# Probiotic Culture Survival and Implications in Fermented Frozen Yogurt Characteristics

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## ABSTRACT

Low-fat ice cream mix was fermented with probiotic-supplemented and traditional starter culture systems and evaluated for culture survival, composition, and sensory characteristics of frozen product. Fermentations were stopped when the titratable acidity reached 0.15% greater than the initial titratable acidity (end point 1) or when the pH reached 5.6 (end point 2). Mix was frozen and stored for 11 wk at –20°C. The traditional yogurt culture system contained the strains *Streptococcus salivarius* ssp. *thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. The probiotic-supplemented system contained the traditional cultures as well as *Bifidobacterium longum* and *Lactobacillus acidophilus*. We compared recovery of *Bifodobacterium* by three methods, a repair-detection system with roll-tubes and plates on modified bifid glucose medium and plates with maltose + galactose reinforced clostridial medium.

Culture bacteria in both systems did not decrease in the yogurt during frozen storage. The roll-tube method with modified bifid glucose agar and repair detection system provided at least one-half $\log_{10}$ cfu/ml higher recovery of *B. longum* compared with recoveries using modified bifid glucose agar or maltose + galactose reinforced clostridial agar on petri plates. No change in concentrations of lactose or protein for products fermented with either culture system occurred during storage. Acid flavor was more intense when product was fermented to pH 5.6, but yogurt flavor was not intensified. The presence of probiotic bacteria in the supplemented system seemed to cause no differences in protein and lactose concentration and sensory characteristics.

(**Key words:** frozen yogurt, probiotic, bifidobacteria, acidophilus)

**Abbreviation key: MBGA** = modified bifid glucose agar; **MGRCA** = maltose + glucose reinforced clostridial medium.

## INTRODUCTION

Frozen yogurt is a low acid product, a shift from the high acidity in frozen yogurt products of the late 1970's (4). Schmidt et al. (22) reported a pH range of 5.76 to 6.72 and a titratable acidity (expressed as lactic acid) range of 0.20 to 0.43 for 11 commercial vanilla-flavored frozen yogurt products. No federal standards for frozen yogurt have been established, but several states have definitions for frozen yogurt, including a minimum titratable acidity. In general, industry practice is to achieve a minimum titratable acidity of 0.30% (4, 18) with a minimum of 0.15% titratable acidity resulting from fermentation by yogurt bacteria (18). This titratable acidity may be achieved by fermentation with a mixture of *Lactobacillus delbrueckii* ssp. *bulgaricus* (subsequently, *L. bulgaricus*) and *Streptocuccus salivarius* ssp. *thermophilus* (subsequently, *S. thermophilus*) or by standardization of the titratable acidity to the specified level by addition of yogurt with ice milk mix.

Consumer interest in frozen yogurt stems from the desirable nutritional properties attributed to the product (4, 8, 18). In addition to low-fat formulation, frozen yogurt supplemented with probiotic bacteria, such as *Lactobacillus acidophilus* and *Bifidobacterium longum*, provides additional health benefits. Health aspects attributed to the consumption of fermented dairy products supplemented with probiotic bacteria include improved lactose utilization, anticarcinogenic activity, control of intestinal infections, and improved flavor and nutritional quality (2, 3, 4, 6, 16). The utilization of lactose during fermentation can make the product more easily digested by lactose intolerant people (6, 18).

The frozen yogurt environment is not optimum for survival of bacteria. The freezing process of the mix may cause a loss of ½ to 1 log cycle in viable counts (2, 4). The fluctuation in temperature, causing ice crystal formation during the 6 to 12-mo shelf-life, may rupture bacterial cells and reduce viability. The concentration

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of sweeteners in the product inhibits growth of yogurt bacteria (14). Bifidobacteria are sensitive to oxygen and acid, conditions found in frozen yogurt (19). Viability of bifidobacteria in the dairy medium is important because the value of the probiotic-supplemented dairy product depends on presence of viable cells (13). A minimum bacterial count of $10^7$ cfu of *Bifidobacterium*/ml in fresh dairy products is recommended (13). The selection of traditional lactic acid starter bacteria in the culture with bifidobacteria is important to balance acid development and flavor.

Activity of yogurt culture and probiotic bacteria cause specific changes in the chemistry of the product that affect sensory characteristics of the product. Carbonyl compounds, such as lactic and acetic acids, acetaldehyde, acetone, and diacetyl result from fermentation of lactose and proteins and contribute to sensory attributes in frozen yogurt (4). Compared to low-fat ice cream, frozen yogurt has an acidic flavor contributed primarily by lactic acid (18). Acetaldehyde, which is produced primarily by *L. bulgaricus* in the first 1 to 2 h of incubation, is the most important aroma compound in yogurt (18). However, industry perception is that acetaldehyde flavor in frozen yogurt should be limited for consumer preference (18). The sensory characteristics of dairy products cultured with bifidobacteria are different from yogurts that use conventional cultures (16). Flavor and aroma of the yogurt are reportedly milder. Very limited information has been reported on the effects of probiotic cultures in frozen yogurt and sensory quality of the product (8, 10).

