A Fermentor for Study of Sauerkraut Fermentation*

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A fermentor was designed and constructed for study of the physical, microbiological, and chemical changes that occur during the sauerkraut fermentation. The fermentor has some essential features that include restriction in volume of the sauerkraut bed, construction of clear plastic to permit visual determination of liquid-level changes as a result of gas entrapment within the sauerkraut bed, and a gas-lift device for use in nitrogen purging of the fermenting brine. Fermentations exhibited two distinct stages, the first one gaseous and the second nongaseous. The gaseous stage was characterized by rapid CO_2 and acid production due to growth by heterofermentative lactic acid bacteria with resultant gas entrapment within the sauerkraut bed and a rise in liquid level. Also, rapid disappearance of fructose and rapid appearance of mannitol occurred during this stage. The nongaseous stage was characterized by growth of homofermentative lactic acid bacteria with little or no CO_2 production and a gradual increase in lactic acid until all fermentable sugars were metabolized. Nitrogen purging appeared to offer several potential advantages, including a means for brine circulation, removal of CO_2 from the brine, and anaerobiosis to ensure retention of ascorbic acid, desirable color, and other oxygen-sensitive traits in sauerkraut.

INTRODUCTION

Fermentation has been used as a natural means for preserving cabbage and other vegetables for centuries. The fermentation of shredded cabbage to produce sauerkraut (kraut) remains an important means of preservation today because of the unique flavors produced and low energy requirements for storage and processing. The per-capita consumption of sauerkraut in the United States has steadily declined since the 1930s.[1] Some consumers prefer the strong, acidic flavor of sauerkraut as traditionally produced, but it has been suggested that milder flavored kraut could increase consumer demand.[2] Also, the flavor and other quality attributes of kraut are subject to wide variability due to vagaries in the fermentation. Uniformity and high quality are thought to be essential for significant expansion of kraut usage in today's institutional and consumer markets. The sauerkraut fermentation has been well characterized microbiologically and technologically.[3,4] However, the basic methods for fermenting sauerkraut have not changed greatly over centuries.

Sauerkraut is produced in the United States in bulk tanks ranging in capacity from 45 to 150 tons. Shredded cabbage is dry salted and conveyed into the tanks. The cabbage is quickly surrounded by brine that is formed by osmotic release of water from the shreds that are contacted by salt. The shredded, salted cabbage is then covered with plastic sheeting that is draped over the tank wall and is weighted down with water or weak brine. This covering prevents contact of the kraut with air, which can be a cause of quality loss. During the first week after packing, the kraut is subject to the problem of "heaving," which is characterized by an increase in volume of the kraut due to an early gaseous fermentation. As the kraut expands vertically, the plastic sheeting is displaced, and air is no longer excluded from the kraut. The tank may require partial draining to lower the kraut bed, followed by leveling and re-covering. Alternatively, the kraut may be weighted down with stones to reduce the problem of heaving. The kraut undergoes fermentation until all of the fermentable sugar is converted to acids and other end products or the kraut is removed for processing. Processors may remove kraut for processing as early as a few weeks after tanking when an acceptable level

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of acidity is reached or may hold it in inexpensive bulk storage for a year or until needed. This variability in time of holding the kraut before processing results in variability in the amount of acid formed since fermentation may continue if sugars remain. The kraut may become excessively acid upon prolonged fermentation. The product may be diluted to the desired acidity when it is removed for processing, but this dilution results in variability of desirable flavor compounds and nutrients such as vitamin C.

Other methods have been used in the tanking of sauerkraut to maintain product quality, in addition to that in current commercial use. The Chinese fermentation jar, with a cover that fits into a water-filled moat to exclude air, has been in home use for over 2000 years.[5] Noel et al.[6] pointed out that heterogeneity in salt concentration through the kraut caused by the dry salting methods used can result in variations in microbial fermentation, some of which may be undesirable. They reported that circulation of the brine overcame problems caused by such heterogeneity. An anaerobic fermentor with a means for liquid circulation and a vacuum system to remove CO_2 has been patented.[7]

Objectives of this study were to design, construct, and test a laboratory fermentor for use in characterization of the physical, microbiological, and chemical changes that occur during sauerkraut fermentation. In addition, a nitrogen gas-lift device was incorporated into one fermentor to study the potential benefits of nitrogen purging of the brine on properties of sauerkraut. Data obtained from the two fermentations reported herein provide rationale for further studies in our efforts to develop controlled fermentation methods for the complex sauerkraut fermentation.

