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

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SURVIVAL OF <u>Lactobacillus acidophilus</u> AND

<u>Bifidobacteria bifidum</u> IN ICE CREAM

FOR USE AS A PROBIOTIC FOOD

by

Sharareh Hekmat

A thesis submitted in partial fulfillment
of the requirements for the degree

of

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in

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Sharareh Hekmat

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ABSTRACT

Survival of <u>Lactobacillus</u> <u>acidophilus</u> and

<u>Bifidobacteria</u> <u>bifidum</u> in Ice Cream

for Use as a Probiotic Food

by

Sharareh Hekmat, Master of Science

Utah State University, 1991

Major Professor: Donald J. McMahon
Department: Nutrition and Food Sciences

Ice cream mix (12% fat, 11% milk solids nonfat, 12.5%
sugar, and 4.5% corn syrup solids) was fermented with
<u>Lactobacillus</u> <u>acidophilus</u> and <u>Bifidobacterium</u> <u>bifidum</u>.
Half of the mix was heat treated at 82°C for 30 minutes and
cooled to 40-41°C. The other half was warmed to 40-41°C
and inoculated with the starter cultures. Both were made
into ice cream and stored at -29°C. Survival of <u>L.</u>
<u>acidophilus</u> and <u>B.</u> <u>bifidum</u> and of ß-galactosidase activity
were monitored during 17 weeks of frozen storage.
Reinforced clostridial medium was used to enumerate culture
bacteria. Colony counts, after fermentation, for both <u>L.</u>
<u>acidophilus</u> and <u>B.</u> <u>bifidum</u> were about 5×10^8. The
population of cultures decreased less than one log cycle
after initial freezing. After 17 weeks storage the
bacterial counts were 1×10^7 for <u>B.</u> <u>bifidum</u> and were
4×10^6 for <u>L.</u> <u>acidophilus</u>. During the same period,

ß-galactosidase activity decreased only 31%. Therefore, frozen fermented dairy products provide a good vehicle to supply ß-galactosidase enzymes to people who are lactose maldigestors.

Frozen fermented ice cream was prepared at four different pH's (5.0, 5.5, 6.0, 6.5) by blending fermented mix with unfermented mix and then was frozen to produce samples for sensory evaluation. All samples were strawberry flavored. These were then evaluated by 88 judges. The preferred pH, based on overall acceptance, was 5.5. A second sensory evaluation was conducted to compare heat-treated with non-heat-treated ice cream. There were no significant differences in appearance, texture, flavor, and overall acceptance between the two samples.

Our study shows that ice cream is a suitable vehicle for delivering these beneficial microorganisms and enzymes to consumers.

(70 Pages)

INTRODUCTION

Fermented milk products are consumed in many
countries. Of these fermented milk products, yogurt has
gained widespread consumer acceptance in the United States,
but overall consumption of fermented milk products is still
much less than in many European countries (36,47).

An important aspect of maintaining good health
includes assuring an optimum "balance" of microbial
organisms in the intestine. Certain bacteria, such as
lactobacilli and bifidobacteria, help maintain such a
favorable balance (3). Bifidobacteria are the predominant
organisms in the large intestine of breast-fed infants and
in the intestinal tract of the adult human (39,42). In
breast-fed infants, bifidobacteria account for about 99% of
the cultivated flora (52). As a person gets older, the
number of intestinal bifidobacteria decreases while the
number of clostridia, streptococci and coliforms increases.
Lactobacilli are the predominant organisms in the small
intestine, and they play an important role in metabolic
activities (45).

Lactobacillus acidophilus lives in excellent symbiosis
with Bifidobacterium bifidum. Each bacterium produces
metabolic products which are needed by the other (26).

When humans consume fermented milk products containing
these beneficial microorganisms, the bacteria will stay in
the intestinal tract. Both of these bacteria are resistant

to the bile salts common to the intestinal tract. Therefore, milk products fermented with these microorganisms would have applications as therapeutic foods (32).

LITERATURE REVIEW

Characteristics of Bacteria

Lactobacillus acidophilus are rod-shaped bacteria, gram-positive, nonsporing and thermophilic (30). The pattern of fermented carbohydrates by L. acidophilus is shown in Table 1.

Bifidobacterium bifidum are rods of various shapes, gram-positive, nonacid fast, nonspore-forming and nonmotile. The fermentative characteristics of B. bifidum (Table 2) distinguish it from other species of the genus Bifidobacterium (29).

Growth Characteristics of Bifidobacterium bifidum. Most of the bifidobacterial species require growth promoters for optimal growth. The best growth promoters are yeast extract and bovine casein digest. Casein digest may act as both the nitrogen source and source of an unknown growth factor. The unknown growth factors are most likely mixtures of various peptides and other unknown substances that may be bound to casein or bovine serum albumin (41). Human milk stimulates growth of B. infantis, B. longum and B. bifidum (6).

Bifidobacteria produce both acetic acid and lactic acid. This may limit their growth although their growth is uncoupled from acid production. About 55 to 75% of the lactate and acetate are produced during the stationary growth phase (14).

4

TABLE 1. Pattern of carbohydrate fermentation by <u>L. acidophilus</u> (30).

Carbohydrates	Response
Amygdalin	+
Arabinose	−
Cellobiose	+
Esculin	+
Fructose	+
Galactose	+
Glucose	+
Gluconate	−
Lactose	+
Maltose	+
Mannitol	−
Mannose	+
Melezitose	−
Sorbitol	−
Salicin	+
Rhamnose	−
Ribose	−
Sucrose	+
Xylose	−

Symbols: +, 90% or more strains positive; − 90% or more strains negative.

TABLE 2. Pattern of carbohydrate fermentation by <u>B. bifidum</u> (29).

Carbohydrates	Response
D-Ribose	−
L-Arabinose	−
Lactose	+
Cellobiose	−
Melezitose	−
Raffinose	−
Sorbitol	−
Starch	−
Gluconate	−

Symbols: +, 90% or more strains positive; − 90% or more strains negative.

Nutritional Benefits of Fermented Milk Products

It has been suggested that cultured dairy products are more digestible and nutritious because the proteins, carbohydrates and fats are predigested by the bacterial cultures (34).

Protein. Ayebo and Shahani (5) claimed that in cultured dairy products, digestibility and absorption of protein are improved because of predigestion of protein by starter bacteria. Breaslaw and Kleyn (7) performed in vitro digestion experiments where digestibility of milk and yogurt was compared. They concluded that yogurt protein was twice as digestible as milk protein. It took only three hours to attain more than 70% digestion of yogurt protein, compared with six hours for milk.

Since milk protein is partially hydrolyzed in cultured dairy products, the content of free amino acids and peptides increases. Also, this partial hydrolysis of the protein enhances action of digestive enzymes (48).

Lactic acid. Whereas fresh milk contains very small quantities of lactic acid, fermenting milk converts some of the lactose into lactic acid. Lactic acid acts as a preservative for the product, gives a mildly sour taste, influences the physical property of casein curd to improve digestibility, and enhances the utilization of calcium and other minerals (43).

<u>Vitamins</u>. Lactic acid bacteria require vitamins (mostly B vitamins) for growth but are capable of synthesizing certain vitamins. According to Shahani and Chandan (48), cultured products, in comparison to milk, contain increased concentrations of folic acid, niacin, biotin, pantothenic acid, B6 and B12. Many strains of bifidobacteria (especially <u>B. bifidum</u>) are important for vitamin synthesis in the human gastrointestinal tract. They may synthesize thiamine, nicotinic acid, folic acid, pyridoxine and vitamin B12 (13).

Among the possible factors that can affect vitamin content of milk and, in turn, vitamin content of the cultured products are breed of cattle, diet, climate, geographical location and stage of lactation (23).

<u>Calcium</u>. Calcium is better absorbed from cultured dairy products than from unfermented milk. This is because calcium absorption is increased in the presence of acid and more digestible milk protein (1). Dupuis (15) conducted a study on the efficiency of absorbance and retention of calcium in rats fed yogurt and in rats fed a normal diet containing calcium in other forms. He found that retention and absorbance of calcium supplied by yogurt was better than the calcium in the normal diet. Yogurt is a good source of calcium for middle-aged women who suffer bone deformity due to calcium deficiency (12).

Health Benefits of Consuming
Fermented Milk Products

During fermentation, L. acidophilus and B. bifidum
produce metabolites such as antibiotics, anticarcinogenic
compounds, anticholesteremic compounds and enzymes (48).
Therefore, the dairy products cultured with these organisms
may have not only high nutritional qualities but
therapeutic properties as well (10).

Control of Intestinal Pathogens. When L. acidophilus
and B. bifidum are consumed together, a more complete,
favorable intestinal microflora is achieved (3). Ingestion
of bifidobacteria with lactobacilli inhibits growth of
harmful bacteria in the intestine (19,25). Both L.
acidophilus and B. bifidum produce antibiotics and organic
acids (such as lactic acid and acetic acid) that are
inhibitory toward gram-negative bacteria (45). They also
out-compete other bacteria through competition for
nutrients and attachment sites to epithelial surfaces.
Lactobacilli, for example, consume certain B-vitamins,
thereby, making them unavailable to other microorganisms
(44). Gordon et al. (22) found that L. acidophilus
inhibits or controls staphylococcal growth in the
intestine. Gilliland and Speck (18) reported that L.
acidophilus inhibits the growth of S. aureus, S.
typhimurium and enteropathogenic Escherichia coli when
grown in associated cultures. Rasic and Kurman (44) showed
that B. bifidum can be used as therapeutic treatment for

some intestinal disorders. Mehta et al. (37) reported that
L. acidophilus produces an inhibitory material that is
active against most of the common food-borne and intestinal
pathogens (Table 3).

TABLE 3. Broad spectrum inhibition produced by L.
acidophilus AC1 (37).

Test Organisms	Inhibition
Staphylococcus aureus	+
Bacillus subtilis	+
Bacillus cereus	+
Bacillus megaterium	+
Escherichia coli	+
Aerobacter aerogenes	−
Salmonella typhosa	+
Serratia marcescens	−
Shigella flexnerii	−
Pseudomonas aeruginosa	+

Consumption of L. acidophilus and B. bifidum in
fermented milk products results in improvement of liver
cirrhosis patients. This is accomplished by reduction in
ammonia and free phenols in the blood. B. bifidum and L.
acidophilus ferment carbohydrates in the intestine and
produce an acid environment. At lower pH, ammonia is found
mostly as ammonium ion which is nonabsorbable. Therefore,
these patients could increase their protein intake to about
70 grams per day (26).

Anticarcinogenic Action. Some lactic acid bacteria
have been shown to have anticarcinogenic properties
(40,46). In epidemiological studies (28) it was observed

that people who consume high levels of cultured dairy products have lower incidence of colon cancer. The mechanism of this action is not fully understood but there are two possibilities: by lactobacilli inhibition of formation of carcinogenic compounds formed in the gastrointestinal (GI) tract or by antagonistic action towards microorganisms that convert procarcinogens into carcinogens (35).