The positive health effects documented for *Bifidobacterium* only occur when the bacteria are viable and active. Manufacturing or storage processes that cause death to *Bifidobacterium* cells will eliminate the benefits; however, injury to cells may not have as great a detrimental impact. Injured bacteria are unable to grow on media that are selective for them, even though they are metabolically active (1). However, if given adequate time and proper conditions they can repair their injuries. Recovery of injured cells in frozen yogurt is important to determine the number of cells that may be available to inhabit the intestine.

A method of recovery, known as the repair detection system, in combination with the roll-tube method has been developed to aid in recovery of injured cells (12). The method involves anaerobic inoculation of a nonselective layer of medium, followed by a 2-h repair period then anaerobically overlaying it with a selective layer of the same medium containing selective agents. Arany et al. (1) demonstrated that injured *Bifidobacterium* could be recovered by this method from water and melted frozen yogurt inoculated with *Bifidobacterium* and *L. acidophilus*. This method provides an environment for cells to repair injury and can be used as an alternative to conventional methods of recovery, which do not account for injured cells.

In this study, two culture systems, a system with traditional yogurt cultures and a probiotic-supplemented system, were used to ferment low-fat ice cream mix to two end points, a developed titratable acidity of 0.15% or pH 5.6. Project objectives were to evaluate probiotic and traditional yogurt culture survival, to determine recovery of *Bifidobacterium* using several methods, to measure compositional changes, and to evaluate sensory characteristics of frozen yogurt over the 11 wk of storage (–20°C).

## MATERIALS AND METHODS

### Source of Bacteria

Low-fat ice cream mix was fermented with one of two culture systems. Traditional yogurt cultures, *L. bulgaricus* and *S. thermophilus* (UltraGro, Sanofi Bio-Industries, Waukesha, WI) were used to ferment the control product (subsequently termed traditional). The second product (hereafter termed supplemented) was fermented with traditional culture and probiotic bacteria using selected strains of *Bifidobacterium longum*, *Lactobacillus acidophilus, S. thermophilus*, and *L. bulgaricus* (BATL-1, Sanofi Bio-Industries, Waukesha, WI). The system was supplemented with a second culture mix (BA-61 Sanofi Bio-Industries, Waukesha, WI) which contained only *Bifidobacterium longum*-6 and *Lactobacillus acidophilus*-1, strains recognized as human origin, to increase concentrations of the probiotic bacteria.

### Frozen Yogurt Manufacture

Raw milk, obtained from the bulk tank at Virginia Polytechnic Institute and State University (VPI&SU) dairy farm, was separated into cream and skim fractions in a pilot plant-scale separator (Elecrem Separator Model 1G, Bonanza Industries, Calgary, AB, Canada). Low-fat ice cream mix (4% fat) was manufactured with 1157.7 g of cream, 1198.6 g of nonfat dry milk, 12076.4 g of skim milk, 1847.8 g of granulated sucrose, 1539.1 g of corn syrup, and 140.7 g of stabilizer. The ice cream mix was mixed until no clumps were present, batch pasteurized at 68°C for 30 min, cooled to 3.3°C, and stored for approximately 24 h.

Ice cream mix was preheated to 40°C in a hot-water bath and inoculated with appropriate cultures. Mix was inoculated with the traditional culture system at 0.2% of mix weight. Supplemented mix was inoculated with the four-culture mix and the two-culture probiotic mix at 0.2% of mix weight and 0.02% of mix weight, respec-

tively. Two containers of ice cream mix (3246 g each) were inoculated for each culture treatment. Cultures were mixed into the ice cream mix, which was then fermented at 40°C to two end points. End point 1 was reached when the titratable acidity was 0.15% greater than the initial titratable acidity value. End point 2 was established as pH 5.6.

The fermented mix was frozen in a batch freezer (Emory Thompson Freezer 2HSC A, Emory Thompson Machine and Supply Co., New York, NY) with an overrun of 55 to 60%. Product was frozen and stored at –20°C.

### Enumeration of Bacteria

*Streptococcus thermophilus* was enumerated with M-17 medium. M-17 medium was made of the following (g/L of $dH_2O$): phytone peptone, 5; polypeptone, 5; lactose, 5; beef extract, 5; yeast extract, 2.5; L-ascorbic acid, 0.5; sodium glycerophosphate, 19; $MgSO_4*7H_2O$ 1*M* solution, 1 ml; and agar, 12. Medium was adjusted to pH 5.4, dispensed into 150-ml screw-cap bottles, sterilized (20 psi, 20 min) and stored in the dark until used.

