Survival of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in Ice Cream for Use as a Probiotic Food

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ABSTRACT

Probiotic ice cream was made by fermenting a standard ice cream mix with *Lactobacillus acidophilus* and *Bifidobacterium bifidum* cultures and then freezing the mix in a batch freezer. Survival of the *L. acidophilus* and *B. bifidum*, as well as β-galactosidase activity, was monitored during 17 wk of frozen storage at –29°C. After freezing of the fermented mix, bacterial counts were 1.5×10^8 cfu/ml for *L. acidophilus* and 2.5×10^8 cfu/ml for *B. bifidum*. Seventeen weeks after freezing, these counts had decreased to 4×10^6 and 1×10^7 cfu/ml, respectively. During the same period, β-galactosidase activity decreased from 1800 to 1300 units/ml.

Probiotic ice cream was prepared at pH 5.0, 5.5, and 6.0 to determine consumer preferences and was compared with standard Utah State University "Aggie" ice cream. All samples were strawberry-flavored and were evaluated by 88 judges. The preferred pH of probiotic ice cream, based on overall acceptance, was pH 5.5.

We demonstrated that probiotic ice cream is a suitable vehicle for delivering beneficial microorganisms such as *L. acidophilus* and *B. bifidum* to consumers. The bacteria can be grown to high numbers in ice cream mix and remain viable during frozen storage.

(Key words: *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, ice cream)

Abbreviation key: ONPG = *o*-nitrophenyl-β-D-galactopyranoside, RCA = reinforced clostridial agar.

Received October 8, 1991.
Accepted January 27, 1992.

INTRODUCTION

Although yogurt has gained widespread consumer acceptance in the United States, the overall consumption of fermented milk products is still much less than in many European countries (12, 19). This may change with the increased interest in the dairy food industry (9) in a concept of probiotics, the use of bacteria to enhance health.

An optimal balance of microbial organisms in the intestine is suggested to be an important aspect of maintaining good health. Certain bacteria, such as lactobacilli and bifidobacteria, that help maintain such a favorable balance (6) are considered to be probiotics. Fuller (4) defined probiotics as the use of a live microbial feed supplement that beneficially affects the host animal by improving its intestinal microbial balance. As a person ages, the number of intestinal bifidobacteria decrease, and the numbers of clostridia, streptococci, and coliforms increase (22). Both *Lactobacillus acidophilus* and *Bifidobacterium bifidum* produce antibiotics and organic acids (such as lactic acid and acetic acid) that are inhibitory toward Gram-negative bacteria (17). Some lactic acid bacteria also have anticarcinogenic properties (16, 18). Goldin and Gorbach (5) studied the influence of *L. acidophilus* on the activity of enzymes produced by intestinal bacteria that can convert procarcinogens into carcinogens. The studied enzymes were β-glucuronidase, nitroreductase, and azoreductase. They found reduced concentrations of each enzyme when milk supplemented with *L. acidophilus* was consumed.

These beneficial microorganisms also improved lactose digestibility. Some people do not produce sufficient β-galactosidase in their small intestines and, therefore, are unable to digest lactose adequately. In contrast, some lactose maldigestors may consume cultured milks without intestinal disturbances. This is

because of reduced lactose content and the presence of β-galactosidase from the starter bacteria in the cultured product (20, 21).

Danielson and Gustafon (3) also suggested that gastrointestinal microorganisms play a role in the metabolism of cholesterol. Evidence to support this was reported by Harrison and Peat (7), who found that serum cholesterol was significantly reduced in people who ingested acidophilus milk. It has been concluded from these studies that consumption of *L. acidophilus* interferes with cholesterol absorption from the intestine.

The *L. acidophilus* and *B. bifidum* in fermented milk products are consumed because of their resistance to intestinal bile salts. Therefore, milk products fermented with these microorganisms could have applications as therapeutic foods (11). The aim of this study was to manufacture a probiotic ice cream containing high levels ($\geq 10^6$ cfu/ml) of *L. acidophilus* and *B. bifidum* and to determine how long these bacteria would remain viable during frozen storage of the ice cream.

