Effects of Anions and Cations on Sugar Utilization in Cucumber Juice Fermentation

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ABSTRACT: The extent of glucose and fructose utilization during cucumber juice fermentation was affected differentially by the addition of 10 to 360 mM of selected anions (chloride, nitrate, sulfate, phosphate, acetate, lactate, and citrate) and cations (Na^+, K^+, NH_4^+, Ca^{2+}, Mg^{2+}, and Mn^{2+}). Inorganic anions generally suppressed utilization of both sugars, particularly at higher concentrations. Acetate and lactate increased utilization of fructose, but not glucose, while citrate increased utilization of both sugars. Of the cations tested, 10-60 mM Mn^{+2} significantly ($P < 0.05$) increased utilization of both sugars, but higher concentrations reduced utilization, as compared with the control. Evidence indicates that brine composition can significantly influence sugar utilization during cucumber fermentation, and may be important in developing controlled fermentation strategies for brined cucumbers.

Keywords: *Lactobacillus plantarum,* cucumber, sugar utilization, anions, cations

Introduction

VEGETABLE FERMENTATIONS TYPICALLY INVOLVE LACTIC ACID PROduction by lactic acid bacteria (LAB) naturally present on the raw materials. *Lactobacillus plantarum* is the most acid-tolerant LAB in vegetable fermentations (Pederson and Albury 1961; Fleming 1982). It is the organism that predominates during the final stage of natural cucumber fermentation. The comparatively low growth-limiting internal pH and ability to maintain a pH gradient across the cell membrane at high organic acid concentration may contribute to its acid-tolerance (McDonald and others 1990). However, the bacterium is frequently inhibited prior to complete conversion of cucumber sugars to lactic acid mainly due to low pH from massive acid production during the fermentation. Incomplete fermentation may allow fermentative yeasts to grow and produce carbon dioxide, which can cause bloater formation in the products, resulting in texture defects and economic losses to the pickle industry (Fleming and others 1995).

Acetate buffer has been added to cucumber fermentation brine to maintain a desirable pH range, and hence to prolong the activity of the starter culture and increase sugar utilization in cucumber fermentation (Etchells and others 1973; Fleming and others 1988). However, addition of acetate buffer does not always result in complete fermentation (Passos and others 1994; McDonald and others 1993), suggesting that factors other than pH may also affect the ability of the starter culture to ferment all cucumber sugars. Certain types and concentrations of buffers may have some adverse effect on the growth of the fermenting LAB. Russell (1992) and Berason and others (1997) suggested that intracellular accumulation of weak acid anions is toxic to bacterial cells. Certain acid anions may be more toxic than others.

In addition to weak acid anions, other ions may affect growth and metabolism by LAB. Like all other bacteria, LAB require certain chemical elements for biosynthesis, enzyme activities, and transport systems (Gottschalk1986). In nature, most of the bioelements occur as salts, and they are readily taken up by LAB as anions such as chloride and sulfate, and cations such as sodium, potassium, and ammonium, respectively. Several studies have shown that manganese ions stimulate growth, enhance lactic acid production, and reduce the fermentation time by *L. plantarum* (Moller 1939; Raccach and Marshall 1985; McDonald and others 1991). Cations including Na^+, K^+, Ca^{2+}, and Mg^{2+} are cofactors of many enzymes involved in transport and metabolic processes (Lehninger 1950; Raccach 1985; Yamada 1987). Axelsson (1993) reported that some LAB at low internal pH (pH_{in}) exchange intracellular protons for extracellular potassium ions, thereby increasing pH_{in} and maintaining pH homeostasis in lactic fermentation. Some studies (Abrams 1965; Abrams and Baron 1968) have shown that binding of H^+-translocating ATPase to the membrane was dependent on multivalent cations, such as Mg^{2+}, Ca^{2+}, and Mn^{2+}. The types and concentrations of anions and cations in the medium affect the growth and activities of LAB. Generally, $Cl^{!}$, $NO_3^{!}$, $SO_4^{2!}$, $PO_4^{3!}$, Na^+, K^+, NH_4^+, Ca^{2+}, and Mg^{2+} are required in large amounts by the organisms, while others such as Mn^{2+} and Zn^{2+} are needed only in small or trace amounts. No comprehensive research has been published on the effects of various ions on the growth and fermentative activity of LAB associated with vegetable fermentations.

The objective of this study was to investigate the effects of 7 anions (chloride, nitrate, sulfate, acetate, phosphate, lactate, and citrate) and 6 cations (Na^+, K^+, NH_4^+, Ca^{2+}, Mg^{2+}, and Mn^{2+}) on sugar utilization in cucumber juice by a strain of *L. plantarum* being evaluated for controlled fermentation of cucumbers. The results provided valuable information for developing a controlled fermentation system to assure complete fermentation for microbial stability of the products.