*Lactobacillus bulgaricus* was enumerated with reinforced clostridial agar (BBL Company, Cockeysville, MD). Rehydrated media was adjusted to pH 5.4, dispensed into 150-ml screw-cap tubes, sterilized (121°C, 20 psi, 20 min), and stored in the dark until used.

*Lactobacillus acidophilus* was enumerated on MRS agar made by addition of 1.2% agar to MRS broth (BBL Becton-Dickinson Microbiological Systems, Cockeysville, MD). The medium was adjusted to pH 5.6, dispensed into 150-ml screw-cap bottles, sterilized (20 psi, 20 min) and stored in the dark until used.

### Enumeration of Bifidobacterium

Bifidobacterium were enumerated with a modified Mara and Oragui's (modified bifid glucose medium, **MBGA**) medium (1). This medium contained (g/L of $dH_2O$): glucose, 10; polypeptone, 10; yeast extract, 20; Casamino Acids, 8; sodium chloride, 3.2; and L-cysteine, 0.5. Dyes were added in the following amounts: 3 ml of methylene blue, and 40 ml of phenol red. The medium was dispensed into 25- × 142-mm roll-tubes in 7- and 10-ml aliquots with anaerobic roll tube equipment (Bellco, Inc., Vineland, NJ) following procedures outlined in the Anaerobe Laboratory Manual (12). Granulated agar (0.02 g/ml) was dispensed into each roll tube (Bellco, Inc., Vineland, NJ) before the medium was added. Medium pH was adjusted to 7.1 (±0.1), prior to being autoclaved and tubes were subsequently stored in the dark. Prepared media was used within 2 wk.

A solution of selective agents was prepared (g/10 ml of $dH_2O$) nalidixic acid (0.056), kanamycin monosulfate (0.085), and polymyxin B (0.00215) for use with the repair detection technique (1, 5). The solution was filter-sterilized with a sterile 0.2-$\mu$m acrodisc (Gelman Sciences, Ann Arbor, MI) and a 30-cc syringe. The sterilized solution was then placed in the freezer at –4°C until used. Prior to use, the solution was stored (4°C) for 24 h to allow proper thawing.

The second medium, maltose + galactose reinforced clostridial medium (MGRCA) was also used to enumerate *B. longum*. The ingredients were as follows (g/L of $dH_2O$): trypticase peptone, 10; beef extract, 10; galactose, 5; maltose, 5; sodium chloride, 5; yeast extract, 3; sodium acetate, 8; starch, 1; L-cysteine, 0.5; granulated agar, 13.5. The medium pH was adjusted to 5.4 (±0.1) and dispensed into 150-ml screw-cap bottles and autoclaved (121°C, 20 psi, 20 min). Medium was stored in the dark until used.

***Dilution blanks.*** Two types of dilution blanks were produced. The first was an aerobically produced prereduced peptone dilution blank. The dilution blanks contained 0.01% (wt/vol) peptone (Difco-Bacto Peptone, Difco Laboratories) and 0.05% L-cysteine (Sigma Cell Culture Reagents). To 500 ml of distilled, deionized water, 0.50 g of peptone and 0.25 g of L-cysteine were weighed and added. Solution pH was adjusted to 7.0 with 1N NaOH. Nine-milliliter aliquots were dispensed into 15-ml screw-cap tubes. Tubes were then capped, sterilized (121°C, 20 psi, 20 min), cooled, and stored in the dark until used. These dilution blanks were used in conjunction with MGRCA.

A second set of dilution blanks was used with the roll-tube method and repair detection system. The ingredients were identical to aerobically produced blanks; however, methylene blue (0.3 ml/500 ml) was added as an oxygen indicator. The blanks were produced following the VPI&SU Anaerobe Laboratory Manual instructions (12). The methodology used to produce the anaerobic blanks involved the injection of $CO_2$ into the tubes to facilitate the removal of the oxygen. The solutions were dispensed into small roll-tubes, which were stoppered as opposed to screw-capped.