Shahani et al. (49) conducted a study on the influence of L. acidophilus on tumor cells in rats. They concluded that L. acidophilus produced something during growth that was antagonistic toward the proliferation of tumor cells. Likewise, rats receiving sour milk had lower numbers of colon tumor cells than those that received no sour milk products (53). Goldin and Gorbach (20) studied the effects of dietary L. acidophilus on incidence of chemically induced colon cancer in rats. Those rats fed grain diets for 36 weeks had a 31% incidence of colon cancer versus 83% in rats fed beef diets. However, rats given beef plus L. acidophilus had a lower incidence of colon cancer (73%).

Goldin and Gorbach (21) further 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 ß-glucuronidase, nitroreductase and azoreductase. They found reduced levels of each enzyme when milk supplemented with L. acidophilus was consumed.

Kimura et al. (33) reported that after two to four days of intravenous adminstration of B. bifidum, these organisms selectively localize and proliferate in tumor-bearing tissues of mice. The cancer lesions are believed to have low oxygen tension. No B. bifidum was found in healthy organs such as liver, spleen, kidney, lung, blood, muscle and bone marrow which are oxygen-rich tissues. Therefore, B. bifidum may prove useful as a tool for diagnosis of cancer.

Lipotechoic acid (LTA) is a common surface antigen within the genus Bifidobacterium. LTA has immunogenic properties because it binds to antibodies and chemicals. Therefore, these chemicals accumulate in tumor cells with low oxygen tension, and immunocytotoxic reactions associated with LTA would take place (8).

Lactose Digestibility. Lactase enzymes (ß-galactosidases) are necessary for hydrolysis of lactose into galactose and glucose. Some people do not produce sufficent ß-galactosidase in their small intestine and, therefore, are unable to adequately digest lactose. Some lactose maldigestors may consume cultured milks without intestinal disturbances. This is because of reduced lactose content and the presence of ß-galactosidase from the starter bacteria (16,50,51).

ß-galactosidases are produced by Streptococcus salivarius ssp. thermophilus and Lactobacillus delbruekii

ssp. <u>bulgaricus</u> during manufacture of yogurt (31).
However, these bacteria are not bile resistant and thus can
not survive in the intestinal tract.   In contrast, <u>L.</u>
<u>acidophilus</u> is resistant to bile acids.   Therefore, it can
survive passage through and colonize the GI tract and
produce more ß-galactosidase (32).   <u>B.</u> <u>bifidum</u> is also
resistant to bile salts.   The best proof that these
bacteria can survive passage through the GI tract is the
fact that they can be found in human faeces (26).

   <u>Control of Serum Cholesterol</u>.   High levels of serum
cholesterol have been associated with heart disease.   A
reduction in serum cholesterol level therefore reduces the
risk of heart attack.   Serum cholesterol level can be
influenced by intestinal microflora.   Danielson and
Gustafson (11) suggested that gastrointestinal
microorganisms play a role in the metabolism of
cholesterol.   Serum cholesterol is significantly reduced in
people who ingest acidophilus milk (27).   They concluded
that consumption of <u>L.</u> <u>acidophilus</u> interferes with
cholesterol absorption from the intestine.
Grunewald (24) conducted a study on the influence of
acidophilus milk on serum cholesterol in rats.   Rats with
fermented milk had lower serum cholesterol (Table 4).
She also concluded that <u>L.</u> <u>acidophilus</u> produces compounds
that lower serum cholesterol level.   The same conclusion

TABLE 4. Serum cholesterol level in rats following four weeks on diets with and without milk fermented by L. acidophilus (24).

Treatment Group	Serum Cholesterol (mg/dl)
No milk control	78
Milk control	79
L. acidophilus milk	65c

c Significantly lower than other two (P<.05).

can be drawn from in vitro experiments. L. acidophilus can

remove cholesterol from laboratory media when grown under

appropriate conditions. This includes anaerobic

environment, presence of bile salts and a growth medium

that contains a source of cholesterol (17).

OBJECTIVES

The objectives of this research were to

1) Produce a fermented ice cream that contains viable <u>L. acidophilus</u> and <u>B. bifidum</u> cultures.

2) Determine survival of <u>L. acidophilus</u> and <u>B. bifidum</u> upon freezing the ice cream.

3) Determine viability of <u>L. acidophilus</u> and <u>B. bifidum</u> cultures during storage of the ice cream.

4) Determine ß-galactosidase activity in the frozen fermented product.

5) Examine ß-galactosidase activity during storage of the ice cream.

MATERIALS AND METHODS

Preparation of Starter Cultures

A schematic diagram of starter culture preparation is given in Figure 1. Each culture was grown separately before being added to the ice cream mix.

To prepare mother culture for B. bifidum, a solution of 7% whey, .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 40-41°C. Commercial freeze dried B. bifidum (10LF,946745101) was obtained from Chr. Hansen Laboratory Inc. (Milwaukee,Wisconsin). One percent inoculum of B. bifidum was added to the whey base solution and incubated anaerobically at 40-41°C for 15 h.

Eleven percent reconstituted non fat dry milk (NFDM) was prepared. It was autoclaved at 121°C for 15 min and then cooled to 40-41°C. Two percent inoculum of B. Bifidum from the mother culture was added to the milk and incubated anaerobically at 40-41°C for 15 h.

Commercial freeze dried L. acidophilus (10LF,946744A) was also obtained from Chr. Hansen Laboratory Inc. One percent inoculum of freeze dried L. acidophilus was added directly to the sterilized NFDM. Then, it was incubated anaerobically at 40-41°C for 15 h.

Whey, Yeast Extract, Cysteine, Trimagnesium Phosphate

11% NFDM

Autoclave at 121°C for 15 min.

Autoclave at 121°C for 15 min.

Cool to 40-41°C

Cool to 40-41°C

freeze dried
B. bifidum

freeze dried
L. acidophilus

1% Inoculum

Incubate Anaerobically at 40-41°C for 15 h

Mother Culture

2% Inoculum

1% Inoculum

Incubate Anaerobically at 40-41°C for 15 h

Incubate Anaerobically at 40-41°C for 15 h

B. bifidum
Starter Culture

L. acidophilus
Starter Culture

Figure 1. Schematic diagram of starter culture preparation.

Manufacturing Procedure of
Frozen Fermented Ice Cream

Standardized ice cream mix with 12% fat, 11% non fat dry milk, .32% stabilizer/emulsifier (Party Pride, Safe Way Stores Incorporated), 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 28-30 s, homogenized at 17.5 MPa and then aged overnight at 4°C. Half of the mix was given an additional heat treatment. This was termed the "HEATED" sample, while the sample that received a pasteurization treatment but no additional heating was termed "NON-HEATED". The "HEATED" sample was heat-treated at 82°C for 30 min and cooled to 40-41°C. The "NON-HEATED" mix was just warmed to 40-41°C prior to inoculating it with the starter cultures.

The ice cream mix was then placed in a hot water bath at 43-44°C. Four percent inoculum of each starter culture was added, mixed well and fermented for approximately five hours until desired pH (pH = $4.9 \pm .05$) 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. A schematic diagram of this manufacturing procedure is given in Figure 2.

```
┌─────────────────────────────────┐
│ Standardized Ice Cream Mix      │
└─────────────────────────────────┘
            │
┌─────────────────────────────────┐
│ Pasteurize at 79.4°C for        │
│ 28-30 s                         │
└─────────────────────────────────┘
            │
┌─────────────────────────────────┐
│ Homogenize at 17.5 MPa          │
└─────────────────────────────────┘
            │
┌─────────────────────────────────┐
│ Aged Overnight                  │
└─────────────────────────────────┘
            │
┌─────────────────────────────────┐
│ *Heat at 82°C for 30 min.       │
└─────────────────────────────────┘
            │
┌─────────────────────────────────┐        ┌──────────────────────────┐
│ Cool to 41-42°C                 │────────│ Add 4% each of L.        │
└─────────────────────────────────┘        │ acidophilus and B.       │
            │                               │ bifidum cultures         │
┌─────────────────────────────────┐        └──────────────────────────┘
│ Ferment for approx. 5 h         │
│ or Until Desired pH Reached     │
└─────────────────────────────────┘
            │
┌─────────────────────────────────┐
│ Cool in Ice Bath to 5°C         │
└─────────────────────────────────┘
            │
┌─────────────────────────────────┐        ┌──────────────────────────┐
│ Freeze in Batch Freezer         │────────│ *Add Flavoring           │
└─────────────────────────────────┘        └──────────────────────────┘
            │
┌─────────────────────────────────┐
│ Package                         │
└─────────────────────────────────┘
            │
┌─────────────────────────────────┐
│ Harden in Freezer at -29°C      │
└─────────────────────────────────┘
```
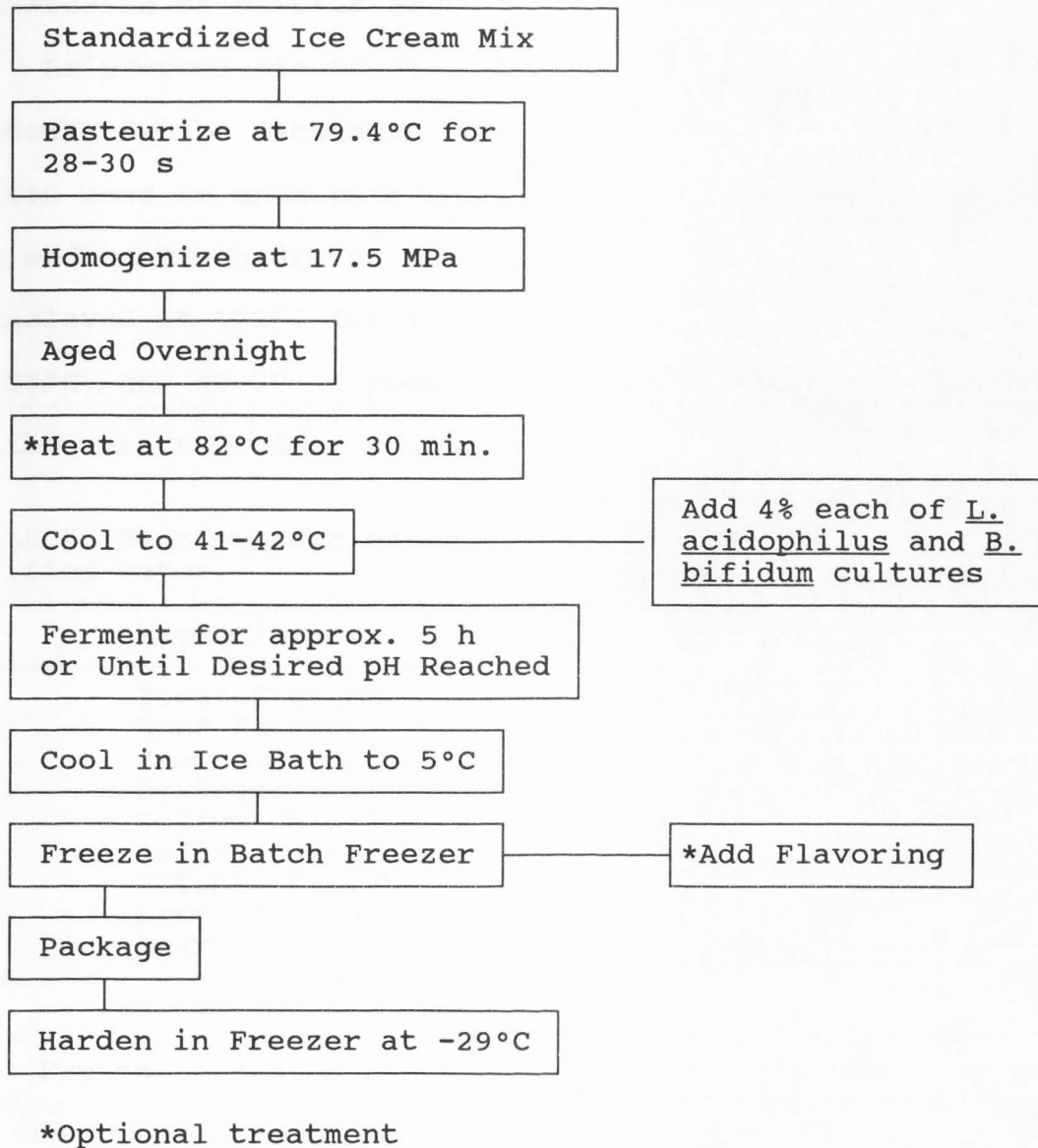
*Optional treatment