Materials and Methods

Cucumber juice

Cucumber juice from size 2 fruits (diameter = 2.7 to 3.8 cm) was used as a model fermentation system in the study. Fresh pickling cucumbers from the cultivar, Cross Country, were obtained from a local farmer (near Mt. Olive, N.C., U.S.A.). The cucumbers were carefully sorted for uniformity of size and shape and the absence of mold growth or mechanical damage before being 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 form a homogeneous slurry (Daeschel and others 1988). The juice was collected by filtering the slurry through cheesecloth, distributing into jars of convenient sizes, and then storing at - 20 °C. When needed, the juice was removed from the freezer and thawed. The thawed juice was heated in a water bath to 80 °C, and then rapidly cooled to room temperature. After centrifugation at 10,000 × g for 20 min, the supernatant juice was collected. This juice was diluted to 55% (by volume) with deionized water to yield the cucumber juice broth (CJB) used in this study. This CJB contained 32 mM glucose and 36 mM fructose.

Stock solutions

Stock solutions (2.0 M) of acids (hydrochloric, nitric, sulfuric, phosphoric, acetic, lactic, and citric acids) were prepared for studying anion effects. Stock solutions (2.0 M) of cation chlorides (NaCl, KCl, NH_4Cl, $CaCl_2$, $MgCl_2$, and $MnCl_2$) were prepared for studying cation effects. A stock NaOH solution (2.0 M) and a stock sugar solution (containing 8.33% glucose and 8.33% fructose) were also prepared. All chemicals used in the study were from Aldrich Chemical Company, Inc. (Milwaukee, Wis., U.S.A.).

Fermentation media for studying ion effects

Cucumber juice was supplemented with additional glucose and fructose by adding an appropriate amount of stock sugar solution to achieve concentrations of 50 mM glucose and 55 mM fructose. The purpose of supplementing medium with sugars was to determine the extent of glucose and fructose utilization in cucumbers that may contain sugar concentrations higher than the model cucumber juice used in this study. For study of anion effects, appropriate amounts of each acid stock acid solution were slowly added to the juice along with NaOH stock solution to control the pH of the juice within the range of 3.0 to 4.7. After all of a stock solution was added, the juice was adjusted to pH 4.74 ± 0.09 and the volume adjusted with deionized water. A pH of 4.8 was chosen because that is the pH that was proposed for controlled fermentation of cucumbers in anaerobic tanks (Fleming and others 1988). Maintenance of a pH of 4.8 or lower (actually 4.6 or lower according to FDA 1979) is required of acidified foods as assurance against growth and toxin production by *Clostridium botulinum*. The acetate buffer added to the brine is sufficient to maintain this pH until growth and acid production by the added starter culture. The added anion concentration in CJB varied between 10 to 360 mM. Supplemented CJB was sterilized by filtration through a 0.22 ìm filter (Costar, Cambridge, Mass.,U.S.A.). There was no control in the study of anion effects because the natural pH of cucumber juice was 5.96 and there was no way to decrease the pH to 4.8 (the initial pH for all treatments) without introducing any acid anions.

For study of cation effects, appropriate amounts of cation chlorides were added to the glucose/fructose-supplemented CJB, adjusted to pH 4.74 ± 0.08 with 3.7% HCl, and then filter-sterilized. The added salt concentrations in the media were in the range of 10 to 360 mM.

Culture

A malolactic-deficient strain of *L. plantarum,* MOP3-M6 (obtained by the procedure of Daeschel and others 1984), was obtained from the culture collection maintained by the U.S. Food Fermentation Laboratory (Raleigh, N.C., U.S.A.). This strain does not produce CO_2 from malic acid (a natural acid present in cucumbers) and is being investigated for commercial use. The culture was stored at - 84 °C in MRS broth (Difco Laboratories, Detroit, Mi.,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 days, 1 isolated colony was picked and transferred into 5 mL of MRS broth supplemented with 2% NaCl. After growth at 30 °C for 24 h, 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, De., U.S.A.) at 3000 × g for 10 min at 10-15 °C and then resuspended in 100 mL of sterile 0.85% NaCl.

Inoculation and fermentation

The inoculum (1% by volume) was added to give an initial cell level approximating 10^6 colony-forming units per mL (CFU/mL) in the brine/cucumber mass. After inoculation, all CJB treatments were incubated at ambient temperature (23 oC). All treatments were in triplicate. A sample (1 mL) was taken aseptically from each medium immediately after inoculation and on d 45 and d 60 of incubation. Only data for the 60-d sampling are reported in this paper. These data were found to be nearly identical to the 45-d sampling data, thus indicating completion of the fermentation. All samples were stored at - 20 °C for later analyses.