MATERIALS AND METHODS

Fermentors

Two fermentors were designed as shown in Fig. 1 and constructed of materials summarized in Table I. Only one of the fermentors included the exterior eduction tube for nitrogen introduction and brine circulation. A metric measuring tape was secured to the outside wall to aid in determining changes in the liquid and decreases in the kraut bed volumes. The basic fermentation vessel is similar geometrically to the laboratory fermentor for kraut depicted by Steinbuch.[8] However, the present fermentor differs functionally in that it is constructed of clear Plexiglas, which permits visual-

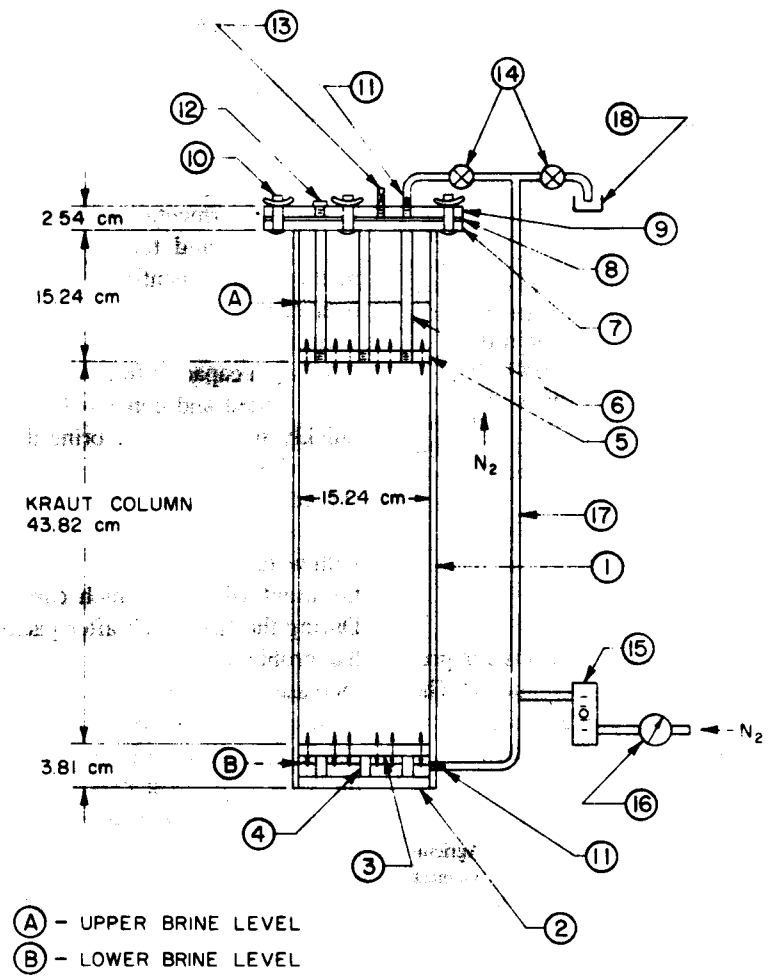

Figure 1. Design of sauerkraut fermentor. See Table I for description of components.

Table I. Component list for fermentor illustrated in Figure 1.

Component	Description
1	fermentation vessel; cylindrical Plexiglas, clear, 15.24 cm i.d., 0.64 cm wall, by 108 cm long
2	bottom end plate; Plexiglas, 1.27 cm thick, bonded to cylinder wall
3	bottom retainer plate; Plexiglas, 1.27 cm thick, perforated with 40 holes, 3.2 mm diameter to allow free movement of brine between kraut bed and bottom reservoir, removable from cylinder
4	support rods (3); Plexiglas, 1.27 mm diameter × 1.27 cm long, bonded to bottom retainer plate
5	top retainer plate; identical to bottom retainer plate, prevents kraut bed from expanding vertically
6	top restraining rod (3); Plexiglas, 1.27 cm diameter, prevents top retaining plate from rising in cylinder
7	locking flange; Plexiglas, 1.27 cm thick, bonded to outside cylinder wall
8	gasket; rubber, 3.2 mm thick, seals top cover to locking flange
9	top cover; Plexiglas, 1.27 cm thick by 22.86 cm diameter, removable to allow access to cylinder
10	bolt and wing nut (6); nylon, 9.5 mm diameter for attaching top cover
11	barbed fitting; PVC, for 6.25 mm tygon tubing, threads into Plexiglas
12	sample port for brine via hypodermic syringe; rubber stopper, tapered, 4 mm diameter at small end
13	fermentation gas lock (Presque Isle Wine Cellars, North East, PA 16428) to allow venting of gases; lock is filled with saturated NaCl brine
14	cutoff valves; PVC, to direct brine to return to fermentor or to exterior for measurement of liquid flow rate
15	flowmeter for nitrogen gas
16	pressure regulator for nitrogen supply
17	exterior eduction tube; tygon, 9.5 mm diameter, into which N_2 is injected and through which brine is circulated by gas lift
18	measuring cup to determine liquid flow rate

ization of changes in the kraut bed and expressed liquid levels, restricts the kraut bed to a maximum volume, provides a means for brine circulation and CO_2 removal by nitrogen injection, and ensures anaerobiosis of the headspace above the kraut and expressed liquid.

The packed fermentors were placed inside a controlled-temperature incubator. Light was excluded from the fermentors except for brief sampling periods.