MATERIALS AND METHODS

Preparation of Cultures

To prepare a mother culture for *B. bifidum*, a 500-ml solution containing 7% whey powder, .5% yeast extract, .05% cysteine, and 1.5% trimagnesium phosphate was made. It was autoclaved at 121°C for 15 min and then cooled to 41°C. Commercial freeze-dried *B. bifidum* (10LF, 946745101; Chr. Hansen's Lab., Inc., Milwaukee, WI) was added at the rate of 1% to the whey-based medium and incubated anaerobically at 41°C for 15 h.

Reconstituted NDM (11% total solids) was prepared. It was autoclaved at 121°C for 15 min and then cooled to 41°C. *Bifidobacterium bifidum* as the mother culture was added to the milk at the rate of 2% and incubated anaerobically at 41°C for 15 h.

Commercial freeze-dried *L. acidophilus* (10LF, 946744A; Chr. Hansen's Lab., Inc.) was added directly to sterilized reconstituted NDM at a rate of 1%. Then, it was incubated anaerobically at 41°C for 15 h.

Procedure for Manufacturing Probiotic Ice Cream

Standardized ice cream mix with 12% fat, 11% milk solids nonfat, .32% stabilizer-emulsifier (Continental Colloids Inc., Chicago, IL), 12.5% sugar, and 4.5% corn syrup solids was obtained from the Utah State University Dairy Products Laboratory. The mix was pasteurized at 79.4°C for 28 s, homogenized at 17.5 MPa, and then aged overnight at 4°C. Half of the mix was then given an additional heat treatment at 82°C for 30 min. This was termed the "heated" sample; the sample that was pasteurized only was termed "unheated." The heated sample was cooled to 41°C after heating, and the unheated mix was warmed to 41°C prior to inoculating both mixes with the starter cultures.

The ice cream mixes were then inoculated with 4% of each starter culture, mixed well, and fermented for approximately 5 h at 42°C until the desired pH (pH = 4.9 ± .05) was reached. The mix was then cooled in an ice bath to 5°C. A batch ice cream freezer was used to freeze the ice cream mix. Ten percent strawberry flavoring was added at the end of freezing. The ice cream was then packaged and placed in a hardening room at –29°C. Two replications of fermented ice cream were made.

Enumeration of Starter Bacteria

Reinforced clostridial agar (RCA) (BBL Microbiology Systems, Becton Dickinson and Co., Cockeysville, MD) was used to enumerate *L. acidophilus* and *B. bifidum* (14). Frozen fermented ice cream was thawed and then diluted 10^6 and 10^7 in autoclaved .85% saline. One-tenth milliliter of each dilution was spread over RCA plates. The plates were then incubated in an anaerobic environment (BBL Gas Pak, Becton Dickinson Microbiology Systems) at 41°C for 48 h. The total number of *L. acidophilus* and *B. bifidum* was determined based on their colony morphology when grown on RCA (14). *Lactobacillus acidophilus* produces pinpoint-sized colonies, but *B. bifidum* produces large colonies. Viable numbers of *L. acidophilus* and *B. bifidum* were determined after 1, 5, 9, 13, and 17 wk of frozen storage. The data were then analyzed using a three-way split-plot factorial randomized complete block for species, heat treatment, and storage time, using FCI (Rex L. Hurst, Utah State University, Logan) for IBM personal computers. The main plot included species and heat treatment; the subplot included storage time.

Lactase Assay

β-Galactosidase activity was measured using a chromogenic substrate *o*-nitrophenyl-β-D-galactopyranoside (ONPG) (2, 21). One milliliter of frozen fermented ice cream was added to 50 ml of .1 *M* phosphate buffer (pH 7.0) containing .001 *M* $MgSO_4$ and .05 *M* β-mercaptoethanol. Then, 1 ml of the diluted sample was withdrawn, and two drops of chloroform and one drop of .1% sodium dodecyl sulfate were added to it. This assay mixture was vortexed for 10 s and then placed in a water bath at 28°C for 5 min. The reaction was started by adding .2 ml of ONPG (4 mg/ml) to the assay mixture and vortexing for 10 s. After 10 min, the reaction was stopped by adjusting the solution to pH 11 by adding .5 ml of 1 *M* Na_2CO_3. At this pH, β-galactosidase is inactivated (13, 21).

