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GROWTH RATE OF COLIFORM ORGANISMS IN COTTAGE
CHEESE AND RECONSTITUTED NON-FAT MILK

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
Wendell Ray Skelton
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GROWTH RATE OF COLIFORM ORGANISMS IN COTTAGE CHEESE
AND RECONSTITUTED NON-FAT MILK

by

Wendell Ray Skelton

AN ABSTRACT

Submitted to the College of Agriculture of Michigan
State University of Agriculture and Applied
Science in partial fulfillment of the
requirements for the degree of

MASTER OF SCIENCE

Department of Food Science

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ABSTRACT

The objective of this study was to determine the population trends of representative strains of Escherichia coli and Aerobacter aerogenes in cottage cheese and reconstituted non-fat milk (skim milk). Inoculated milk samples were stored at 0, 4, 10 and 32 C for a maximum of 130, 76, 119, and 11 days, respectively. The inoculated cheese samples were stored at 4, 10, and 13 C for 80 days. Coliform populations of the inoculated samples were determined at various intervals by plating on violet red bile agar. In milk at 10 and 32 C the generation time of E. coli during the logarithmic growth phase varied from 516 to 642 minutes and from 33 to 39 minutes, respectively. The corresponding generation times for A. aerogenes were 540 to 648 minutes and 29 to 33 minutes. With both coliform species there was a decrease in numbers at 0 and 4 C and the rate was greater at 4 C.

In cottage cheese the population of both coliform species increased at 13 C and decreased at 4 C, but at 10 C the numbers of E. coli increased while those of A. aerogenes decreased. During the first twenty days at 13 C, the generation times of E. coli ranged from 31.5 to 42.5 hours while those of A. aerogenes varied 33 to 47 hours. At 10 C the generation times of E. coli ranged from 48 to 120 hours. At both temperatures the coliforms increased most rapidly during the first five days of incubation. At 4 C the diminution of coliforms in cottage cheese was negligible throughout the normal shelf-life of 15 to 20 days. Reconstituted non-fat milk was superior to cottage cheese as a growth medium for E. coli at 10 C.

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INTRODUCTION

Cottage cheese and skim milk are nutritious foods which are increasing in popularity. Between 1950 and 1960 the per capita consumption of cottage cheese increased from 3.1 to 4.8 pounds (27) and per capita sales of skim milk increased from 14.7 to 23.1 pounds (26).

Initial coliform populations of less than one per milliliter or gram in processed fluid milk and cottage cheese products have shown significant increases during storage and movement through sales outlets. The increase in population is attributed to the ability of coliforms to grow at temperatures normally encountered in the distribution of dairy products.

Coliform bacteria are an index of sanitation in milk and milk products, and may be responsible for undesirable fermentations. In nature they may be associated with intestinal pathogens. Proper pasteurization destroys these organisms; therefore their presence in milk is due to post-pasteurization contamination. The normal growth range of the coliforms is 10 to 45 C, but these organisms have been known to survive or even increase in milk products stored at 1.6 to 7.2 C. Since they are capable of growth at refrigeration temperatures, it is important that the rate of increase be known.

The purpose of this study was to determine the population trends and generation time of typical strains of Escherichia coli and Aerobacter aerogenes, when grown in cottage cheese and reconstituted non-fat milk (skim milk) at various refrigeration temperatures. The growth pattern and generation time of the organisms were also determined at the optimum temperature of 32 C.

REVIEW OF LITERATURE

Coliform Organisms in Milk

The coliform group of bacteria consists of the genera Escherichia and Aerobacter. E. coli and A. aerogenes, the most important members of this group, have an optimum temperature for growth of 30 to 37 C and a normal growth range of 10 to 45 C.

Many investigators have been concerned with the importance and source of the coliform organisms in pasteurized milk. In a study of four processing lines, Glenn and Olson (13) found the bottle filler was the most common source of coliform contamination in commercially pasteurized milk.

The heat resistance of coliforms in milk has been studied by several workers. Paley and Isaacs (33) found no survivors after an E. coli population of one million per milliliter was heated in milk at 61.7 C for 20 minutes. According to findings of Norrgren (29) Bact. (E.) coli cells heated in milk were destroyed in 10.8 seconds at 72 C, but were not destroyed in 10.8 or 25.2 seconds at 68 C. Glenn and Olson (13) tested the heat resistance of 67 coliform cultures isolated from raw and processed milks. The cultures were heated individually in milk at 61.7 C for 30 minutes. In milk samples containing over 300 million per ml., three of 67 cultures survived the heat treatment but in milk containing approximately 300 per ml., no coliforms were present following the heating trial.

Futschik and Sachslehner (12) demonstrated that coliforms in pasteurized milk need not be due to post-pasteurization contamination. Milk samples collected at the cooler exit of various heat exchangers and stored at 4 C yielded negative counts, but coliforms were detected in samples stored at 10, 15, and 20 C for 24 and 48 hours. These workers suggested that the presence of the coliforms in samples stored above 10 C was due to a

reactivation of thermally damaged organisms.

In recent years several workers have isolated members of the coliform group from pasteurized milk products which had been held at refrigeration temperatures. Dahlberg (9) demonstrated that coliform bacteria maintain a constant population in pasteurized milk stored for four days at 1.7 to 4.4 C; however, a cell count of one or less per milliliter increased to 100 per ml. in several samples incubated for three days at 7.2 to 10 C. The coliform organisms which Glenn and Olson (13) isolated from pasteurized milk grew very slowly at 7.2 C, slowly at 10 C and very rapidly at 12.8 C. There was no increase in coliform bacteria in concentrated or recombined milk stored at 1, 4, and 7 C according to findings of Olson et al. (30).

Harmon et al. (17) studied the shelf-life of commercial homogenized milk. They found an initial coliform count of 15 or less per ml. reached a population of one million per milliliter in six days at 11.7 C.

Weber (37) reported an analysis of 10 thousand coliform tests collected over a three year period for three health departments. These data showed that during the period of January, February and March 21.5 per cent of all pasteurized milk samples were coliform positive; whereas 48 per cent of the samples were positive during July, August and September. The higher incidence of coliforms in milk during the warm months was attributed primarily to a greater degree of contamination of the milk from equipment which is exposed to flies, insects and air-borne sources.

Dahlberg (10) found that six per cent of fresh commercially pasteurized milk samples contained coliforms in October; whereas in July and August, 35 per cent of the samples were coliform positive. Also after the pasteurized milk samples were stored at 7.7 to 10 C for 4 days the coliform bacteria represented 5.55 per cent of the total count in October

and 88 per cent in July and August. Burgwald and Josephson (6) demonstrated that coliform counts increased in pasteurized milk stored for seven days at 2.2 to 5.5 C in summer, but no increase was noted during the same interval at 2.8 to 4.0 C in winter months.

Coliform bacteria were isolated by Schultze and Olson (35, 36) as one of the psychrophilic types from numerous samples of dairy products. The samples had been stored for one week at 4 C. Of 586 cultures isolated, sixty-one (10.8 per cent) were classified as coliforms, but the biochemical characteristics exhibited by many of the coliform isolates were quite different than those shown by a typical coliform. Panes and Thomas (34) reported that coliforms increased in raw milk samples stored at 3 to 5 C for 72 hours. Klebsiella (Aerobacter) cloacae and K. (A.) aerogenes were predominant, but only six of 102 cultures were classified as E. coli. Audrey and Frazier (2) showed that some strains of A. aerogenes isolated from bulk tank milk, which had been maintained at 3.3 C for two days, were capable of showing growth in pasteurized milk at the same temperature.

Generation Times of Coliform Organisms

Generation time is defined as the time (minutes or hours) necessary for the number of cells to double, assuming all are dividing. The generation times of coliform organisms grown in broth media have been determined by several workers. Barber (3) used a single cell isolation technique to study the generation time of Bacillus (Escherichia) coli with respect to different temperature and time intervals. He found the maximum rate of growth occurred at 37.5 C with a minimum generation time of 17.2 minutes. The generation time at 10 C was 750 minutes and

at 32 C, 25 minutes were required. Jennison (21) grew two strains of E. coli and one strain of A. aerogenes in nutrient broth which was incubated at 32 C. After 24 hours the cultures contained 200 to 280 million cells per ml. He determined that the generation times of the E. coli strains were 33 and 26 minutes and that of A. aerogenes was 21 minutes. Mason (25) compiled a list of the minimum generation times of various bacteria observed when grown under optimal conditions. In a broth medium the generation times of E. coli varied from 16 to 17 minutes and A. aerogenes ranged from 17 to 30 minutes. He concluded that the coliforms are the most rapidly growing group of bacteria. Ingraham (19) reported that the generation times of E. coli, (strain K-12), grown in a broth medium were 21 minutes at 36, 42, and 44 C, 30 minutes at 32 C and 1200 minutes at 10 C.

Coliform Organisms in Cottage Cheese

The presence of coliform bacteria in creamed cottage cheese has been reported by several investigators. Lyons and Mallmann (23) examined samples of cottage cheese packaged at commercial dairy plants and found 64 per cent contained 220 or more coliforms per hundred g. of cheese. Hedrick (18) reported that 89 per cent of samples collected from nine Baltimore companies contained more than 50 coliforms per g. Martin et al. (24) examined 142 samples of cottage cheese, representing 27 manufacturers, and reported that 71.2 per cent of the samples contained 10 or more coliforms per g. It was found that the percentage of samples containing 10 or more coliform organisms per g. was higher in summer

than winter. More than 100 thousand coliforms per g. were found in 7.7 per cent of the samples examined. Overcast et al. (32) investigated the coliform population of cottage cheese purchased at retail outlets and found the counts varied from < 1 to 715 thousand per g.

The source of coliforms in cottage cheese is important. Harmon and Smith (15) found that air, cream, contaminated equipment and improperly pasteurized milk were sources of coliforms. Lyons and Mallmann (23) reported that pipes, pumps, vats, improperly handled cream, and faulty packaging methods contributed a significant number of coliforms to cottage cheese.

