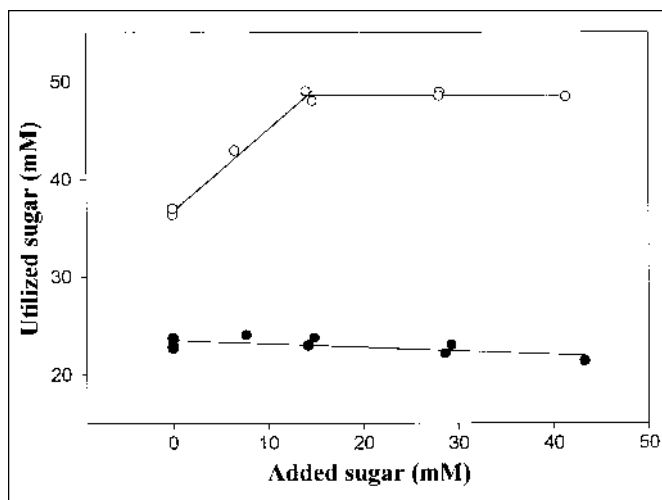
Fig 2—Effect of added sugars on glucose and fructose utilization in cucumber juice fermentation by MOP3-M6. ●, glucose; ○, fructose. All the values represent the means of triplicate incubations.
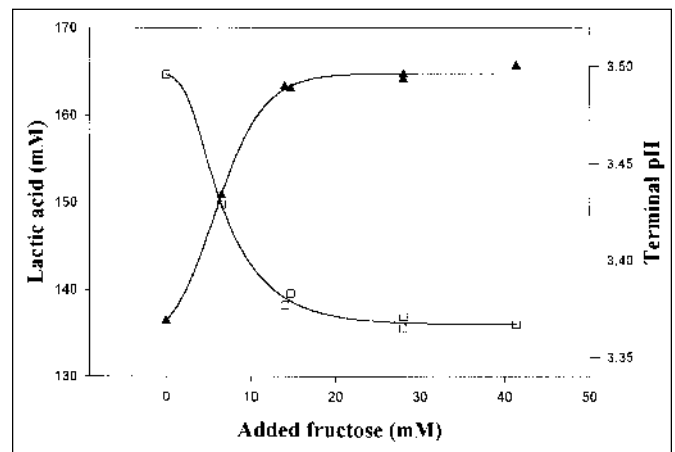

Figure 3—Effect of added fructose on lactic acid production and terminal pH in cucumber juice fermentation by MOP3-M6. ▲, lactic acid; □, terminal pH. All values represent the means of triplicate samples.