Cabbage Handling

Fresh cabbage, cv. Green Boy, was trimmed of outer leaves. The trimmed cabbage (average of 803 g/head) was sliced 1 mm thick with a Hobart food slicer. The sliced cabbage was mixed with granular food-grade salt to obtain an equalized concentration of about 2% NaCl. The salted, sliced cabbage was packed by hand into the fermentors. The packing characteristics of the fermentors are given in Table II. After packing, the fermentors were incubated at 18.3°C for the course of the fermentation.

Microbiology

Samples (50 g) of raw cabbage, in duplicate, were blended with an equal weight of sterile 0.85% NaCl and serially diluted for plate counting. Samples of brine were taken aseptically with a sterile syringe with a needle that protruded through the fermentor top to approximately mid-depth of the kraut bed. Samples of fresh cabbage extract and of brine were enumerated for total aerobic microorganisms (Plate Count Agar, Difco), lactic acid bacteria (MRS Agar, BBL, with 0.02% sodium azide), total *Enterobacteriaceae* (violet red bile agar, Difco, +1% glucose), and yeasts (dextrose agar, Difco, +5 mL 10% tartaric acid/100 mL) according to general procedures described or referred to previously.[9] Pour plates were duplicated and incubated at 30°C. Brines and isolated colonies were observed by phase-contrast microscopy.

Chemical Analyses

Samples of the fresh cabbage were blended with an equal volume of water, filtered through filter paper, and then centrifuged at 12,800 *g* prior to high-performance liquid chromatography (HPLC) injection. Brine samples from the sauerkraut fermentations were centrifuged and diluted with water, as needed, before injection into the HPLC (Waters Associates, Milford, MA).

Sucrose, glucose, fructose, and mannitol were analyzed by HPLC using a Sugar Pak I column (7.8 × 250 mm, Waters Associates). The elution solvent was water. The column was held at 70°C in a water bath. Malic acid, lactic acid, acetic acid, and ethanol were also analyzed by HPLC using a Techsil C_{18} reversed-phase column (4.6 × 250 mm, Phenomenex, Rancho Palos Verdes, CA) with 0.05*M* phosphoric acid solution adjusted to pH 2.5 with NH_4OH.

Table II. Characteristics of packed sauerkraut fermentors.

Parameter	Fermentor	
	Purged	Nonpurged
Cabbage added, g	8987	8704
Salt added, g	180	174
Kraut bed, initial		
Height, cm	43.8	43.8
Volume, cm^3	7992	7992
Mass,[a] g	7895	7829
Density, g/cm^3	0.988	0.979
Brine volume		
Percentage of initial bed volume (day 0)	13.1	10.6
Percentage of initial bed volume (day 4)	24.9	23.9
Entrapped gas volume[b]		
Percentage of initial bed volume (day 4)	11.8	13.4
Changes in kraut bed volume, % initial		
Day 6	100	100
Day 7	96.7	100
Day 150	85.6	94.9

[a] Note that kraut bed mass, which is calculated value, is less than total mass added to fermentor because of liquid that was initially expressed from salted cabbage. This liquid was forced above top retaining plate during packing of fermentor and is accounted for as "brine volume: percentage of initial bed volume (day 0)."

[b] Calculated as difference in brine volume as percentage of initial bed volume at 0 and 4 days.

Separation of these compounds was carried out at room temperature (25°C). A refractive index detector was used for all compounds. Concentrations were determined relative to an external standard for each compound.[10]

Ascorbic acid concentrations were determined by a HPLC procedure similar to that described by Ashoor et al.,[11] except that a Techsil C_{18} reversed-phase column, as described in the preceding, was used for the separation. Samples of sauerkraut brine were immediately diluted 10-fold with 0.2*N* H_2SO_4, filtered through a 0.22-μm filter and analyzed the same day. Ascorbic acid was measured with a UV detector set at the absorption maximum of 245 nm. Concentrations were determined relative to a freshly prepared 20-μg/mL solution of ascorbic acid in 0.2*N* H_2SO_4. The possibility that significant amounts of dehydroascorbic acid might be present in the sauerkraut brines was checked by treating samples with dithiothreitol to convert any dehydroascorbic acid to ascorbic acid.[12] A 1.0-mL sample of diluted sauerkraut brine was transferred to a 1.5-mL centrifuge tube, 25 μL 1.0% bromcresol purple dissolved in 0.05*N* NaOH, 100 μL 10*mM* dithiothreitol, and 100 μL 0.2*M* K_2HPO_4 were added in order to the tube. The pH was adjusted to approximately 6.1 by addition of 2.0*N* NaOH. This was indicated when the solution turned purple. This solution was immediately injected into the liquid chromatograph. No increase in ascorbic acid concentration was found after reduction with dithiothreitol, which indicated that dehydroascorbic acid was not present in the sauerkraut in significant amounts.

Dissolved CO_2 in the brines was measured by the Harleco Micro CO_2 assay kit.[13] Brine samples (2 mL) were taken by syringe and injected into a sealed vial containing 0.5 mL 2*N* NaOH. Subsamples (0.1 mL) were then analyzed by the Harleco method.