Optical density at 420 nm was recorded using a Beckman DU-65 spectrophotometer (Seattle, WA). To eliminate light scattering, the samples were centrifuged at 16,266 × *g* for 15 min before measuring optical density. The following formula was used to determine units of enzyme activity per milliliter.

$$\beta\text{-Galactosidase (units/ml)} = 1000 \left(\frac{A_{420}}{tv}\right)$$

where t is time of reaction in minutes, v is volume of sample used in the assay, and A is absorbance at 420 nm.

Sensory Evaluation

Frozen fermented ice cream was prepared at pH 5.0, 5.5, and 6.0 by mixing fermented mix with unfermented mix. These were then compared with a sample at pH 6.5 made from standard Utah State University "Aggie" ice cream mix. All samples were strawberry-flavored. Sensory evaluation was conducted by 88 untrained judges. The judges were asked to indicate their most and least preferred samples and to evaluate flavor, texture, and overall acceptance of the product using a hedonic scale of 1 to 9 (1). In addition, they were asked to describe their consumption of yogurt and frozen yogurt. The statistical analysis of data from the taste panels was made using JMP™ software for Macintosh computers (SAS Institute, Cary, NC).

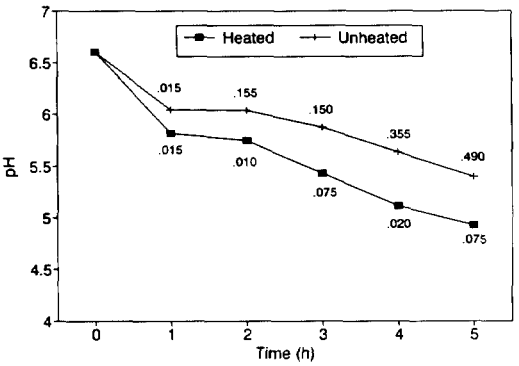

Figure 1. Comparison of acid production during fermentation of ice cream mix by *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in heated and unheated fermented ice cream during fermentation process. Numbers indicate standard deviation of the mean for two replicates; the heated sample was pasteurized and received heat treatment (82°C) for 30 min; the unheated sample was pasteurized but received no additional heat treatment.

RESULTS AND DISCUSSION

Acid Production

Lactobacillus acidophilus and *B. bifidum* were able to grow and produce acid in the ice cream mix. The rate of acid production in the heated ice cream mix was faster and more consistent than in the unheated mix (Figure 1), probably because the additional heat treatment at 82°C for 30 min released some free amino acids and other stimulating substances. This would allow the culture to begin acid production more quickly, as was observed. It also provided a semi-sterile environment for the growth of culture bacteria; therefore, competition for nutrients from other nonlactic bacteria was reduced.

Microbial Counts

The differentiation of *L. acidophilus* and *B. bifidum* has been a problem in cultured dairy foods. The difficulty of cultivating bifidobacteria in milk, because of lack of acid tolerance or oxygen sensitivity, was not encountered in these experiments. In our study, *B. bifidum* and *L. acidophilus* grew to high numbers in ice cream mix. Even the high solids level of the ice cream mix did not prevent growth of either *L. acidophilus* or *B. bifidum* when a high percentage (4%) of inoculum was used. In-

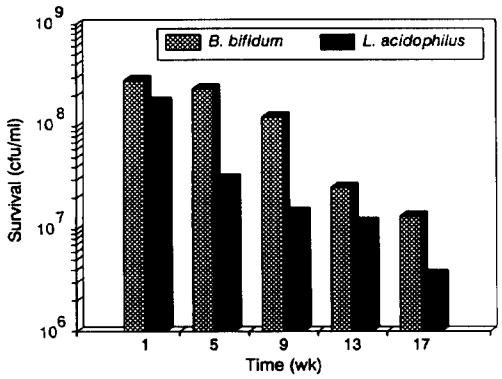

Figure 2. Mean survival of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in fermented ice cream over 17 wk of frozen storage.

sufficient growth occurred with 1 and 2% inoculum. The total colony counts after fermentation of the ice cream mix to pH 4.9 were 5×10^8 cfu/ml for both types.