Analyses

The pH was measured with a pH meter (model 825 MP, Fisher Scientific Co., Pittsburgh, Pa., U.S.A.). Sugars and organic acids were determined by the HPLC method of McFeeters (1993). Each sample was centrifuged at 12,000 × g for 3 min in a microcentrifuge (Eppendorf 5415, 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 rate 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.

Statistical analysis

The 1-way analysis of variance procedure of SAS (SAS Institute, Inc., Cary, N.C.) was used to compute the statistics used to make inferences.

Results and Discussion

THE EFFECTS OF INORGANIC ACID ANIONS ON GLUCOSE AND FRUCtose utilization are shown in Figure 1a and 1b, respectively. When CJB contained 10 mM of added inorganic anions, sugar utilization in different treatments was almost the same. About 12 to14 mM glucose and 23 to 26 mM fructose were utilized. At elevated anion concentration (60 to 360 mM), glucose and fructose utilization decreased, except that at 60 to 180 mM chloride or 60 mM phosphate, fructose utilization increased slightly. The decrease in sugar utilization varied with specific anions and their concentrations. Addition of nitrate always resulted in lower sugar utilization than for other anions tested. At the same anion concentration, the ionic strength of nitrate was only one-third that of sulfate (Table 1). Lactic acid production as well as sugar utilization in CJB containing nitrate was lower than CJB containing sulfate which had much higher ionic strength, suggesting that the inhibitory effect on the starter culture was due more to the specific anion effect than to ionic strength. Generally, *L. plantarum*

Table 1—Anion effects on terminal sugar utilization, lactic acid production and pH in cucumber juice fermentation[a]

Anion		Initial		Utilized		Produced	
	mM	Ionic strength[b]	Glucose (mM)	Fructose (mM)	Citrate (mM)	Lactate (mM)	Terminal pH
Chloride	10	0.01	12.46	26.17		96.90	3.14
Chloride	60	0.06	13.98	33.13		112.79	3.07
Chloride	180	0.18	12.40	30.03		97.66	3.08
Chloride	360	0.36	8.61	24.00		77.81	3.13
Nitrate	10	0.01	12.62	22.60		86.92	3.20
Nitrate	60	0.06	12.08	20.56		81.48	3.18
Nitrate	180	0.18	7.31	11.44		51.56	3.32
Nitrate	360	0.36	0.59	5.55		26.23	3.54
Sulfate	10	0.03	14.71	25.58		94.34	3.16
Sulfate	60	0.18	11.35	24.72		91.03	3.16
Sulfate	180	0.54	6.59	20.37		74.38	3.22
Sulfate	360	1.08	3.53	22.67		78.74	3.19
Phosphate	10	0.01	13.09	26.73		99.30	3.15
Phosphate	60	0.06	14.22	29.09		98.90	3.20
Phosphate	180	0.18	6.39	17.70		71.08	3.43
Phosphate	360	0.36	7.12	16.00		65.93	3.59
Acetate	10	0.01	12.17	29.21		109.04	3.20
Acetate	60	0.03	13.22	41.53		132.29	3.47
Acetate	180	0.10	12.37	48.00		144.38	3.93
Acetate	360	0.19	13.05	55.70		157.05	4.23
Lactate	10	0.01	12.32	32.04		110.57	3.54
Lactate	60	0.05	9.97	33.07		110.59	3.53
Lactate	180	0.16	6.16	38.04		150.69	3.83
Lactate	360	0.32	1.42	39.23		141.14	4.05
Citrate	10	0.02	12.12	31.03	9.05	123.53	3.36
Citrate	60	0.12	18.85	43.37	32.69	163.18	3.79
Citrate	180	0.37	28.40	52.30	34.74	199.13	4.13
Citrate	360	0.75	26.12	22.59	16.48	118.40	4.54

[a]Cucumber juice (55%) with sodium salts of anions listed was inoculated with *L. plantarum* MOP3-M6 and incubated at 23 EC for 60 d. The initial pH was adjusted to 4.74 ± 0.08 with NaOH.
[b]The initial ionic strength was calculated based on initial pH 4.80.

strains are not able to reduce nitrates. Rare strains may reduce nitrates provided the concentration of glucose in the medium is limited and the pH is poised at 6.0 or higher (Kandler and Weiss 1986). Since there was residual glucose (~40 mM) present at the end of fermentation and final pH was between 3.2 and 3.5, it was not likely that the starter culture would reduce nitrate to nitrite. Therefore, the inhibitory effect of nitrate could not be explained by nitrite toxicity.