***Plating procedures: repair-detection procedure with roll-tubes.*** The roll-tube method used in this study followed procedures outlined in the Anaerobe Laboratory Manual (12). The 7-ml media tubes were used for inoculating with dilutions of frozen yogurt. Media in the 10-ml tubes were used as the overlay. Tubes were autoclaved and stored for no longer than 2-wk prior to use. Prior to inoculation, tubes were steamed (100°C, 10 min) to facilitate melting of media and tempered in a 47°C water bath. Anaerobic dilution blanks were unstoppered under $CO_2$ canula and serial dilutions were made to the highest dilution required. Nonselective (7-ml) tubes were unstoppered under $CO_2$

canula and inoculated from the anaerobic dilution blank corresponding to desired dilution. Tubes were then stoppered, spun until solid, inverted, and incubated for 2 h at 37°C to allow injured cells to repair. Once the incubation time passed, tubes were reopened and placed under the canula. A 10-ml tube of tempered MBGA then was opened under $CO_2$ canula, 0.1 ml of selective agent was added, and the contents of the tube were added to the unstoppered 7-ml tube. The overlayed tube was then stoppered, spun until solid, inverted and incubated, at 37°C for up to 96 h.

***Plating procedures: repair detection system with plates.*** MBGA also was used with plates which were inoculated with anaerobic dilution blanks. Tubes that contained the medium were steamed and tempered as described above. The 7-ml tubes were unstoppered under the canula, inoculated, and poured onto plates (nonselective layer), allowed to solidify, and placed in an anaerobe jar with a GasPak and an indicator strip. The plates in the anaerobe jar were then incubated at 37°C for 2 h to allow repair of injured cells. After incubation, the jar was removed from the incubator and opened. Ten-milliliter MBGA tubes were unstoppered under canula, 0.1 ml of selective agent was added, and the contents were poured over the inoculated plates (selective overlay), allowed to solidify, placed in anaerobe jar with a fresh GasPak and indicator strip, and returned to the incubator for up to 96 h.

***Plating procedures: maltose + galactose reinforced clostridial medium.*** Bottles of media were steamed (100°C, 10 min) and tempered at 46°C prior to use. Serial dilutions were made with liquefied frozen yogurt in aerobic dilution blanks. The plates were then inoculated using 1 ml of dilution and the pour-plate method (approximately 10 ml of medium/plate). The plate was slowly swirled to facilitate mixing, and medium was then allowed to solidify. Plates were inverted, then placed in an anaerobe jar with a GasPak and indicator strip. The plates in the anaerobe jar were placed in a 40°C incubator for 72 h, and colonies were counted subsequent to removal of plates and tubes from the incubator.

## Biochemical Analyses

Protein content of the frozen yogurt was determined spectrophotometrically (Spectronic 1001 Split Beam Spectrophotometer, Milton Roy Co., Rochester, NY) based on the Bradford method of the Bio-Rad protein assay (Bio-Rad, Hercules, CA). Lactose and D-galactose concentrations in the frozen yogurt were determined spectrophotometrically based on the methodology for examination of low-fat ice cream in the Boehringer-Mannheim Lactose-D-Galactose Test Kit (Gene-Trak, Framingham, MA).

## Descriptive Sensory Evaluation

***Panelist training.*** Seven panelists, students, and staff from the VPI&SU Food Science and Technology Department were selected based on willingness to participate in this project. Panelists participated in six one-half hour training sessions. During these sessions, product descriptors were selected and defined, and training in identification and intensity ratings of each descriptor was completed. Six attributes (acidity, smoothness, yogurt flavor, freshness, vanilla flavor, and sweetness) were selected for evaluation based on importance to the project objectives (Table 1). Definitions for each attribute were developed based on group discussion. The attributes selected represented those characteristics most likely to be affected by the experimental variables.

Prior to initiating the product evaluation, panelists were tested for the ability to distinguish attributes and repeat measurements. Panelists independently evaluated three different samples of laboratory-manufactured frozen yogurt products. Two of the products were the same and evaluations were used to determine if panelists could replicate their own judgment. One-ounce samples in plastic soufflé cups (Solo Cup Co., Urbana, IL) with lids were coded with 3-digit random numbers. Samples were presented such that each panelist received all samples, one at a time, in a random order. Each sample appeared in every position an equal number of times across all panelists. Samples were stored at –10°C in a counter-top freezer (Arctic Star of Texas model AS3, Arlington, TX) to maintain sample integrity during sensory analyses. Panelists evaluated one sample, then waited at least 30 s before presentation of the next sample. Panelists rated each product for intensity of each attribute, and marked the perceived intensity, with a hash mark, on 15-cm unstructured line scales with anchor terms. Distance (cm) from the origin to the hash mark was used as a measurement of intensity. Evaluations were completed in individual booths under fluorescent white light. Panelists were instructed to rinse the palate between samples.

***Product evaluation.*** Descriptive analyses were completed on five frozen yogurt products. Panelists evaluated each of the four samples of manufactured product (i.e., two end points of each of two treatments) and a commercial brand of frozen yogurt (Crowley's Vanilla Frozen Yogurt) for intensity of each of the six attributes. All panelists received the same set of samples, in random order, at each sensory session. Products were presented for evaluation as previously described.