Initial freezing of the ice cream mix in the batch freezer followed by hardening at –29°C caused a reduction of less than one log cycle in total colony counts. After 1 wk of frozen storage, *L. acidophilus* was at a level of 1.5×10^8 cfu/ml, whereas *B. bifidum* was at 2.5×10^8 cfu/ml. Then, during 17 wk of frozen storage, the *L. acidophilus* decreased by two log cycles to 3×10^6 cfu/ml, whereas *B. bifidum* decreased by only one log cycle to 1×10^7 cfu/ml (Figure 2).

In a recent survey of commercial soft serve frozen yogurt (unpublished data), viable number of lactic acid bacteria ranged from 10^5 to $<10^3$ cfu/ml. Our study shows that ice cream mix can be fermented with *L. acidophilus* and *B. bifidum* and still have higher numbers of viable organisms after 17 wk of storage. Holcomb et al. (8) studied viability of *L. acidophilus* and *B. bifidum* in soft serve frozen yogurt. Their study indicated that both bacteria were able to survive and grow in frozen yogurt before and after freezing. Also frozen storage (for 6 h at –5°C) caused no adverse effect on bile resistance of either bacteria. Modler et al. (15) studied survival of bifidobacterium in ice cream over 70 d of frozen storage and found approximately 90% survival of these bacteria during the storage period. They (15) also suggested that ice cream is an excellent vehicle for delivering bifidobacteria into the human diet.

Table 1 shows no significant differences in colony counts (after fermentation to pH 4.9) between heated and unheated treatments ($P \leq .05$). However, the total colony counts for *B. bifidum* were significantly different from those for *L. acidophilus* ($P < .0029$), and the effect of storage time was significant ($P < .0000$). The viable numbers of *L. acidophilus* and *B. bifidum* were significantly different at wk 1, 5, 9, and 17 (LSD = .4077). There was no significant difference in viable number of *B. bifidum* for wk 1, 5, and 9 (LSD = .2998). There was also significant interaction between type of culture bacteria and length of frozen storage ($P = .0130$), which is shown in Figure 2 as the different rates at which bacterial viability decreased for the two types of cultures. *Lactobacillus acidophilus* sharply decreased in the first 5 wk of storage, but, for *B. bifidum*, the largest decrease occurred between wk 9 and 13.

β-Galactosidase Activity

To determine the influence of *L. acidophilus* and *B. bifidum* in ice cream on lactose digestibility, actual measurement of the activity of β-galactosidase in the ice cream rather than lactose, glucose, or galactose content is necessary. Measuring sugar content of a fer-

TABLE 1. Three-way split-plot factorial randomized complete block ANOVA for $\log_{10}$ number of bacteria in fermented ice cream over 17 wk of frozen storage for *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in both heated and unheated samples.

Source	df	MS	*F*	*P*
Replication	1	.1106		
Heat	1	.2338	2.8461	.1902
Species	1	6.5233	79.3783	.0029
Heat × species	1	.4645	5.6530	.0978
Error (a)[1]	3	.0821		
Week	4	3.1079	77.5279	.0000
Week × heat	4	.0436	1.0887	.3952
Week × species	4	.1788	4.4603	.0130
Week × heat × species	4	.0687	1.7161	.1955
Error (b)[1]	16	.0400		
Total	39	.5594		

[1]Error term of the whole plot (a) and the subplot (b).

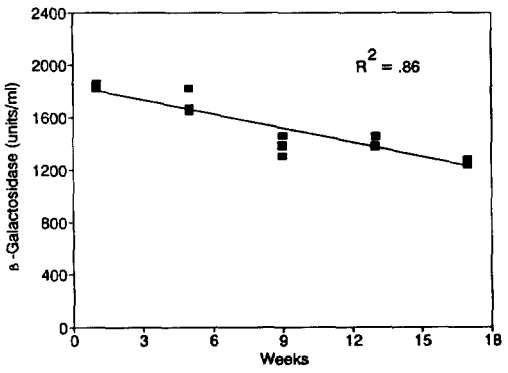

Figure 3. Effect of frozen storage on β-galactosidase activity in ice cream fermented to pH 5 with *Lactobacillus acidophilus* and *Bifidobacterium bifidum*

mented dairy product is not an accurate index of β-galactosidase activity, because first glucose and then galactose are rapidly metabolized to lactic acid by culture bacteria (10).