The effects of organic acid anions on glucose and fructose utilization are shown in Figure 2a and 2b, respectively. When 10 mM of organic anions was present in CJB, regardless of type, glucose utilization (12 mM) was similar to, but fructose utilization (29-32 mM) was higher than those when 10 mM inorganic acid anions were present. Interestingly, these organic anions had distinctly different effects on glucose utilization (Figure 2a). At elevated concentrations (60 to 360 mM), addition of citrate significantly (P 0.05) increased glucose utilization; addition of acetate did not affect glucose utilization, and addition of lactate decreased glucose utilization. At 60 to 180 mM, all of these organic anions increased fructose utilization (Figure 2b). Further increasing concentrations of acetate and lactate to 360 mM resulted in further utilization of fructose, while 360 mM citrate decreased fructose utilization sharply. In most cases, much more fructose than glucose was metabolized by the starter culture. These results suggest that the metabolism of glucose and fructose are regulated by different mechanisms. A detailed discussion is given in a separate paper (Lu and others 2001).

The maximal sugar utilization (28 mM glucose and 52 mM fructose) and lactic acid production (199 mM lactic acid) were achieved in CJB containing180 mM citrate, but the starter culture was still unable to utilize all the natural glucose (32 mM) in the CJB. Citrate concentration decreased during the fermentation (Figure 3). Accordingly, acetic acid production increased proportionally (Figure 3), indicating that citrate was co-metabolized with cucumber sugars during the fermentation. Co-metabolism of glucose and citrate by *Leuconostoc mesenteroides* was observed by Cogan (1987). He reported that the co-metabolism resulted in a growth advantage relative to growth on glucose alone. It was not previously known that the starter culture used in this study was able to co-metabolize citrate. Generally, citrate metabolism produces lactate, acetate, and CO_2 (Gottschalk1986; Starrenburg and Hugenholtz 1991; Marty-Teysset and others 1996). Cselovszky and others (1992) reported that succinate also was formed when carbohydrates were limited. We did not detect succinate, perhaps because carbohydrates were in excess in our study. The production of CO_2 was not measured in this study, but would be undesirable because it contributes to bloater formation in the fermented product.

It was noted that the terminal pH increased with increasing concentration of organic acid anions (Table 1). This apparently was due to the buffer effect associated with these organic acid anions. However, high buffer capacity and high terminal pH did not always result in high sugar utilization and lactic acid production. CJB containing 360 mM citrate had a much higher buffer capacity and terminal pH (4.5), but its fructose utilization (Figure 2) and acid production (Table 1) were much lower than that in CJB having 180 mM citrate. This suggested that pH was not the only factor influencing the fermentative activity of the starter culture, and buffer agents themselves at a high concentration had inhibitory effects on the starter culture.

Table 2—Cation effects on terminal sugar utilization, lactic acid production, and pH in cucumber juice fermentation[a]

Added salt			Utilized		Produced	
		Ionic	Glucose	Fructose	Lactate	Terminal
	mM	strength[b]	(mM)	(mM)	(mM)	pH
Control	0	0.00	16.74	31.92	98.5	3.13
NaCl	10	0.01	17.96	32.49	93.1	3.12
NaCl	30	0.03	17.18	31.42	96.5	3.10
NaCl	60	0.06	17.86	30.92	96.0	3.11
NaCl	180	0.18	15.94	32.79	94.2	3.05
NaCl	360	0.36	10.73	22.54	65.9	3.16
KCl	10	0.01	17.97	32.17	98.1	3.12
KCl	30	0.03	16.42	34.25	96.9	3.08
KCl	60	0.06	18.40	31.27	98.1	3.11
KCl	180	0.18	14.91	31.84	93.6	3.09
KCl	360	0.36	13.77	30.14	79.8	3.11
NH_4Cl	10	0.01	18.26	32.59	96.2	3.11
NH_4Cl	30	0.03	19.21	34.91	98.5	3.08
NH_4Cl	60	0.06	20.09	33.39	99.7	3.10
NH_4Cl	180	0.18	17.49	32.20	90.2	3.07
NH_4Cl	360	0.36	13.37	27.52	74.1	3.11
$CaCl_2$	10	0.03	18.94	33.14	100.7	3.07
$CaCl_2$	20	0.06	20.28	34.52	102.1	3.04
$CaCl_2$	60	0.18	17.08	28.03	84.2	2.99
$CaCl_2$	120	0.36	13.54	24.27	73.6	2.92
$CaCl_2$	180	0.54	12.36	20.51	61.7	2.90
$CaCl_2$	360	1.08	7.27	10.56	31.1	3.00
$MgCl_2$	10	0.03	16.45	31.59	98.1	3.08
$MgCl_2$	20	0.06	15.72	30.91	97.7	3.05
$MgCl_2$	60	0.18	14.84	27.14	86.1	3.02
$MgCl_2$	120	0.36	13.72	27.08	82.9	2.92
$MgCl_2$	180	0.54	11.62	21.87	70.1	2.92
$MnCl_2$	10	0.03	23.93	38.42	119.9	2.97
$MnCl_2$	20	0.06	23.68	40.36	122.8	2.91
$MnCl_2$	60	0.18	20.56	41.56	121.6	2.80
$MnCl_2$	120	0.36	17.74	31.45	101.1	2.75
$MnCl_2$	180	0.54	14.91	21.79	73.9	2.77

