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Evaluation of Frogurt: A New Product Prepared by Fermentation of Ice Cream Mix

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EVALUATION OF FROGURT: A NEW PRODUCT PREPARED BY FERMENTATION OF ICE CREAM MIX

by

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Morteza Mashayekh

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Evaluation of Frogurt: A New Product Prepared by

Fermentation of Ice Cream Mix

by

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Department: Nutrition and Food Sciences

Ice cream mix was fermented with yogurt cultures of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* to four different pH's then frozen in a batch ice cream freezer. A consumer panel of 120 people tasted samples of strawberry flavored product with pH's of 4.4, 4.7, 5.1, and 5.4 and commercial frozen yogurt as a standard. Results from the panel were used to predict a preferred pH of 4.9. Another panel of 181 people compared product at pH 4.9 with 10, 15 and 20% strawberry flavoring. There was not a statistically significant difference among levels of flavoring.

Starter culture populations and lactase activity were monitored for one month both in yogurt and in the frozen fermented ice cream mix. Lactase activity and colony counts progressively decreased in refrigerated yogurt at 4°C during the 30–day period. In frozen fermented ice cream mix, lactase activity and colony counts decreased slightly. The fermented ice cream mix can be held frozen for more than one month with active lactase activity, and viable colony counts, but refrigerated yogurt does not have a shelf life of more than one month.

(71 pages)

LITERATURE REVIEW

Yogurt, ice cream, and frozen yogurt are three types of dairy products available for consumers.

Yogurt

Yogurt is a product resulting from culturing pasteurized milk with or without added milk solids-not-fat (SNF), partially skimmed milk or skim milk with a yogurt culture. The culture is usually a mixture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Wilcox, 1971; Bradley and Winder, 1977; Code of Federal Regulation, 1985).

The process for making yogurt is as follows: low fat milk, cream, skim milk, SNF, and stabilizer are mixed together. The ingredients are pasteurized at 85°C for 30 min, then homogenized at 79.5°C for 28 sec and cooled to a temperature of 43.3°C. Yogurt culture is added and the mix is incubated at 43.3°C to the desired pH, and then is cooled (Reed, 1982). Mann (1977) reports that in a survey of twenty yogurt—manufacturing plants, the pH of yogurt varies from 4.0–5.7.

The flavor and texture of yogurt results from growth of starter and production of acid. During freezing, ice crystals form. When frozen yogurt is stored, ice crystals become larger and that making it difficult to eat when taken out of the freezer. Large ice crystals are formed in frozen yogurt because the amount of total solids is low and there is a large quantity of free water present.

Ice Cream

Ice cream in the U.S. is defined by U.S. government standards (Arbuckle, 1986). It must contain not less than 10% milk fat and 20% total milk solids. When bulky flavors are used, fat and total milk solids must not be less than 8 and 16%.

The process for making ice cream is as follows: Ice cream mix typically contains milk fat, MSNF, added sugar and additives like emulsifier, stabilizer, and flavor and color substances. The mix is always homogenized. It is made into ice cream by freezing rapidly to -4 to -6°C while beating in air (Walstra and Jenness, 1984).

During freezing of ice cream, small ice crystals form. Because of the presence of ice crystal inhibitors, ice crystals do not grow to make big crystals. In ice cream there is more fat and sugar when compared to yogurt, and that makes it more creamy and improves the texture.

Frozen Yogurt

Frozen yogurt can be either soft or hard; its physical state resembles ice cream rather than yogurt (Arbuckle, 1986; Tamime and Deeth, 1980), but is similar to yogurt in chemical composition and manufacturing process up to the freezing stage (Tamime and Deeth, 1980; Code of Federal Regulation 1985).

Some different processes and formulations for production of frozen yogurt are as follows:

Peck et al. (1977) proposed a final mix for production of frozen yogurt consisting of 50% yogurt and 50% ice cream mix.

Therrein et al. (1982) proposed adding 16% dried milk and a 1:1:2 mixture of *L. bulgaricus*, *Lactobacillus jugurt* and *S. thermophilus* to skim milk, incubating until 1.3% lactic acid is obtained, then adding carboxymethylcellulose (0.05%) and gelatin (0.2%), homogenizing, and freezing in an ice cream freezer.

Aries (1977) proposed that yogurt mix be pasteurized (90°C for 3 min.), homogenized and cooled to 42.8°C, 4–6% yogurt starter added and the mix incubated, cooled to 8.3–10°C and stored in aging tanks. It would then be frozen in a continuous ice cream freezer using nitrogen instead of atmospheric air for overrun (Bradley and Winder, 1977; Mann, 1977; Aries, 1978).

Aries (1978), proposed that milk and dry skim milk be blended, pasteurized, homogenized and cooled to 43.3°C. Yogurt culture is added and incubated in the processing vat at 43.3°C to 1–1.1% titratable acidity. A mix containing sugar solution, emulsifier, stabilizer and water would then be pasteurized and pumped in quickly with agitation and cooled to 4.4°C. The mix would then be frozen in a continuous freezer (Speck, 1977; Bradley and Hekmati, 1982; Mann, 1977).

Bradley and Winder (1977) proposed a formulation to make soft-serve frozen yogurt from a blend of ice milk, ice cream, and yogurt (Table 1). They also proposed a formulation to make hard frozen yogurt from a blend of ice cream, yogurt, and sugar syrup (Table 2).

Bray (1981) proposed a formulation to make yogurt ice cream from the mix of ice cream, syrup, and yogurt (Table 3). He also proposed a formulation for fruit yogurt ice cream from the mix of ice cream, syrup, and yogurt (Table 4).

Frozen yogurt does not have as high proportion of solids as ice cream, resulting in larger ice crystals that make it more firm when taken out of the freezer.

Ingredients' Functions

To make frozen yogurt these ingredients must be added: stabilizer, emulsifier, culture, flavoring and air. Function of each of those ingredients is as follows:

Stabilizer. Stabilizer is used to produce smoothness in body and texture, impart gel structure, and reduce wheying off during syneresis. It also reduces ice crystal growth during storage, and resists melting. The stabilizer increases shelf life and provides a reasonable degree of uniformity to the product. A stabilizer's function is to form gel structures in water, leaving less free water for syneresis. The amount used may be in the range of 0.0 to 0.5%, but generally is from 0.2 to 0.3% (Arbuckle, 1986; Reed, 1982; Jochumsen and Hoyer, 1977).

Table 1. Manufacture of soft serve frozen yogurt (Bradley and Winder, 1977).

	Mixes				
Ingredients (%)	Ice Mik	Ice Cream	Yogurt	IM/Y	IC/Y
milk fat	4.0	10.0	2.0	3.0	6.0
SNF	12.5	10.0	13.0	12.75	11.5
sugar	10.0	12.0	---	5.0	6.0
corn sweetener	10.0	6.0	---	5.0	3.0
stabilizer	0.5	0.5	---	0.25	0.25
total solids	37.0	38.5	15.0	26.0	26.0

Table 2. Manufacture of hard frozen yogurt (Bradley and Winder, 1977).

	Mixes			
Ingredients (%)	Ice Cream	Yogurt	Sugar Syrup	40:40:20 Blend (IC/Y/S)
milk fat	10.0	2.0	---	4.8
SNF	10.0	13.0	---	9.2
sugar	12.0	---	36.0	12.0
corn sweetener	6.0	---	37.0	9.6
stabilizer	0.5	---	---	0.2
total solids	38.5	15.0	73.0	35.8

Table 3. Formulation for manufacture of yogurt ice cream (Bray, 1981).

Base A		Syrup B		Yogurt	
milk fat	7.1%	water	13.5%	fat	3.0%
skim milk	18.5%	saccharose	70.0%	SNF	9.0%
saccharose	18.5%	corn syrup	15.0%		
corn syrup	5.1%	stabilizer	1.5%		
emulsifier	0.6%				
water	50.2%				
Base A			35.%		
Syrup B			15.0%		
Yogurt			50.0%		

Table 4. Formulation for manufacture of fruit yogurt ice cream (Bray, 1981).

Base A		Syrup B		Yogurt	
milk fat	6.7%	saccharose	60.0%	fat	3.0%
skim milk	18.2%	fruit	30.0%	SNF	9.0%
saccharose	19.8%	corn syrup	9.0%		
corn syrup	4.0%	stabilizer	1.0%		
emulsifier	0.5%				
water	50.8%				
Base A			30.0%		
Syrup B			20.0%		
Yogurt			50.0%		

The types and use level of different stabilizers are recommended as follows:

gelatin	0.3–0.5%
sodium alginates	0.22–0.3%
carageenans	0.08–0.12%
carboxy methyl cellulose	0.15–0.18%
pectin	0.15%

Moss (1955) stated that, compared to ice cream that does not contain a stabilizer, properly stabilized ice cream has a heavier body, does taste as cold, and will melt down to a creamier consistency.

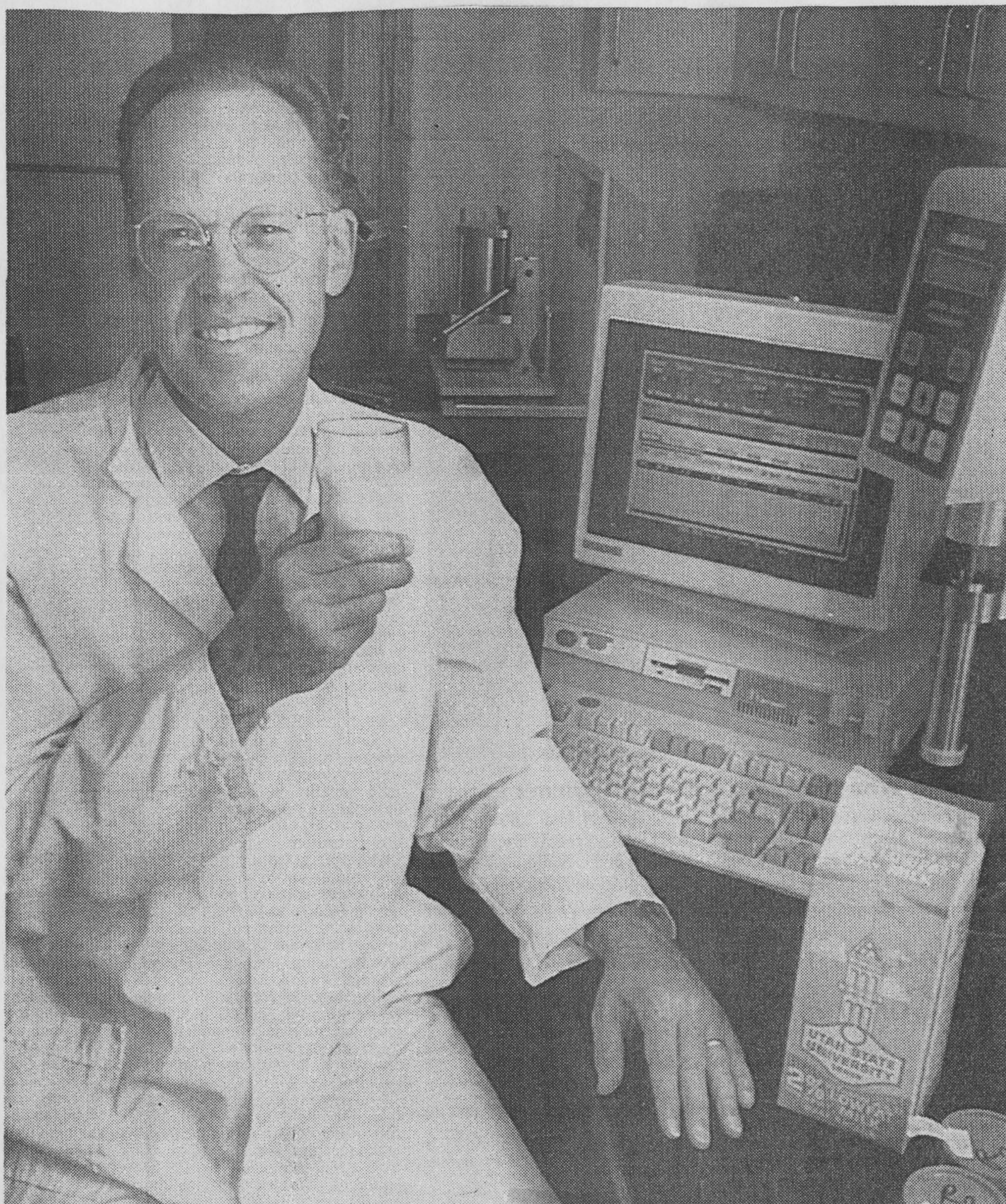
Emulsifier. Emulsifier is used in manufacture of ice cream to produce a finished product with a smooth texture and stiff body. It reduces whipping time and gives the mix a uniform whipping quality (Arbuckle, 1986). It helps maintain the best overrun of mix during freezing (Jochumsen and Hoyer, 1977) and helps to produce small ice crystals that are distributed evenly, giving small air cells and a drier ice cream with smooth body and texture (Webb et al., 1974). Different types of emulsifiers used are as follows: mono and diglycerides, egg yolks, lecithin, poly oxyethylene derivatives of hexahydric alcohol, glycol, and esters (Arbuckle, 1986; Bassett, 1988).