Using the method described in this paper, the fermented ice cream gave a positive reaction with ONPG, and its characteristic yellow color developed in about 10 min. β-Galactosidase activity of the probiotic ice cream after hard freezing was about 1800 units/ml. This enzyme activity declined 31% over the 17 wk of frozen storage (Figure 3). In comparison, β-galactosidase activity is lost more quickly in refrigerated yogurt than in a frozen yogurt. Mashayekh and Brown (12) observed that 20% of β-galactosidase activity in yogurt was lost after 30 d of refrigeration, whereas in a frozen fermented ice cream only 11% activity was lost during the same time. In that experiment, the ice cream mix had been fermented by *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus salivarius* ssp. *thermophilus*. In our experiments, in which the ice cream was fermented using *L. acidophilus* and *B. bifidum*, there was only an estimated 8% loss on β-galactosidase activity during the first 30 d of frozen storage. Speck and Geoffrion (21) also found about 50% reduction in lactase activity of unfrozen yogurt during a 20-d period, but there was no decrease in lactase activity of frozen yogurt. Therefore, freezing has only a minimal effect on β-galactosidase activity. Thus, frozen fermented foods provide the best means of delivering β-galactosidase enzymes to people who are lactose maldigestors but who wish to consume dairy foods. *Lactobacillus acidophilus* and *B. bifidum* are bile-resistant and can survive and grow in the intestinal tract. β-Galactosidase, because it is intracellular, is also able to survive passage through the gastrointestinal tract and supplement in vivo secretion of β-galactosidase (6, 11). Therefore, lactose maldigestors may be able to consume these fermented milk products even though they still contain significant amounts of lactose.

Sensory Evaluation

In recent years, consumption of frozen yogurt has increased drastically because many consumers associate yogurt with good health. In our study, the preferences for ice cream at pH 5.0, 5.5, 6.0, and 6.5 were affected by the panelist's pattern of yogurt consumption. However, consumption of frozen yogurt did not significantly affect sample preference. This is not surprising, considering the types of frozen yogurt presently available on the retail market. Some frozen yogurts have been fully fermented. These have a pH of about 4.5 to 5.0. However, some frozen yogurts are not fermented but are actually a soft serve ice cream (sometimes mixed with 5 to 10% yogurt). These have very little, if any, acidity and are typically in the pH range 6.3 to 6.7. Therefore, this dichotomy in frozen yogurt composition confounds the pattern of frozen yogurt consumption as a predictor of consumer preferences for fermented dairy products.

Figure 4 shows that acceptance for the pH 5.0 sample increased as yogurt consumption rate was higher. However, at the same time that the pH 5.0 sample was receiving high scores as "most liked" sample, it was also scored frequently as "most disliked" (Figure 5). Those who consume yogurt once a year or less prefer ice cream at pH 6.0 and strongly dislike ice cream at pH 5.0. The large range of like and dislike for pH 5.0 and 6.5 samples indicated that the preferred pH, based on overall acceptance, was pH 5.5. There was a significant (P = .05) overall preference for pH 5.5 versus pH 5.0 samples, as shown by the separate 95% comparison circles of their means, but not between any other sample pairs (Figure 6). The flavor mean score of pH 5.5 and pH