[a]Cucumber juice (55%) with chloride salts of cations listed was inoculated with *L. plantarum* MOP-M6 and incubated at 23 EC for 60 d. The initial pH was adjusted to 4.74 ± 0.08 with HCl.
[b]The ionic strength was calculated based on the concentration of added salt.

In contrast to organic acid anions, inorganic acid anions have no or little buffer action. As lactic acid was produced, the pH of CJB decreased rapidly. When a similar amount of lactic acid was produced, the terminal pH was lower in CJB containing inorganic acid anion than those in CJB containing organic acid anions (Table 1). It was observed that the terminal pH in CJB increased with phosphate concentration. However, this increase was mainly due to the decrease in lactic acid production. The buffer effect of phosphate was not appreciable during the fermentation because the pK_as (2.12 and 7.21) of phosphate were too far (more than 1 pH unit) away from initial pH (4.8) and terminal pH in CJB. The fact that the terminal pH in CJB increased slightly with nitrate concentration was simply due to lower lactic acid production.

The cation effects on glucose and fructose utilizations are shown in Figure 4 and 5, respectively. The dotted lines in the figures indicate glucose or fructose utilization in the control (without added cations). As compared to the control, addition of 10-20 mM Mn^{2+} in the medium significantly ($P > 0.05$) increased the utilization of glucose (from 17 to 24 mM) and fructose (from 32 to 40 mM) and consequent lactic acid production (from 98 to 123 mM) (Table 2).

Generally, cucumbers contain about 0.014 mM Mn^{2+} (USDA 1998) which is only 4% of Mn^{2+} (0.33 mM) present in MRS broth for cultivation of lactic acid bacteria. Addition of 10 to 20 mM Mn^{2+} into the media provided sufficient Mn^{2+} for the growth of the starter culture and, hence, greatly increased sugar utilization and lactic acid production. McDonald and others (1991) observed pronounced changes in cell density and lactic acid production by *L. plantarum* when 10 mM Mn^{2+} was added in the modified HHD medium.

Mn^{2+} plays several important roles in the activity of LAB. It is a cofactor of several kinases and an enolase in the EMP glycolysis pathway (Lehninger 1950; Thomas 1976) and may also be an activator of phosphoenolpyruvate phosphotransferase system (PEP:PTS) in sugar transport (Raccach 1985). In addition, Mn^{2+} is involved in an important defense mechanism against oxygen toxicity. Most LAB are able to accumulate large concentration of intracellular Mn^{2+}. Mn^{2+} at high intracellular concentration (10 to 25 mM) is functionally equivalent to superoxide dismutase (SOD) in scavenging destructive superoxide radicals (Archibald and Fridovich 1981a, 1981b; Stanier and others 1986). *Lactobacillus plantarum* uses millimolar levels of Mn^{2+} to scavenge the superoxide radical, much as most other organisms use micromolar levels of SOD (Archibald and Fridovich 1981a, 1981b). Mn^{2+} is also a component of Mn-containing pseudocatalase produced by some LAB, including *L. plantarum,* to mediate the decomposition of H_2O_2 to H_2O and O_2 (Kono and Fridovich 1983a, 1983b; Stanier and others 1986). Moreover, Mn^{2+} was specifically required in RNA and protein syntheses (Eichhorn and others 1980; Stetter and Zillig 1974). It may also play a role in stabilizing the native