**Table 1.** Description of attributes.

| Attributes | Description |
|---|---|
| Acidity | Early taste which is noticed on the sides, tip, and back of tongue. May produce a "dry" feeling on the tongue. A sour flavor. |
| Smoothness | Lack of granular texture, mouth-feel is of even consistency, with no "crunching" if the sample is chewed rather than allowed to melt in the mouth. |
| Yogurt flavor | Fermented flavor; "cheese-cake" taste. |
| Sweetness | Candy-like flavor, sensation is like cake-icing sweetness. |
| Freshness | Absence of "freezer" flavor; lacks stale-air taste; "cardboard", "old" flavor. |
| Vanilla | Has flavor like vanilla beans, can be easily tasted and smelled. "Alcohol" type taste can be sensed when excessive amounts of vanilla are used. |

Products were evaluated within 48 h after manufacture and at 2-wk intervals for 11 wk.

### Statistical Analysis

The complete experiment was replicated twice. Data analysis was conducted on least square means of the values using a general linear model procedure. A complete block design, with replications as the block, was used for analysis using SAS (Statistical Analysis System, Cary, NC). Differences in culture system, fermentation end point, and weeks of storage (treatments) were determined.

Statistical analysis for enumeration of bifidobacteria was conducted on log transformation of bacterial counts. A model was developed to evaluate differences in recovery of *B. longum* from frozen yogurt fermented to two end points by three recovery methods. Analysis of variance on SAS (Statistical Analysis System, Cary, NC) was used to analyze the data.

## RESULTS AND DISCUSSION

### Bacterial Survival

Figures 1 and 2 compare frozen yogurt fermented with the two culture systems on recovery of the traditional fermentation organisms, *L. bulgaricus* and *S. thermophilus*, respectively, found in both culture systems. Initial bacterial counts recovered from the frozen yogurt for *L. bulgaricus* and *S. thermophilus* did not decline over the 11-wk storage period ($P > 0.05$). Recovery of *S. thermophilus* was significantly higher ($P < 0.05$) from the product supplemented with probiotic cultures for wk 5 to 11 than that recovered from the product with traditional cultures only (Figure 2). Supplementation with probiotic bacteria had no influence on number of *L. bulgaricus* or *S. thermophilus*. Both *L. bulgaricus* and *S. thermophilus* were recovered at approximately 6.5 to 7 $\log_{10}$ cfu/ml.

Thompson and Mistry (25) studied the effects of cold storage on survival of *L. bulgaricus* and *S. thermophilus* in frozen yogurt and found no significant decrease in bacterial numbers. In contrast, Miles and Leeder (20) found that *L. bulgaricus* and *S. thermophilus* concentrations decreased an average of 1.5 and 0.5 $\log_{10}$ cfu/ml, respectively, when stored for 2 wk at –28.9°C in frozen yogurt. Lopez et al. (17) observed only a slight decline in lactic acid bacteria in three batches (pH = 4.32, 5.09, and 5.53) of commercial frozen yogurt stored at –23°C for 1 yr. They suggested that, because of the close link of streptococci and lactobacilli in fermentation, both types of organisms were important in survival of the bacterial cultures. Baig and Prasad (2) reported, in frozen yogurt, a 1 $\log_{10}$ cfu/ml reduction in *S. ther-*

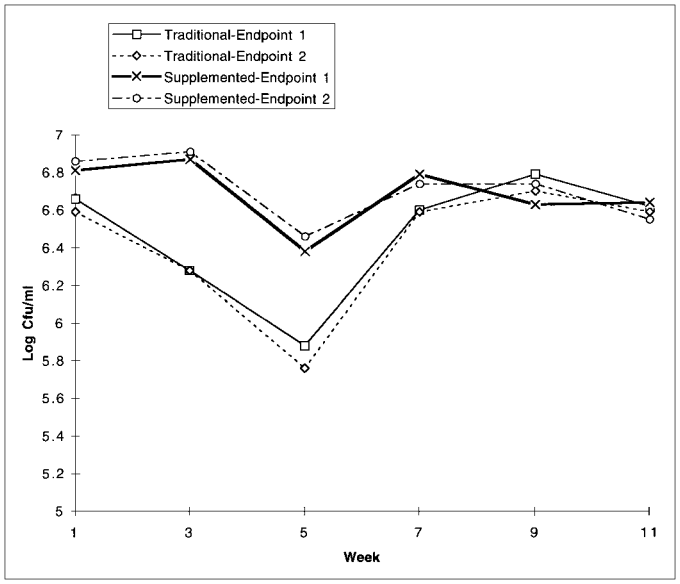


**Figure 1.** Recovery of *Lactobacillus bulgaricus* from frozen yogurt fermented with two different culture systems to two end points and stored for 11 wk (–20°C). Traditional culture includes *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Supplemented culture includes *Lactobacillus acidophilus, Bifidobacterium longum, Streptococcus thermophilus*, and *Lactobacillus bulgaricus.* Endpoint 1 = titratable acidity of 0.15% greater than initial titratable acidity. Endpoint 2 = pH 5.6.