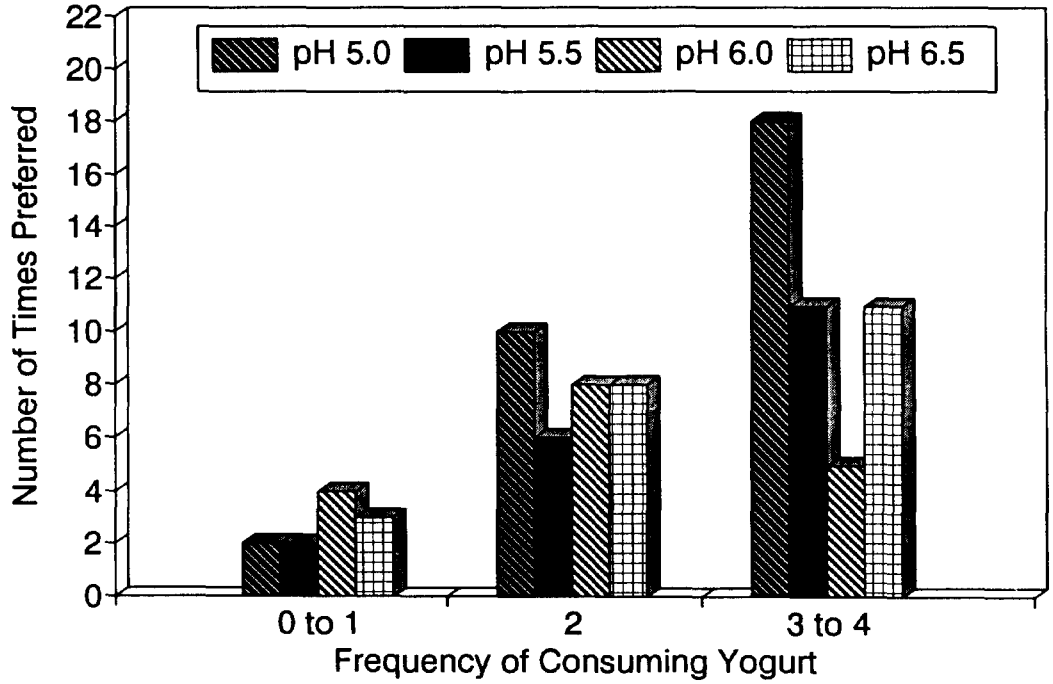

Figure 4. Frequency distribution showing most preferred pH of fermented frozen ice cream, segregated by how often judges consumed yogurt. The judges were divided into three groups based on their yogurt consumption (0 to 1 = once a year or less, 2 = once a month, and 3 to 4 = once a week or more).