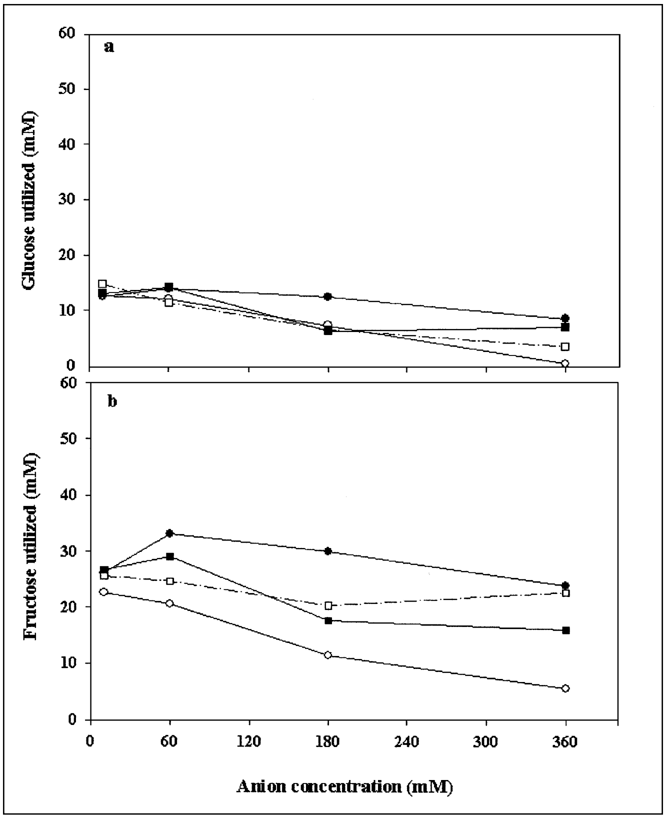

Figure 1—Effect of inorganic acid anions on utilization of glucose (a) and fructose (b) in cucumber juice fermentation: ● = chloride, □ = sulfate, ■ = phosphate, ○ = nitrate. The initial pH was adjusted to 4.8 with NaOH. Values represent the mean values for three replicates.

conformation of ribosomes (Lyttleton 1960; Tal 1969), cell membranes and cell walls (Chipley 1974). It is possible that some other unknown mechanisms may also be involved.

It was noted that increasing Mn^{2+} concentration from 10 to 60 mM caused slightly greater fructose utilization, but reduced glucose utilization. Addition of 120 mM Mn^{2+} resulted in similar sugar utilization to that in the control. Further increasing Mn^{2+} concentration resulted in a decrease in utilization of both sugars, suggesting that high concentration of Mn^{2+} had an inhibitory effect on the starter culture. Raccach (1985) reported that 0.145 mM was in the range of Mn^{2+} providing maximum stimulation of the lactic fermentation by frozen-thawed *L. plantarum* in modified All Purpose Tween broth. Further work is needed to determine the minimum concentration of manganese required for complete sugar utilization in cucumber fermentation. It was observed that the pH of fermented CJB containing Mn^{2+} was below 3.0, indicating a strong acid stress encountered by the starter culture in the fermentation. It may be possible to reduce the acid stress by addition of buffer to the medium and, thus, to further increase sugar utilization. More research is needed to better understand the mechanism of manganese influencing sugar utilization and to learn the combined effect of Mn^{2+} and buffer on sugar utilization. In addition, it may be useful to use plant materials or their extracts as a source of Mn^{2+}. Spices such as clove, cardamom, ginger, celery seed, cinnamon, and turmeric were found to strongly stimulate acid production by LAB due to their high Mn^{2+} content (Zaika and others 1978; Zaika and Kissinger 1984).

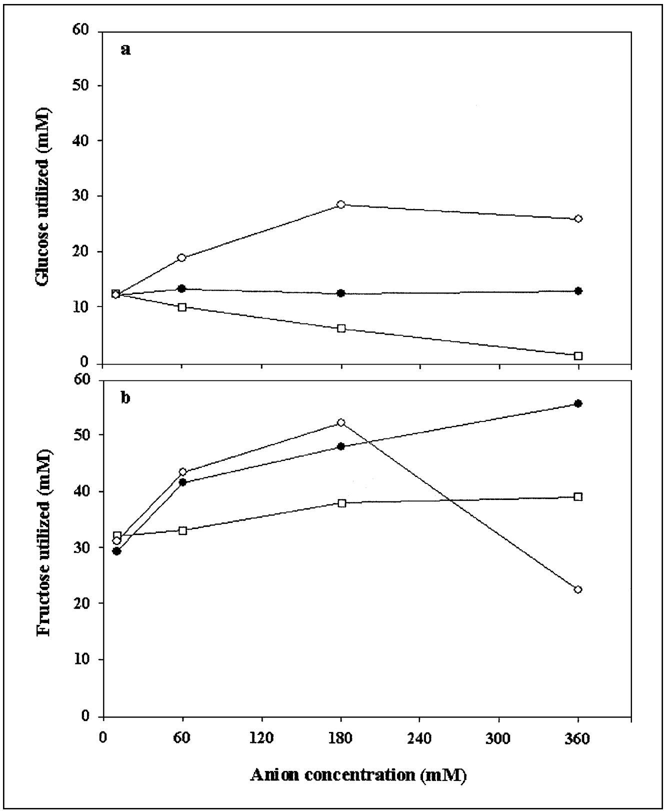

Figure 2—Effect of organic acid anions on utilization of glucose (a) and fructose (b) in cucumber juice fermentation: ○ = citrate, ● = acetate, □ = lactate. The initial pH was adjusted to 4.80 with NaOH. Values represent the mean values for three replicates.