Figure 2. Schematic diagram of manufacturing procedure.

Enumeration of Starter Bacteria

Reinforced clostridial agar (RCA)(BBL Microbiology
Systems, Becton Dickinson and Co. Cockeysville, MD.) (Table
5) was used to enumerate L. acidophilus and B. bifidum.
The medium was hydrated as directed, mixed well and
autoclaved at 121°C for 15 min. Then, the agar was cooled
to 55°C, and 20-30 ml poured into sterile plates. The
plates were stored at 4°C.

TABLE 5. Reinforced clostridial agar formula per liter of
purified water.

Ingredients	Grams
Yeast Extract	3.0
Beef Extract	10.0
Pancreatic Digest of casein	10.0
Dextrose	5.0
Sodium Chloride	5.0
Sodium Acetate	3.0
Soluble Starch	1.0
L-Cysteine.HCl	.5
Agar	13.5

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 agar plates.
The plates were then incubated in an anaerobic environment
(BBL Gas Pak, Becton Dickinson Microbiology Systems,
Cockeysville, MD.) at 40-41°C for 48 h.

The total number of L. acidophilus and B. bifidum was
determined based upon the differences in colonial
appearances of the two bacteria. L. acidophilus produced
pin-point sized colonies while B. bifidum produced large

colonies. Viable numbers of <u>L.</u> <u>acidophilus</u> and <u>B.</u> <u>bifidum</u> were determined after 1, 5, 9, 13 and 17 weeks of frozen storage. Statistical analysis of data from the taste panels were made using the costat program (CoHort Software) for IBM computers (Berkeley, California).

Taste Panel

Frozen fermented ice cream was prepared at four different pH's (5.0, 5.5, 6.0, 6.5) by mixing fermented mix with unfermented mix in the appropriate ratios. The sample at pH 6.5 was standard ice cream mix with no addition of fermented ice cream mix. All samples were strawberry flavored. A hedonic taste panel (4) was conducted. The judges were asked to indicate their most preferred and their least preferred samples. In addition, they were asked to describe their consumption of yogurt and frozen yogurt (Figure 3).

The hedonic scale method measures the level of acceptance for foods and relies on the panelist's ability to report a feeling of like or dislike (2). This method has nine categories, as follows: like extremely, like very much, like moderately, like slightly, neither like nor dislike, dislike slightly, dislike moderately, dislike very much and dislike extremely. The judges evaluated flavor, texture and overall acceptance of the product. The ice cream cups were coded 24 hours prior to taste panel sensory evaluation to allow ink aroma to be dissipated.

Please evaluate the samples in the order listed.
Use the following scale.

> 9=like extremely
> 8=like very much
> 7=like moderately
> 6=like slightly
> 5=neither like nor dislike
> 4=dislike slightly
> 3=dislike moderately
> 2=dislike very much
> 1=dislike extremely

On the line beside the sample's number, enter the number
(from the above scale) that best describes how you feel
about flavor, texture and overall.

Sample #	Flavor	Texture	Overall
505	_____	_____	_____
498	_____	_____	_____
923	_____	_____	_____
611	_____	_____	_____

Please rank the samples in the order of your preference by
placing the sample code number beside the scale below.

Sample like best _____
Sample like least _____

What is the best description of how often you eat yogurt
(choose only one).

------ Once a day or more
------ Once a week
------ Once a month
------ Once a year
------ Never

How often do you eat frozen yogurt?

------ Once a day or more
------ Once a week
------ Once a month
------ Once a year
------ Never

Figure 3. Form used for first taste panel.

Judges were served four samples and requested to rinse
their mouths between samples. A paired-comparisons taste
panel between "HEATED" and "NON-HEATED" frozen fermented
ice cream was also conducted (Figure 4). Statistical
analysis of data from the taste panels was made using JMP
software for Macintosh computers from SAS (Raleigh, North
Carolina).

Lactase Assay

The chromogenic substrate O-nitrophenyl-ß-D-
galactopyranoside (ONPG) was used to measure ß-
galactosidase activity (9,51). Chromogenic substrates are
colorless compounds that are hydrolyzed to produce colored
products. For example, in the presense of ß-galactosidase,
ONPG is converted to D-galactose and O-nitrophenol. In
solution, O-nitrophenol is yellow and can be measured by
its absorption at 420 nm. If ONPG concentration is
sufficiently high, the amount of O-nitrophenol produced is
proportional to the amount of ß-galactosidase present (38).

ß-galactosidase activity was measured by adding 1 ml
of frozen fermented ice cream to 50 ml of .1$\underline{M}$ phosphate
buffer (pH 7.0) containing .001$\underline{M}$ $MgSO_4$ and .05$\underline{M}$ ß-
mercaptoethanol, and mixing well. One milliliter of the
diluted sample was withdrawn and two drops of chloroform
and one drop of .1% sodium dodecyl sulfate was 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

Please evaluate the samples in the order listed.
Use the following scale.

    9=like extremely
    8=like very much
    7=like moderately
    6=like slightly
    5=neither like nor dislike
    4=dislike slightly
    3=dislike moderately
    2=dislike very much
    1=dislike extremely

On the line beside the sample's number, enter the number
(from the above scale) that best describes how you feel
about appearance, flavor, and texture.

Sample #	Appearance	Flavor	Texture
909	_____	_____	_____
725	_____	_____	_____

Please rank the samples in the order of your preference by
placing the sample code number beside the scale below.

Sample like best _____

Sample like least _____

Figure 4. Form used for second taste panel.

started by addition of .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 $1\underline{M}$ Na_2CO_3. At this pH, ß-galactosidase is inactivated (38,51).

Optical density at 420 nm was recorded using a Beckman DU-65 spectrophotometer. The 420 nm reading is actually a combination of absorbance by O-nitrophenol and light-scattering by cell debris. Rather than correcting for influence of light-scattering by measuring absorbance at 550 nm where O-nitrophenol does not absorb [as was done by Mashayekh (36)], it was eliminated by spinning down the cell debris. The additional absorbance reading at 550 nm was then not required. The assay mixture was centrifuged (Sorvall Instruments, Du Pont) at 10,000 RPM (16,266g) for 15 min and its optical density measured at 420 nm. The following formula was used to determine units of enzyme activity.

$$\text{Units (ß-D-galactosidase)} = 1000 * \frac{A420}{t * v}$$

where, t = time of reaction in minutes and v = volume (ml) of culture used in the assay.

RESULTS AND DISCUSSION

Acid Production

L. acidophilus and B. bifidum were able to grow and
produce acid in the ice cream mix. The rate of acid
production in heated and non-heated ice cream mix during
fermentation is shown in Figure 5. Initial acid production
was faster and more consistent in the "HEATED" ice cream
mix than in the "NON-HEATED" mix. This was 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. It also provided a semi-sterile
environment for the growth of culture bacteria and there
was, therefore, less competition for nutrients from other
non-lactic bacteria.