Bonner and Harmon (4) determined the salt tolerance and heat resistance of E. coli isolated from cottage cheese. This organism survived a temperature of 48.9 C in skim milk (pH 6.7) for five minutes but was completely destroyed at 61.7 C under the same conditions.

Overcast and Britton (31) demonstrated that a high quality cottage cheese could be manufactured from fresh skim milk pasteurized at 62.2 C for 30 minutes. An initial count revealed that only three of 64 samples of cheese contained more than 10 coliforms per g. After the samples had been stored at 5 C for 11 days, the coliform count exceeded 10 per g. in only two samples. Harmon and Smith (15) reported on the relationship between temperature and keeping quality of cottage cheese. They demonstrated that by lowering the storage temperature from 10 to 5.6 C, the shelf-life of samples averaged 51 per cent longer. Bonner and Harmon (4) showed that E. coli isolated from cottage cheese grew in trypticase soy broth in seven days at 3 C. Growth also occurred at 10, 20, and 35 C in both three and seven days, but there was no

growth at 50 C during the same interval of time.

Overcast and Britton (31) manufactured cottage cheese which contained terminal coliform counts of 10 or less per g. after storage for 11 days at 4.4 C. They concluded that under carefully controlled conditions, cottage cheese could be manufactured with a shelf-life of at least 15 days.

Collins (8) demonstrated the necessity for a cooking temperature of at least 54 C and a holding time of at least 18 minutes to destroy spoilage organisms found in cottage cheese. The D values of representative strains of E. coli and A. aerogenes were 11.7 and 1.9 minutes, respectively, when these organisms were heated in cottage cheese whey (pH 4.6) at 51.5 C. He indicated that 23.7 minutes would be needed at 54 C to reduce a post-pasteurization contamination of 10 thousand E. coli per g. to one cell per 10 g. of cheese. Bonner and Harmon (4) reported that E. coli isolated from cottage cheese was destroyed when heated for 15 minutes at 48.9 C in whey at pH 4.55.

The effect of chemical germicides on coliform bacteria has been reported by several workers. Collins (7) found that 3 parts per million (ppm) of residual chlorine destroyed cultures of E. coli and A. aerogenes. The bactericidal efficiency of the chlorine solutions decreased as the pH increased from 6.0 to 10.0 and as the temperature decreased from 21 to 4.4 C. E. coli cells isolated from cottage cheese withstood hypochlorite and iodophor treatments of 25 ppm for 10 minutes at 15 C at the respective pH ranges of 7.4 to 7.6 and 5.1 to 5.8 according to Bonner and Harmon (4). However, concentrations of 50 ppm for one minute under the same conditions were destructive. Results of the same study showed that E. coli withstood quaternary concentrations of 50 ppm at

pH 6.8 to 7.2 for five minutes, but were destroyed in ten minutes. Mueller (28) reported that 25 ppm of iodine compared favorably with 100 ppm of chlorine in killing E. coli, in the presence of hard water and added organic matter.

The survival of coliform bacteria in cottage cheese seems to be closely linked to pH. Martin et al. (24) examined 130 samples and found the pH values ranged from 4.0 to 5.5 with over 63 per cent between pH 4.5 and 5.0. Lyons and Mallmann (23) reported that the pH of 150 samples ranged from 4.7 to 5.5 with over 80 per cent between 5.0 and 5.5. When the samples were incubated at 37 C the pH dropped rapidly, and in 96 hours coliform organisms were destroyed at a pH of approximately 4.0, but when cottage cheese samples were stored at temperatures ranging from 4.4 to 10 C coliforms survived for about 182 hours with the pH dropping to a minimum of 4.6. Harmon and Smith (14) also found an inhibitory effect of low pH on coliforms. They reported a decrease in coliform numbers in samples possessing a terminal pH of 4.8 or less and held at 5.6 C. Cottage cheese samples which possessed an initial and terminal pH of above 5.1 exhibited continuous increases in coliform populations when the samples were stored at 5.6 C.

PROCEDURE

Preparation of Milk

Low heat non-fat dry milk powder was reconstituted to nine per cent serum solids and steamed for one hour at 100 C in an autoclave. The steaming process was repeated three times at 24 hour intervals. Between heatings, the reconstituted milk was stored at room temperature. After the last heat treatment, the samples were adjusted to pH 6.6 to 6.7 with a 10 per cent solution of sterile trisodium phosphate and stored at 4 C until used. Sample bottles containing 100 ml. of the sterile reconstituted non-fat milk were tempered at 0, 4, 10, and 32 C for 24 hours before the initial coliform inoculum was added.

Manufacture of Cottage Cheese

The cottage cheese was prepared by the short set method from skim milk which was pasteurized at 71.7 C for 17 seconds. During a period of ninety minutes the temperature of the cheese was raised from 32.2 to 57 C which allowed an average increase of 0.56 C (1 F) in temperature every two minutes. The cheese was held in the whey at 57 C for 20 minutes to reduce the number of psychrophiles in the curd to non-detectable levels. Following the cooking process the curd was washed three times with water containing 15 ppm of a hypochlorite solution. The chlorinated water was held overnight at 0 C. During and after the manufacturing process the cheese curd was handled carefully to minimize contamination.

A creaming mixture containing 10.5 per cent milk fat was steamed at 100 C for one hour and sodium chloride equal to 3.5 per cent by weight was added. A sufficient quantity of the creaming mixture was blended with the cheese curd to give a concentration of three per cent milk fat in the cottage cheese.

Isolation and Identification of Coliform Organisms

Coliform bacteria were isolated from raw bulk tank milk received at the Michigan State University Dairy Plant. The coliform organisms were isolated initially on violet red bile agar plates incubated at 32 C. Brilliant green bile (two per cent solution) was used to determine gas production. The ability of the organisms to utilize citrate as a sole source of carbon, produce acid from dextrose (methyl red reaction), form acetyl methyl carbinol from dextrose (Voges-Proskauer reaction), and produce indole from tryptophan was determined using suitable media and reagents described in the Difco Manual (11). Four strains of E. coli and three strains of A. aerogenes were retained for use after isolation and purification. The characteristics of these cultures agree with those reported by Breed et al. (5) and are summarized in Table 1. Eighteen hour cultures, grown on tryptone glucose yeast agar slants, were inoculated into the test medium and incubated at 32 C for the appropriate length of time for each individual test.

Propagation of Coliform Organisms

Stock cultures of E. coli and A. aerogenes were carried on tryptone glucose yeast agar slants. A loop of the stock culture of each organism was transferred to nine milliliters of sterile reconstituted non-fat milk. Active cultures were maintained by transferring the cultures daily. An 18 hour culture was diluted with buffered sterile water to give the desired initial population and added to the reconstituted non-fat milk or creaming mixture. Initial coliform populations of 10 or less per ml. were added to the milk samples stored at 10 and 32 C; whereas 1500 to 2000 coliforms per ml. were added to the milk samples stored at 0 and 4 C. One thousand to 1500

coliforms per ml. were added to the creaming mixture to give an initial population in the cottage cheese of 100 to 250 per g. The reduction was partially due to the dilution of the creaming mixture with the cheese curd. The remaining reduction was attributed to change in environment.

Preparation of Cottage Cheese Samples

The coliform organisms were uniformly dispersed in the creaming mixture which was then mixed with the curd at a ratio of 2.5 parts of curd to one part of creaming mixture. Four lots of creamed cottage cheese were prepared and inoculated with coliform organisms. Each trial included three groups of cheese samples: (a) control, (b) inoculated with E. coli and (c) inoculated with A. aerogenes. The creamed cottage cheese was measured into sterilized bottles and incubated at 4, 10, and 13 C.

Bacteriological Analyses of Samples

Control samples of the cottage cheese and reconstituted non-fat milk were initially analyzed for total, coliform, mold, yeast, and psychrophile populations.

Equal volumes of cottage cheese and two per cent sodium citrate solution were weighed into a sterilized Waring blender jar and mixed for three minutes at slow speed. A two gram sample of this homogenate was added to a 99 ml. sterile buffered distilled water blank. The water blanks were used to dilute both the cheese and milk to proper dilutions. Appropriate dilutions of both cheese and milk were plated on violet red bile agar and incubated at 35 and 32 C, respectively, for 24 hours. The higher incubation temperature of 35 C was used to minimize psychrophilic growth which may have developed in the cottage cheese. Procedures

outlined in Standard Methods (1) were used in determining all bacterial counts.

The coliform count of control and inoculated samples of the reconstituted non-fat milk and cottage cheese was determined at various intervals. The plating interval depended upon the storage temperature of the samples. The reconstituted non-fat milk stored at 32 C was sampled and plated every six hours. The samples stored at 10 C were plated every 24 hours until the population had reached the declining phase then the interval was extended. The plating interval of the milk samples stored at 0 and 4 C was one to four days for the first 46 days and less frequently thereafter. The cottage cheese samples, incubated at 4, 10, and 13 C, were plated initially when inoculated, then on the second and seventh days and thereafter the coliform population was determined once a week.

Calculation of Generation Times

The generation times were calculated according to the following formula:

$$\begin{aligned} \text{generation time} &= \frac{(T_2 - T_1) \times \log 2}{\log b - \log B} \\ (T_2 - T_1) &= \text{interval of time (minutes or hours)} \\ b &= \text{bacterial population at time } (T_2) \\ B &= \text{bacterial population at time } (T_1) \end{aligned}$$

pH Determinations

All pH determinations were made with a Beckman Model H-2 pH meter equipped with glass electrodes.

RESULTS

Identification of Coliform Organisms

The data in Table 1 show the results of various tests used in identifying the coliform organisms isolated from raw milk. The organisms were classified as either E. coli or A. aerogenes according to the characteristics listed in Breed et al. (5). Cultures which did not give typical biochemical reactions were discarded.