Tomato, cabbage, and asparagus juices also stimulate the growth of LAB (Snell and Lewis 1953; Stamer and others 1964).

Addition of 10 to 60 mM of other cations had a slight or no stimulatory effect on glucose or fructose utilization (Figure 4 and 5) and lactic acid production (Table 2) as compared to the control. Addition of Mg^{2+} at concentrations tested (10-180 mM) decreased sugar utilization. It is possible that Mg^{2+} inhibited certain enzymes involved in sugar transport and or metabolism.

Besides the specific cation effect on the cells, addition of any tested cations at high concentration (180 to 360 mM) decreased overall sugar utilization, indicating that high concentration of the cations had inhibitory effects on the starter culture. High concentration of cations (in the form of salts) results in high osmotic pressure, which alters the content and composition of cell walls and membranes and consequently the nutrient transport system (Csonka 1989). High cation concentration may also inhibit essential enzymes activity and thus cell growth (Neidhard and others 1990). The results in Figures 4 and 5 show that both glucose and fructose utilizations were lower when 180 mM of divalent cations (Ca^{2+} or Mg^{2+} or Mn^{2+}) were added than when the same concentration of any monovalent cations (Na^+ or K^+ or NH_4^+) was added, possibly due to the higher ionic strength of the former. It was shown that when ionic strength of Mn^{2+} was higher than 0.36 or ionic strength of other cations was higher than 0.20, sugar utilization was lower than that in the control (Table 2, Figures 4 and 5).

The control and CJB containing added cations all had terminal pH values lower than 3.2 (Table 2). Fermented CJB containing high concentration of cations, especially bivalent cations, had low acid concentrations and also low terminal pH. Apparently, the low pH was not totally due to the acid produced in the fermentation. It was partially attributed to high ionic strength in the medium, which altered hydrogen ion activity. Evidence indicates that brine composition can significantly influence sugar utilization during fermentation of cucumber juice. The findings may be important in developing controlled fermentation systems for brined cucumbers. Perhaps the combination of manganese with acetate buffer could further increase sugar utilization by the starter culture. Further research is needed to determine

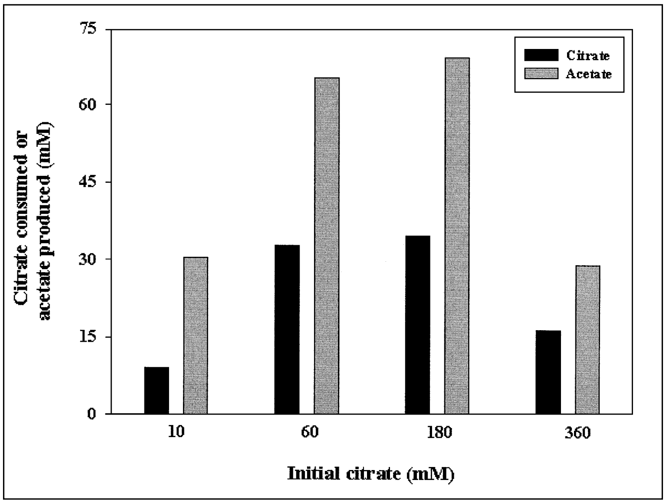

Figure 3—Citrate consumption from added citrate and acetate production from the metabolism of citrate and sugars in cucumber juice fermentation. Values represent the mean values for three experiments.