Microbial Counts

The total number of L. acidophilus and B. bifidum
fermented in the ice creams were determined based upon
their colony morphology when grown on RCA. L. acidophilus
produced pin-point sized colonies while B. bifidum produced
large colonies (Figure 6). The total colony counts after
fermentation of the ice cream mix to pH 4.9 were 5×10^8
for both types of bacteria. Initial freezing of the ice
cream in the batch freezer caused a reduction of less than
one log cycle in the total colony counts. The final colony
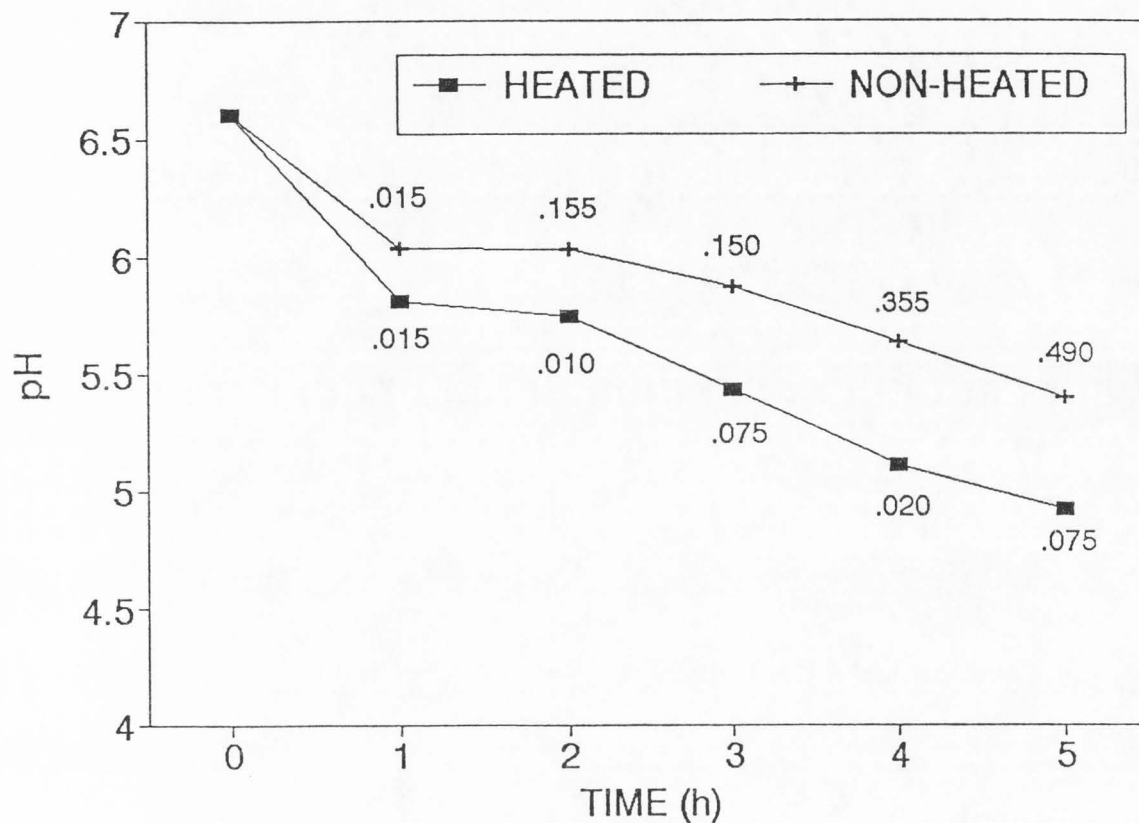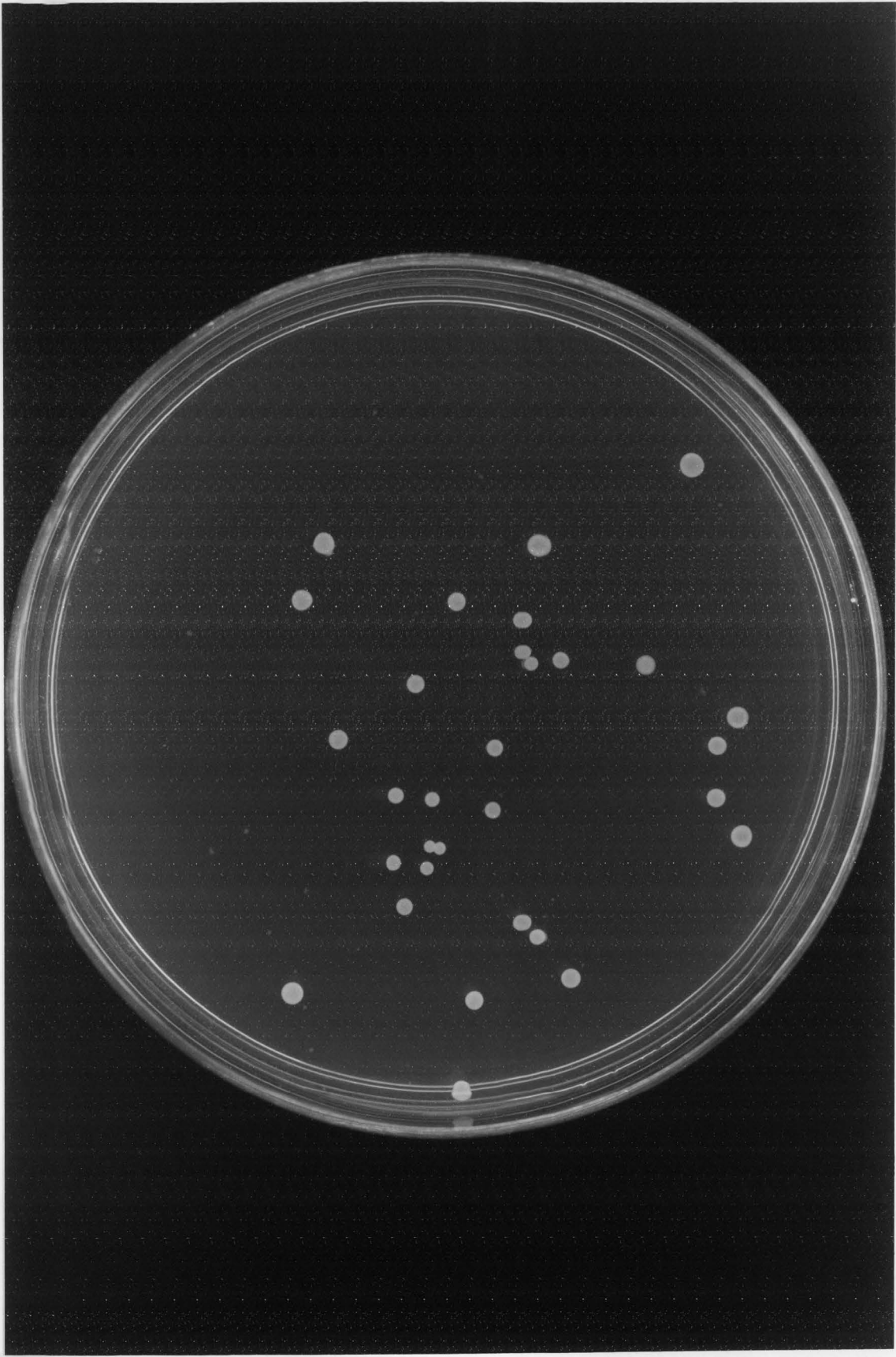counts, after 17 weeks of frozen storage, for B. bifidum

Figure 5. Comparison of acid production in "HEATED" and "NON-HEATED" fermented ice cream during fermentation process (numbers indicate standard deviation of the mean for two replicates).

Figure 6. Photograph showing colony morphology of
L. acidophilus and B. bifidum.

had been reduced by one log cycle and L. acidophilus by two
log cycle to 1 x 10^7 and 3 x 10^6 respectively.

Figures 7 and 8 depict the total plate counts over 17
weeks of frozen storage of the ice cream. These data were
analyzed using a three-way analysis of variance randomized
complete blocks for storage time, species and heat
treatment (Table 6). There were no significant differences
in colony counts between "HEATED" and "NON-HEATED"
treatments at P ≤ .05 confidence level. However, the total
colony counts for B. bifidum were significantly different
from L. acidophilus (P < .0000). The interaction between
type of culture bacteria and length of frozen storage was
also significant (P = .0009).

TABLE 6. Three-way analysis of variance randomized complete
blocks for number of bacteria in fermented ice cream over
17 weeks of frozen storage for L. acidophilus and B.
bifidum in both "HEATED" and "NON-HEATED" samples.

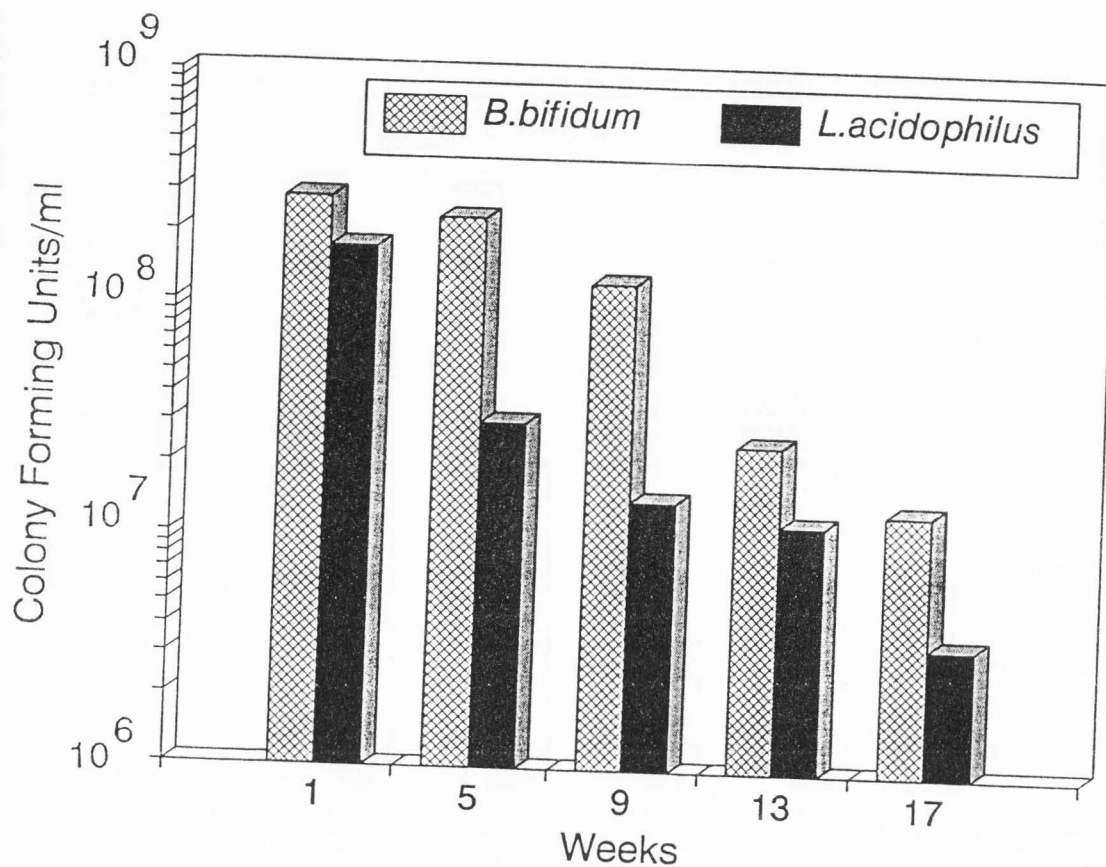| Source	df	MS	F	P
Reps	1	2.15 x 10^{15}	.63	.4350
Main Effects				
Weeks	4	8.38 x 10^{16}	24.80	.0000*
Species	1	1.52 x 10^{17}	44.99	.0000*
Heat	1	3.35 x 10^{15}	.99	.3317
Interaction				
Week * Spec	4	2.51 x 10^{16}	7.42	.0009*
Week * Heat	4	2.02 x 10^{15}	.59	.6696
Spec * Heat	1	1.21 x 10^{16}	3.57	.0742
Week * Spec * Heat	4	4.07 x 10^{15}	1.20	.3420
Error	19	3.38 x 10^{15}		
Total	39			

Figure 7. Mean survival of L. acidophilus and B. bifidum in "HEATED" fermented ice cream over 17 weeks of frozen storage (two replications).