Growth of Coliform Organisms in Reconstituted Non-fat Milk

The generation times of four strains of E. coli and three strains of A. aerogenes grown in reconstituted non-fat milk containing nine per cent serum solids and incubated at 10 and 32 C are shown in Table 2. The generation times of E. coli and A. aerogenes incubated in milk at 32 C represent a logarithmic average of two and six trials, respectively, while the results at 10 C were obtained from a single trial. During the logarithmic growth phase, the generation times were usually computed for the interval between the second and ninth days of incubation in milk at 10 C and within the first 12 hours in milk stored at 32 C. The data in this table also include the generation times which were computed during the interval from inoculation to maximum population. The interval was 24 hours for all coliforms incubated in milk at 32 C., and from 13 to 21 days for samples held at 10 C.

The curves representing the trends in population of the four strains of E. coli and three strains of A. aerogenes in reconstituted non-fat milk containing nine per cent serum solids and incubated at 0, 4, 10, and 32 C are shown in Figs. 1 to 7 inclusive. These curves depict data from one trial with each coliform organism at 0, 4, and 10 C. However, the curves representing the data for the coliforms incubated at 32 C are a logarithmic

Table 1

Morphological, physiological, and biochemical characteristics of coliform organisms isolated from milk

organism	morphology	Gram stain	gas production ^a	indole	methyl red	Voges-Proskauer	citrate
<u>E. coli</u> strain 1	rods, 0.5 x 1.5 μ	-	+	+	+	-	-
"	2 rods, 0.5 x 1.5 to 2.0 μ , irregular lengths	-	+	+	+	-	-
"	3 rods, 0.5 x 2.0 μ lengths up to 3.5 to 4.0 μ	-	+	+	+	-	-
"	4 rods, 0.5 x 2.0 to 2.5 μ	-	+	+	+	-	-
<u>A. aerogenes</u> strain 1	rods, 0.5 x 1.0 to 1.5 μ	-	+	-	-	+	+
"	2 rods, 0.5 x 1.0 to 1.5 μ	-	+	-	-	+	+
"	3 rods, 0.5 x 1.0 μ occasionally longer	-	+	-	-	+	+

^a two per cent solution of brilliant green bile

Table 2

Generation times of coliform organisms in reconstituted non-fat milk containing nine per cent serum solids, incubated at 10 and 32 C

		incubation temperature			
		10 C		32 C	
		generation time during logarithmic growth phase	generation time during interval from inoculation to maximum population	generation time during logarithmic growth phase	generation time during interval from inoculation to maximum population
		(min.)	(min.)	(min.)	(min.)
<u>E. coli</u>					15
strain 1		516	942	34	52
" 2		546	744	34	54
" 3		522	774	33	53
" 4		642	1038	39	56
<u>A. aerogenes</u>					
strain 1		540	870	33	53
" 2		648	852	34	50
" 3		636	1014	29	48

average of two trials with A. aerogenes and six trials with E. coli. Non-inoculated control samples incubated at the same temperatures and subjected to the same analyses as the inoculated samples consistently contained <1 coliform per ml.

With the storage temperature at 32 C there was no perceptible lag period with any of the coliforms. The logarithmic growth phase terminated after the 12th hour of incubation and the maximum population was reached at 24 hours. These values are relative, rather than absolute, because the bacterial population was determined at six hour intervals. During incubation at 32 C an initial coliform inoculum of 10 or less per ml. reached a maximum number of 880 to 890 million cells per ml. in one day (Figs. 1 to 7). The population decreased only slightly through the third day, but a definite diminution in cell numbers was noted on the fourth day. On the 11th day, the cell count ranged from < 1 to 3,000 per ml. There was one exception; the population of A. aerogenes, strain 3, decreased to < 1 per ml. on the seventh day.

The data in Figs. 1 to 7 also show that at 10 C initial numbers of 10 or less E. coli per ml. attained a maximum population of 170 to 500 million per ml. within 13 to 21 days. Initial inoculations of 10 or less A. aerogenes per ml. reached a maximum count of 150 to 260 million per ml. in 15 to 17 days.

The logarithmic growth phase usually began between 24 and 48 hours after inoculation with most strains of coliforms held in milk samples at 10 C, but A. aerogenes, strain 2, (Fig. 6) and E. coli, strains 2 and 3, (Figs. 2 and 3) showed no definite lag phase. The initial inoculum of E. coli, strain 4, (Fig. 4) was < 1 per ml. and on the third day after inoculation the sample contained only three coliforms per milliliter.

This lower initial inoculum of E. coli accounted for what appeared to be a longer lag phase and may be responsible for the longer time required for the strain to reach maximum population. In samples inoculated with the other coliform strains the initial counts of one to 10 per ml. increased to 40 to 700 per ml. by the third day. In general at 10 C the most rapid growth of the coliforms occurred between the second and ninth days. An exception was E. coli, strain 4, which received less inoculum.

At 10 C the stationary growth phase prevailed for 10 to 15 days with E. coli and 12 to 15 days with A. aerogenes. A decline in population began on the 23rd to 26th day with all strains of coliforms and continued until the samples contained 10 or less per ml. after 119 days.

An average initial coliform population of 1700 per ml. was added to reconstituted non-fat milk which was stored at 0 and 4 C. There was no increase in cell count at either temperature. For the first 16 to 25 days after inoculation the decrease in numbers of coliforms in milk stored at 0 and 4 C were similar. In subsequent incubation the coliforms decreased to approximately 10 per ml. by the 30th and to < 1 per ml. by the 50th day in the samples stored at 4 C. There were minor variations among the different strains. At 0 C, following the 16 to 25 day period, the number of coliforms decreased very slowly for the next 60 to 70 days. The samples usually contained 50 or less cells per ml. after 90 days and five or less per milliliter after 130 days with minor fluctuations among the different strains.

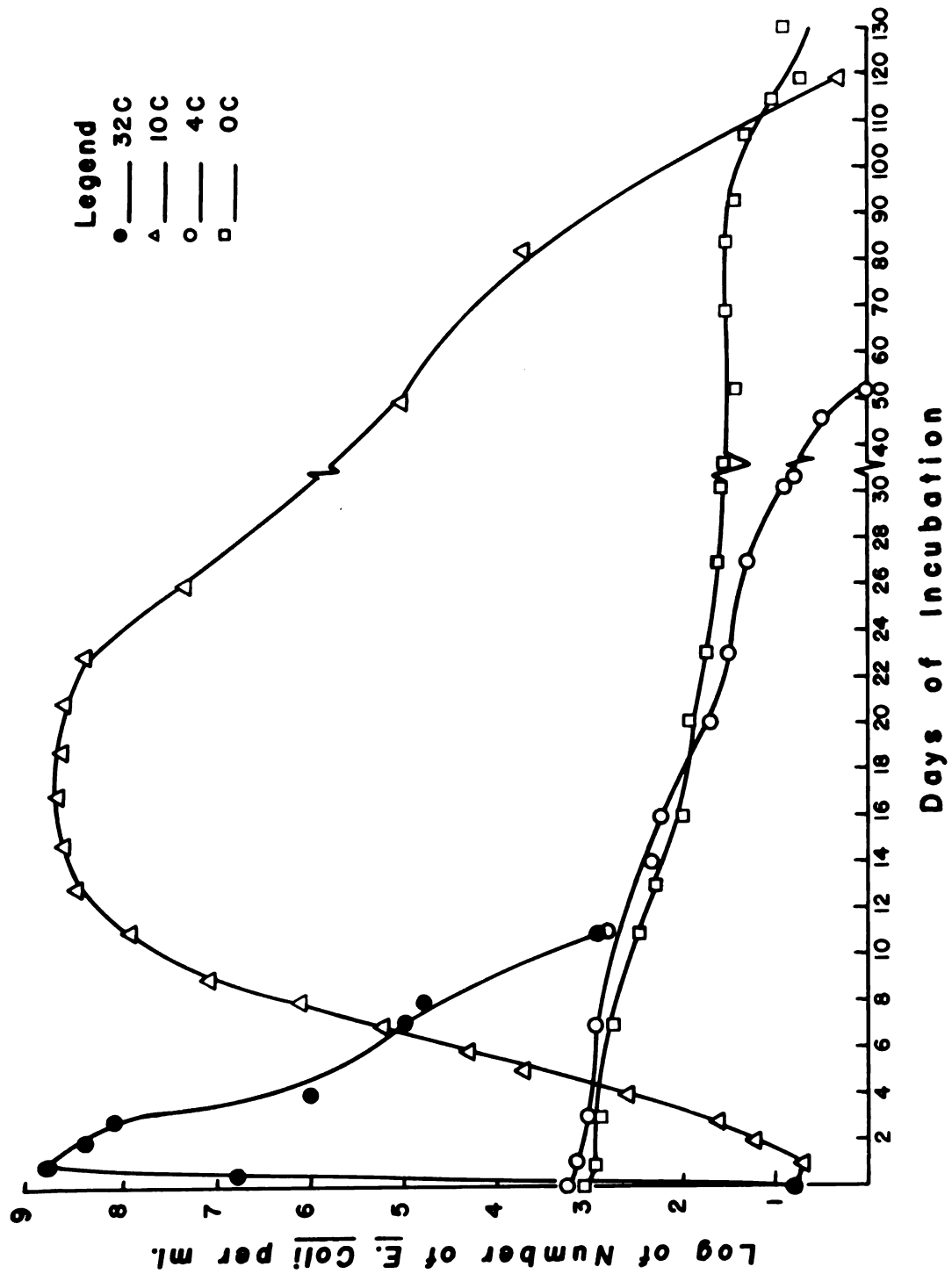


Figure 1. Population curves of *Escherichia coli* (strain 1) in reconstituted non-fat milk containing 9% serum solids and incubated at 0, 4, 10, and 32 C.