Culture. Culture in yogurt manufacturing is for production of flavor compounds and lactic acid. They affect the texture which results from growth of starter and acid production. The starter culture consists of *L. bulgaricus* and *S. thermophilus*, and these should be in approximately equal numbers in the finished product (Speck, 1979; Reed, 1982; Foster et al.,1957; Kosikowski, 1982).

These two organisms have a symbiotic relationship during manufacture of yogurt, with the ratio of *S. thermophilus* to *L. bulgaricus* constantly changing (Bautisa et al., 1966). *S. thermophilus* grows quickly at first, using essential amino acids produced by

W., November 25, 1992

The New York Times



L. bulgaricus. *S. thermophilus* produces lactic acid, which lowers the pH to a more optimal level for growth of *L. bulgaricus*, along with lesser amounts of formic acid that stimulates growth of *L. bulgaricus* (Tamime, 1980). Growth of *S. thermophilus* then slows while *L. bulgaricus* reduces the pH even further by producing lactic acid (Tamime and Robinson, 1976).

S. thermophilus produces some diacetyl, which gives yogurt its creamy or buttery flavor, whereas *L. bulgaricus* produces acetaldehyde which helps to give yogurt its characteristic sharp flavor (Davis et al., 1971; Merilainen, 1986).

Flavoring. Values of frozen desserts are mainly based on their pleasing flavor, cooling, and their refreshing effects (Sommer, 1932). Among the flavoring substances that play an important part in frozen desserts are vanilla, chocolate and cocoa, fruits and fruit extracts, nuts, spices and sugars (Arbuckle, 1986).

Air. Air is a necessary ingredient of ice cream, since without it the mix would freeze to a hard mass. The increase in volume is secured by whipping air into the mix during freezing. The usual range of overrun is from 80 to 100% (Lampert, 1947). The calculation for obtaining overrun, could be based on volume or weight and the formulation is as follows:

Calculation by volume:

$$\frac{\text{Vol of ice cream - Vol of mix}}{\text{Vol of mix}} \times 100 = \%\ \text{Overrun}$$

Calculation by weight:

$$\frac{\text{Wt. / volume of mix - Wt. / volume of ice cream}}{\text{Wt. / volume of ice cream}} \times 100 = \%\ \text{Overrun}$$

Lactose Intolerance

Lactose, the natural sweetener in milk and milk products, may be hydrolyzed to D–galactose and D–glucose with mineral acids, with cation exchange resins in the acidic form, and with β–D–galactosidases (EC 3. 2. 1. 23), often called lactases. Lactase is an enzyme produced in the small intestine to hydrolize lactose (Simoon, 1973; Walstra and Jenness, 1984).

In the absence of lactase, "sweet" milk may, though it does not always, precipitate symptoms of digestive–tract distress–cramps, flatulence, or diarrhea (Bayless et al., 1975). In the U. S. about 13.4% to 35.1% of the population suffer from lactose intolerance (Houts, 1988).

Lactose intolerant individuals were able to digest yogurt, and other milk fermented products without the adverse effects experienced when regular milk is consumed (Gallagher et al., 1974; Speck, 1977; Speck, 1979; Speck and Geoffrion, 1980).

Health Benefits

Yogurt cultures produce certain metabolites during their growth in yogurt manufacture that allow milk proteins to be digested and absorbed more readily. Metabolism is mainly due to enzymic activity of lactase in yogurt (Breslaw and Kleyn, 1973; Simhaee and Keshavarz, 1974). Certain of these metabolites (Reddy and Shahani, 1971) also have an antagonistic effect against foodborne pathogens (Speck, 1971) which, if allowed to enter yogurt, could be inhibited by the metabolites and prevent it from having a damaging effect on the host (Speck and Geoffrion, 1980; Gilliland and Kim, 1984).

Killara and Shahani (1976) concluded that lysing of yogurt culture cells, as occurs in the intestinal tract, releases lactase from the cells which hydrolyzes lactose contained in the dairy product that has been consumed. Lactose is hydrolized inside the bacterial cell by the enzyme β–D–galactosidase to D–glucose and D–galactose (Wierzbicki and

Kosikowski, 1973; Blankenship and Wells, 1974; Killara and Shahani, 1976; Rao and Dutta, 1977 and 1978). Permi et al. (1972), reported a second enzyme, β–D–phosphogalactosidase, that yields D–glucose and D–galactose–6–phosphate from lactose phosphate, is present in *L. bulgaricus*, and (Somkuti and Steinlberg 1978) in *S. thermophilus*.

Results of studies by Metchnikoff on prolongation of life, theorize that people in the Balkan countries enjoy long life because of the large quantities of cultured milk which they consume (Speck, 1977). Some of these people eat yogurt as their principle food, as much as six pounds per day.

Processing of Mix

The steps in processing of frozen yogurt are pasteurization, homogenization, heat treatment, cooling, aging and freezing. The function of each of these processing steps is as follows:

Pasteurization. It is required to pasteurize all yogurt and ice cream mixes because pasteurization destroys pathogenic or disease causing organisms, safeguarding the health of the consumer. Pasteurization (1) renders the mix free of pathogenic bacteria, (2) aids in blending the ingredients, (3) improves flavor, (4) improves keeping quality, and (5) produces a more uniform product. The standard pasteurization is at 79.4°C for 25 sec or batch pasteurized at 68.3°C for 30 min for ice cream mix (Arbuckle, 1986).

Homogenization. The main purpose of homogenization is to make a permanent and uniform suspension of fat and to reduce fat droplet size to a small diameter, preferably not more than 2 mm. A pressure of 13.78–17.23 MPa with one valve or 17.23–20.68 MPa on the first stage and 3.44 MPa on the second stage is needed for homogenization (Arbuckle, 1986).

Heat treatment. Heat treatment of the mix at 85°C for 30 min or equivalent is an important step in yogurt manufacturing. The heat treatment (1) produces a nearly sterile medium for better growth of starter, (2) affects thermal breakdown of milk constituents (especially proteins, releasing peptones, sulfhydryl groups which provide nutrition and anaerobic conditions for the starter), and (3) denatures milk albumins and globulins which enhance viscosity of the product (Reed, 1982).

Cooling. Cooling is a critical step in yogurt production. It is carried out directly after the product reaches the desired acidity. The objective is to reduce metabolic activity of the starter culture and to control the acidity of the yogurt. Yogurt starters *L. bulgaricus* and *S. thermophilus* have limited growth at 10°C (Tamime and Deeth, 1980).

Aging. Experimental work shows that appreciable benefit results from aging the mix before freezing, although little is gained by aging longer than 4 h. The following changes happen during aging: (1) fat is solidified, (2) if gelatin has been used as a stabilizer it swells and combines with water, (3) the protein of the mix may change slightly, and (4) viscosity is increased (Arbuckle, 1986).

Freezing. The mix, after aging for a few hours in the refrigerator, can be frozen to ice cream by either batch freezer or continuous freezer. The function of freezing is to freeze a portion of the water of the mix. This involves lowering the temperature of the mix to the freezing point, while incorporating air into the mix and freezing a portion of the water (flavor can be added to the mix while it is freezing). Fast freezing is essential for a smooth product, because ice crystals formed quickly are smaller than those formed slowly. After freezing, ice cream is transferred to a hardening room at -29°C (Lampert, 1947; Judkins and Keener, 1960).

Effect of Temperature and Storage

Typical growth curves for populations of *L. bulgaricus* and *S. thermophilus* show an increase in number of yogurt organisms during and immediately after manufacture followed by a decrease during refrigerated storage. This initial increase and later decrease are accelerated at 10°C as compared to 5°C (Hamann and Marth, 1984).

Freezing of yogurt results in a slight reduction in total counts of starter cultures. Storage of frozen yogurt at -32°C maintains the original level of lactase activity, and colony counts decrease only slightly (Speck and Geoffrion, 1980).

Davis (1970) using LAB Medium, found few changes over a 28–day period in viable counts of *S. thermophilus* and *L. bulgaricus* in yogurt stored at 5°C. However, at 15°C viable counts decreased markedly after 14 d. Lusiani and Bianchi-Salvadari, (1978), Lusiani et al. (1974) observed that all types of yogurt still contain more than 10^7 viable lactic acid bacteria / ml after 40 d at 5°C, although the numbers decreased to less than 10^5 / ml after 16 d when yogurt was held at 22°C. Luisiani et al. (1974) concluded that yogurt has a shelf life of about one month stored continuously at refrigerated temperature. If held for more than 30 d, it has an unacceptable taste (Choi and Kosikowski, 1985).

OBJECTIVES

The purpose of this study was to produce a new fermented product that is as rich and creamy as ice cream without the characteristic large ice crystals of commercial frozen yogurt. Effects of refrigerated storage and freezing for one month on colony counts and lactase activity of yogurt and frogurt were compared.

MATERIALS AND METHODS

Frogurt Making

Ice cream mix was prepared with 12% fat, 11% SNF, 0.32% stabilizer/emulsifier, 12.5% sugar and 4.5% corn syrup solids obtained from Utah State University Dairy Products Laboratory. The mix was pasteurized at 71.7°C for 30 min, cooled to 60°C, then heat treated at 79.4°C for 28 sec, and homogenized at 17.23 MPa. The mix was aged for at least 4 h at 4°C. Cultures of *L. bulgaricus* and *S. thermophilus* were grown separately in 600–700 ml erlenmeyer flasks containing autoclaved skimmed milk. After cooling the milk, *L. bulgaricus* and *S. thermophilus* were added to the flasks. The cultures were mixed and placed in a hot water bath at 43.3°C overnight.

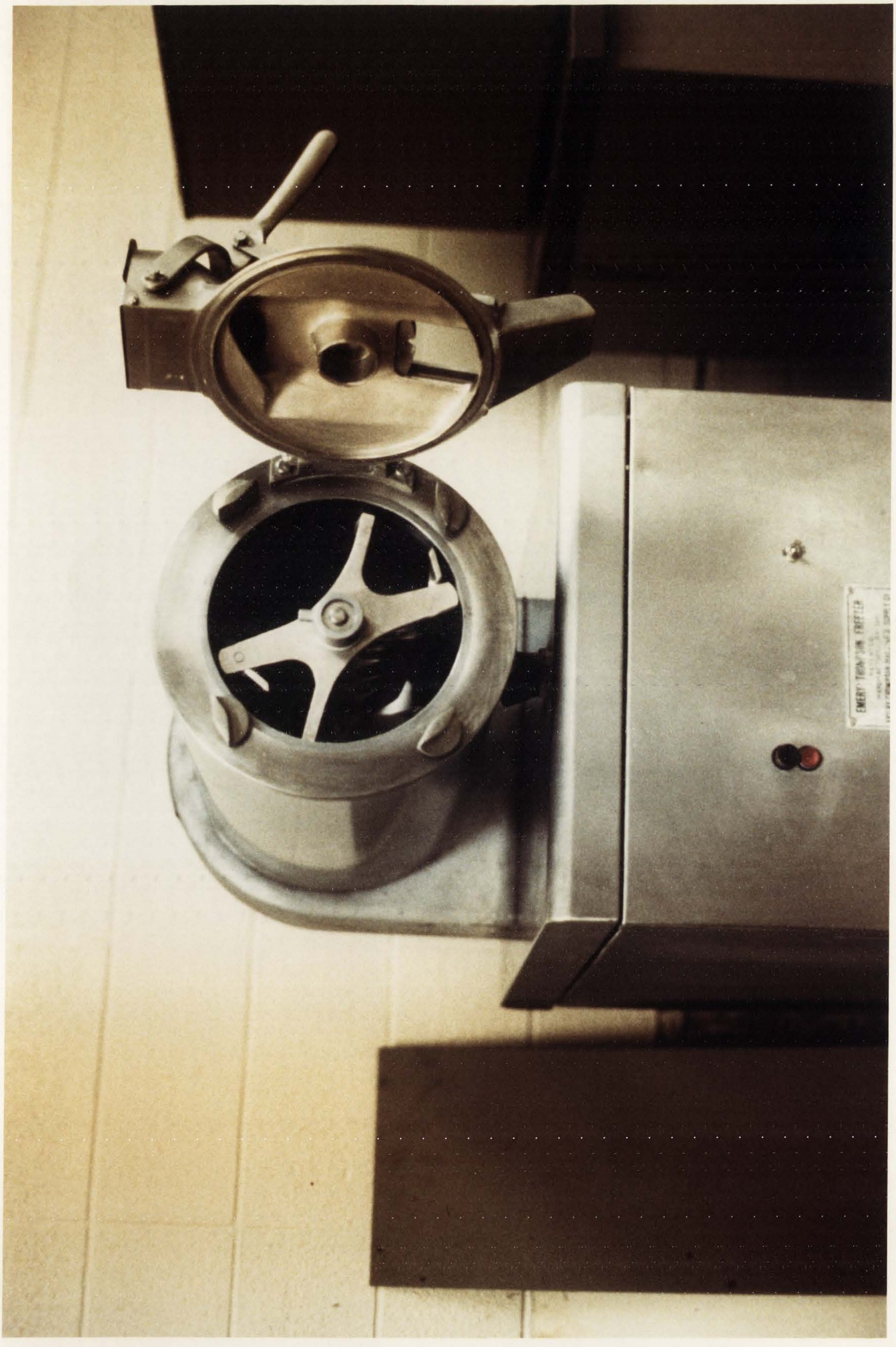
The ice cream mix was transferred to a hot water bath at 43.3°C. One percent inoculum was added, mixed well, and allowed to ferment. After 4–5 h a sample was taken and pH was checked. When the mix achieved the desired pH, it was cooled in an ice bath with agitation to below 10°C and aged overnight at 4°C.