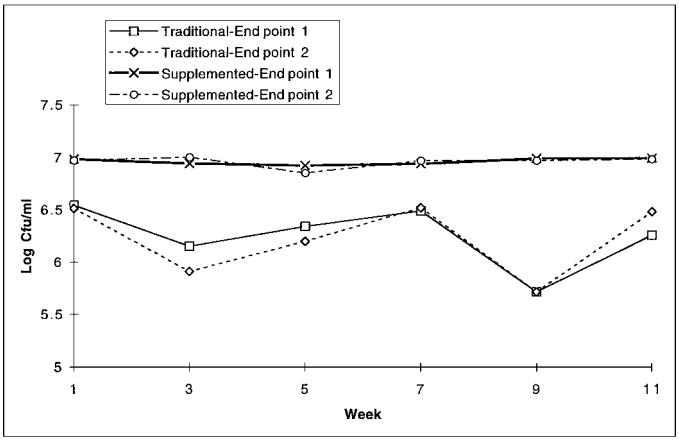


**Figure 2.** Recovery of *Streptococcus thermophilus* from frozen yogurt fermented with two different culture systems to two endpoints and stored for 11 wk (–20°C). Traditional culture includes *Streptococcus thermophilus* and *Lactobacillus bulgaricus.* Supplemented culture includes *Lactobacillus acidophilus, Bifidobacterium longum, S. thermophilus*, and *L. bulgaricus*. Endpoint 1 = titratable acidity of 0.15% greater than initial titratable acidity. Endpoint 2 = pH 5.6.

*mophilus, L. bulgaricus*, and *B. bifidum* during a 90-d storage period at –20°C. This decrease in viable counts was in addition to a 1 $\log_{10}$ cfu/ml reduction that occurred during the freezing process.

Increased acidification, when fermentation was allowed to proceed to the second end point, had no significant effect on the recovery or survival of *L. bulgaricus* (Figure 1), *S. thermophilus* (Figure 2), or *L. acidophilus* (Figure 3). Hekmat and McMahon (10) found that *L. acidophilus*, inoculated in a standard ice cream mix and allowed to ferment, exhibited a 2 $\log_{10}$ cfu/ml decrease when stored at –29°C for 17 wk. Holcomb et al. (11) observed no evidence of freeze injury to *L. acidophilus* when exposed to –5°C temperatures for 6 h.

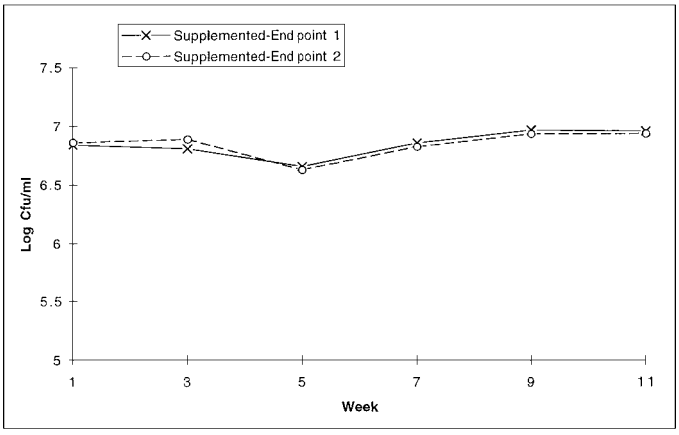


**Figure 3.** Recovery of *Lactobacillus acidophilus* from frozen yogurt fermented with two different culture systems to two endpoints and stored for 11 wk (–20°C). Traditional culture includes *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Supplemented culture includes *Lactobacillus acidophilus, Bifidobacterium longum, S. thermophilus*, and *L. bulgaricus*. Endpoint 1 = titratable acidity of 0.15% greater than initial titratable acidity. Endpoint 2 = pH 5.6.