RESULTS AND DISCUSSION

Fermentor Performance

The fermentors functioned as anticipated and provided a means for studying the problem of heaving that is common in sauerkraut fermentation. Characteristics of the packed fermentors are summarized in Table II. The liquid level rose as fermentation became evident (1–2 days) in both the purged and nonpurged fermentors (Fig. 2). The rise in brine level was accompanied by an increase in the CO_2 concentration in both the purged (Fig. 2A) and nonpurged (Fig. 2B) fermentations. The rise in brine level reached a maximum at 4 days and amounted to about 11.8 and 13.4% of the initial kraut bed volume in the purged and nonpurged fermentors, respectively (Table II). The rise was assumed to be due to evolution of CO_2 within the kraut, which formed gas pockets, thus expressing liquid from the restricted kraut bed. The phenomenon is thought to be analogous to bloater formation in brine-fermented cucumbers, where CO_2 and other gases collect within the cucumber, causing liquid to be expressed as gas pockets are formed.[14,15] By day 7, however, the brine level in the purged fermentor had receded below the initial level and was accompanied by a decrease in CO_2 concentration. The kraut bed volume also decreased, as indicated by a drop in the level of the top retaining plate of the kraut bed (Fig. 2A). The kraut bed volume decreased further in the purged fermentor and was 85.6% of the initial bed volume after 150 days (Table II). In contrast, the brine level, CO_2 concentration, and kraut bed volume remained comparatively high over the 150-day period in the nonpurged fermentor (Fig. 2B; Table II). In the nonpurged fermentor gas pockets apparently remained within the kraut bed and prevented liquid from returning to the bed.

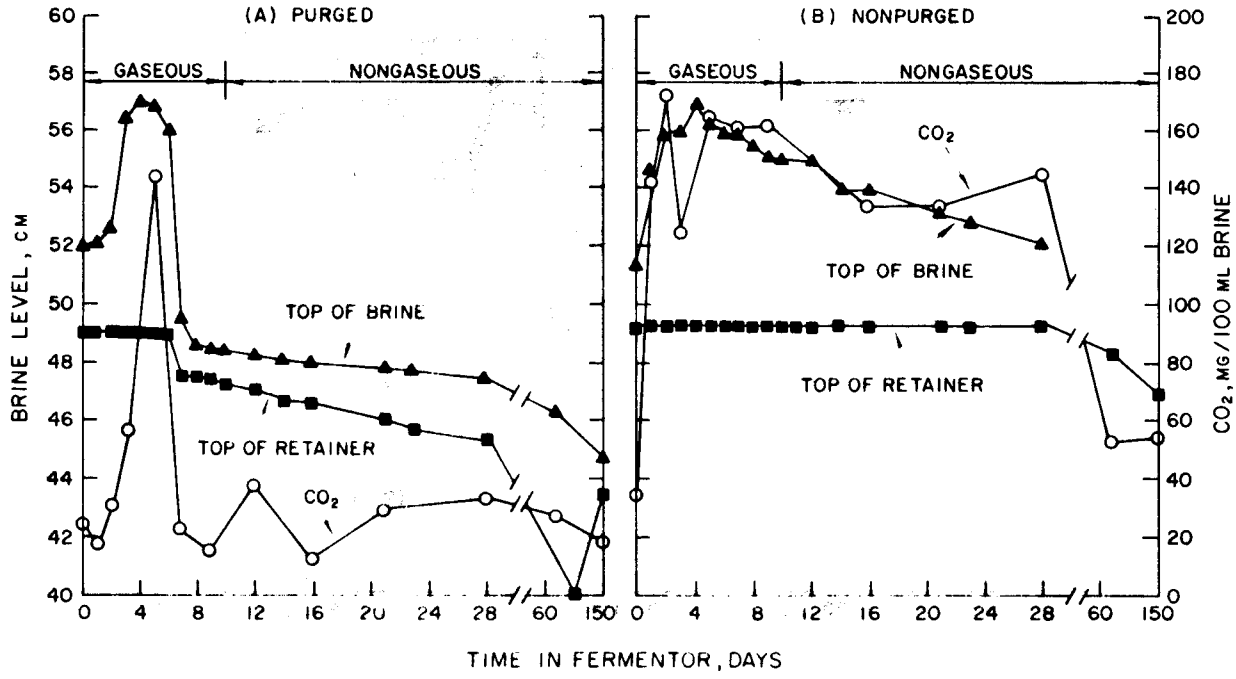

Figure 2. Changes in liquid and kraut bed levels and CO_2 concentration during fermentation.

Purging of fermenting kraut brine with resultant brine circulation appeared to relieve pressure within the kraut bed. However, the brine circulation rate used in this study apparently was insufficient to remove enough CO_2 from the kraut bed to completely exclude gas entrapment within the kraut bed. This conclusion was made on the basis of the rise in brine level and intermittent cessation in brine circulation. The brine circulation was about 90 mL/min for the first 2 days at a nitrogen flow rate of 400 mL/min but then essentially stopped for a period of 5 days, after which the brine circulation started again and varied in flow rate thereafter. Thus, it was speculated that CO_2 entrapment impeded liquid movement through the kraut bed. Studies are underway to determine parameters involved in liquid circulation through the kraut bed and alternative methods for effecting the circulation.