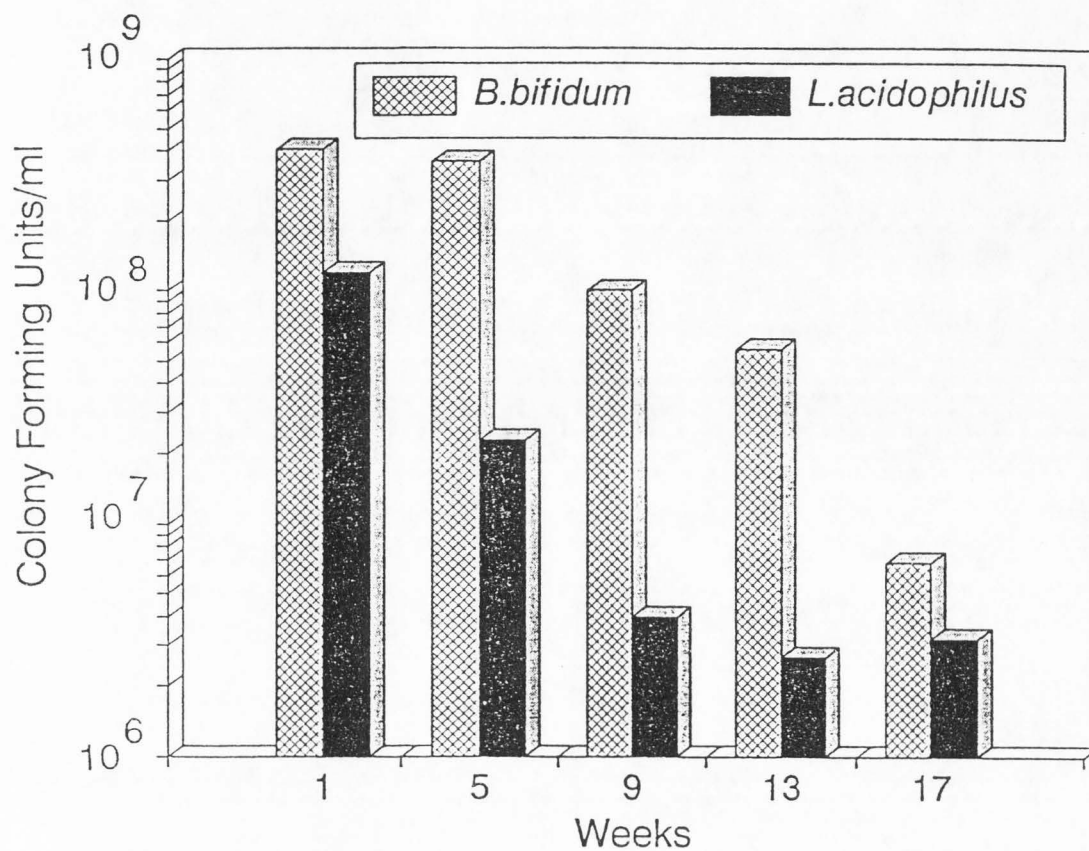Figure 8. Mean survival of L. acidophilus and B. bifidum in "NON-HEATED" fermented ice cream over 17 weeks of frozen storage (two replications).

The total colonial counts for both bacteria decreased by two log cycle in both treatments over 17 weeks of frozen storage. However, the rate of reduction of L. acidophilus was quicker than for B. bifidum, especially with the "NON-HEATED" sample. There was a sharp reduction in colonial counts for L. acidophilus during the first nine weeks of storage to <3 x 10^7 colony forming units/ml and then no further drop. In the "HEATED" sample the L. acidophilus decreased continuously over the 17 weeks of frozen storage.

Our study shows that ice cream mix can be fermented with L. acidophilus and B. bifidum and still have viable organisms (especially B. bifidum) after 17 weeks of frozen storage.

Taste Panel

To produce samples for sensory evaluation with pH's 5.0, 5.5, 6.0 and 6.5, fermented ice cream mix was blended with unfermented mix and then frozen. These were then evaluated by 88 judges. It was found that the preferences for ice cream at these pH's was affected by the panelist's pattern of yogurt consumption (Table 7). Consumption of frozen yogurt did not significantly affect sample preference. This is not surprising when one considers the types of frozen yogurt that are presently available on the retail market. There are some frozen yogurts that have been fully fermented. These would have pH values of about 4.5-5.0. On the other hand, some frozen

TABLE 7. Flavor mean score for all categories of yogurt consumption at four different pH's and the significance of yogurt consumption on sample preference.

Yogurt Cons.	Num. of Panelist	Flavor Mean				Prob>F
		5.0	5.5	6.0	6.5	
Never	2	4.0	7.5	7.5	7.0	0.2481
Once a year	9	6.3	7.4	7.7	7.8	0.0789
Once a month	32	6.6	7.4	7.2	6.7	0.1367
Once a week	41	6.6	7.2	6.9	6.6	0.2058
Every day	4	5.0	6.7	7.5	6.7	0.5861

yogurts are not fermented but are actually a soft serve ice cream (sometimes mixed with 5-10% yogurt). These have very little, if any, acidity and are typically in the pH range of 6.3-6.7. This dichotomy in frozen yogurt composition therefore confounds one's ability to use it as a predictor of consumer's preferences for liking or disliking fermented dairy products.

Figure 9 shows that acceptance for the pH 5.0 sample increased as yogurt consumption rate was more frequent. However, at the same time as the pH 5.0 sample was receiving high scores as "most liked" sample, it was also being scored frequently as "most disliked" (Figure 10). 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. There were no significant differences (at 95% confidence levels) on flavor mean score between samples for each of the individual categories based on yogurt consumption (Figures 11-15). The large range of like and dislike for pH 5.0 and 6.5 samples made the preferred pH, based on
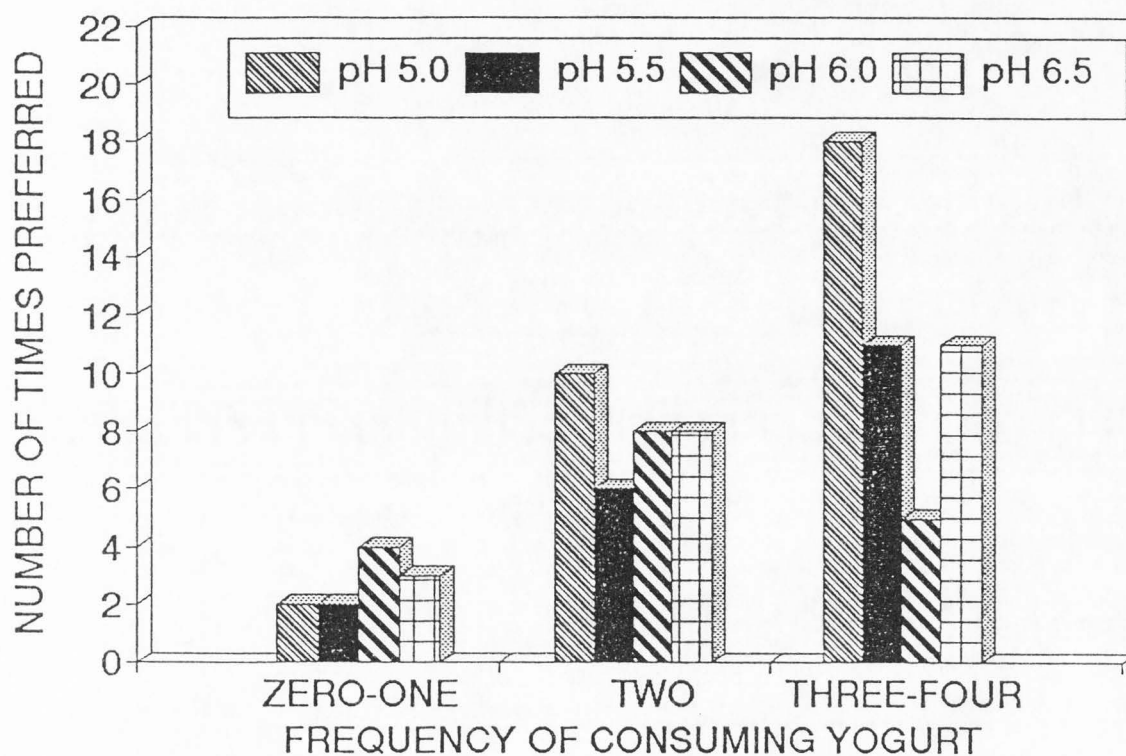
Figure 9. Frequency distribution showing response to question on which of the samples was most preferred and segregated by how often panelists consumed yogurt. The panelists were divided into three groups based on their yogurt consumption (Zero-One =Once a year or less, Two =Once a month, Three-Four =Once a week or more).
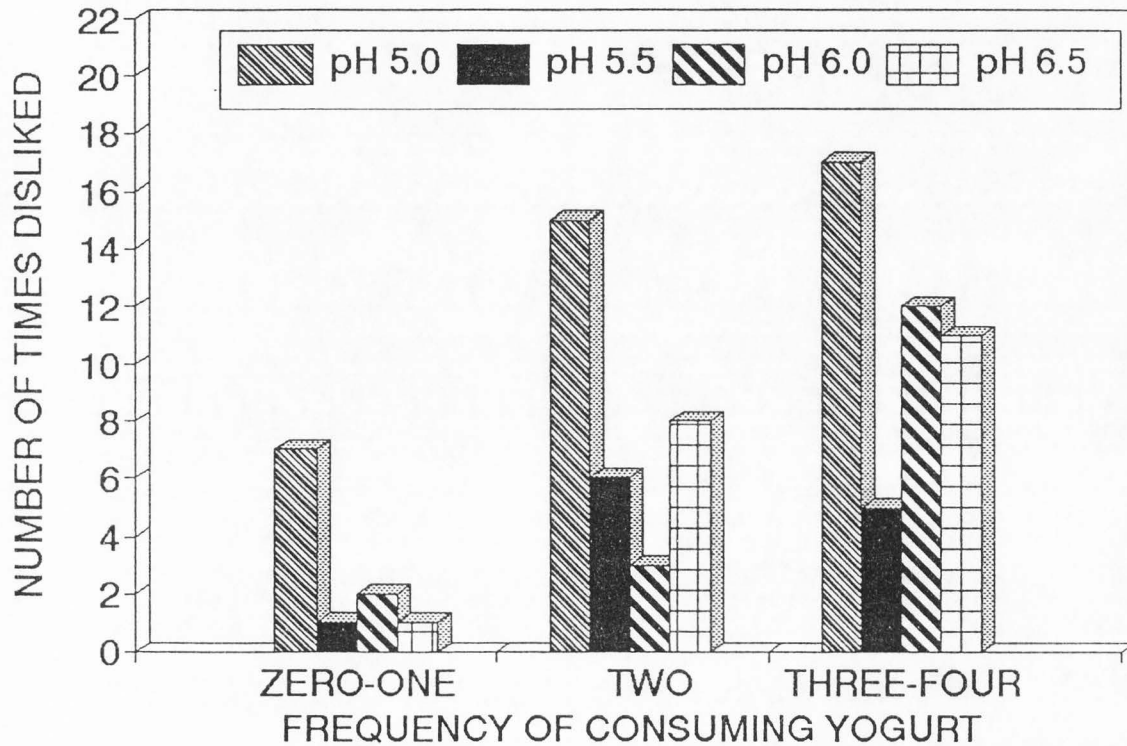
Figure 10. Frequency distribution showing response to
question on which of the samples was most
disliked and segregated by how often
panelists consumed yogurt. The panelists
were divided into three groups based on
their yogurt consumption (Zero-One =Once a
year or less, Two =Once a month, Three-Four=
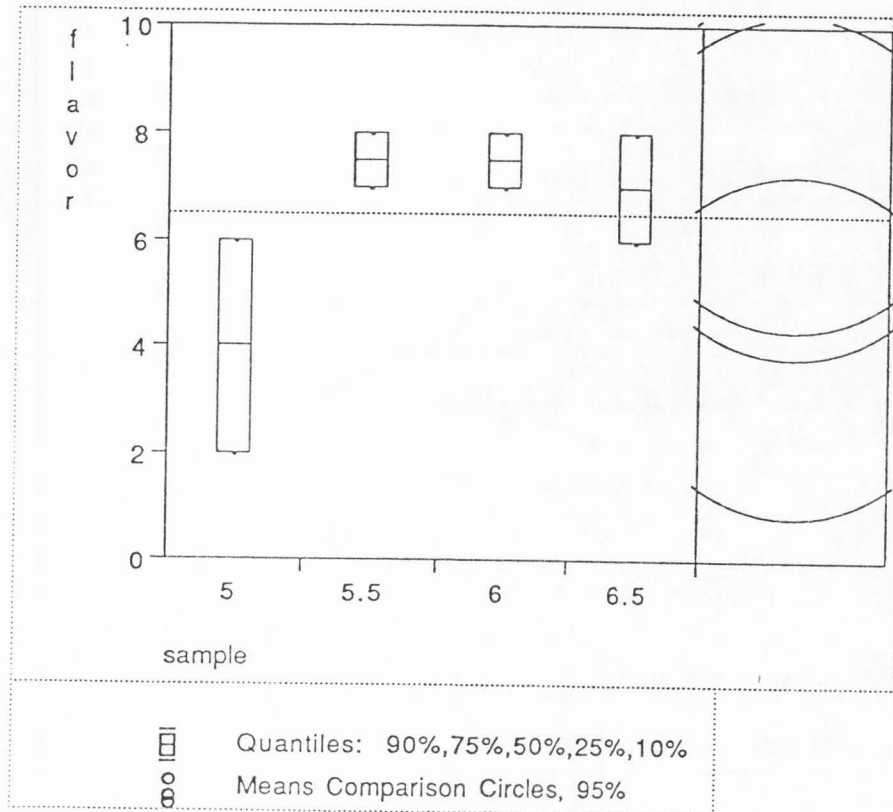Once a week or more).