## Recovery of *Bifidobacterium*

There was no difference ($P > 0.05$) in *Bifidobacterium* (6.75 to 7.25 $\log_{10}$ cfu/ml) in the frozen yogurt fermented with the supplemented culture system to the two end points (Figure 4). Hekmat and McMahon (10) observed a 1 $\log_{10}$ cfu/ml loss in *B. longum* cells in frozen yogurt when stored at –29°C for 17 wk. Modler and Villa-Garcia (21) reported a 2 $\log_{10}$ cfu/ml loss in *B. longum* concentrations from acidification of frozen yogurt caused by fermentation and refreezing. Holcomb et al. (11) observed no decrease in *B. longum* in frozen yogurt that was frozen at –5°C for 6 h. Bifidobacteria are sensitive to lactic acid production by traditional starter cultures; selection of lactic acid starter culture strains with lower acid production provides a better culture system for maintaining bifidobacteria viability (13).

Recovery of *B. longum* ($P < 0.05$) on MBGA roll-tubes with the repair-detection method was increased compared with the MBGA plates and MGRCA plates for products fermented to both end points (Figure 4). The MBGA roll-tube with repair-detection system recovered cells at levels from ¼ to ½ $\log_{10}$ cfu/ml higher than the

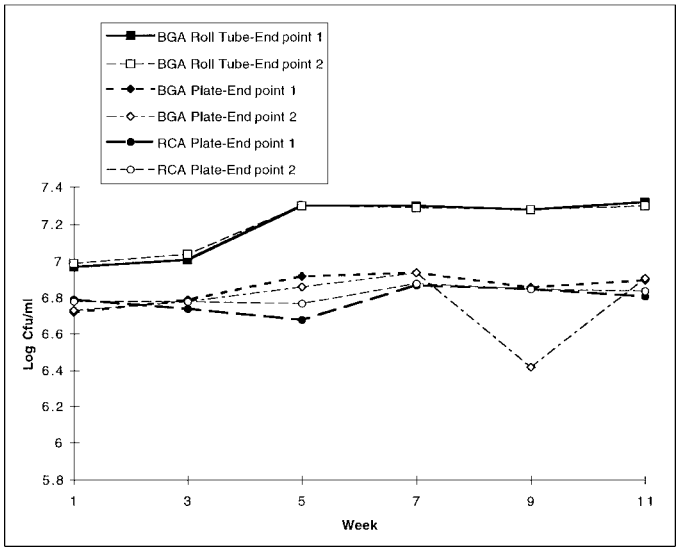


**Figure 4.** Comparison of methods of recovery of *Bifidobacterium longum* from frozen yogurt fermented with two different culture systems to two end points and stored for 11 wk (–20°C). Traditional culture includes *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Supplemented culture includes *Lactobacillus acidophilus, Bifidobacterium longum, S. thermophilus*, and *L. bulgaricus.* Endpoint 1 = titratable acidity of 0.15% greater than initial titratable acidity. Endpoint 2 = pH 5.6.

MBGA and MGRCA plates. No decrease in bacterial numbers was observed over the storage period for any of the three methods of recovery. The repair detection system provided injured cells with an environment and opportunity to repair their injury, thus maximizing bacterial counts. The ingredients in the medium may or may not have been responsible for this increase in recovery; but rather, the method itself may be the reason for enhanced recovery due to the constant anaerobic environment, i.e., from dilution blanks to inoculation.

### Biochemical Changes

Lactose concentrations were monitored in all products to determine if additional organisms (supplemented culture) increased lactose hydrolysis. Lactose concentrations were also evaluated to determine if the increase in acidity (i.e., two end points) would cause a significant change in the amount of lactose in the frozen yogurt samples. Lactose concentration did not change over the 11-wk period, remaining in the range of 1.7 to 2.1 g of lactose/100 ml of mix. No differences were observed in lactose concentration due to the difference in levels of acidity at the two end points. Thompson and Mistry (25) observed no significant changes in lactose concentrations in frozen yogurt mix when stored frozen for 1 and 3 mo. Gilliland and Kim (7) found that lactose decreased from 6.26% in uninoculated yogurt mix to 4.23% in inoculated. Schmidt et al. (22) reported a range of lactose (2.31 to 4.25%), based on an HPLC analysis, among 11 commercial brands of vanilla-flavored frozen yogurt. Protein concentration did not change when comparing culture systems at the same end point or the same culture system at both end points.

### Sensory Evaluation of Frozen Yogurt

The commercially produced brand of frozen yogurt had a smoother texture than the pilot plant manufactured samples but had similar intensities for vanilla flavor and sweetness. Freshness declined slightly over the 11-wk storage period for all products. Perceived acidity in the products corresponded to the development of acidity during the fermentation (Figure 5). However, the second end point of the supplemented system showed significantly higher scores, approaching "extreme," compared with the other treatments. The second end point of the traditional treatment also had higher acidity scores than the products fermented to the first end point, although statistical significance ($P < 0.05$) was observed only on a few occasions. The products fermented to the first end point had low to moderate acid flavor. The commercially produced frozen yogurt was consistently evaluated to be the lowest-acid product.