Data obtained from the two fermentations reported herein generally typify physical, microbiological, and chemical changes that we have observed in several runs using the fermentors. It is apparent from Fig. 2 that the kraut fermentations exhibited two stages based on CO_2 production. A *gaseous* stage occurred during the first few days followed by a *nongaseous* stage. Subsequent data will reveal the nature of other changes that distinguish these stages.

Microbiological Changes

The fresh cabbage contained 1.3×10^5 total aerobes, 3.9×10^3 total *Enterobacteriaceae*, 4.2×10^1 lactic acid bacteria, and <10 yeasts/g cabbage (averages of duplicate samples). Microbiological changes during fermentation are summarized in Fig. 3. Similar trends were observed in purged and nonpurged fermentations. The gaseous stage of the fermentation was characterized by rapid increase in total and lactic acid bacteria and a rapid increase followed by a rapid decrease in *Enterobacteriaceae*. Lactic acid bacteria isolated (20 isolates) during the gaseous stage (day 5) were all gas-forming cocci, typical of *Leuconostoc mesenteroides*. After reaching a maximum population at 4 days, the numbers of lactic acid bacteria decreased by a factor of 100 (Fig. 3) at 9 days.

The nongaseous stage of the fermentation was characterized by an increase in the numbers of lactic acid bacteria, which approximated the total numbers of bacteria. The 20 lactic acid bacteria isolates taken at the 28th day were all non-gas-forming rods, typical of *Lactobacillus plantarum*. *Enterobacteriaceae* were not detected (<100/mL) during this stage.

Extensive characterization of the kraut fermentation has indicated that the heterofermentative *L. mesenteroides* predominates the early stages, and *L. plantarum* predominates the later stages.[3,4] The present data are consistent with this well-established observation. Yeasts were not observed (<100/mL) in either fermentation during the 28-day period of examination.

The brine samples taken represent only the microorganisms suspended in the brine taken from the kraut bed at the time of sampling. Perhaps the higher numbers of lactic acid bacteria enumerated in the nonpurged, as compared to the purged, fermentation were influenced by differences in liquid movement.

Chemical Changes

The composition of the raw cabbage used in the fermentation is summarized in Table III. Glucose and fructose constituted most of the fermentable sugars in the cabbage leaves, with sucrose constituting only a comparatively small

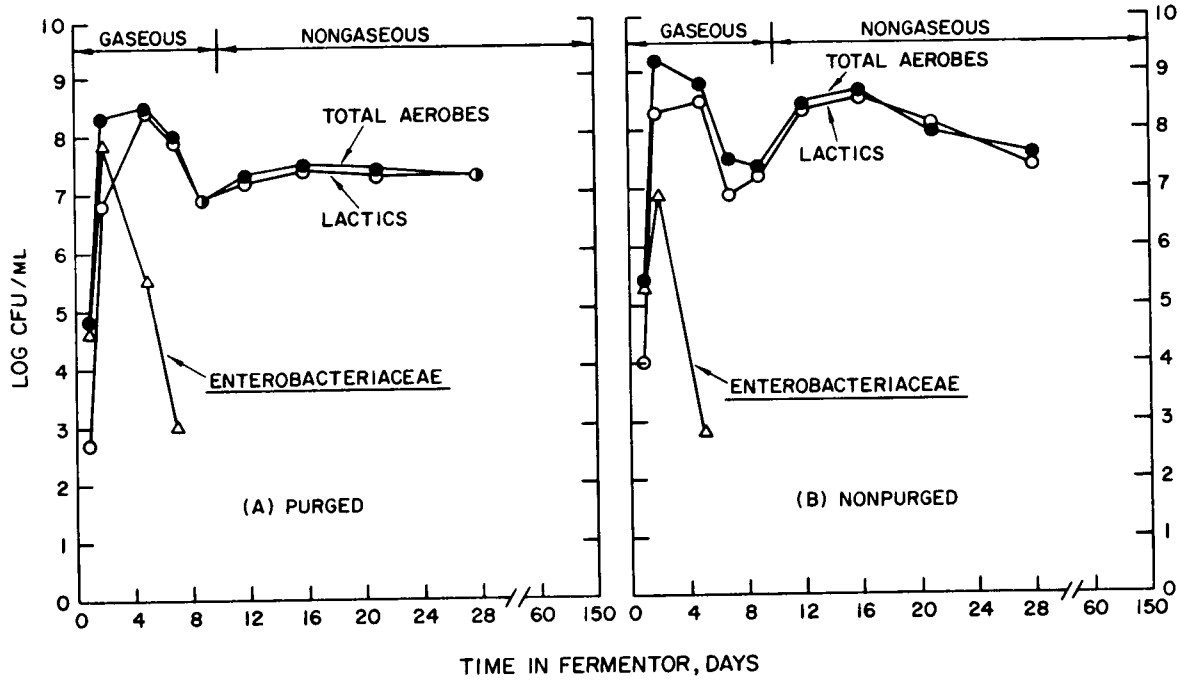

Figure 3. Microbiological changes during sauerkraut fermentation.