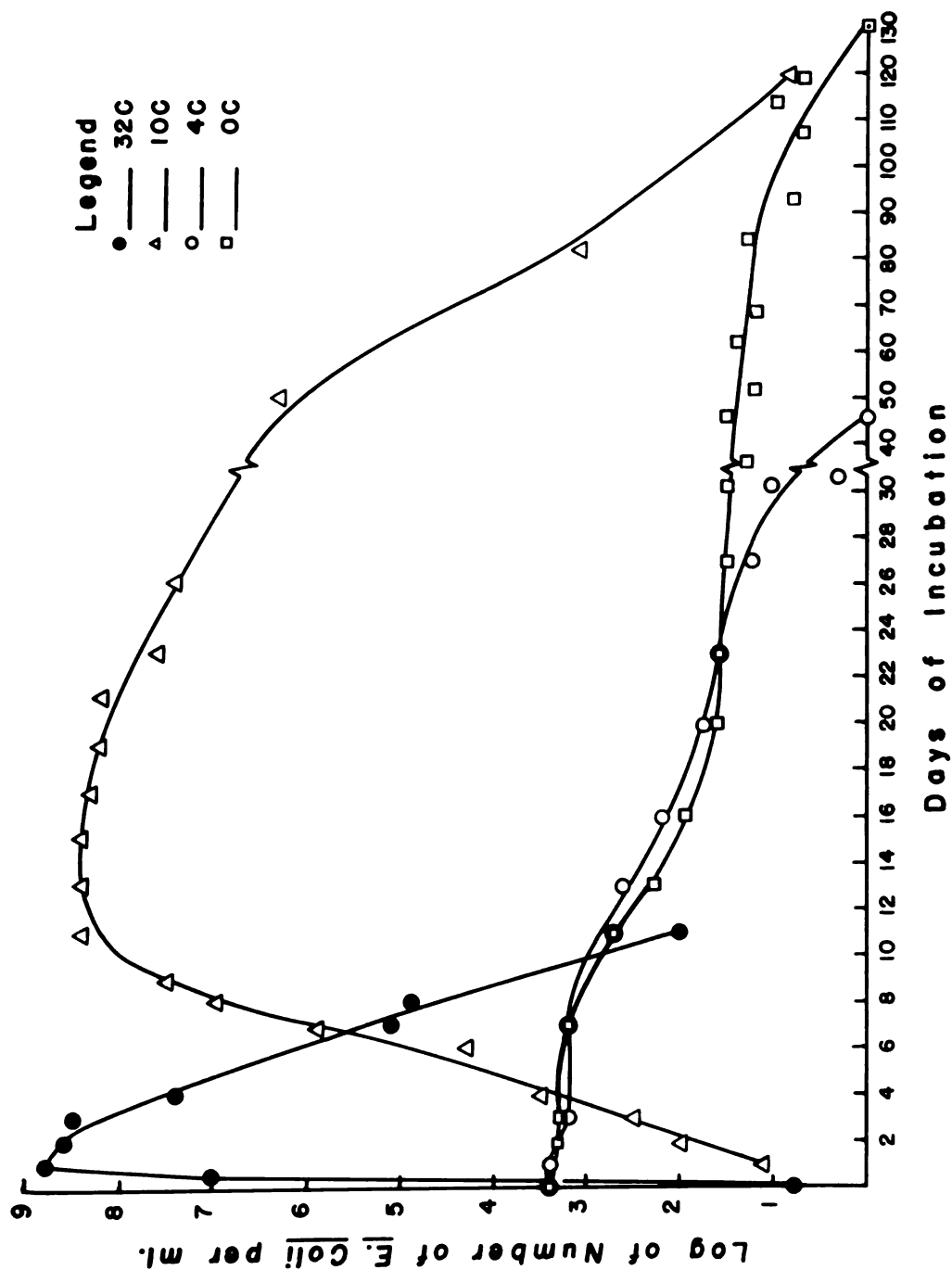


Figure 2. Population curves of *Escherichia coli* (strain 2) in reconstituted non-fat milk containing 9% serum solids and incubated at 0, 4, 10, and 32 C.

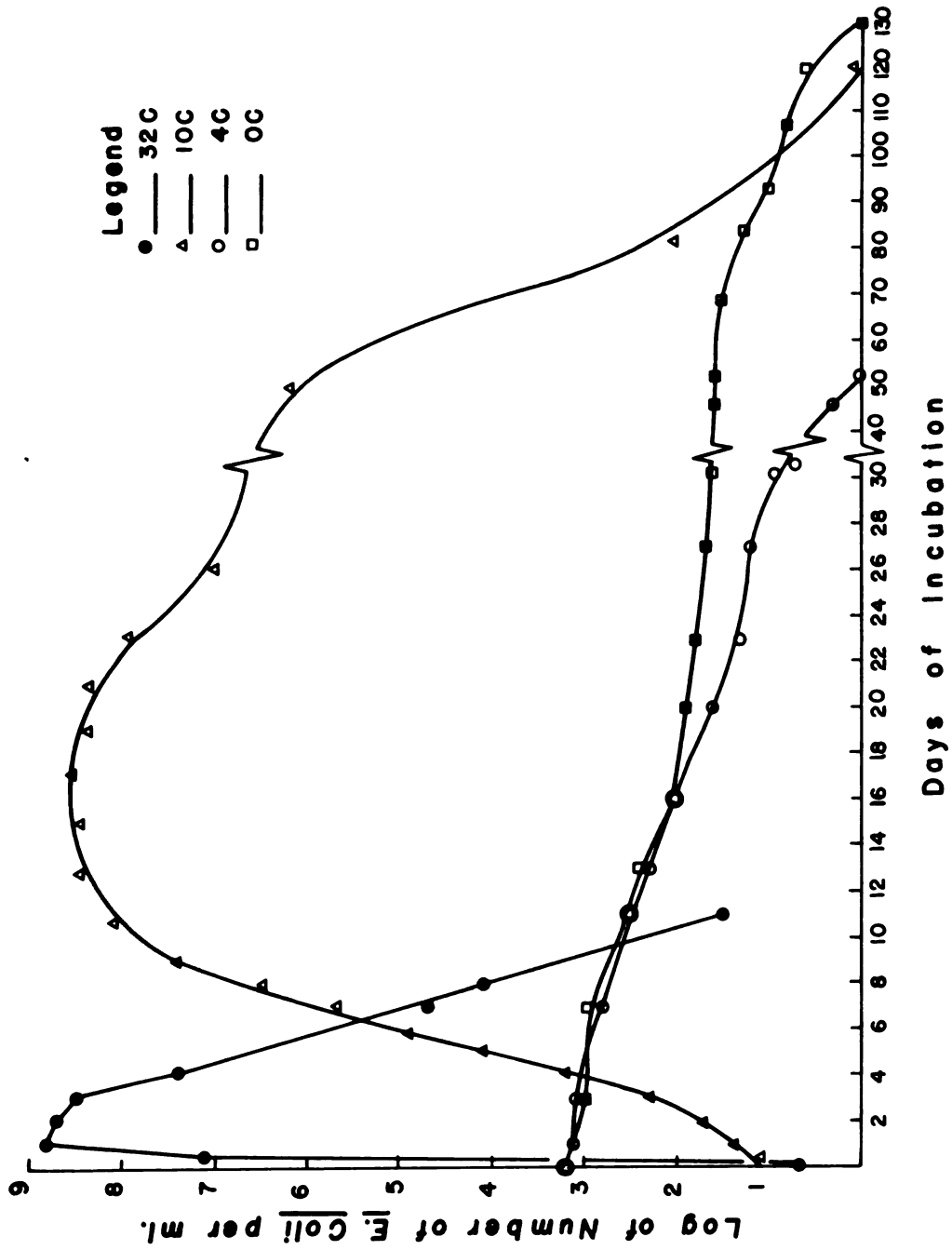


Figure 3. Population curves of *Escherichia coli* (strain 3) in reconstituted non-fat milk containing 9% serum solids and incubated at 0, 4, 10, and 32 C.