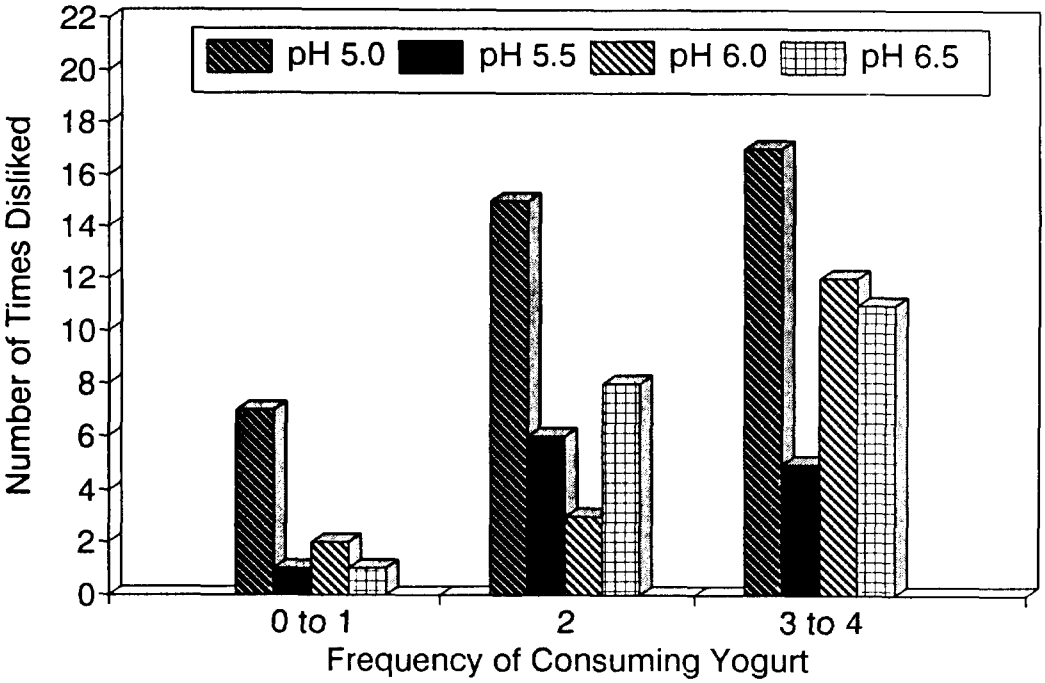

Figure 5. Frequency distribution showing least preferred pH of fermented frozen ice cream segregated by how often judges consumed yogurt. The judges were divided into three groups based on their yogurt consumption (0 to 1 = once a year or less, 2 = once a month, and 3 to 4 = once a week or more).

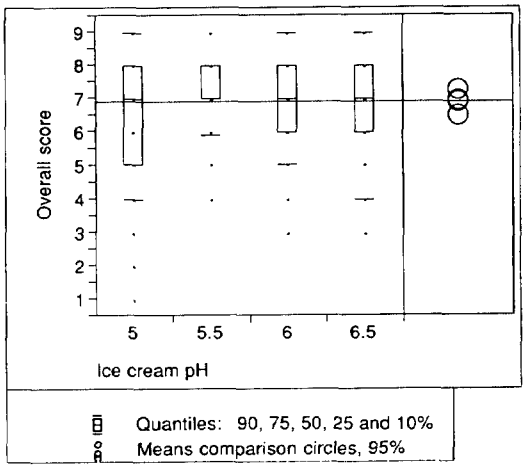

Figure 6. Overall scores from all judges of strawberry-flavored probiotic ice creams (pH 5.0, 5.5, and 6.0) compared with unfermented ice cream (pH 6.5) on a hedonic scale.

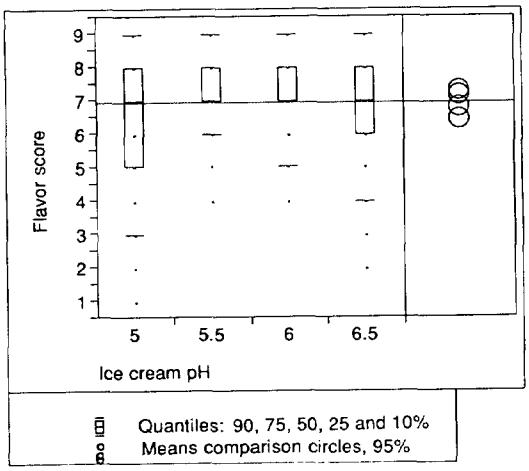

Figure 7. Flavor scores from all judges of strawberry-flavored probiotic ice cream (pH 5.0, 5.5, and 6.0) compared with unfermented ice cream (pH 6.5) on a hedonic scale.

6.0 samples was significantly higher than the flavor mean score of pH 5.0 sample (Figure 7). At a 95% confidence level, there were no significant differences in preference for flavor of the pH 5.5, 6.0, or 6.5 samples.

Although there was no significant difference in preferences between the two heat treatments, the heated sample had a smoother texture with less crystallization than the unheated sample. The unheated sample was firmer, more difficult to scoop, and had more ice crystals. Heating the mix at 82°C for 30 min denatures more whey proteins, which increases their water-binding capacity in the ice cream mix. This is similar to the heating used to increase water-holding capacity of milk protein in the manufacture of yogurt.

CONCLUSIONS

Probiotic ice cream can be manufactured using *L. acidophilus* and *B. bifidum* to ferment ice cream mix. Such an ice cream contains high levels of viable organisms, even after 17 wk of frozen storage. Therefore, ice cream could be used as a good source for delivering these probiotic bacteria to the consumers.

Good textured, fermented ice cream can be made by heat treatment of the ice cream mix. The preferred pH of probiotic ice cream based on overall acceptance was pH 5.5.

ACKNOWLEDGMENTS

This research was supported by the Utah Agricultural Experiment Station and through a Utah State University Faculty Grant FN-11900. Approved as Journal Paper Number 4229.

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