Table III. Composition of raw cabbage used in fermentations.[a]

Compound	Concentration[b] in leaves		Concentration[b] in core	
	m*M*	SD	m*M*	SD
Sucrose	7.0	4.6	53.1	16.4
Glucose	132.5	9.9	75.7	15.9
Fructose	114.2	3.2	60.3	7.3
Malic acid	12.2	1.6	7.1	3.0

[a] SD, standard deviation.
[b] Averages of four replicates.

amount. In the cabbage core, however, sucrose constituted a much greater fraction of the fermentable sugars. Malic acid concentration was higher in the leaves than the core. Overall, the core accounted for 23% and the leaves 77% of the cabbage weight.

During the gaseous stage of fermentation, fructose was depleted rapidly from the brine (Fig. 4). In contrast, glucose concentration increased up to 3–8 days and then gradually decreased. Sucrose was present in relatively small amounts. It should be noted that only the brine was sampled and that this brine was formed by osmotic release of water from the shredded cabbage that was in contact with the added dry salt. Apparently, water was released faster than glucose from the cabbage, accounting for the subsequent increase in glucose concentration. The rapid depletion of fructose was attributed to microbial conversion to end products, primarily mannitol, soon after its release into the brine surrounding the cabbage shreds.

The production of total titratable acidity increased rapidly during the gaseous stage, slowed, and then increased again, but at a slower rate in the nongaseous as compared to the gaseous stage of fermentation. A decrease in pH corresponded generally to the increase in acidity (Fig. 5).

Fermentation products measured included lactic and acetic acids, ethanol, and mannitol (Fig. 6). The pattern of substrate and product changes during the gaseous stage of fermentation were similar to those observed when green beans were fermented with heterofermentative lactic acid bacteria.[16] The rapid appearance of mannitol during the gaseous stage corresponded to the rapid disappearance of fructose. Heterofermentative lactic acid bacteria are known to reduce fructose to mannitol[3] and probably accounted for the rapid decrease in fructose concentration noted. Mannitol formed during the gaseous stage did not decrease in concentration during storage (Fig. 6). In the nitrogen-purged fermentor, the concentration had not changed appreciably after a year of storage at 18.3°C. In contrast, Hughes and Lindsay[17] reported that mannitol concentration decreased after 2–4 weeks in a commercial tank of kraut. It would be interesting to further establish the stability of mannitol in commercial tanks, if special microorganisms can adapt to its metabolism, and if handling conditions of the kraut can

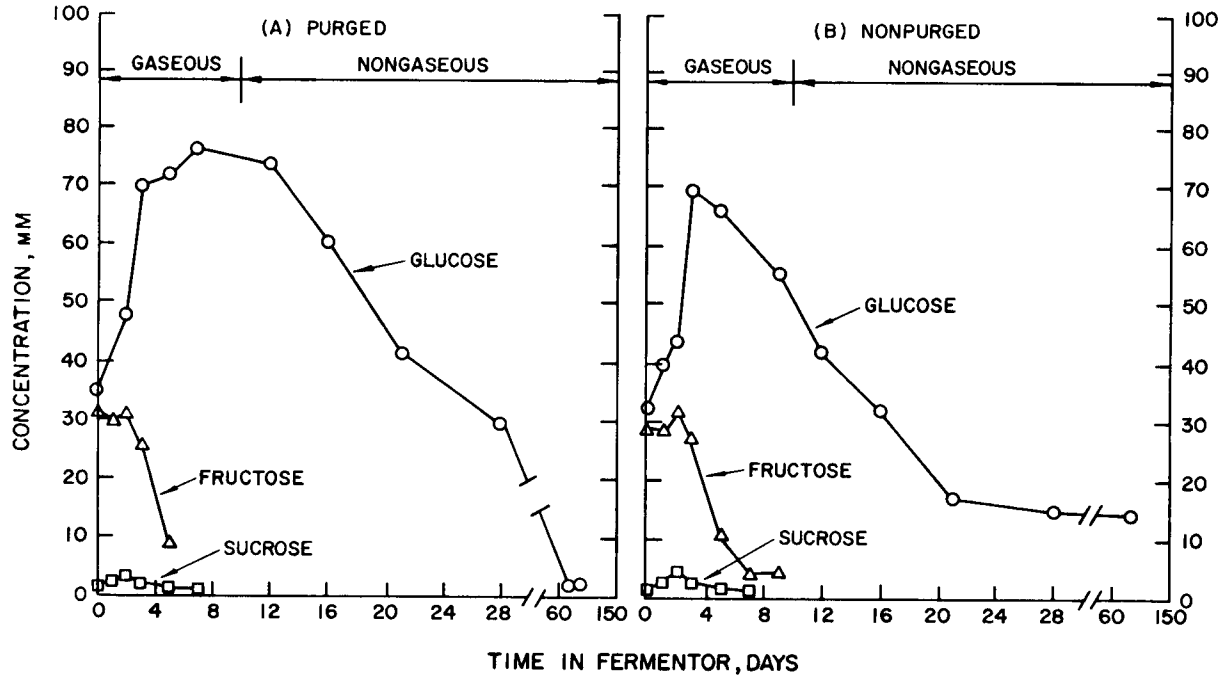

Figure 4. Substrate depletion during sauerkraut fermentation.