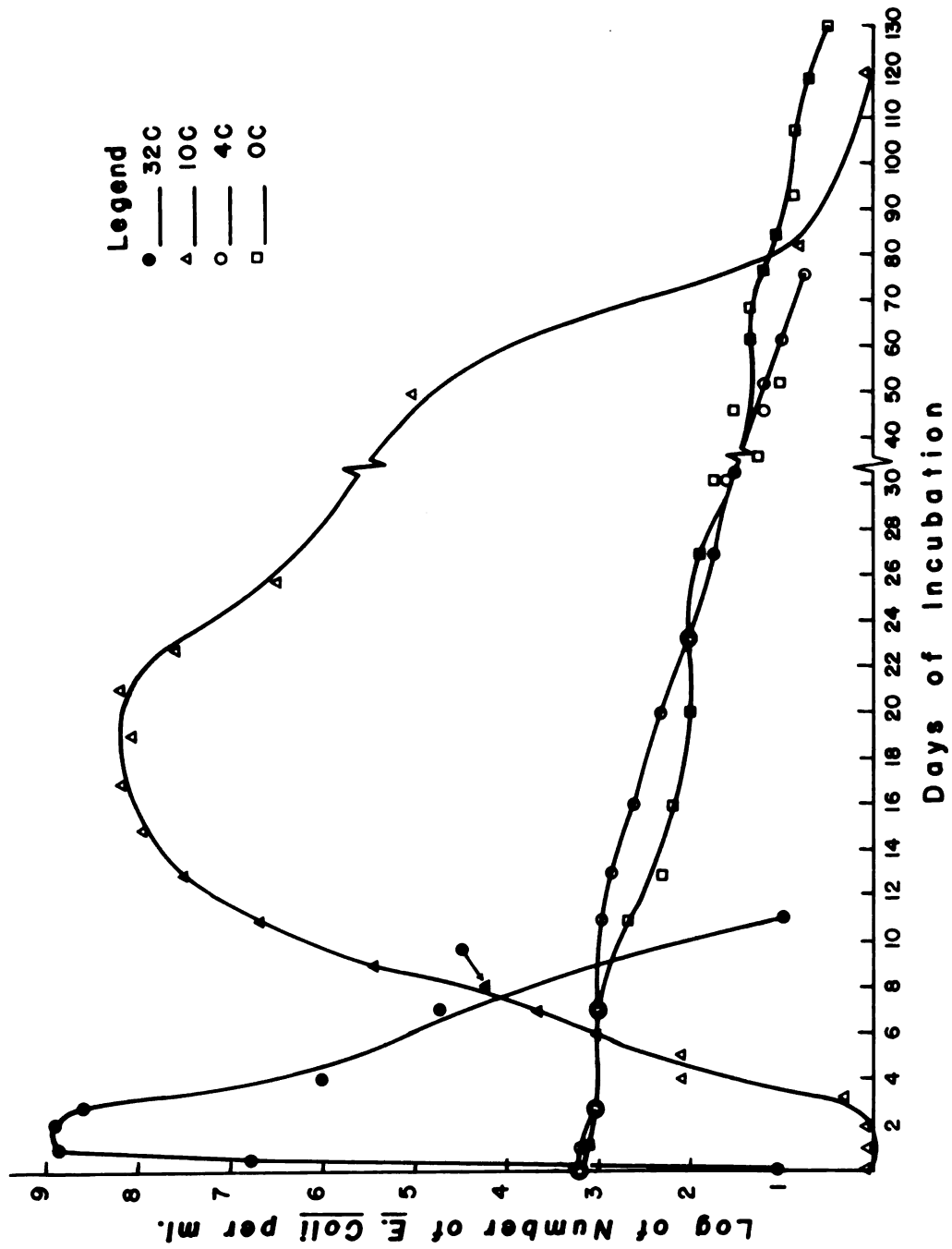


Figure 4. Population curves of *Escherichia coli* (strain 4) in reconstituted non-fat milk containing 9% serum solids and incubated at 0, 4, 10, and 32 C.

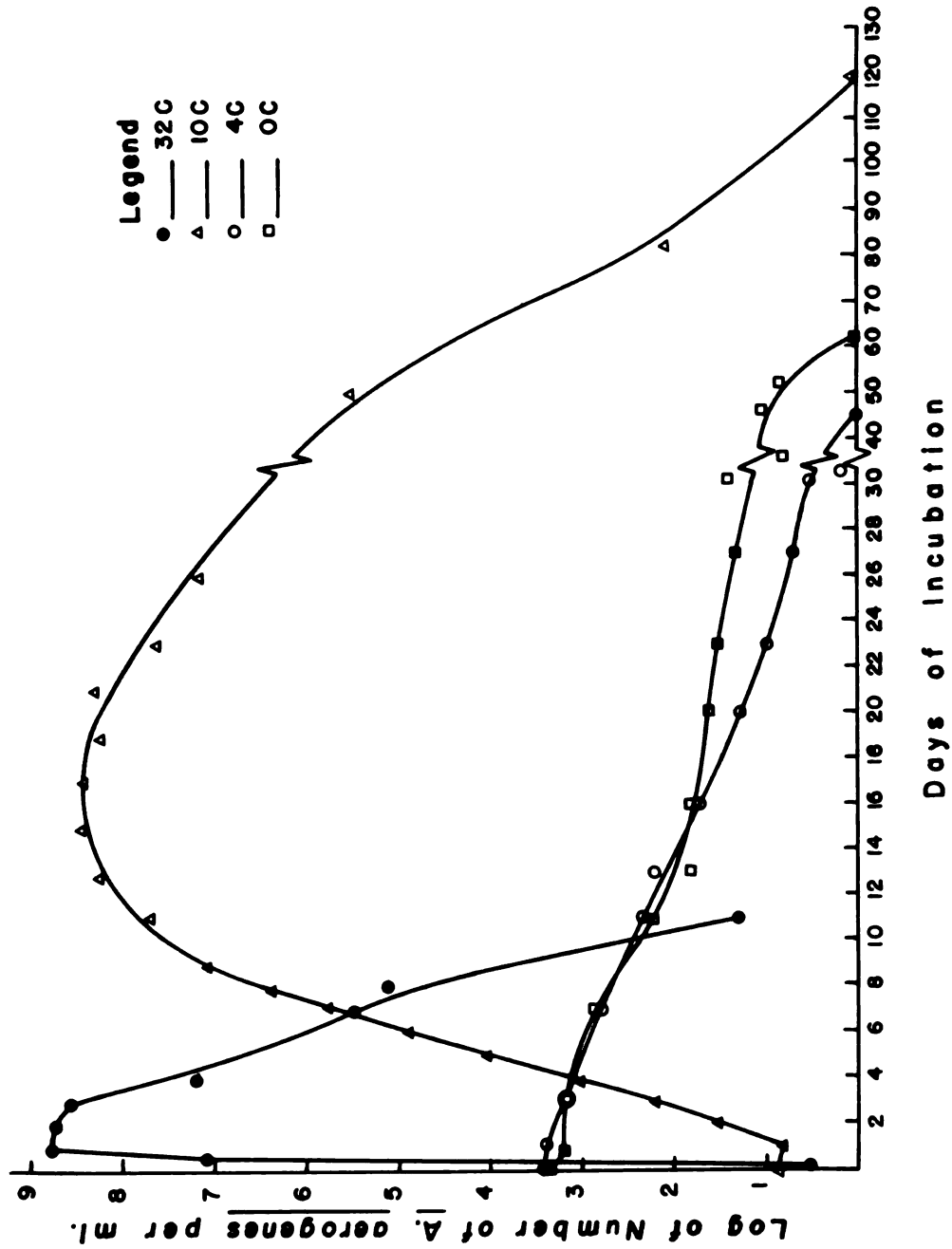


Figure 5. Population curves of Aerobacter aerogenes (strain 1) in reconstituted non-fat milk containing 9% serum solids and incubated at 0, 4, 10, and 32 C.

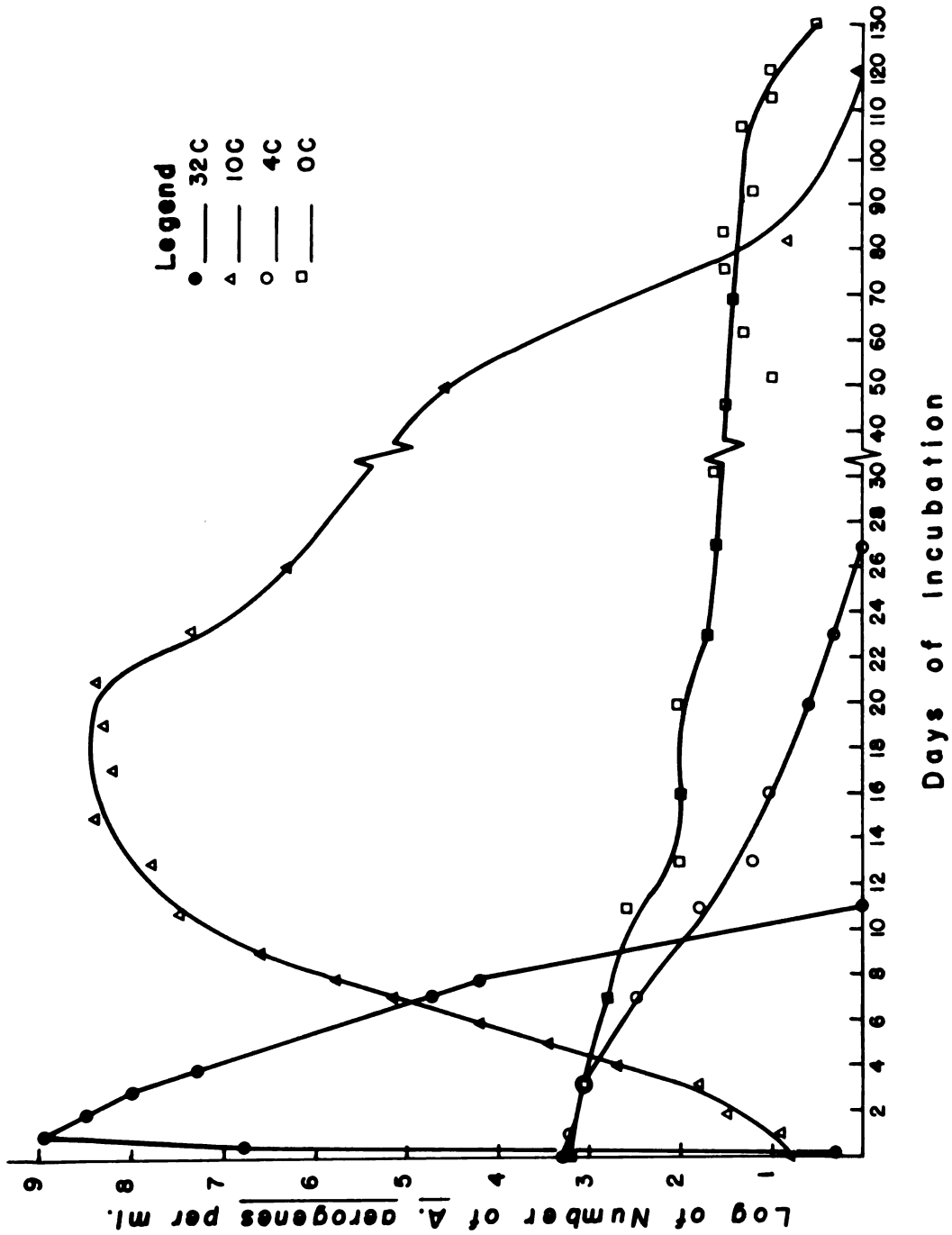


Figure 6. Population curves of *Aerobacter aerogenes* (strain 2) in reconstituted non-fat milk containing 9% serum solids and incubated at 0, 4, 10, and 32 C.