Figure 11. Flavor scores of strawberry flavored
fermented ice cream on a hedonic scale of 1-9
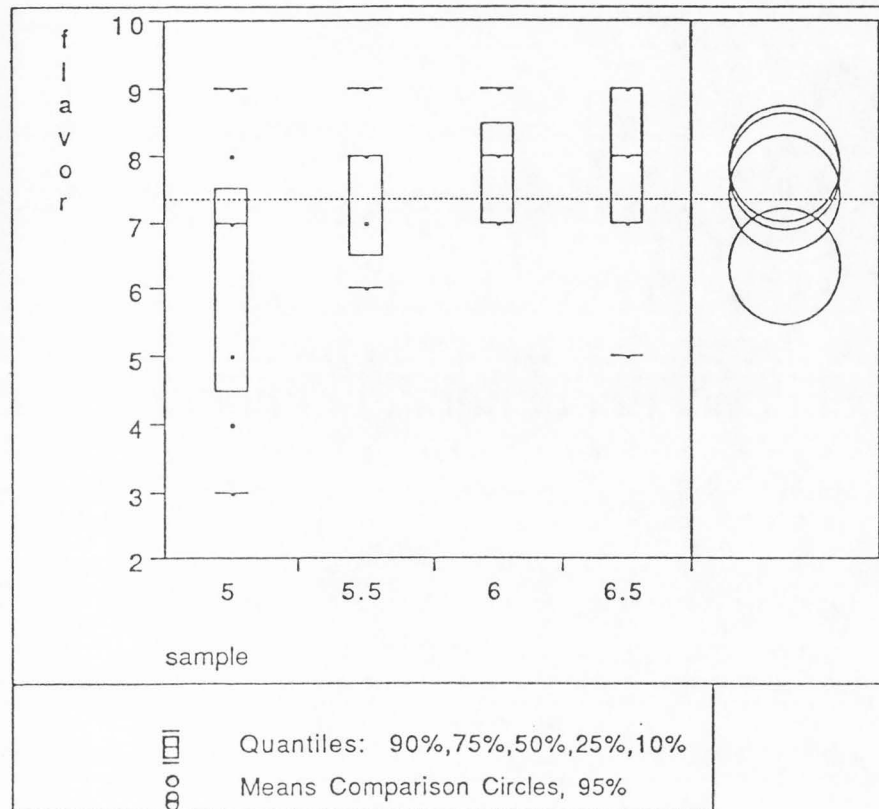for those panelists who reported their
yogurt consumption as never.

Figure 12. Flavor scores of strawberry flavored
fermented ice cream on a hedonic scale of 1-9
for those panelists who reported their
yogurt consumption as once a year.

Figure 13. Flavor scores of strawberry flavored
fermented ice cream on a hedonic scale of 1-9
for those panelists who reported their yogurt
consumption as once a month.

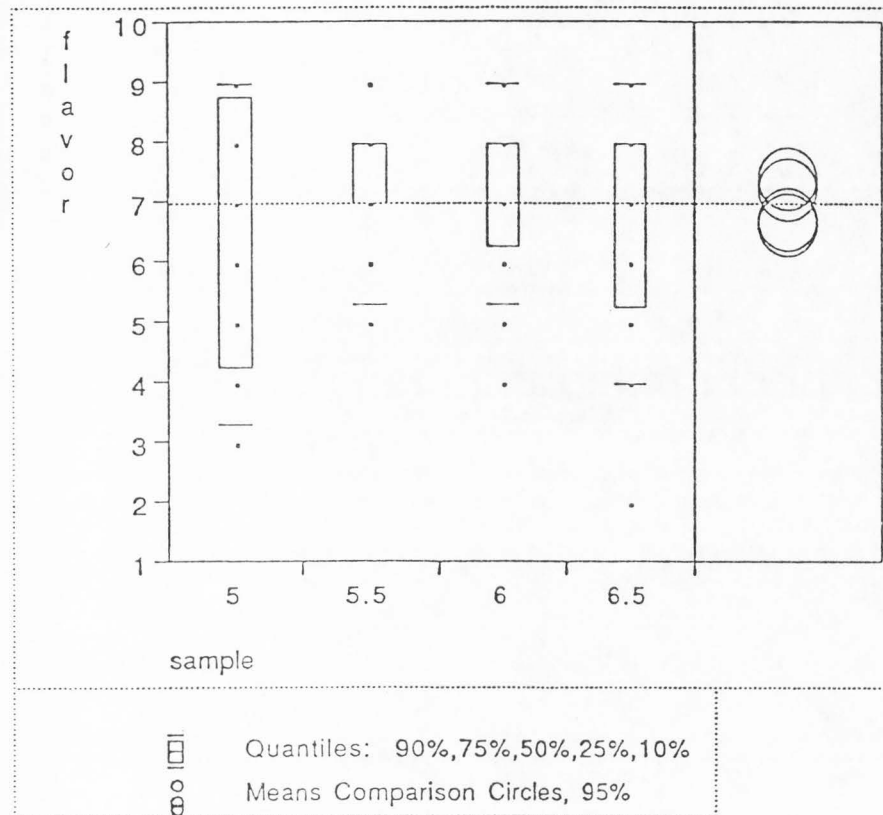Figure 14. Flavor scores of strawberry flavored
fermented ice cream on a hedonic scale of 1-9
for those panelists who reported their yogurt
consumption as once a week.
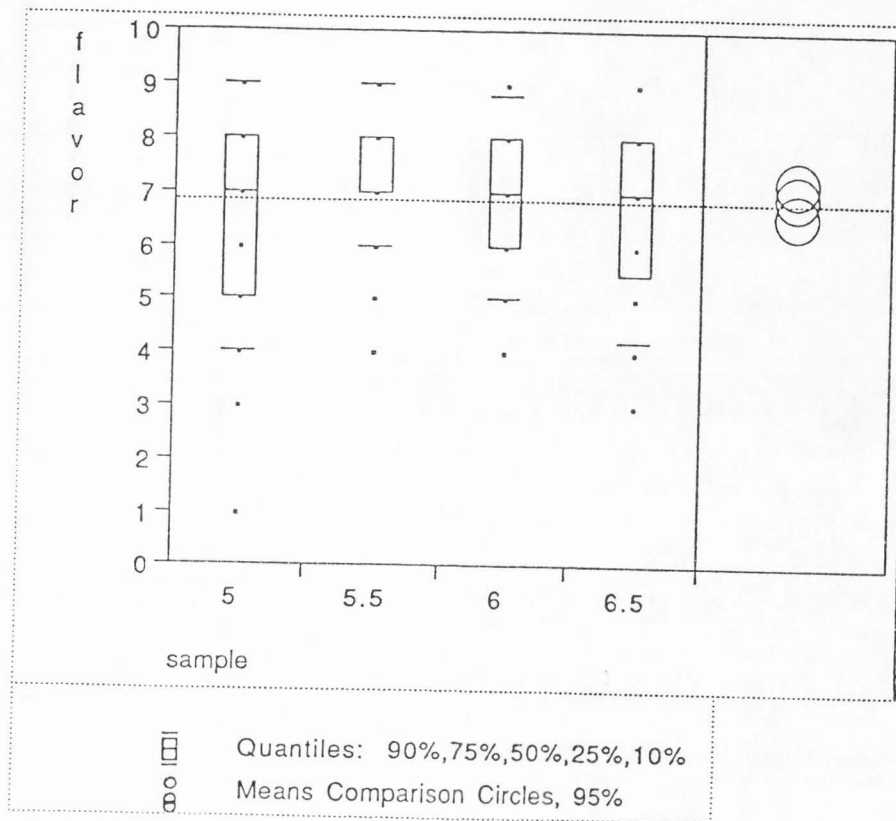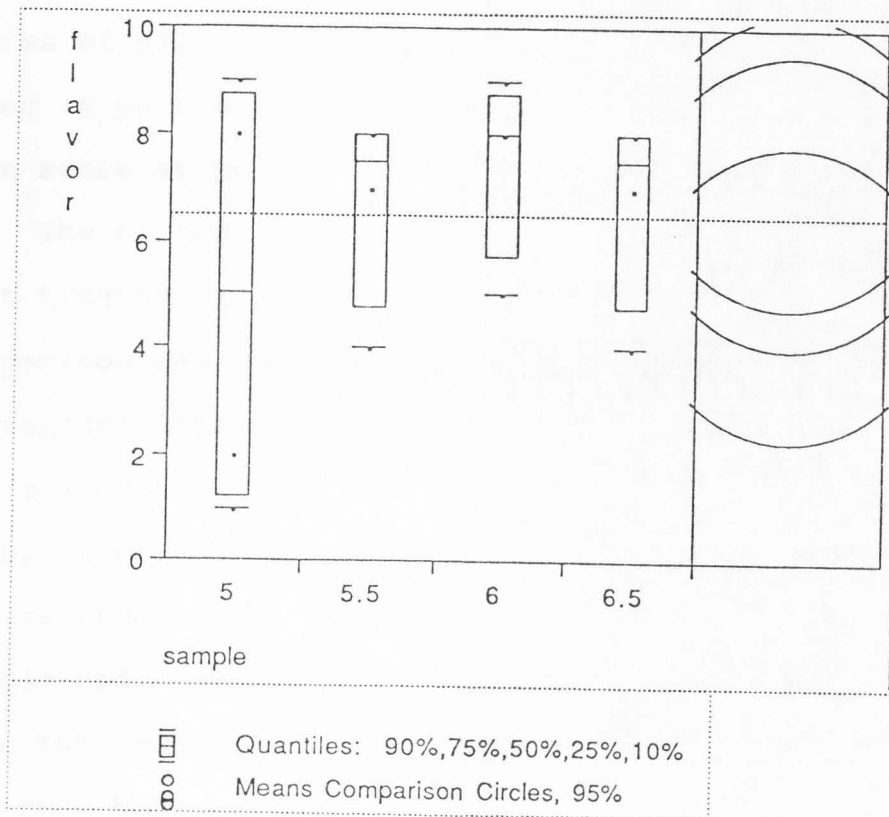
Figure 15. Flavor scores of strawberry flavored
fermented ice cream on a hedonic scale of 1-9
for those panelists who reported their yogurt
consumption as every day.