The mix was transferred to a batch ice cream freezer (Figure 1) and flavor was added. Flavor was added at the end of freezing so flavoring would not prevent the mix from freezing quickly. The ice cream freezer was adjusted to obtain a volume increase of 90–100% which gave a product of a smooth consistency. Then the frogurt was hardened in a hardening room at a temperature of -29°C (Figure 2).

Taste Panel

A hedonic taste panel (Anon., 1984; Anon., 1981) was conducted with four samples at pH 4.4, 4.7, 5.1, and 5.4. Commercial frozen yogurt with a pH of 4.6 was used as a standard (Figure 3). All samples were strawberry flavored. Panelists were asked to rate samples for appearance, texture and mouthfeel, flavor, and overall. Data from the panel were analyzed for most desirable pH. To eliminate visual product differences, pink

Figure 1. Batch ice cream freezer.



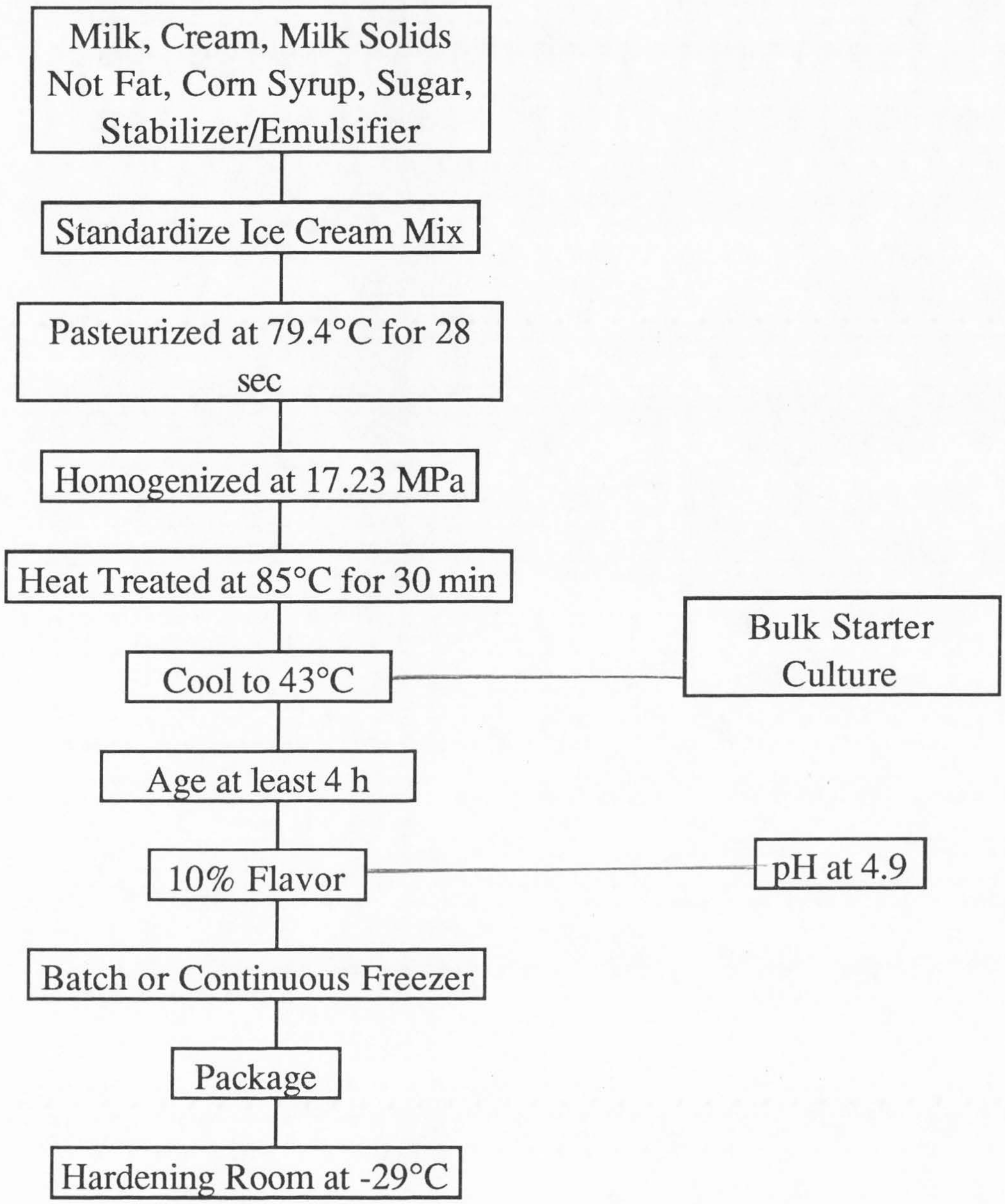


Figure 2. Manufacture of frogurt

Form used for first taste panel

Please evaluate the following samples in the order listed. Give each sample a rating for each attribute using the following scale.

9 = like extremely

8 = like very much

7 = like moderately

6 = like slightly

5 = neither like or dislike

4 = dislike slightly

3 = dislike moderately

2 = dislike very much

1 = dislike extremely

	932	278	512	156	284
appearance					
texture/mouthfeel					
flavor					
overall					

comments

Figure 3. Form used for first taste panel.

lighting was used. Sidel and Stone (1976) reported use of special lighting to mask visual product differences which could have significant impact on the validity of test conclusion.

Another ranking taste panel (Kahan et al., 1973; Anon., 1981) was conducted with three samples (pH 4.9) of 10, 15 and 20% strawberry flavoring. Panelists ranked samples in order of preference (Figure 4).

Enumeration of Starter Bacteria

Starter culture populations were monitored for one month in yogurt and in frogurt. Total numbers of starter bacteria were enumerated on MRS agar (deMan et al., 1960; Speck, 1976; Bracquard, 1981), Rogosa agar (Hamann and Marth, 1984), and lactic agar (Elliker et al., 1958; Speck, 1976). MRS broth (Difco) was rehydrated as directed then 1.5% granulated agar was added. The mix was heated to boiling to dissolve agar completely and 1.32 ml glacial acetic acid was added. The media was mixed thoroughly and distributed in bottles and autoclaved for 15 min at 121°C. Rogosa agar (Difco) was rehydrated as directed, heated to boiling to dissolve completely, then mixed with 1.32 ml glacial acetic acid. Boiling was continued for 2–3 min. Elliker broth (Difco) was rehydrated as directed, 1.5% granulated agar was added, and the mixture was heated to boiling. Agars were distributed in bottles and autoclaved for 15 min at 121°C. The agars were cooled to 45°C, and 20–30 ml were dispensed into sterile plates. Both MRS and Rogosa agars had final pH values of 5.5.

The cultures were plated on agars the first day, second day, then every four days after. Yogurt was diluted to 10^{-6} and 10^{-7} and frogurt was diluted to 10^{-5} and 10^{-6} in autoclaved 0.1% peptone (Richardson, 1985). One tenth millileter of yogurt and frogurt dilutions were spread over three different agar plates, placed in anaerobic jars and flushed with CO_2 (Figure 5). All plates were incubated for two days at 37°C (Speck and

Form used for the second taste panel

Please compare the three different yogurt ice cream, then rank the samples in the order of your preference. Write the code number in the appropriate blanks.

Order of preference

first choice (like best) ----------

second choice ----------

third choice (liked least) ----------

What is it that you liked or disliked about the flavor of each of the samples.

190

264

857

Figure 4. Form used for second taste panel.

Figure 5. Anaerobic jar with color indicator and flushed with CO_2.

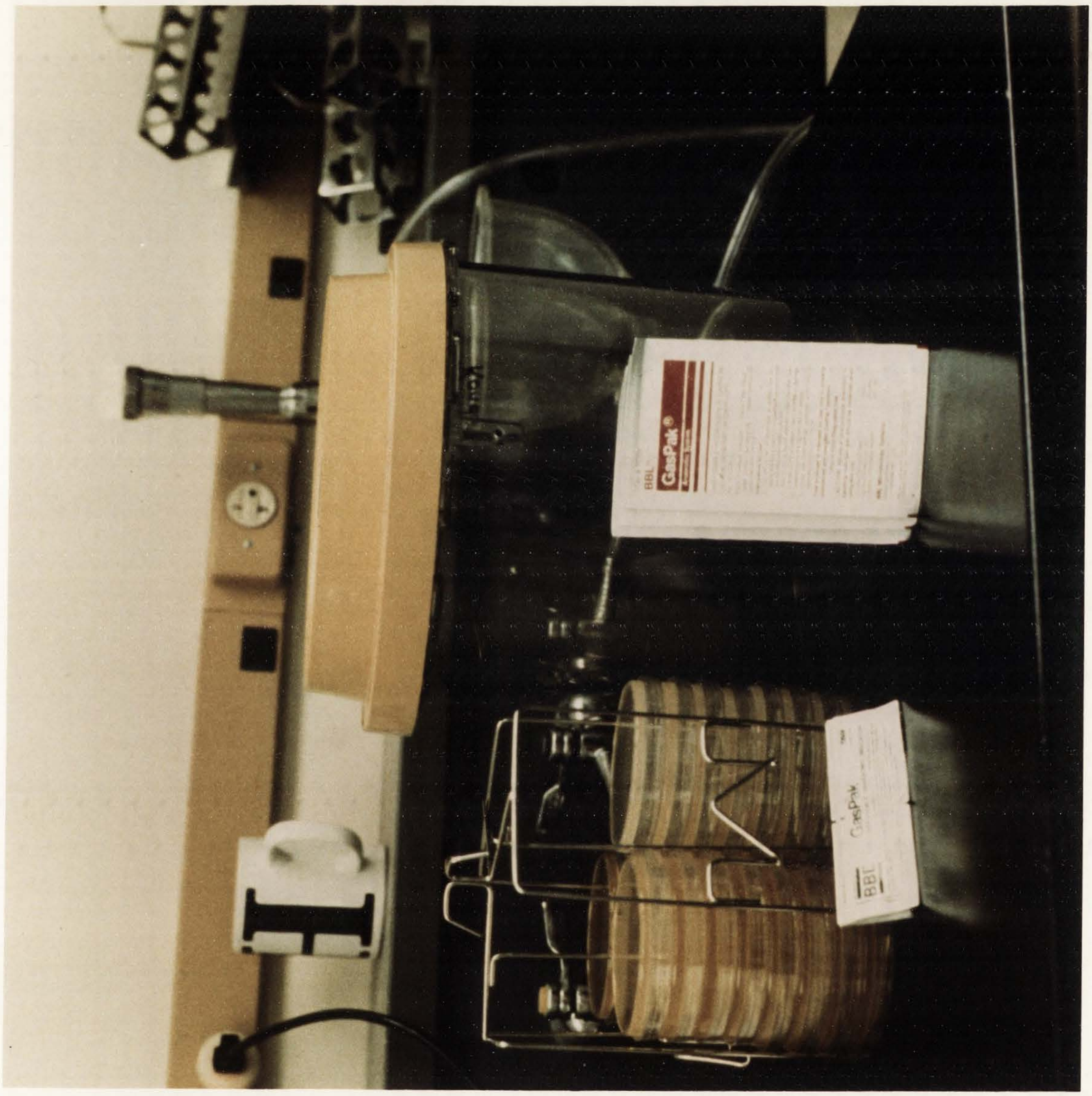
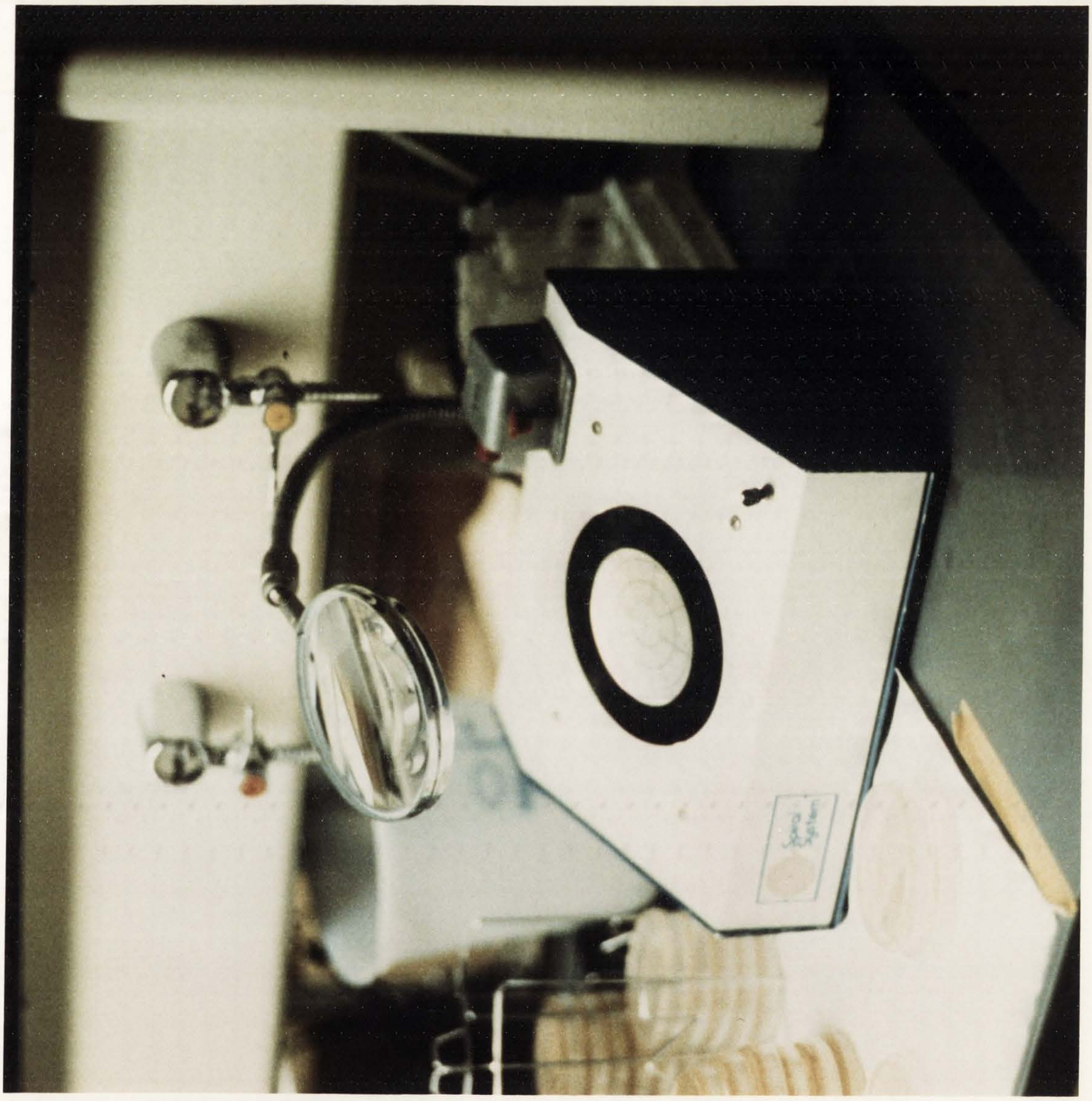