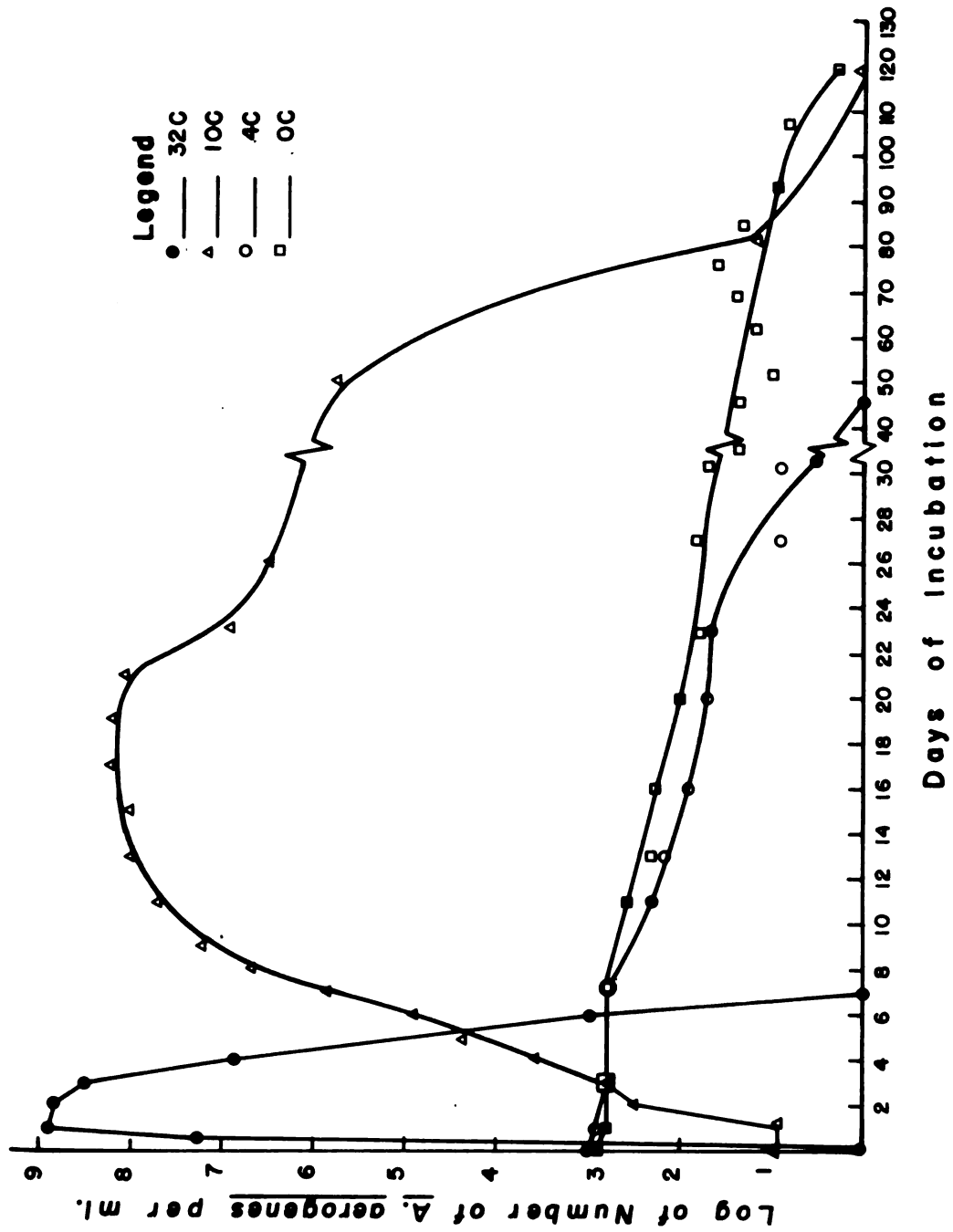


Figure 7. Population curves of *Aerobacter aerogenes* (strain 3) in reconstituted non-fat milk containing 9% serum solids and incubated at 0, 4, 10, and 32 C.

Growth of Coliform Organisms in Cottage Cheese

The data in Figs. 8 and 9 show the population trends of E. coli and A. aerogenes, respectively, when inoculated into cottage cheese and incubated at 4, 10, and 13 C. The population of both organisms increased at 13 C and decreased at 4 C, but at 10 C the numbers of E. coli per gram increased while those of A. aerogenes decreased. At 13 C an initial population of 250 E. coli per g. increased to 3500 per g. at five days and to 28 thousand per g. after 10 days of growth; whereas at 10 C the population increased from 250 to 1400 per g. at five days and to 2800 per g. at 10 days. An initial A. aerogenes population of 100 per g. at 13 C increased to 1600 per g. at five days and to 8,000 per g. at 10 days. Non-inoculated control samples incubated at the same temperatures and subjected to the same analyses as the inoculated samples consistently contained < 1 coliform per g.

The generation times of E. coli and A. aerogenes grown in cottage cheese at 10 and 13 C are shown in Table 3. The shortest generation times were obtained for the first five days of incubation and each five day increase in incubation time yielded a longer generation time. The generation times of the strain of A. aerogenes were slightly longer than those of the E. coli. During the first twenty days at 13 C, the generation times of E. coli ranged from 31.4 to 42.5 hours while those of A. aerogenes varied from 32.8 to 47.3 hours. At 10 C the generation times of E. coli varied from 48.2 to 120.4 hours while the A. aerogenes showed a decrease in population.

A comparison of the generation times of E. coli grown in cottage cheese and in reconstituted non-fat milk incubated at 10 C is shown in Table 4. These data demonstrate the superiority of the reconstituted non-fat milk

over the cottage cheese as a growth medium for coliforms. The most rapid growth of E. coli occurred within the first five days in the cottage cheese, but between the 5th and 10th days in the reconstituted non-fat milk.

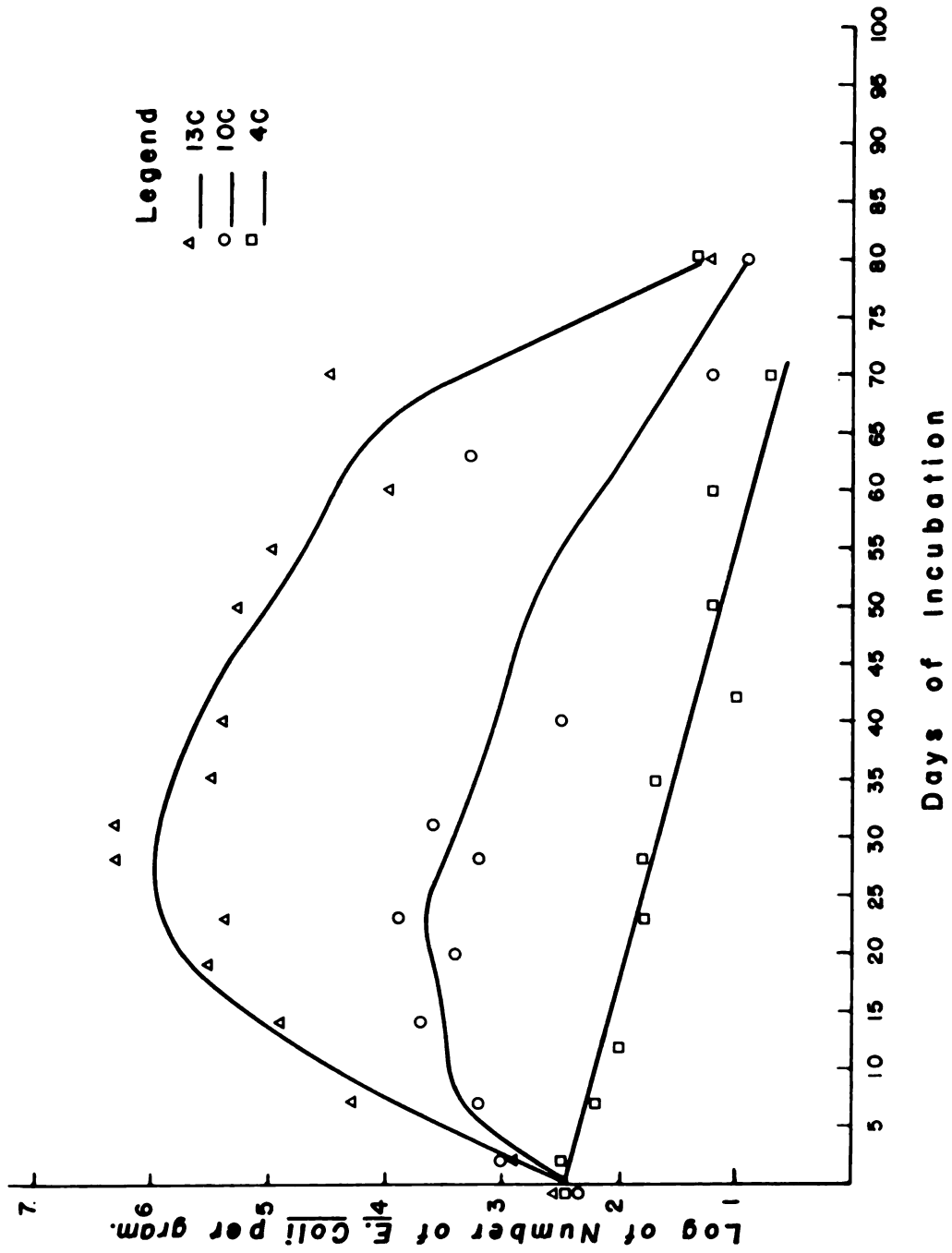


Figure 8. Population curves of Escherichia coli (strain 3) in cottage cheese, incubated at 4, 10, and 13 C.