overall acceptance, to be pH 5.5. There was a significant difference between overall mean score of the pH 5.0 and pH 5.5 samples but not between any other samples (Figure 16). There were no significant differences among texture mean scores of all samples (Figure 17). However, flavor mean score at pH 5.0 was significantly different from flavor mean score at pH's 5.5 and 6.0 (Figure 18).

The second sensory evaluation was conducted to compare heat treated with non-heat treated ice cream. This comparison was done to determine consumer preferences based on texture differences between the two samples. Heating the mix at 82°C for 30 min denatures whey protein which, in turn, increases water holding capacity of the proteins in an ice cream mix. My observation was that the "HEATED" sample had a smoother texture with less crystallization than the "NON-HEATED" sample. The "NON-HEATED" sample had a firmer body with more ice crystals. However, when the panelists judged these samples they indicated no significant preference between the two treatments. Also, no significant differences in appearance, texture, flavor and overall acceptance were observed (Figures 19 and 20). The inability of panelists to detect the textural differences could be due to the fact that some people may detect slightly "cooked" flavor in the heated ice cream and that made them choose the "NON-HEATED" sample. Or it may be that for non-trained consumers, an ice crystal defect in ice cream is not felt to be detrimental.
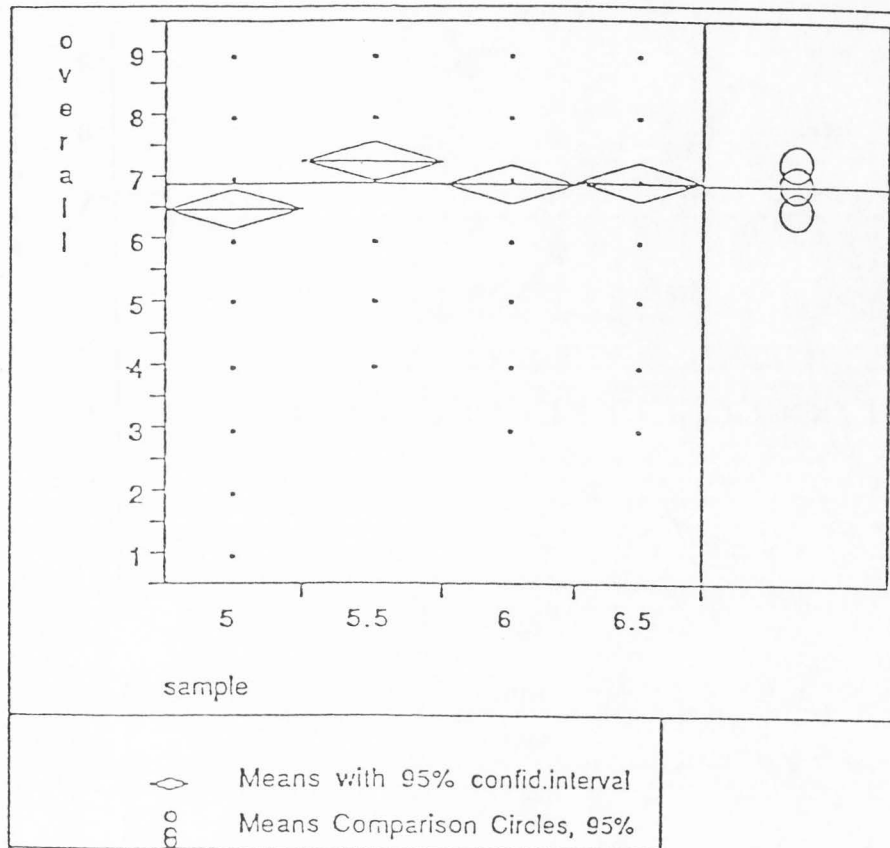
41

Figure 16. Overall scores of fermented ice cream on a
hedonic scale from all judges.

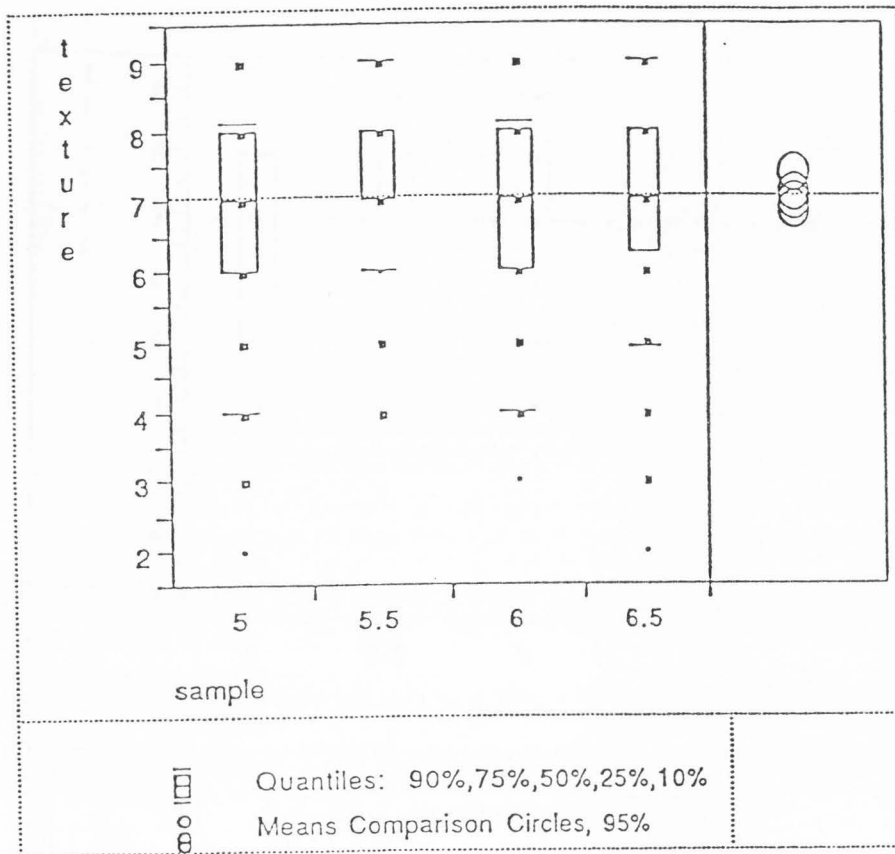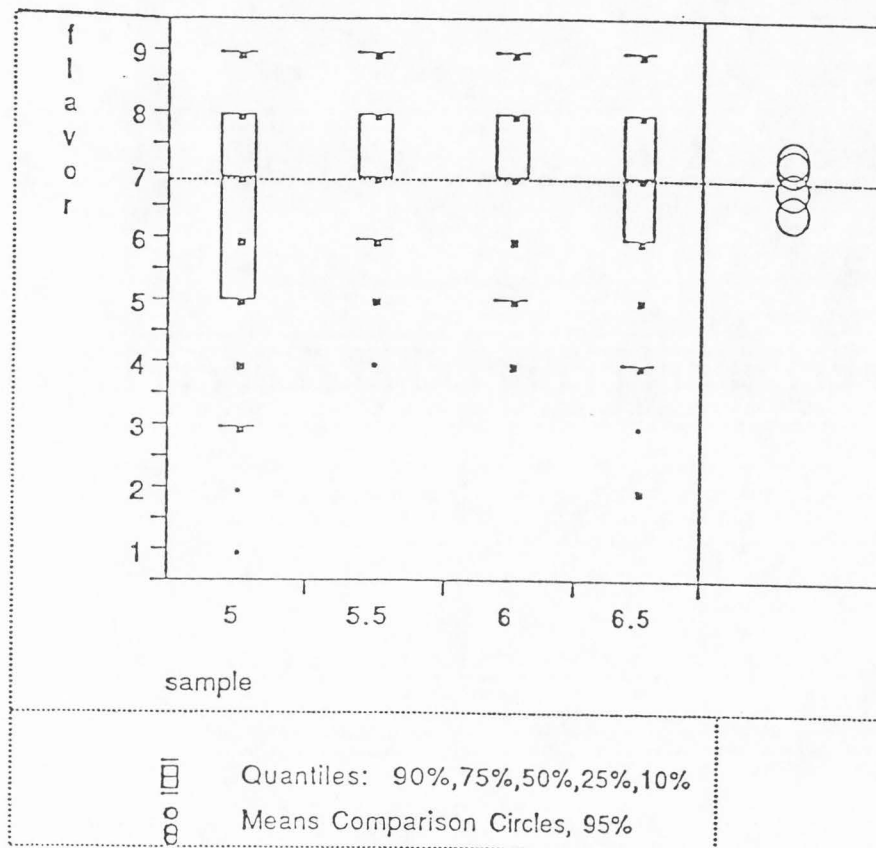Figure 17. Texture scores of fermented ice cream on a hedonic scale from all judges.