BBL
GasPak

Figure 6. Spiral colony counter.



Geoffrion, 1980; Hamann and Marth, 1984) and counted with a Spiral Colony Counter (Figure 6).

Hamann and Marth (1984) reported that Elliker lactic agar gave total numbers of viable organisms. Rogosa agar, and MRS agar gave the numbers of *L. bulgaricus*. The number of *S. thermophilus* was then calculated as the difference between the number of *L. bulgaricus* and the total number of bacteria.

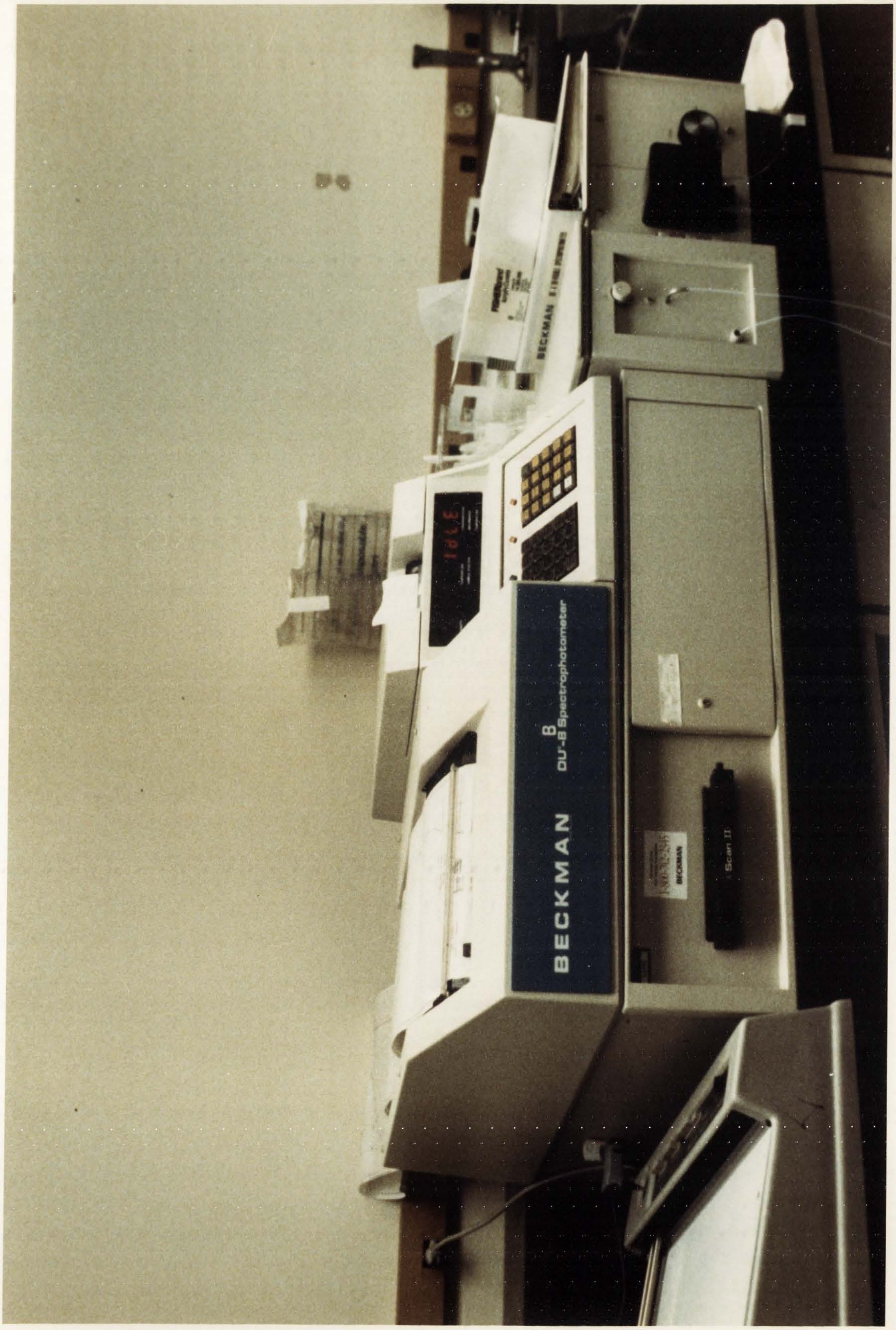
Lactase Assay

Lactase activity was monitored for one month both in frogurt and yogurt. Lactase activity was detected by the yellowish color of o–nitrophenol at 420 nm. O–nitrophenyl β–D–galactoside (ONPG) is a colorless compound, but in the presence of β–D–galactosidase it is converted to D–galactose and o–nitrophenol. If ONPG concentration is high enough, the amount of o–nitrophenol produced is proportional to amount of enzyme present and to the time the enzyme reacts with ONPG.

One milliliter of yogurt and 1 ml of frogurt were placed into 100 and 50 ml erlenmeyer flasks, diluted immediately with pH 7.0 phosphate buffer containing .001 *M* $MgSO_4$ and .05 *M* β–mercaptoethanol, and mixed thoroughly. One milliliter of this diluted culture was withdrawn for lactase assay measurement. Two drops of chloroform and one drop of 0.1% SDS solution were added to each milliliter of assay mixture. The tubes were then vortexed for 10 sec. The tubes were placed in a water bath at 28°C for 5 min. The reaction was started by adding 0.2 ml of ONPG (4 mg/ml) to each tube and vortexing for 10 sec. The reaction was stopped by adding 0.5 ml of 1 *M* Na_2CO_3 solution after 10 min (Killara and Shahani, 1976; Speck and Geoffrion, 1980) when sufficient yellow color had developed (Miller, 1972).

Optical density was recorded at both 420 nm and 550 nm for each tube by a Beckman DU–8B spectrophotometer (Figure 7). The reading at 420 nm was a combination of absorbance by o–nitrophenol and light scattering by cell debris. This

Figure 7. Beckman DU–8B Spectrophotometer.


BECKMAN
DU-8 Spectrophotometer
Scan II

latter component was corrected for by obtaining the absorbance at 550 nm where there is only light scattering (no contribution from o–nitrophenol). The light scattering at 420 nm was proportional to that at 550 nm.

$$A_{420} \text{ (light scattering)} = 1.75 \text{ times } A_{550}.$$

Using this correction factor to compensated for light scattering, true absorbance of o–nitrophenol was computed. The following formula was used.

$$\text{Units } (\beta\text{–D–galactosidase}) = 1000 \times \frac{A_{420} - 1.75 \times A_{550}}{t \times v}$$

A_{420} and A_{550} were read from the reaction mixture.

t = time of reaction in minutes

v = volume of culture used in the assay in ml

RESULTS AND DISCUSSION

Taste Panel

The first sensory evaluation of the new product was done at four different pH's of 4.5, 4.7, 5.1, 5.4 and frozen yogurt control at pH of 4.6 by 120 judges (Figure 3). There was no significant difference in appearance and texture of products, but there were differences in flavor and overall quality (Table 5). Means of the data from all taste panel categories were used to fit a second degree polynomial curve and find the preferred pH's. The preferred pH based on flavor was 4.9 and on overall score was 4.85 (Figures 8, 9). The most desired pH from all analyses (including appearance and texture, Figures 10, 11) combined was 4.9.

The second sensory evaluation was conducted for percent flavoring added to the product. In this ranking taste panel three different amounts (10, 15 and 20%) of strawberry flavoring at pH 4.9 were used. Judges were asked to rank samples in order of their preference (Figure 4). Using chi–square analysis, no significant differences werefound between products, and they were graded equally for their preference by 181 judges (Figure 12).

Both sensory panels preferred the new product (frogurt) strawberry flavored over commercial strawberry frozen yogurt control. Reasons given by panelists for preference of frogurt over yogurt were its rich and texture.

Enumeration

Starter culture populations were enumerated on MRS and Rogosa agar that only allow *L. bulgaricus* to grow, and lactic agar that allows both *S. thermophilus* and *L. bulgaricus* to grow (Figure 13). *L. bulgaricus* are gram positive bacilli (cylindrical or rod shapes Figure 14) producing large circular colonies. *S. thermophilus* are gram positive cocci (spherical shapes Figure 15) producing small colonies on the agars. The number of *S.thermophilus* present are found by difference (APPENDIX).

Table 5. Analysis of variance of the first sensory evaluation for appearance, flavor, overall and texture.