The balance of flavoring systems may be significantly affected by varying levels of organic compounds. Kneifel et al. (15) studied 47 commercially available yogurts and starter cultures by using a sensory panel. Acidity was rated the most important attribute, in terms of perceived flavors, in frozen yogurt. Speck (23) determined that frozen yogurts with the lowest titratable acidity (0.28 to 0.38%) received the highest overall quality scores. The study also determined that of all flavors tested—vanilla, chocolate and coffee—all would receive higher quality scores if the acidity was kept relatively low. Coffee, black currant, apple, cloudberry, grapefruit, pear, and banana/vanilla flavors were found to be unsuitable in frozen yogurt with high acidity (9).

There were no differences in perceived intensity of yogurt flavor among products (Figure 6). Generally, however, the product fermented with supplemented cultures and the commercial product had less yogurt flavor than the other products. The product fermented with the traditional culture to the second end point had consistently higher levels of yogurt flavor. Laroia and Martin (16) suggested a milder flavor and aroma found in yogurt with bifidobacteria.

## CONCLUSIONS

Frozen yogurt can serve as an excellent vehicle for dietary incorporation of probiotic bacteria. Frozen storage of the product has little or no effect on culture survival, and bacterial cultures remained at levels suf-

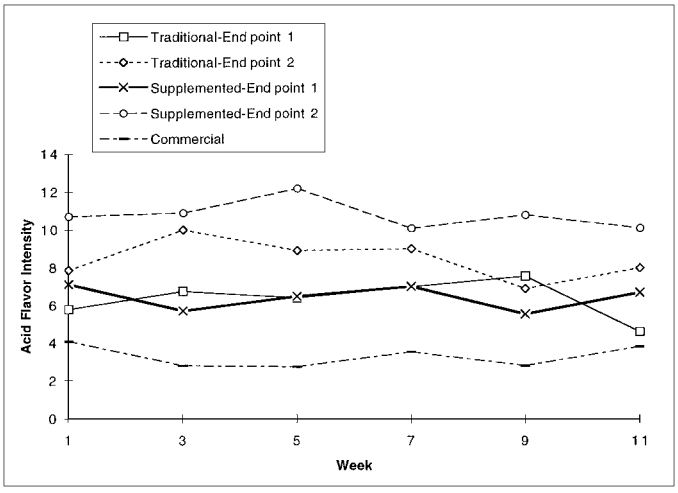


**Figure 5.** Acid flavor intensity in frozen yogurt fermented with two different culture systems to two endpoints and stored for 11 wk (–20°C). Traditional culture includes *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Supplemented culture includes *Lactobacillus acidophilus, Bifidobacterium longum, S. thermophilus*, and *L. bulgaricus*. Endpoint 1 = titratable acidity of 0.15% greater than initial titratable acidity. Endpoint 2 = pH 5.6.

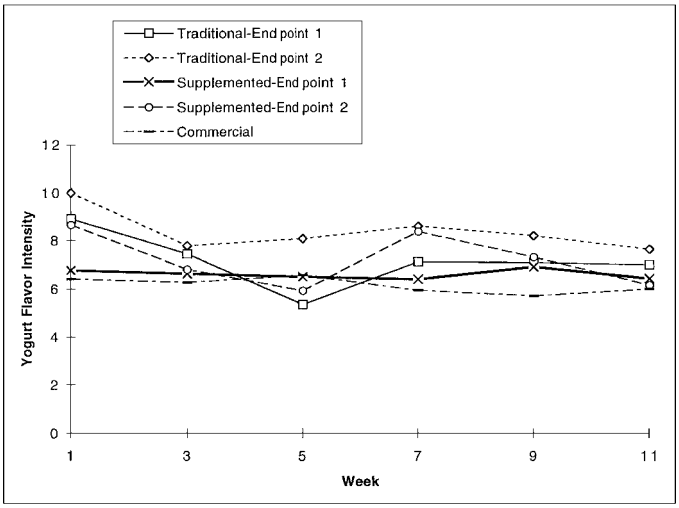


**Figure 6.** Yogurt flavor intensity in frozen yogurt fermented with two different culture systems to two endpoints and stored for 11 wk (–20°C). Traditional culture includes *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Supplemented culture includes *Lactobacillus acidophilus, Bifidobacterium longum*, *S. thermophilus*, and *L. bulgaricus*. Endpoint 1 = titratable acidity of 0.15% greater than initial titratable acidity. Endpoint 2 = pH 5.6.

ficient to offer the suggested therapeutic effects. Supplementation with probiotic bacteria has little effect on flavor or compositional characteristics of frozen yogurt. However, fermentation to a lower pH (5.6) does significantly increase the acid flavor of the product.

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