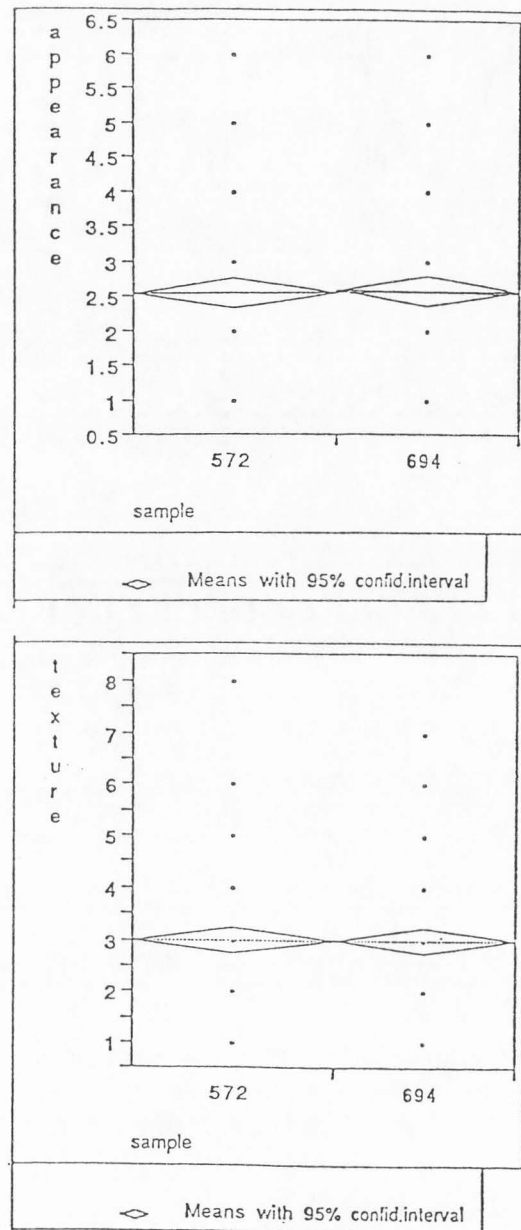Figure 18. Flavor scores of fermented ice cream on a hedonic scale from all judges.

Figure 19. Comparison of appearance and texture means of "HEATED" (572) and "NON-HEATED" (694) fermented ice cream.
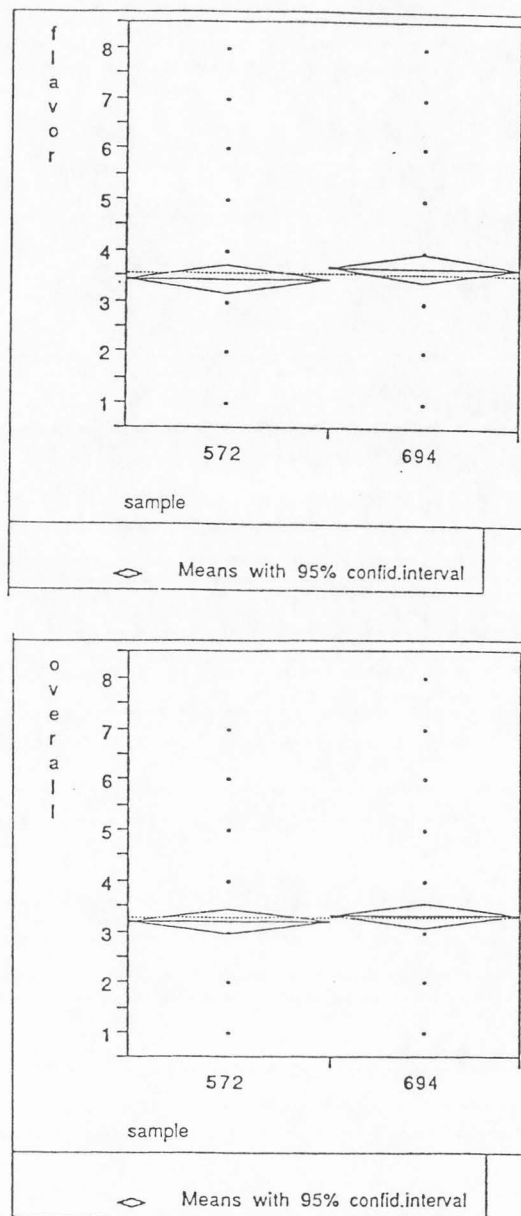
Figure 20. Comparison of flavor and overall means
of "HEATED" (572) and "NON-HEATED" (694)
fermented ice cream.

Lactase Activity

Measuring the glucose or galactose content of a fermented dairy product is not an accurate index of ß-galactosidase activity because first glucose and then galactose are rapidly metabolized to lactic acid by culture bacteria (31). Therefore, to determine the influence of L. acidophilus and B. bifidum in ice cream on lactose digestibility one needs to measure activity of ß-galactosidase in the ice cream.

The fermented ice cream gave a positive reaction with ONPG and the characteristic yellow color was developed in about 10 minutes. The initial enzyme activity was about 1800 units/ml. ß-galactosidase activity of fermented ice cream declined 31% over the 17 weeks of frozen storage (Figure 21). ß-galactosidase activity is lost more quickly in refrigerated yogurt than in a frozen yogurt. Mashayekh (36) observed that 20% of ß-galactosidase activity was lost after 30 days refrigeration while in a frozen fermented ice cream only 11% activity was lost. In that experiment, the ice cream mix had been fermented by L. delbruekii ssp. bulgaricus and S. salivarius ssp. thermophilus. In my experiment where the ice cream was fermented using L. acidophilus and B. bifidum there was only an estimated 8% loss on ß-galactosidase activity during the first 30 days of frozen storage. Speck and Geoffrion (51) also found about 50% reduction in lactase activity of unfrozen yogurt during a 20-day period, but there was no decrease in
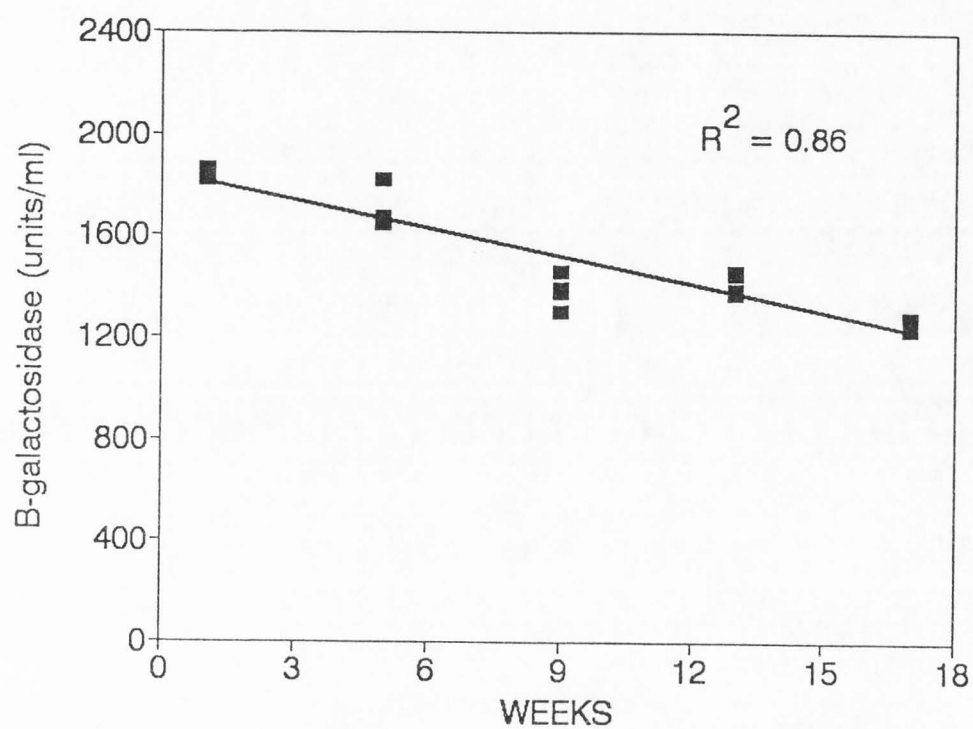
Figure 21. Effect of frozen storage on ß-galactosidase
           activity in ice cream fermented to pH 5 with L.
           acidophilus and B. bifidum.

lactase activity of frozen yogurt. Therefore, freezing has only a small effect on ß-galactosidase activity. Thus it can be seen that frozen fermented foods provide the best means of delivering ß-galactosidase enzymes to people who are lactose maldigestors but desire to consume dairy foods. L. acidophilus and B. bifidum are bile resistant and can survive and grow in the intestinal tract. ß-galactosidase, being intracellular, is also able to survive passage through the gastrointestinal tract. Therefore, lactose intolerant people could consume these fermented milk products.

CONCLUSIONS

The conclusions from this research can be summarized as follows:

1) Initial batch freezing of the fermented ice cream mix decreased the total colony counts by less than one log cycle for both L. acidophilus and B. bifidum.

2) Survival of B. bifidum was slightly affected by frozen storage. There was a decrease of one log cycle (2×10^8 to 1×10^7 cfu/ml) in colony counts of B. bifidum over 17 weeks of frozen storage of the "HEATED" sample.

3) There was a corresponding two log cycle decrease (1×10^8 to 3×10^6 cfu/ml) in colony counts of L. acidophilus over 17 weeks of frozen storage. This shows that L. acidophilus is more labile during frozen storage of ice cream than B. bifidum.

4) Ice cream could be used as a good source for delivering these probiotic bacteria to the consumers.

5) The initial rate of acid production by L. acidophilus and B. bifidum in ice cream mix (12% fat, 40% total solids) was faster when the mix had been heated to 85°C for 30 minutes in addition to being pasteurized at 79°C for 28 s.

6) Personal consumption frequency of yogurt affects sample preference for frozen fermented ice cream.

7) Because of a large range of like and dislike for strawberry ice cream at pH 5.0 and 6.5, the preferred pH for strawberry ice cream, based on overall acceptance, was pH 5.5.

8) The "HEATED" and "NON-HEATED" ice creams were equally preferred even though there were considerable differences in their texture. These differences were not shown to affect consumer preferences.

9) There was a decrease of 31% in ß-galactosidase activity of the ice cream fermented with L. acidophilus and B. bifidum over 17 weeks of frozen storage. Therefore, freezing does not have as much effect on enzyme activity as it has on cell number reduction. Fermented ice cream would provide a good vehicle to supply these enzymes to people who are lactose maldigestors and would enable many of them to include dairy foods in their diets.

RECOMMENDATIONS FOR FURTHER STUDY

Recommendations for further research on growth and survival of L. acidophilus and B. bifidum in dairy products would include:

1) A study to determine growth and survival of L. acidophilus and B. bifidum in fermented ice cream with lower sugar and fat content.

2) A study to examine survival and viability of L. acidophilus and B. bifidum in fermented ice cream using artificial sweeteners.

3) A study to determine survival and viability of L. acidophilus and B. bifidum in refrigerated yogurt.

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