Analysis of variance on appearance

SV	df	SS	MS	F
Sample	4	16.49	4.12	1.91
Error	515	1109.82	2.15	
Total	519	1126.31		

Analysis of variance on flavor

SV	df	SS	MS	F
Sample	4	72.12	18.03	5.99*
Error	515	1550.31	3.01	
Total	519	1622.43		

Analysis of variance on overall

SV	df	SS	MS	F
Sample	4	45.92	11.48	4.52*
Error	515	1309.15	2.54	
Total	519	1355.07		

Analysis of variance on texture

SV	df	SS	MS	F
Sample	4	19.47	4.87	1.83
Error	515	1366.84	2.65	
Total	519	1386.31		

* Significant at P = 0.01

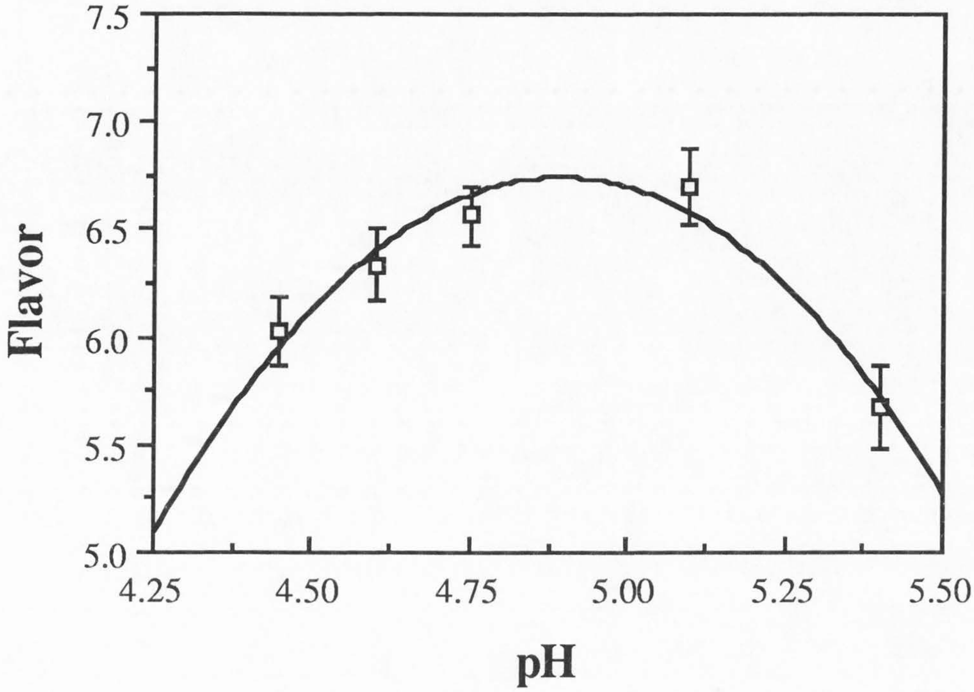


Figure 8. Effect of pH on flavor rating by the first panel.
(Error bars are SE of Mean)

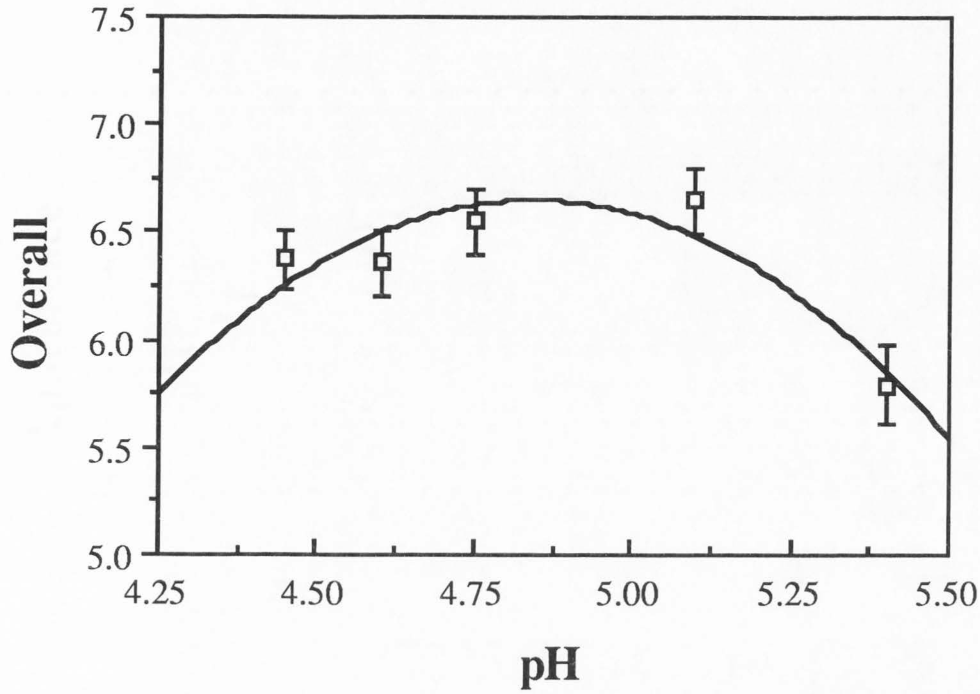


Figure 9. Effect of pH on overall rating by the first taste panel.
(Error bars are SE of Mean)

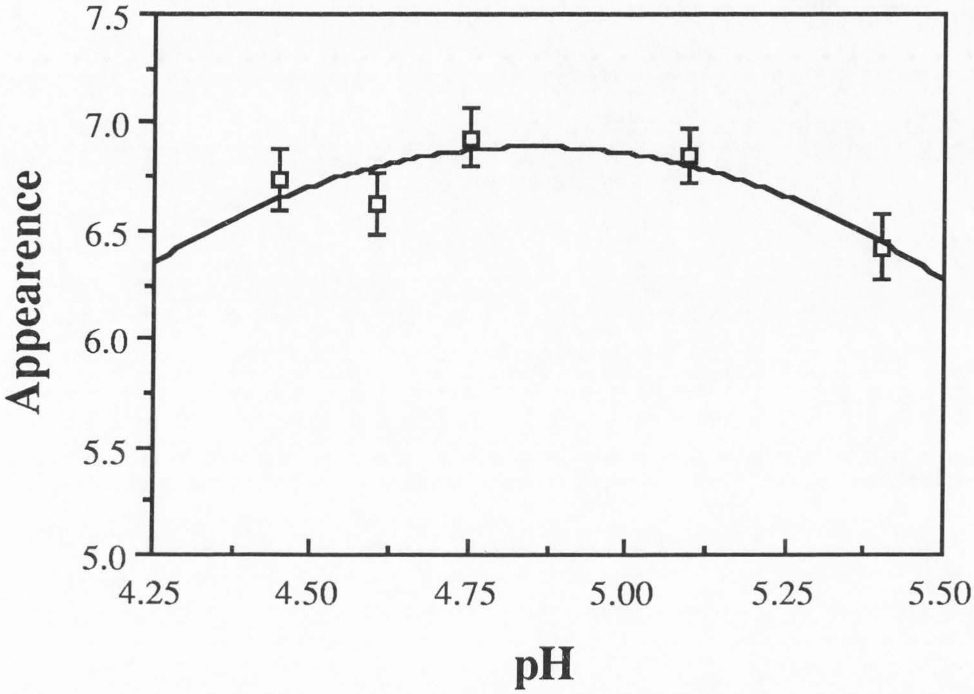


Figure 10. Effect of pH on appearance rating by the first taste panel.
(Error bars are SE of Mean)

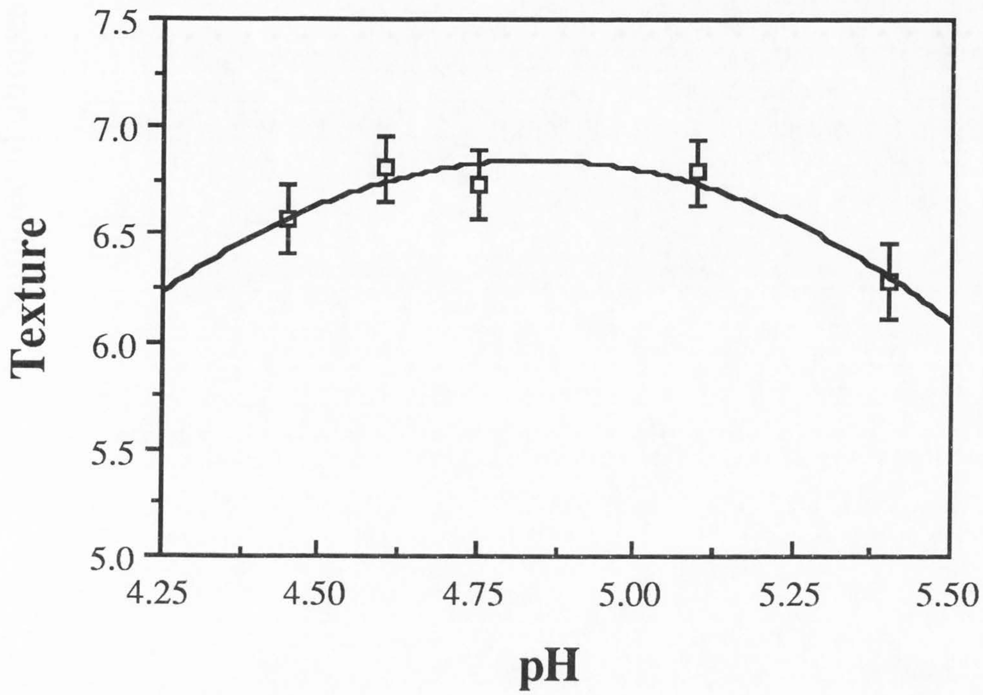


Figure 11. Effect of pH on texture rating by the first taste panel.
(Error bars are SE of Mean)

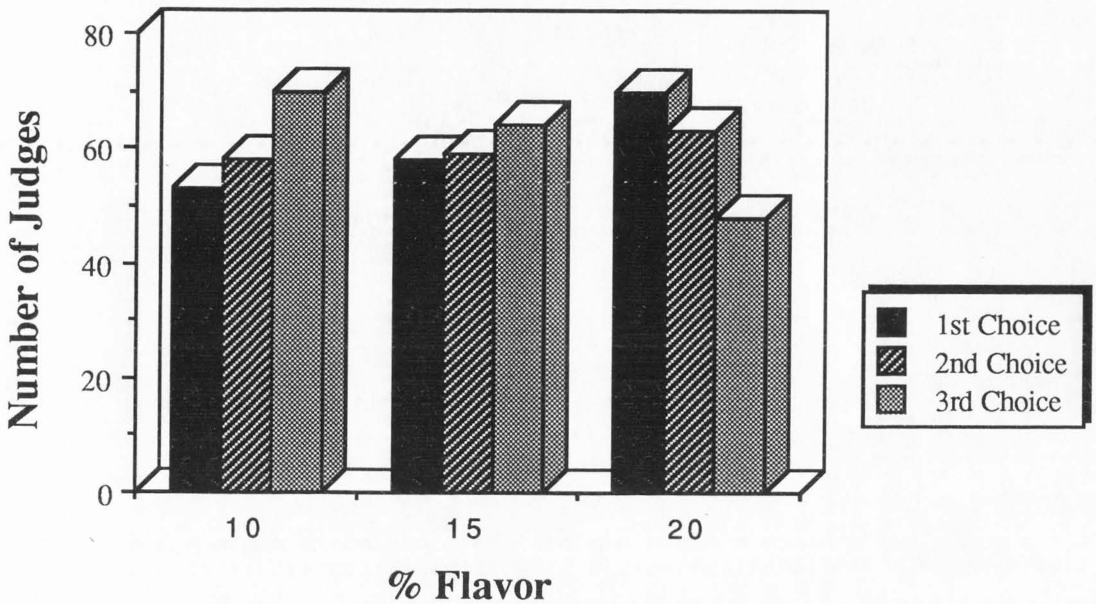


Figure 12. Effect of amount of flavoring on product acceptance by the second taste panel.

Figure 13. Gram positive *Lactobacillus bulgaricus* and gram positive *Streptococcus thermophilus* present in frogurt.

Figure 14. Gram positive *Streptococcus thermophilus*.