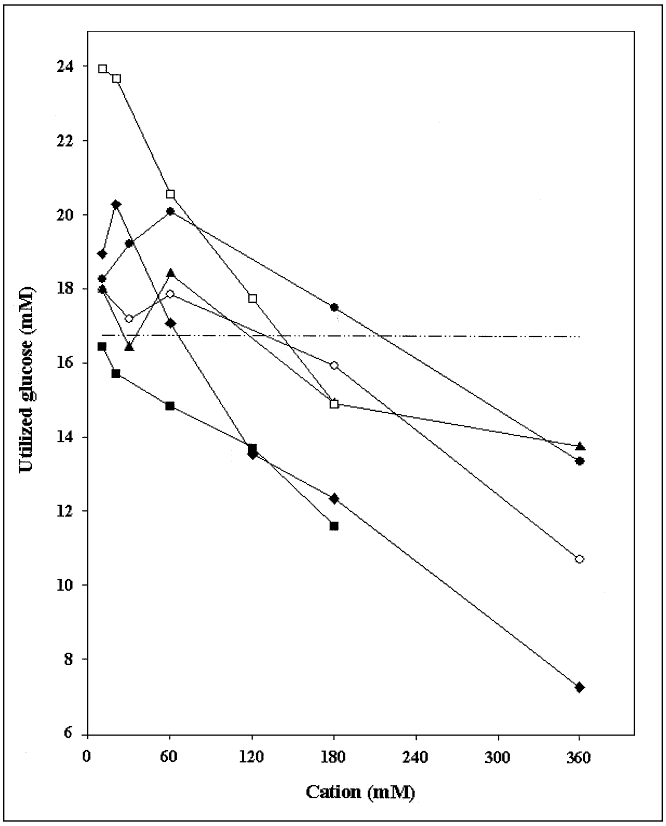

Figure 4—Effect of cations on glucose utilization in cucumber juice fermentation: ○ = sodium, ▲ = potassium, ● = ammonium, ◆ = calcium, ■ = magnesium, □ = manganese. The horizontal dotted line indicates glucose utilization in the control. Values represent the mean values for three experiments.

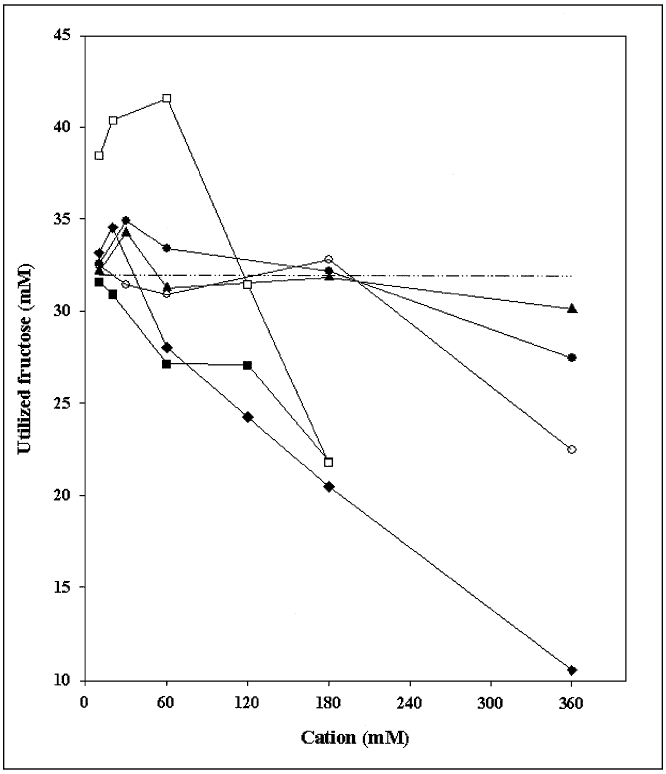

Figure 5—Effect of cations on fructose utilization in cucumber juice fermentation: ○ = sodium, ▲ = potassium, ● = ammonium, ◆ = calcium, ■ = magnesium, □ = manganese. The horizontal dotted line indicates fructose utilization in the control. Values represent the mean values for three experiments.

the optimum composition of the cover brine of cucumbers to achieve complete sugar utilization and the desired terminal pH 3.5 for microbial and textural stability of the products.

Conclusions

SUGAR UTILIZATION BY THE STARTER CULTURE VARIED WITH ANIONS and cations in the fermentation media. Addition of inorganic acid anions showed little or no beneficial effect on sugar utilization. Nitrate had a strong inhibitory effect on the starter culture. Addition of organic acid anions increased fructose utilization but did not consistently increase glucose utilization. Addition of 10 to 20 mM Mn^{2+} significantly increased both glucose and fructose utilizations. Addition of other cations had little or no stimulatory effect on sugar utilization.

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Corrections to 20010049, Effects of Anions and Cations on Sugar Utilization in Cucumber Juice Fermentation

Page 1, Author line: Correct name is S. S. Yoon.

Page 1, right column, line 14: Correct notations are CI^{-}, NO_3^{-}, SO_4^{-2}, PO_4^{-3}

Page 2, Abstract, line 8: Change “for brined cucumbers” to “to assure microbial stability.”

Page 2, left column: Correct space between lines 21 and 22

Make Fermentation media...heading same font size as Stock solutions
Line 32: After an appropriate amount...
Line 34: An initial pH of...
Line 44: 0.22 µm

Page 2, right column, line 16: 23°C

Page 5, right column, line 15: membrane and, consequently,

Page 7, right column: Realign the USDA. 1998 reference:
Namely: “peel, raw. In: USDA food composition...”