tion of glucose to glucose-6-phosphate, which in turn converts to fructose-6-phosphate. Fructose is transported into cells via EII[man] and fructose-PTS (EII[fru]), producing fructose-6-phosphate and fructose-1-phosphate, respectively (Thompson 1987). The study on *Lactococcus lactis* (Thompson and Thomas 1977) suggested that the mannose-PTS catalyses the transport and phosphorylation of sugars in the order: glucose > 2-deoxy-D-glucose > mannose > glucosamine > fructose. Therefore, the transport of glucose is much faster than the transport of fructose via mannose-PTS. Once fructose-1-P and fructose-6-P are converted to fructose-1,6-bisphosphate, the remaining steps of glycolysis are shared by glucose and fructose. It is not clear which steps play a significant role in making the starter culture utilize much more fructose than glucose. Since no more than 23 mM glucose was utilized, it seemed that either glucose transport or the subsequent glucose flow through the pathway was inhibited or shut down at a certain point of glucose catabolism. In *Escherichia coli*, PTS plays a central role in the regulation of carbon metabolism. The activity of enzyme EII in PTS mediates the regulation of the transport of PTS sugars (Fisher 1987). The PTS in *Bacillus subtilis* also appears to be involved in regulating carbon metabolism. In streptococci, the step of sugar transport could be one of the most important sites regulating the rate of glycolysis (Yamada 1987). It is unknown if that is also true in the starter culture used in cucumber fermentation. It is possible that the enzymes necessary for the transport and metabolism of glucose had relatively high optimal pH as compared to that for fructose, and thus their activities were more inhibited by lactic acid produced in the fermentation. Or perhaps fructose-1-phosphate competed for ATP with fructose-6-phosphate (F6P) and made ATP less available for F6P, thereby inhibiting glucose flow. Alternatively, the activities of 1-phosphofructokinase and 6-phosphofructokinase could be regulated by different mechanisms, resulting in the different extents of utilization of the 2 sugars. Which step in sugar metabolism was the most important site regulating glucose and fructose utilization and limiting the ability of the starter culture to utilize more glucose in the fermentation? The question remains fascinating and unanswered. A better understanding of glucose and fructose catabolism and their regulation mechanisms may be useful in developing a procedure to assure the complete sugar utilization during cucumber fermentation by the starter culture.
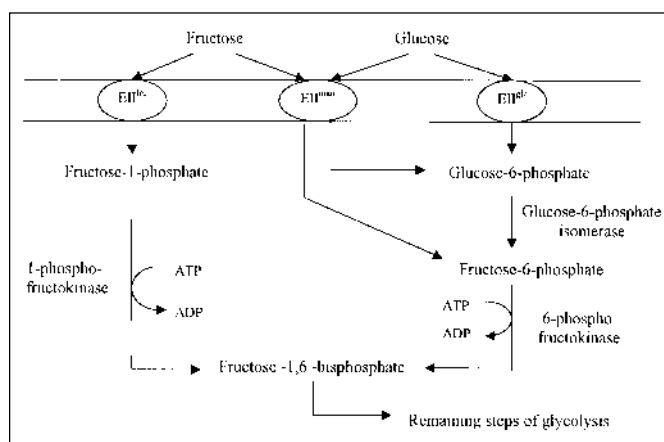
Since the starter culture was able to utilize 50 mM fructose and the presence of glucose did not seem to affect fructose utilization, residual fructose might not be present in most cucumber fermentations because cucumbers generally do not contain such high fructose content. Therefore, complete glucose utilization would be the major concern in developing a system to assure complete sugar utilization by the starter culture.

The starter culture entered the stationary growth phase perhaps due to low pH, product inhibition, nutrient limitation, or a combination of these factors. The acid production from stationary and death phases is probably due to energy generation for the maintenance of cell viability. Cell maintenance involves survival or preservation of cell integrity (or viability) and growth potential (Neijssel and Tempest 1976), which is not directly related to the synthesis of a new cell (Neidhardt and others 1990; Russell and Cook 1995). Several workers demonstrated a maintenance energy requirement by LAB (Giraud and others 1991; Kemp and others 1989).

The fact that malic acid concentration remained the same in the fermentation verified that the starter culture was malolactic-deficient. It is not clear why the carbon recoveries exceeded 100% of the theoretical value from hexoses present in the cucumbers. But the observation was consistent with those reported by Fleming and others (1983, 1988) and McDonald and others (1993).

The extent to which the differential glucose and fructose utilization phenomena observed in this study exists among other strains of *L. plantarum*, as well as other species and genera of LAB, is a subject for future investigation. The fact that the MOP3-M6 culture was achieved by mutagenesis raises the issue of its distinguishing metabolic characteristics from its parent strain, MOP3. When parent and mutant strains were compared, lag and generation times were significantly (P ≤0.05) longer for the mutant strain, slightly less sugar was fermented, slightly less lactic acid was formed, and malic acid was not utilized (McDonald and others 1993). Similar relationships existed between another mutagenized *L. plantarum* strain (WSO-M35) and its parent. In other studies on cucumber juice fermentation by the MOP3 parent strain (Passos and others 1994), the rate of glucose utilization was initially higher than that for fructose, but, as the sugar concentration was reduced during fermentation, the rate of fructose utilization was higher. This effect is similar to the sugar utilization pattern found in the MOP3-M6 mutant strain, as reported in the current study. There seems to be no major physiological differences in the way sugars are utilized by the parent and mutant strains. It would be of interest, however, to learn how the extent of sugar utilization relates to malic acid utilization, and if the same relationship exists with naturally existing malate-negative and -positive strains. Regardless of those fundamental questions, the MOP3-M6 mutant has potential commercial value, which we are continuing to investigate.

Figure 4—The 1st steps of glucose and fructose metabolism in homofermentative LAB (adapted from Thompson 1987).

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