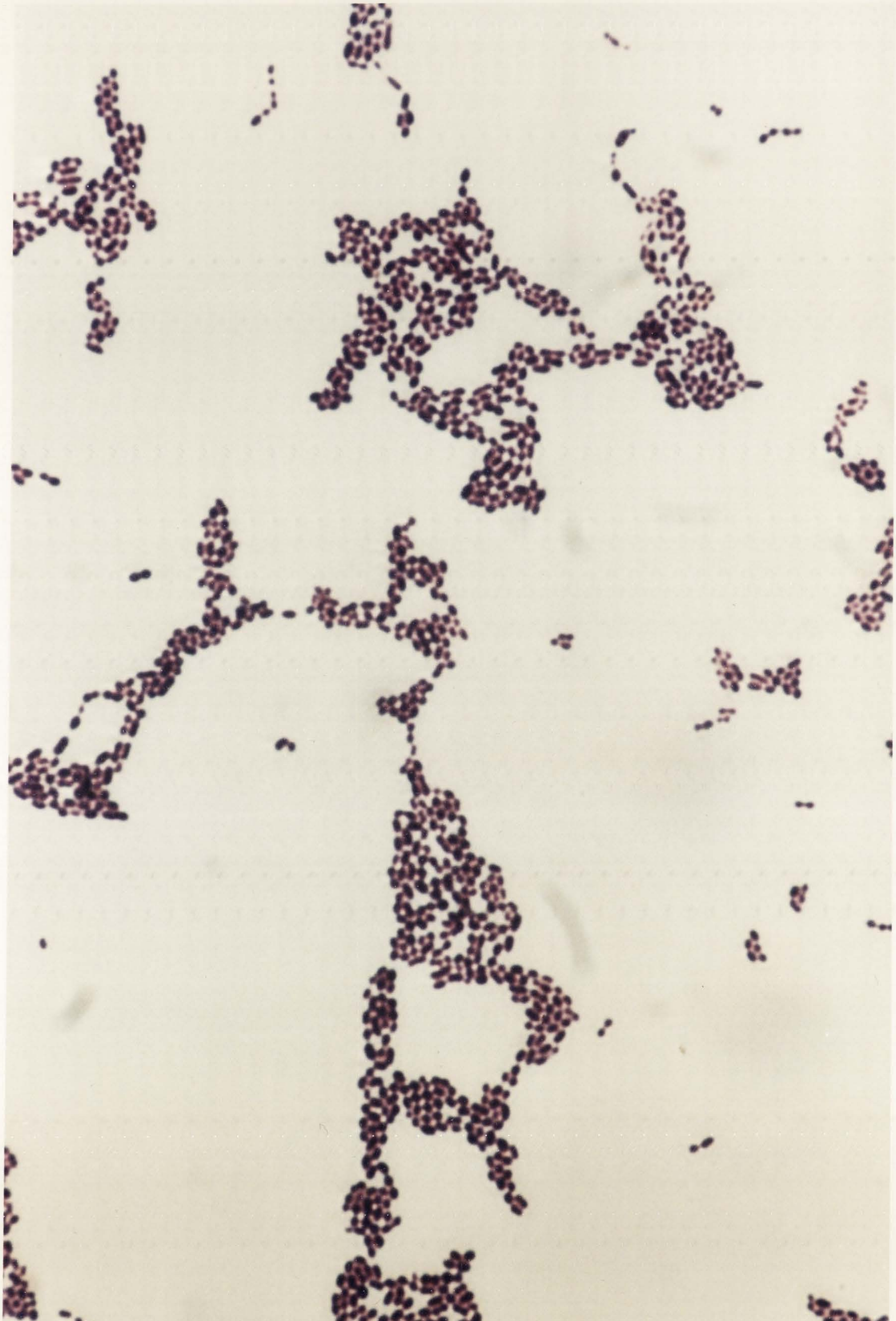
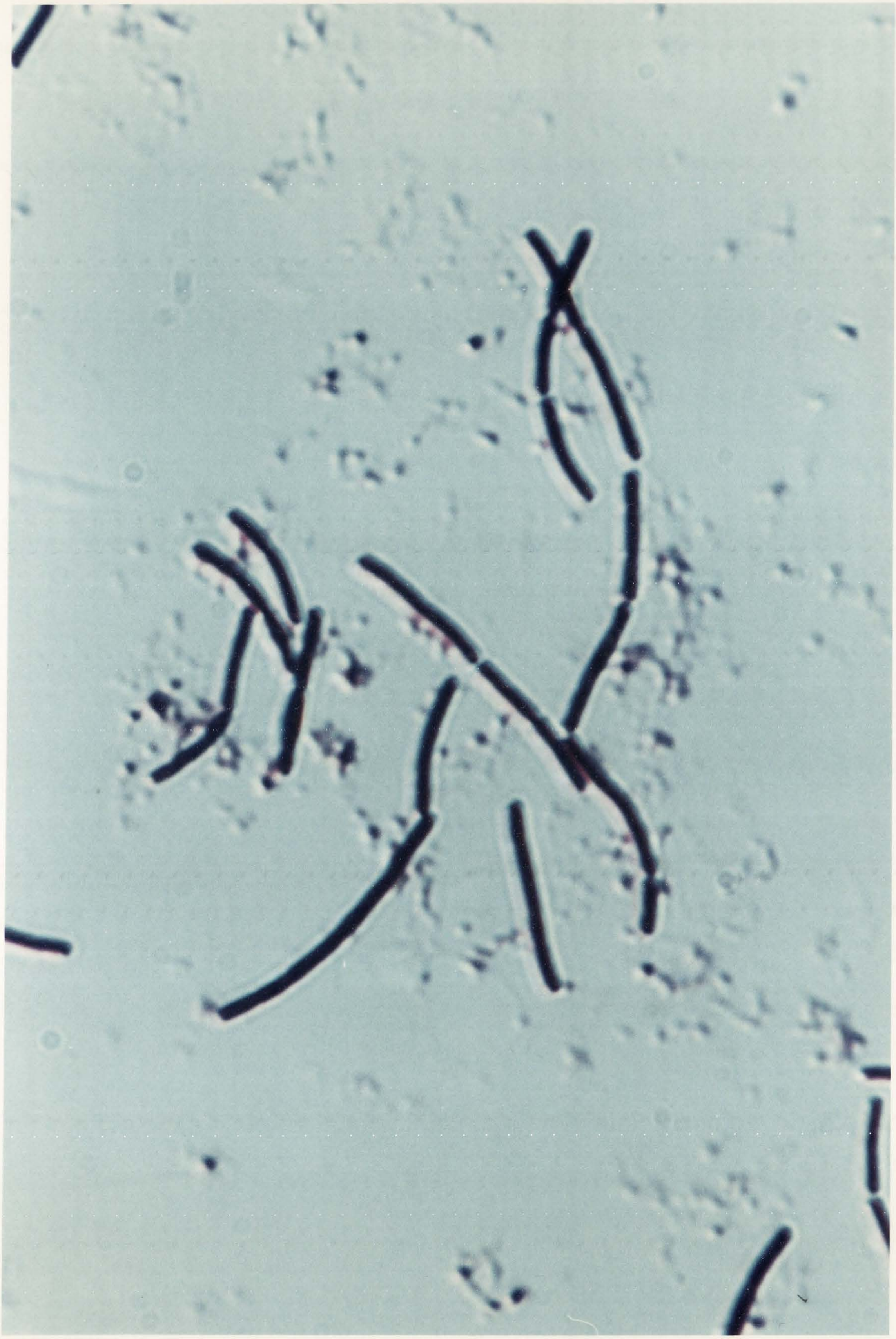
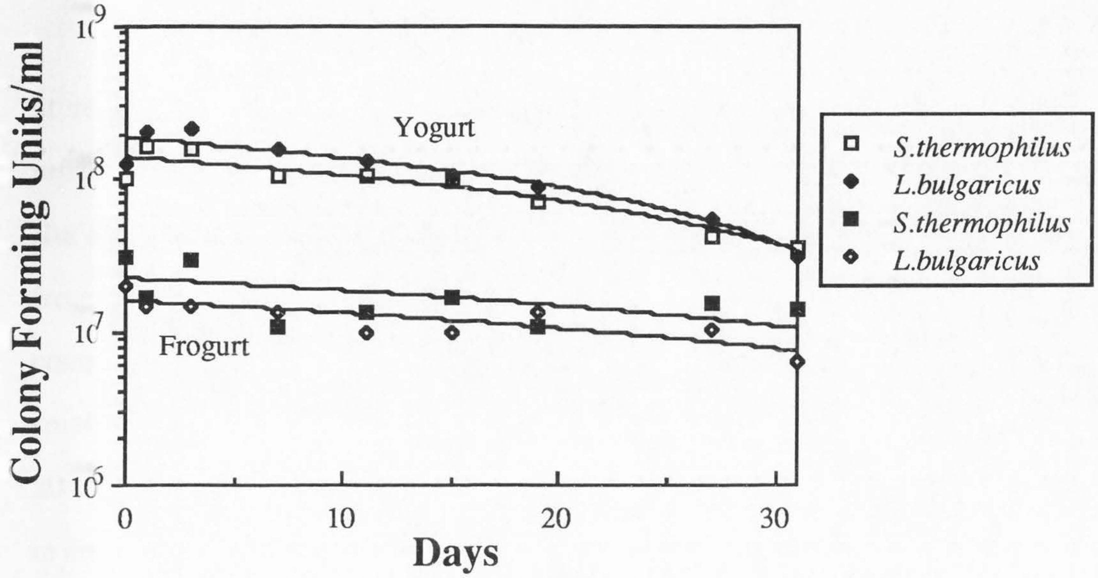


Figure 15. Gram positive *Lactobacillus bulgaricus*.





Figur 16. Effect of 31–day storage on yogurt and frogurt colony counts.
(Error bars are SE of Mean)

These organisms were grown aerobically and incubated for 48 h at 37°C without interruption. After 24 h *L. bulgaricus* on Rogosa and MRS agars had not produced colonies.

During storage, colony counts decreased, and it was more extensive in yogurt stored at refrigeration compared to frogurt stored frozen at -29°C (Figure 16). During refrigeration of yogurt, the population of cultures decreased more than half a log cycle. But reduction of cultures in frogurt was less than half a log cycle. These data show if frogurt had been stored for more than one month it would still have viable colony counts. It is reported by Speck and Geoffrion (1980) that in unfrozen yogurt there is a small decrease and in frozen yogurt there is only a slight decrease in colony counts during 20–day period. The initial freezing of frogurt, caused a reduction of about one log cycle in the number of colonies.

Lactase Activity

Lactase activity of yogurt declined 20.28% and of frogurt only 11.11% in the 30–day period (Figure 17). The decline in yogurt lactase activity was twice as much as frogurt. Speck and Geoffrion (1980) reported they saw a progressive decrease in lactase activity of unfrozen yogurt and there was no decrease in lactase activity of frozen yogurt over period of 20 d. Lactase activity varied among different samples of yogurt. These differences could be due to differences in cultures or to differences in preparing the samples.

The reduction in both colony counts and lactase activity were twice as much in refrigerated yogurt as in hardened frogurt. Yogurt has an unacceptable taste after one month, but frogurt can be held without any adverse effect on flavor and can maintain lactase activity for more than one month.

Toba et al. (1983) reported that lactose content of yogurt mix decreased from 6.53 to 4.22% during fermentation, then decline further in storage at 5°C for 10 d to 3.89%. It was also reported by Gilliland and Kim (1984) that cultured yogurt contained approximately 4.3% lactose compared to about 6.3% for the mix. As a result of this fermentation, the amount of D–galactose increased from 0.04 to 1.46%.

Reduction in percentage of lactose and presence of lactase enzyme in the mix makes it safe for lactose intolerant people to eat yogurt. That would be true also for fermented ice cream, because lactase enzyme due to presence of culture is active. The amount of lactose is reduced when compared to ice cream, and freezing does not have much effect on activity of lactase enzyme.

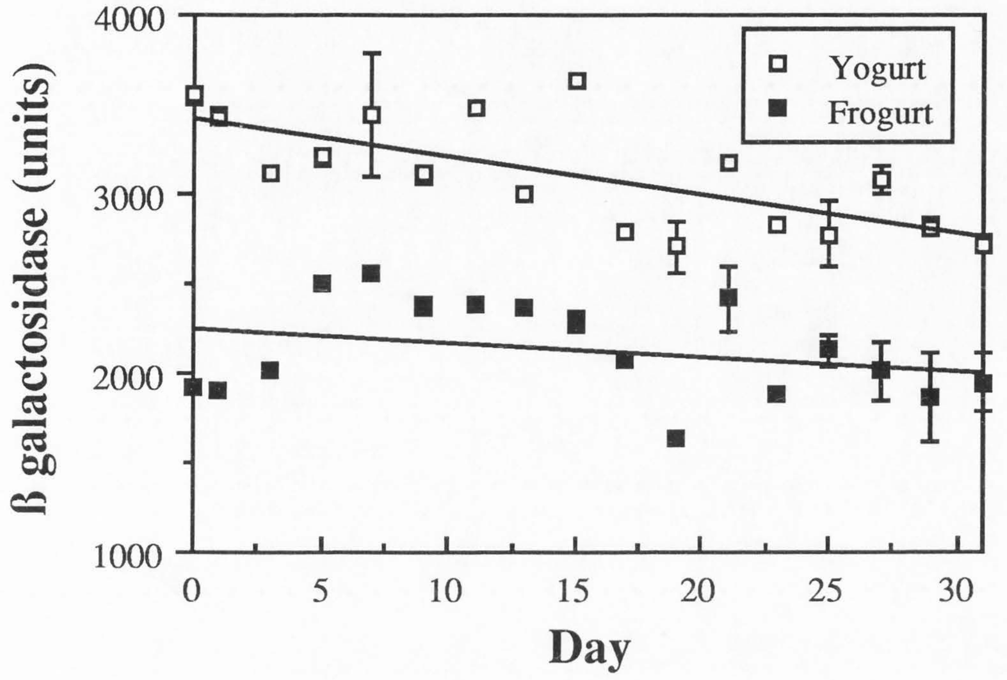


Figure 17. Effect of 31–day storage of yogurt and frogurt on lactase activity.
(Error bars are SE of Mean)

CONCLUSIONS

1. People prefer this new frozen product (frogurt) over frozen yogurt for flavor and overall quality at pH of 4.9.

2. The three different flavoring levels (10, 15 and 20%) are equally preferred.

3. There is a decrease of about 20% in lactase activity and more than half a log cycle in colony counts of refrigerated yogurt during 30 d of storage at 5°C.

4. There is a decrease of about 11% in lactase activity and less than half a log cycle in colony counts of frogurt during 30 d of storage at -29°C.