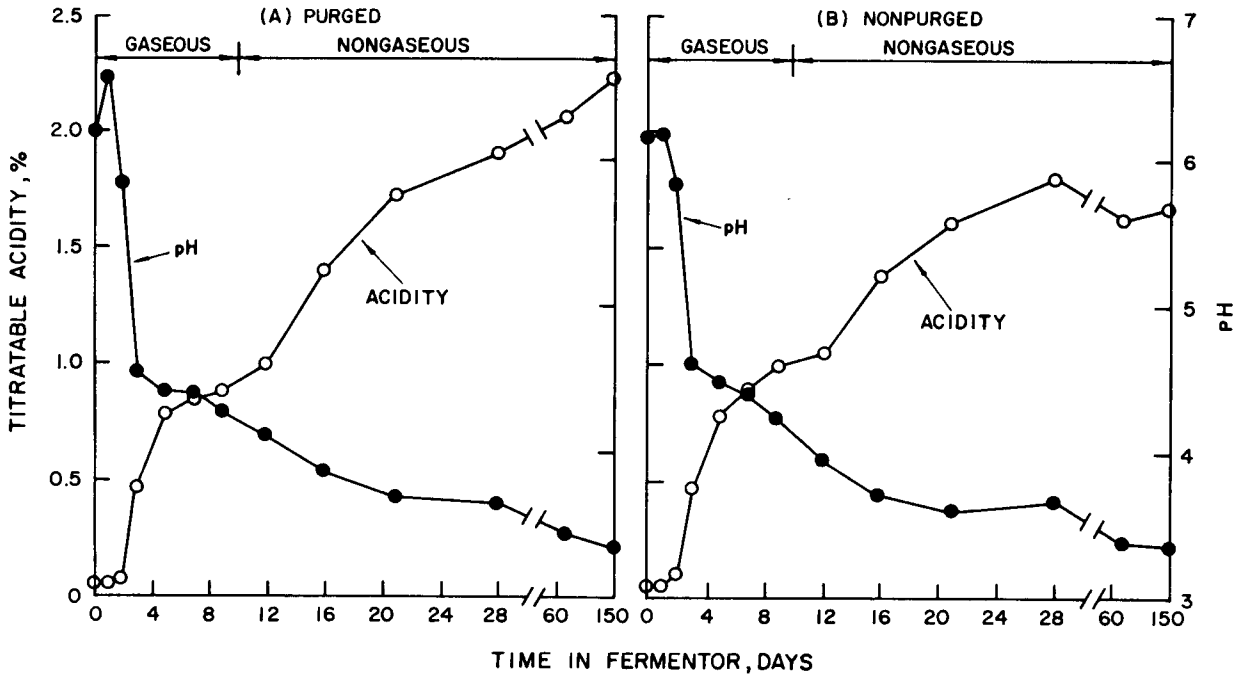

Figure 5. Titratable acidity and pH changes during sauerkraut fermentation.

foster its metabolism. Acetic acid and ethanol also were produced during the gaseous stage of fermentation, giving further evidence of a heterofermentation. Appearance of lactic acid generally followed the bimodal appearance of titratable acidity noted earlier (Fig. 5), with a comparatively rapid increase in concentration during the gaseous stage, followed by a slow increase in the nongaseous stage.

Ascorbic acid concentration was uniformly high throughout the bed of the purged kraut after 150 days, and the kraut had a uniformly bright, light straw color, as desired (Table IV). In contrast, ascorbic acid concentration in the nonpurged kraut was appreciably lower in the top of the fermentor; and this kraut had a gray color. Kraut in the bottom of this fermentor had an ascorbic acid concentration and light straw color comparable to the purged kraut. It was apparent that oxygen had contaminated the headspace of the nonpurged fermentor as mold had grown on the surface of the top retaining plate of the kraut bed. Thus, the presence of oxygen probably was responsible for darkening and loss of ascorbic acid in the nonpurged kraut. The mold growth