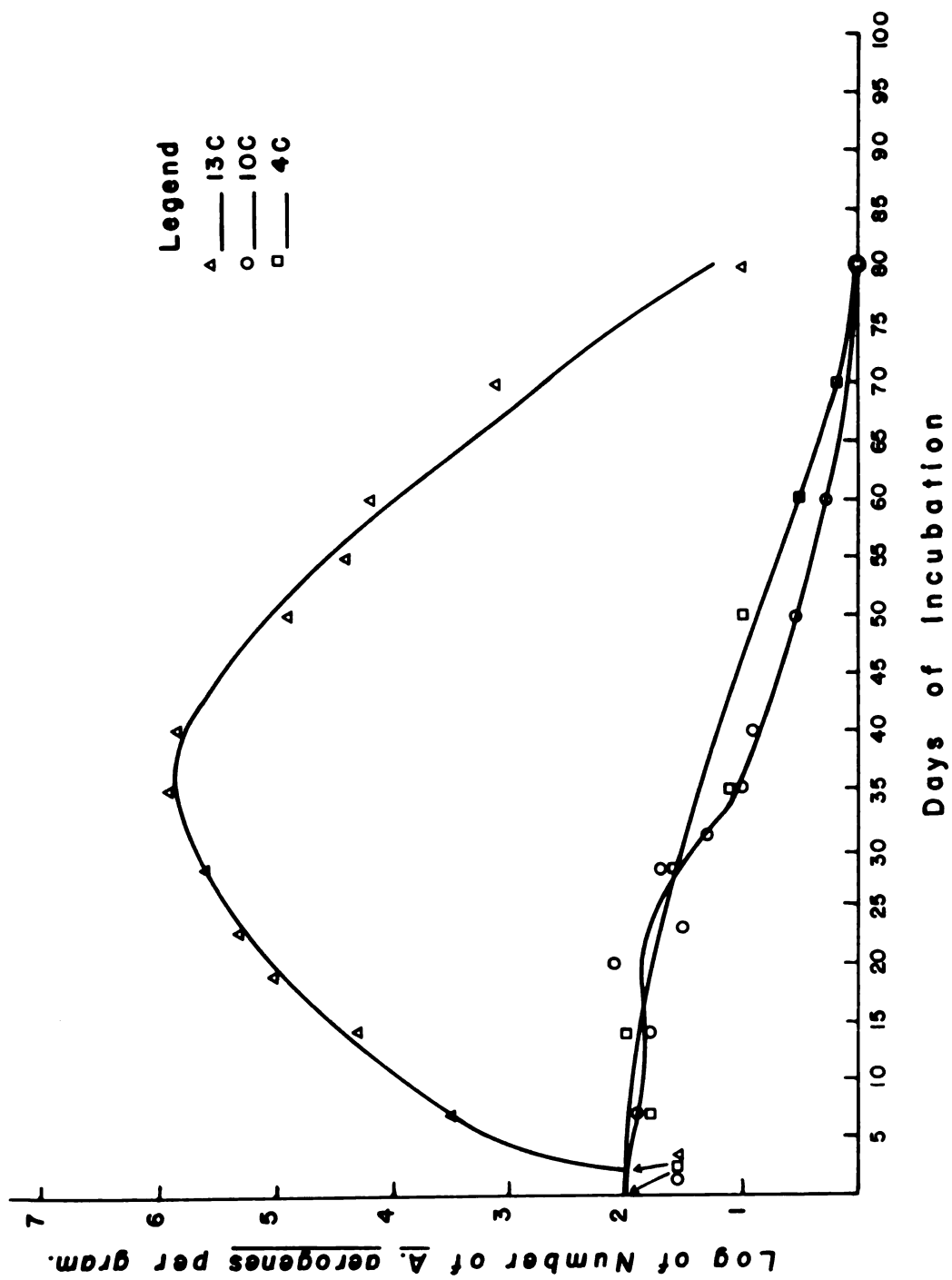


Figure 9. Population curves of Aerobacter aerogenes (strain 3) in cottage cheese, incubated at 4, 10, and 13 C.

Table 3

Generation times of coliform organisms in cottage cheese incubated at 10 and 13 C

		incubation temperature			
		13 C		10 C	
interval in which generation time was obtained (days)		generation time		generation time	
		<u>E. coli</u> (hr.)	<u>A. aerogenes</u> (hr.)	<u>E. coli</u> (hr.)	<u>A. aerogenes</u> (hr.)
0-5		31.4	32.8	48.2	decrease in population
0-10		35.2	38.0	68.8	"
0-15		38.7	43.3	98.5	"
0-20		42.5	47.3	120.4	"

Table 4

Comparison of the generation times of one strain of Escherichia coli in reconstituted non-fat milk incubated at 10 C.

interval for which generation times was obtained (days)	<u>generation time</u>	
	cottage cheese (hr.)	reconstituted non-fat milk (hr.)
0-5	48.2	12.0
0-10	68.8	10.9
0-15	98.5	14.8
0-20	120.4	population in declining phase

DISCUSSION

Growth of Coliform Organisms in Reconstituted Non-Fat Milk

The four strains of E. coli and three strains of A. aerogenes isolated from raw bulk tank milk and subsequently inoculated into sterile reconstituted non-fat milk had a minimum growth temperature between 4 and 10 C. The numbers decreased at 4 C; whereas at 10 C the organisms multiplied moderately. Kereluk et al, (22) obtained similar results with E. coli isolated from frozen meat pies.

Coliform bacteria have been isolated from, or found to grow in, raw milk and commercially pasteurized milk products refrigerated at 1.6 to 7.2 C by Audrey and Frazier (2), Burgwald and Josephson (6), Dahlberg (10), Panes and Thomas (34), and Schultze and Olson (35, 36). Ingraham (20) stated, "Recent experiments have shown that the increase in temperature characteristic of growth of E. coli at low temperatures is a consequence of some metabolic damage. Growth at low temperatures is required for the damage to be fully expressed and subsequent growth at higher temperatures is required to correct the damage". These findings may explain the atypical biochemical properties of certain coliform organisms which were isolated from milk products by Schultze and Olson (36).

The difference between results reported herein and those of other workers may be attributed to (a) different coliform strains were used in this study than were used by previous workers and (b) the symbiotic effect of mixed populations as found naturally in raw and commercially pasteurized milk. One strain of A. aerogenes, which was isolated but not used in this investigation, showed a continuous decrease in population for 16 days when stored in reconstituted non-fat milk at 4 C. After

the 16th day, the cell count increased to 500 thousand per ml. on the 23rd day. The culture did not appear to be a pure strain which suggests that a symbiotic effect between different strains of the same specie or species of different genera may be a reason for growth at a lower temperature.

The ability of pure strains of coliforms to grow at 3.3 to 5.0 C in pasteurized milk and on yeast-dextrose agar has been reported by Audrey and Frazier (2) and Panes and Thomas (34). A more detailed and extensive study of the Michigan State University Dairy raw milk supply would probably yield psychrophilic coliforms capable of growing in milk at 4 C or lower.

There is no exact explanation for the greater decrease in numbers of coliform organisms at 4 than at 0 C. It has been suggested that permease enzymes are inactivated at both 0 and 4 C and since there is some metabolic activity at 4 C toxic products may accumulate which are detrimental to cellular metabolism.

The growth rate was considerably slower at 10 than 32 C. There were minor variations in generation times among the strains within each specie of the coliform group, but there was no apparent difference in growth rate between the E. coli and A. aerogenes strains.

At 10 C the generation time for strains of E. coli from time of inoculation to time of maximum count was similar to the time reported by Barber (3), but shorter than the time observed by Ingraham (19). However, at 32 C the generation times of E. coli during the logarithmic growth phase were comparable to those reported by Barber (3), Ingraham (19) and Jennison (21). The generation time of A. aerogenes as reported by Jennison (21) was 8 to 12 minutes longer than found in this study. Age of inoculum and differences in medium used for propagation may account for these differences.

Growth of Coliform Organisms in Cottage Cheese

The strains of E. coli and A. aerogenes, which were isolated from raw milk, grew in cottage cheese at temperatures commonly used in commercial refrigeration. Harmon et al. (16) made a study of storage temperatures used for cottage cheese. The temperature of cottage cheese samples taken from refrigeration cabinets in retail outlets ranged from 3.3 to 12.2 C. Fourteen of 48 samples (29 per cent) had a temperature above 10 C. Among the cultures used in this study, the strains of E. coli tolerated lower temperatures than A. aerogenes. E. coli grew at 10 and 13 C; whereas A. aerogenes grew at 13 but not at 10 C.

A decrease in cell numbers occurred at 4 C with both of the coliform organisms and the A. aerogenes population declined at 10 C. However, the diminution in cell population was negligible throughout the normal shelf-life of the cottage cheese and coliforms persisted after the cheese had been stored for 15 to 20 days at 4 C. Harmon and Smith (15) reported that the shelf-life of cottage cheese stored at 5.6 C averaged 51 per cent longer than corresponding samples held at 10 C. Results of this study indicate that coliform spoilage would be held to a minimum if samples were stored at 4 C.

In the reconstituted non-fat milk a coliform population of 170 to 500 million per ml. was reached within 13 to 21 days at 10 C. However, in the cottage cheese the maximum count of approximately one million per gram occurred between the 20th and 35th days in samples stored at 13 C. The differences in maximum population between the two products were attributed to the lower pH in cottage cheese and the fact that cottage cheese is not a sterile product. Some lactic acid bacteria from the

starter culture survive the cooking process and are the dominant organisms in fresh cottage cheese. The lactics compete with the coliforms for nutrients and produce acid from the lactose in the cheese. The coliforms are sensitive to pH below 5.0. Harmon and Smith (14) found that the coliform population declined in cottage cheese samples held at 5.6 C which had an initial and terminal pH of 4.8 or less. When the initial and terminal pH was above 5.1 continuous increases in numbers occurred.

The scope of this study did not include the effect of pH on coliforms, but some pH determinations of cottage cheese samples were made. In one trial the initial pH was 5.1 and no change in pH occurred during the first 14 days at 4, 10, or 13 C. This may explain the rapid growth and shorter generation times during the first 10 days of growth. In another trial the initial pH of the cheese was 5.4. At 60 days the pH readings were 5.3, 4.8, and 4.4 at 4, 10, and 13 C, respectively. This indicates that the decline in cell numbers at 4 C was primarily due to the effect of temperatures rather than pH.

SUMMARY AND CONCLUSIONS

The growth rates and generation times of four strains of Escherichia coli and three strains of Aerobacter aerogenes were studied in cottage cheese and reconstituted non-fat milk. Inoculated samples of milk were stored at 0, 4, 10, and 32 C and samples of cheese were stored at 4, 10, and 13 C.