5. Freezing decreases the population of colonies about one log cycle in frogurt.

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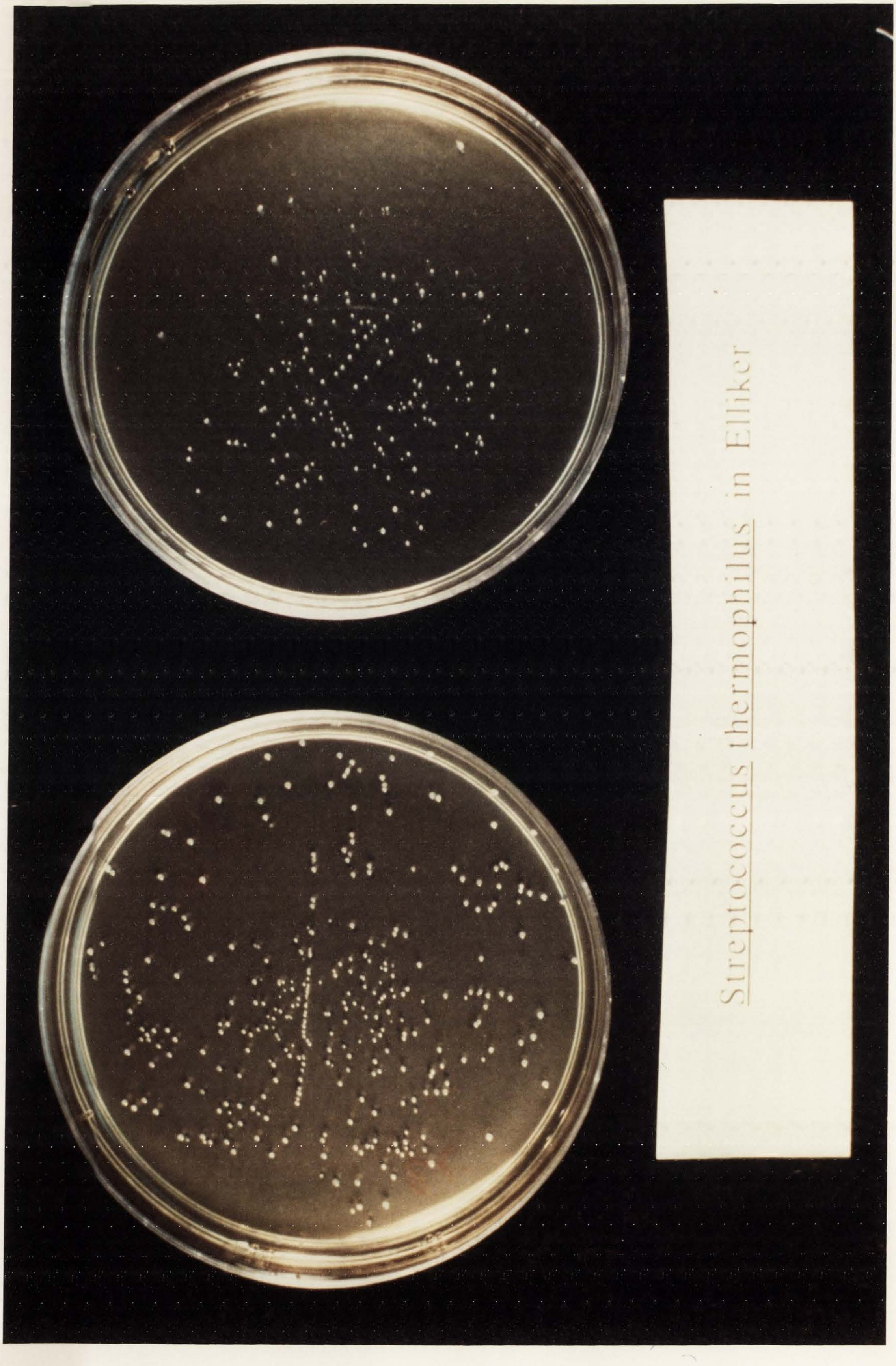
APPENDIX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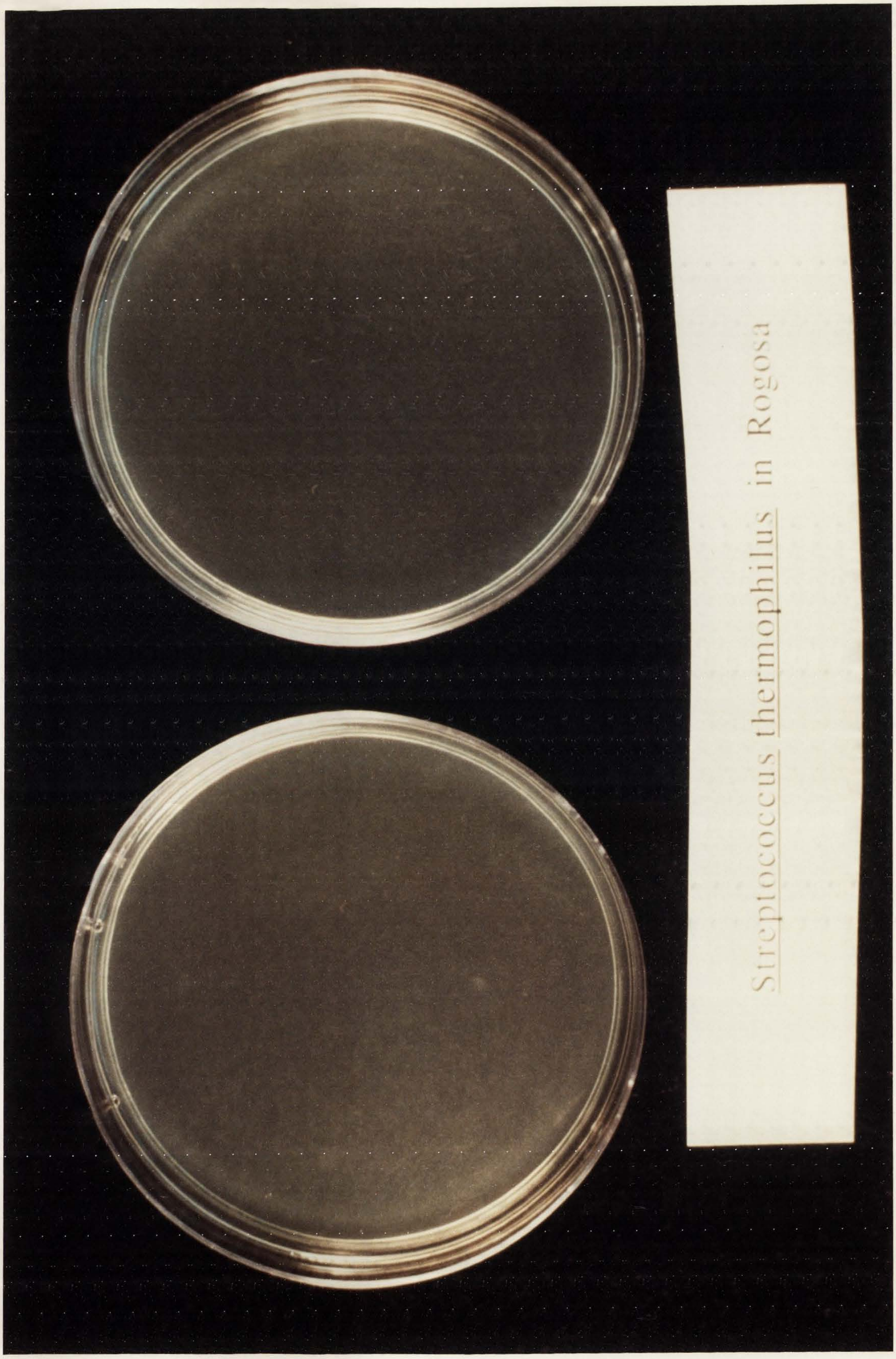
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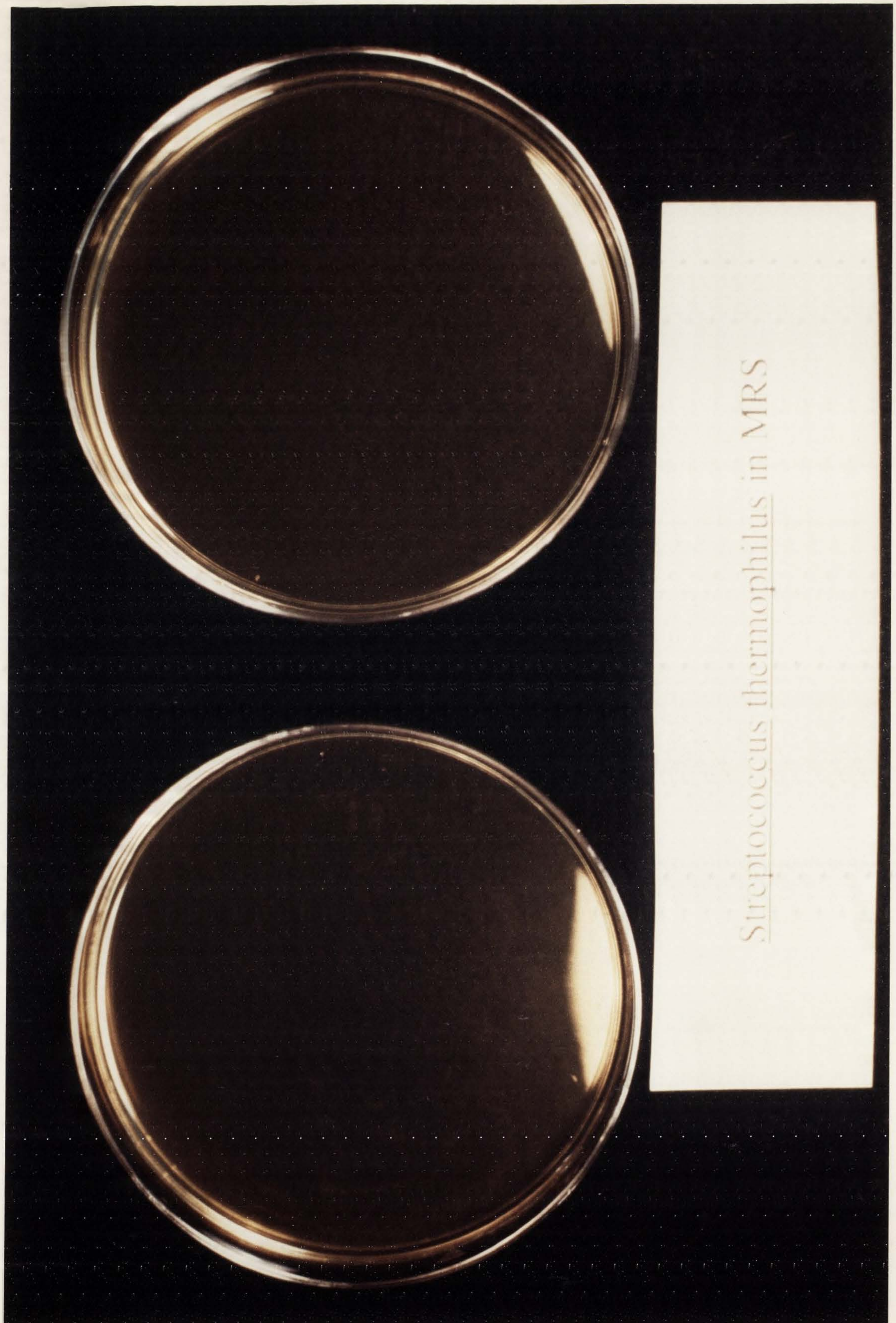