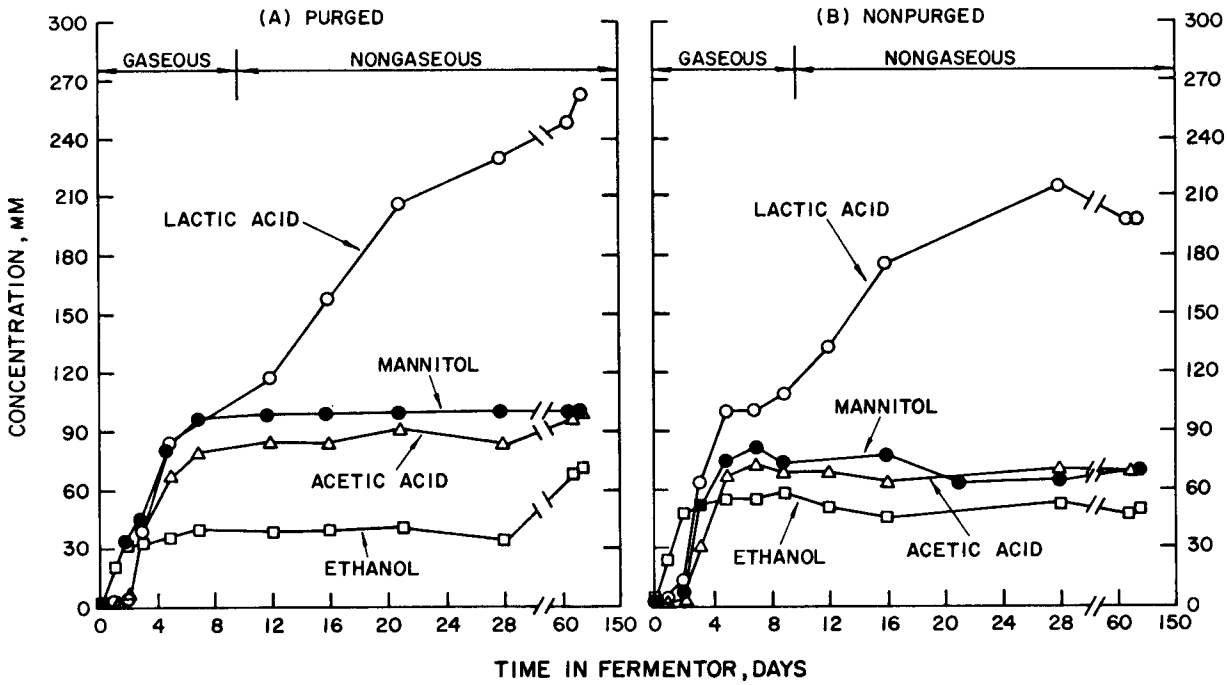

Figure 6. Product formation during sauerkraut fermentation.

Table IV. Ascorbic acid content and color of sauerkraut.[a]

Fermentor type and assay	Fermentor location		
	Top	Middle	Bottom
Purged			
Ascorbic acid, mg/100 mL	54.8 (0.3)	53.3 (0.4)	54.5 (0.2)
Color	light straw	light straw	light straw
Nonpurged			
Ascorbic acid, mg/100 mL	1.2 (0.5)	6.1 (0.1)	59.1 (0.6)
Color	dark gray	light gray	light straw

[a] Determined after 150 days of fermentation. Ascorbic acid values are averages of duplicate HPLC injections. Standard deviations are in parentheses.

and kraut darkening at the top of the nonpurged fermentor were not evident during the first 60 days. During the early stages of fermentation, CO_2 was liberated, which undoubtedly helped to ensure anaerobiosis. Similar problems have been observed in commercial kraut tanks during storage when the plastic cover sheet is dislodged, allowing air to contact the kraut.[3]

CONCLUSIONS

The fermentation of sauerkraut was observed to undergo a gaseous and a nongaseous stage of fermentation. The gaseous stage was characterized by brine unheaval; rapid production of CO_2; growth of heterofermentative lactic acid bacteria such as *L. mesenteroides;* rapid reduction of the pH; rapid increases in lactic and acetic acids, ethanol, and mannitol; and rapid depletion of fructose. The nongaseous stage was characterized by stabilization in brine upheaval; little if any production of CO_2; growth of homofermentative lactic acid bacteria such as *L. plantarum:* further increase in lactic acid until all of the remaining glucose was fermented and there was a gradual decrease in pH; and no significant changes in the acetic acid, ethanol, and mannitol concentrations. The fermentors designed for the study proved valuable in studying the problem of heaving due to gas entrapment within the sauerkraut bed during the gaseous stage of fermentation.

Purging of the sauerkraut brine with nitrogen appeared to serve several useful purposes. It afforded a means of brine circulation by gas-lift action, which could serve to render more uniformity of salt within the sauerkraut and thereby permit more uniformity in microbial activities. Nitrogen purging removed CO_2 from the brine, which attenuated the potential problem of brine upheaval. The nitrogen atmosphere maintained in the headspace of the sauerkraut excluded the presence of oxygen and resulted in preservation of ascorbic acid and a desirable color throughout the sauerkraut and precluded growth of aerobic microorganisms on the surface of the sauerkraut. Brine circulation was limited by the gas-lift mechanism, and other options should be