During incubation at 32 C, milk inoculated with 10 or less E. coli or A. aerogenes per ml. reached a maximum count of 880 to 890 million per ml. within 24 hours. During the interval from inoculation to attainment of maximum population the generation time of four E. coli strains varied from 52 to 56 minutes and the generation time of three A. aerogenes strains ranged from 48 to 53 minutes.

At 32 C there was no lag phase and the logarithmic growth phase was completed during the first 12 hours of incubation with both E. coli and A. aerogenes. During this period of maximum growth the generation time varied from 33 to 39 minutes for the E. coli strains and from 29 to 33 minutes for the A. aerogenes strains.

After 24 hours at 32 C the populations of both organisms showed only a slight decrease through the third day and a definite decrease thereafter. In most of the samples the count was < 1 per ml. by the 11th day.

In milk stored at 10 C, E. coli and A. aerogenes had similar growth rates and generation times. There was an indistinct lag phase of about one day followed by the logarithmic growth phase which extended until about the 10th to 12th day. The approximate generation time during the logarithmic growth phase of E. coli varied from 516 to 642 minutes, while A. aerogenes ranged from 540 to 648 minutes.

Initial coliform populations of 10 or less per ml. reached a

maximum count of 170 to 500 million per ml. within 13 to 21 days at 10 C.

In reconstituted non-fat milk stored at 4 C an initial average population of 1700 per ml. of both E. coli and A. aerogenes declined to approximately 10 per ml. by the 30th day and to < 1 per ml. after about 50 days.

In milk stored at 0 C the number of cells of E. coli and A. aerogenes diminished from 1700 to 100 per ml. between the 16th and 20th days. During the next 60 to 70 days there was little change in population and the samples contained five or less organisms per milliliter on the 130th day.

A. aerogenes decreased and E. coli grew very slowly in cottage cheese stored at 10 C. Both coliform organisms increased in number at 13 C although less rapidly than in reconstituted non-fat milk held at 10 C. The generation time of E. coli grown in cottage cheese incubated at 10 C was four to six times longer than when the cells were grown in reconstituted non-fat milk at the same temperature.

Within the first ten days, an initial E. coli inoculation of 250 per g. increased to 28 thousand per g. in cottage cheese held at 13 C with a generation time of 35.2 hours. During the same interval of time A. aerogenes increased from 100 to 8,000 per g. with a generation time of 38.0 hours.

In cottage cheese stored at 10 C, E. coli increased from 250 to 1400 per g. in five days, and to 2800 per g. in ten days with generation times of 48.2 and 68.8 hours, respectively. The A. aerogenes population remained stationary for 25 to 30 days and then decreased.

In cottage cheese stored at 4 C both genera of coliform organisms declined in numbers. A cell count of 250 E. coli per g. in fresh cottage cheese decreased to 90 per g. by the 20th day. A corresponding population of A. aerogenes decreased to 60 per g. by the 20th day.

Typical E. coli and A. aerogenes strains grew in reconstituted non-fat milk stored at 10 and 32 C, but there was no increase in cell population at 0 and 4 C. When coliforms were incubated in reconstituted non-fat milk the rate of diminution was greater at 4 than at 0 C and no significant differences were found between the growth rate of E. coli and A. aerogenes in reconstituted non-fat milk. At 10 C the reconstituted non-fat milk was a better growth medium than cottage cheese. Among the strains of organisms used in this experiment the E. coli endured a lower temperature in cottage cheese than the A. aerogenes.

REFERENCES CITED

1. American Public Health Association. 1960. Standard methods for the examination of dairy products. 11th ed. New York. 448 p.
2. Audrey, J. and W. C. Frazier. 1952. Psychrophiles in milk held two days in farm bulk cooling tanks. J. Dairy Sci. 42: 1781.
3. Barber, M. A. 1908. Rate of multiplication of Bacillus coli at different temperatures. J. Infect. Dis. 5: 379.
4. Bonner, M. D. and L. G. Harmon. 1957. Characteristics of organisms contributing to spoilage in cottage cheese. J. Dairy Sci. 40: 1599.
5. Breed, R. S., E. G. D. Murray, and A. P. Hitchens. 1957. Bergey's manual of determinative bacteriology. 7th ed. Williams and Wilkins Co., Baltimore. 1094 p.
6. Burgwald, L. H. and D. V. Josephson. 1947. Effect of refrigerator storage on keeping qualities of pasteurized milk. J. Dairy Sci. 30: 371.
7. Collins, E. B. 1955. Factors involved in gelatinous curd defects of cottage cheese. II. Influence of pH and temperature on bactericidal efficiency of chlorine. J. Milk and Food Technol. 18: 189.
8. Collins, E. B. 1961. Resistance of certain bacteria to cottage cheese cooking procedures. J. Dairy Sci. 44: 1989.
9. Dahlberg, A. C. 1945. The keeping quality of pasteurized milk in the New York metropolitan area during cool weather as determined by bacterial counts, presence of coliform bacteria and flavor scores. J. Dairy Sci. 28: 779.
10. Dahlberg, A. C. 1946. Relations of growth of all bacteria and coliform bacteria in pasteurized milk held at refrigeration temperatures. J. Dairy Sci. 29: 651.
11. Difco Laboratories. 1953. Difco manual. 9th ed. Detroit, Michigan. 350 p.
12. Futschik, J. and L. Sachslehner. 1956. Problems of the reactivation of coliforms and proteolytic bacteria in pasteurized milk. Milchwiss, Berichte, Wolfpassing. 6 (2): 59. (Original not seen; abstracted in Dairy Sci. Abstr. 19: 575.)
13. Glenn, W. E. and H. C. Olson. 1959. Sources, heat-resistance, and growth at refrigeration temperatures of coliforms in pasteurized milk. Milk Dealer. 48 (6): 54.
14. Harmon, L. G. and C. K. Smith. 1956a. Influence of microbiological population on shelf-life of creamed cottage cheese. Quart. Bull. Mich. Agr. Expt. Sta. Mich. State Univ. East Lansing. 38: 368.

15. Harmon, L. G. and C. K. Smith. 1956b. Influence of environment and processing on spoilage organisms in cottage cheese. *J. Milk and Food Technol.* 20: 196.
16. Harmon, L. G., G. M. Trout, and M. D. Bonner. 1955. A market survey of cottage cheese. I. Some characteristics influencing consumer acceptance and shelf-life. *Quart. Bull. Mich. Agr. Expt. Sta.* 38: 146.
17. Harmon, L. G., G. M. Trout, and T. I. Hedrick. 1959. Shelf-life of homogenized milk. *Milk Dealer.* 48 (11): 40.
18. Hedrick, T. I. 1963. Cottage cheese problems in production and sanitation-public health aspects. *J. Milk and Food Technol.* 26: 10.
19. Ingraham, J. L. 1958. Growth of psychrophilic bacteria. *J. Bacteriol.* 76: 75.
20. Ingraham, J. L. 1963. Psychrophilic microorganisms (Abstr.) *Soc. Am. Bacteriol. Proc. (Cleveland)* 63: xxiv.
21. Jennison, M. W. 1935. Some quantitative relationships in bacterial population cycle. *J. Bacteriol.* 30: 603.
22. Kereluk, K., A. C. Peterson, and M. F. Gunderson. 1961. Effects of different temperatures on various bacteria isolated from frozen meat pies. *J. Food Sci.* 26: 21.
23. Lyons, P. R. and W. L. Mallmann. 1954. Bacteriological study of cottage cheese with particular reference to public health hazards. *J. Milk and Food Technol.* 17: 372.
24. Martin, W. H., V. D. Foltz, and W. P. Rutz. 1960. Survey of cottage cheese quality. *J. Milk and Food Technol.* 23: 306.
25. Mason, M. M. 1935. Comparison of maximal growth rates of various bacteria under optimal conditions. *J. Bacteriol.* 29: 103.
26. Milk Industry Foundation. 1963. Fluid milk sales show 33.9% gain in 12 year period. July newsletter. Loose leaf. n.p.
27. Milk Industry Foundation. 1962. Milk Facts, Washington, D. C. 31 p.
28. Mueller, W. S. 1955. Bactericidal effectiveness of iodophor detergent-sanitizers. *J. Milk and Food Technol.* 18: 144.
29. Norrgren, O. 1960. Bacteriological testing of plate pasteurizers with the aid of coliform bacteria. *Medd. Maskinprovningar Alnarp.* 240 M (Original not seen; abstracted in *Dairy Sci. Abstr.* 23: 182. 1961.)
30. Olson, J. C., Jr., A. J. Nielsen, E. L. Thomas, and H. A. Morris. 1953. Changes in bacterial count and flavor of concentrated or recombined milk during storage at low temperatures. *J. Dairy Sci.* 36: 817.

31. Overcast, W. W. and J. V. Britton. 1959. Study of microbial flora of cottage cheese during storage. (Abstr.) J. Dairy Sci. 42: 910.
32. Overcast, W. W., J. D. Skean, and J. V. Britton. 1961. Coliform bacteria in milk. (Abstr.) J. Dairy Sci. 44: 970.
33. Paley, C. and M. L. Isaacs. 1941. Effects of pasteurization on Escherichia coli in milk and ice cream mix. J. Dairy Sci. 24: 421.
34. Panes, J. J. and S. B. Thomas. 1959. Multiplication of coli-aerogenes bacteria in milk stored at 3 to 5 C. J. Appl. Bacteriol. 2: 272.
35. Schultze, W. D. and J. C. Olson, Jr. 1960a. Studies in psychrophilic bacteria. I. Distribution in stored commercial dairy products. J. Dairy Sci. 43: 346.
36. Schultze, W. D. and J. C. Olson, Jr. 1960b. Studies in psychrophilic bacteria. II. Psychrophilic coliform bacteria in stored commercial dairy products. J. Dairy Sci. 43: 351.
37. Weber, C. W. 1944. Coliform bacteria in pasteurized milk. New York State Assoc. of Milk Sanitarians Proc. (Ithaca). 21: 39.

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