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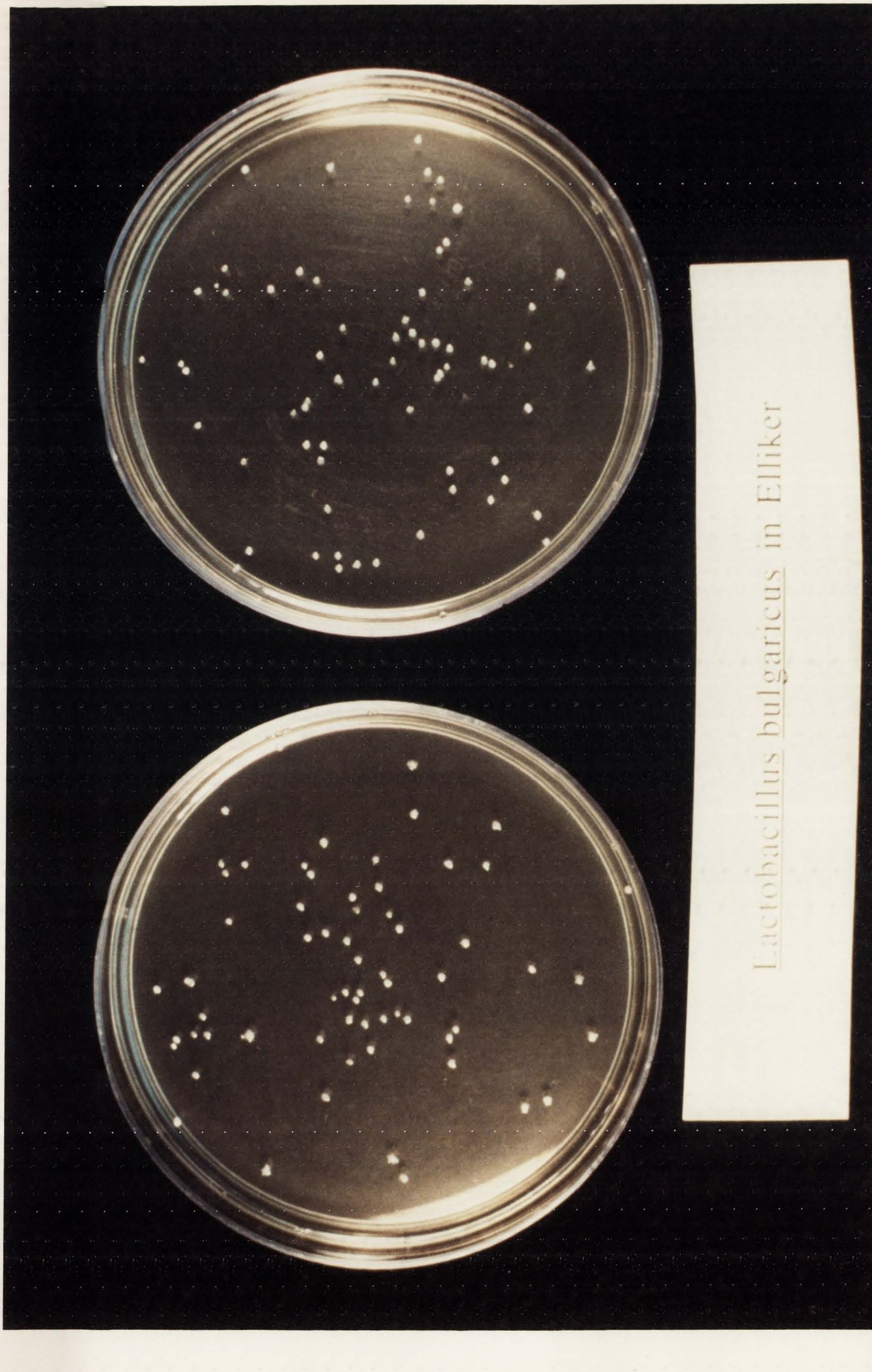
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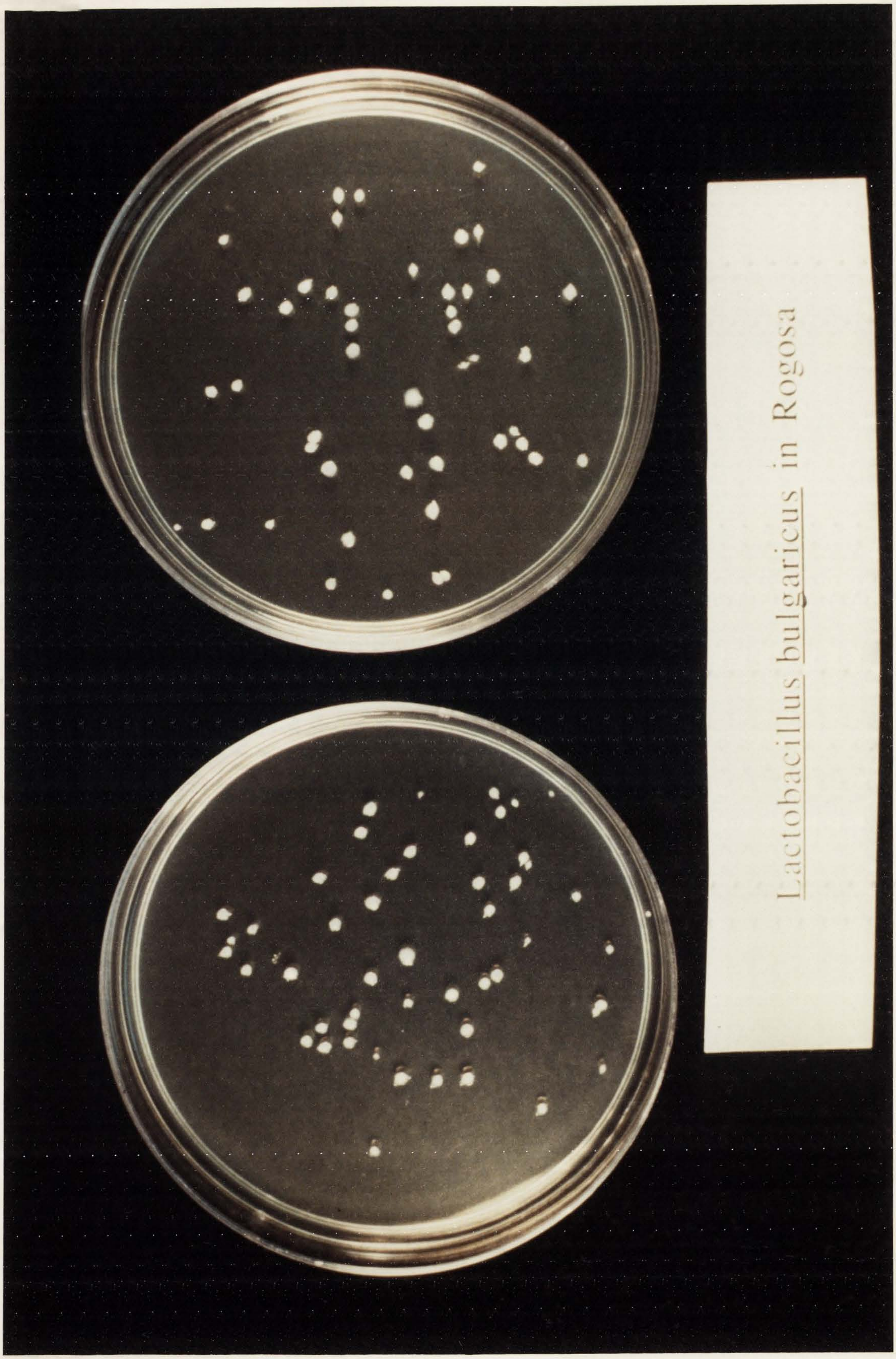
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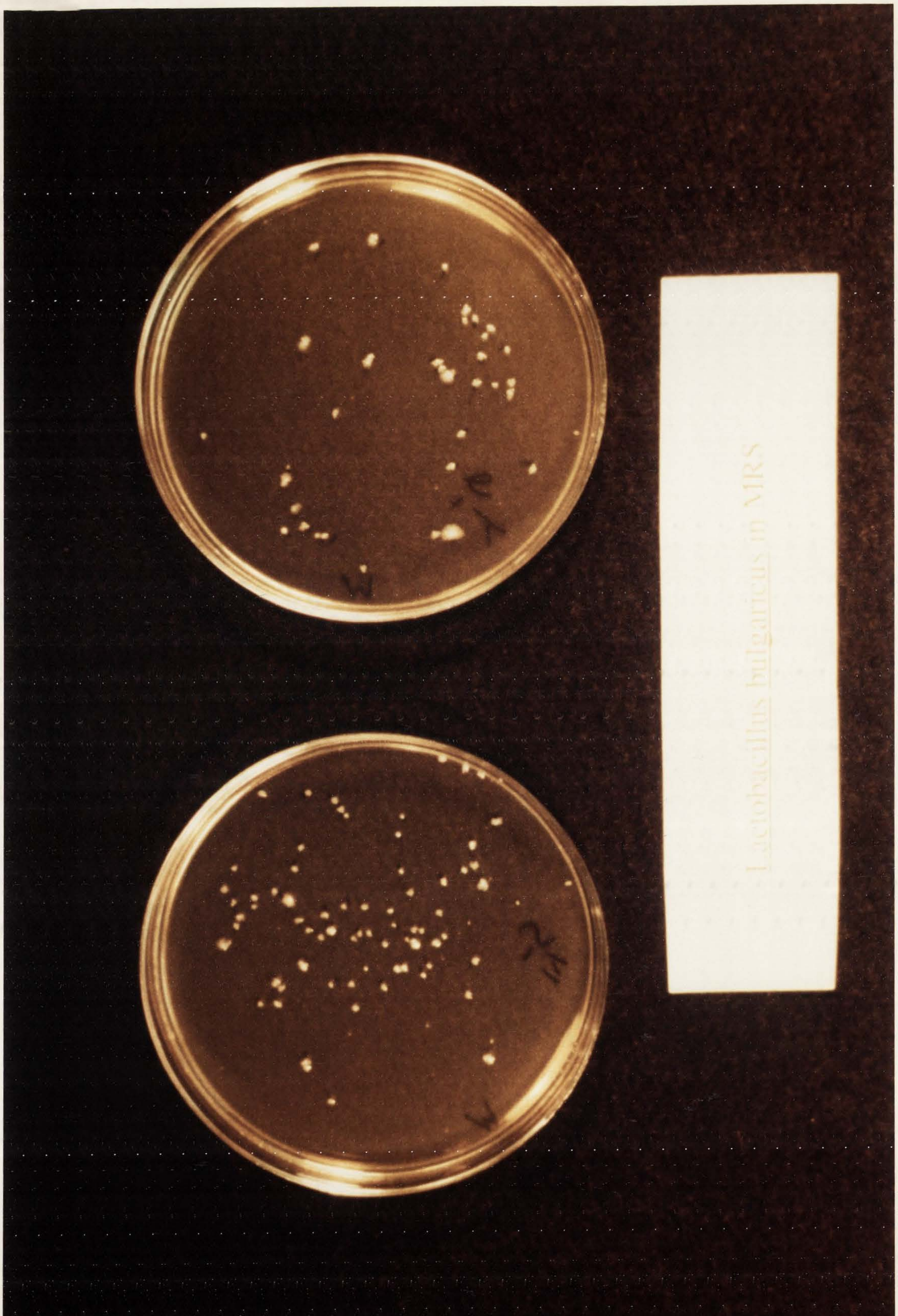
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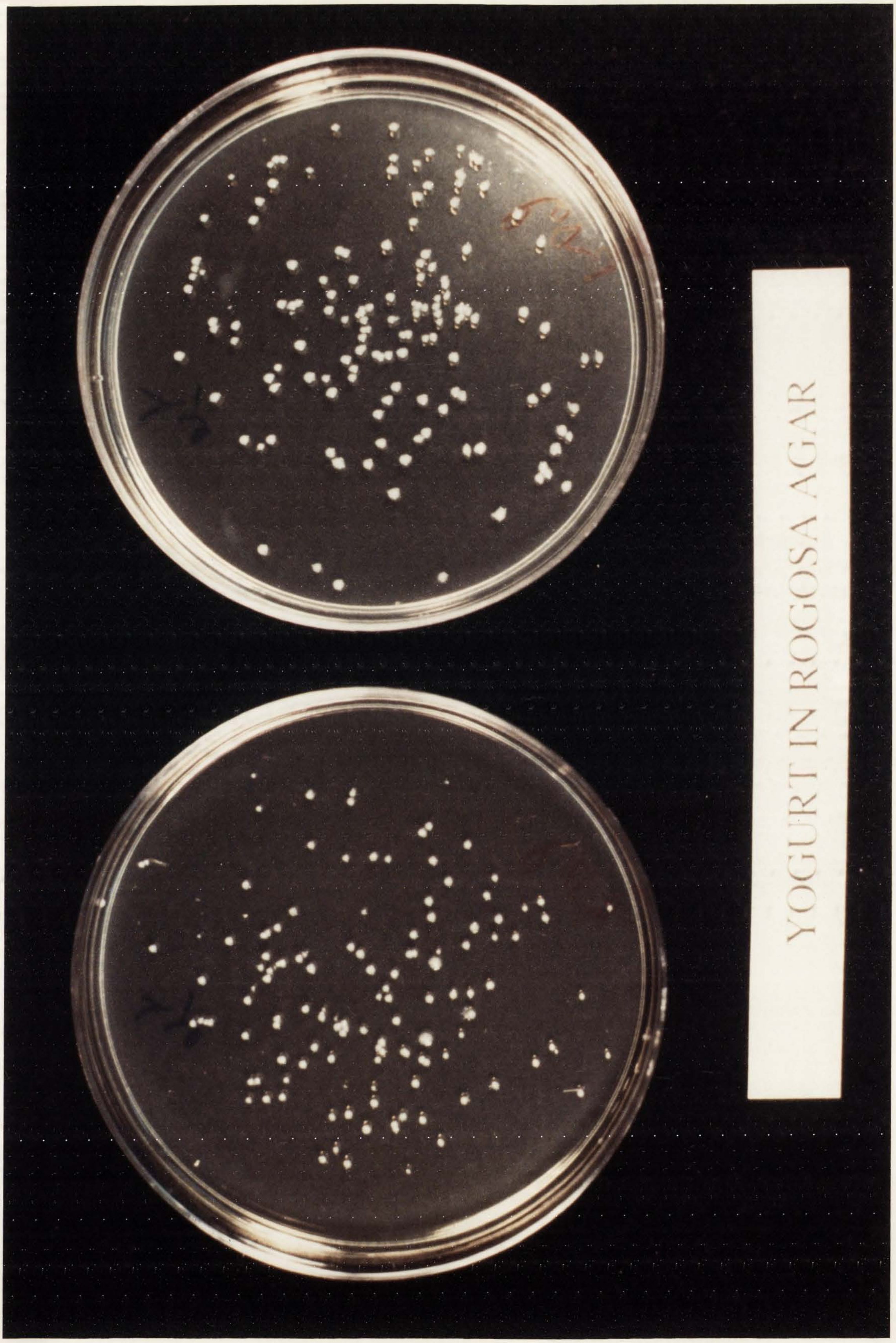
YOGURT IN ROGOSA AGAR


YOGURT IN MRS AGAR