March 8, 1957

To the Graduate Council:

I am submitting herewith a thesis written by James C. Prow, Jr. entitled "The Growth Response of Coliform Bacteria in Milk to an Increase of 5°C after Refrigerated Storage." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Dairying.

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THE GROWTH RESPONSE OF COLIFORM BACTERIA IN MILK TO AN INCREASE OF 5°C AFTER REFRIGERATED STORAGE

A THESIS

Submitted to
The Graduate Council
of
The University of Tennessee
in
Partial Fulfillment of the Requirements
for the degree of
Master of Science

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by

James C. Prow, Jr.

March 1957

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J. C. P.

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INTRODUCTION

The milk industry strives to offer the public safe and nourishing products. Improvements in milk processing have been made through the years and without a doubt will continue to be made in the future. Pasteurization by heating is the present method of assuring that milk is free of pathogenic organisms. The phosphatase test is used for determining whether or not milk has been properly pasteurized.

Even if milk has been properly pasteurized, there is the possibility of recontamination through careless handling of the pasteurized milk before it is bottled. At the present time, the best means of determining recontamination after pasteurization is the test for coliform bacteria.

"The Coliform Group includes all aerobic and facultative anaerobic, Gram-negative, nonspore-forming bacteria which ferment lactose with gas formation" (28). This Group hereafter will be referred to as "coliforms".

There has been considerable work done on coliforms but still there are questions that have not been answered. Studies have been conducted on their resistance to pasteurization and growth at refrigeration temperatures but still their growth in milk has not been fully explained.

The present milk marketing program creates problems that were not faced a few years ago. Every other day pick-up at the farm, three times a week retail delivery to the consumer and five work days a week are present day operations, any of which may add one to four days to the

age of the products. Generally, it is accepted that there will be little or no bacterial growth at 35-40°F during the first 48 hours of storage. Longer storage may result in some bacterial growth. The rate of growth will depend upon the types of organisms present and may be influenced considerably by only a few degrees increase in the storage temperature.

STATEMENT OF PROBLEM

This experiment was conducted to study the growth characteristics of coliforms in relation to storage temperatures and temperature changes and to obtain a survey of the milk in Knoxville in regards to coliform and total count.

REVIEW OF LITERATURE

The coliform test is used to check for recontamination of pasteurized milk. The test is not intended to detect fecal pollution but rather to measure the general care used to minimize bacterial contamination and care used to retard bacterial growth in dairy products. A few coliforms in otherwise properly pasteurized milk, although almost invariably an indication of recontamination, do not necessarily mean the milk is unsafe. Their presence does mean that proper steps should be taken to locate and eliminate all sources of recontamination to prevent the possibility of introduction of pathogenes (1).

Numerous studies have been made on coliforms to see if they survive pasteurization. Most of the reports since 1930 show that pasteurization kills coliforms.

Tanner and Windsor (28) determined that Escherichia coli would be destroyed by exposure to 62.8°C for 30 minutes if in the concentration that it normally occurs in raw milk. The possibility of encountering resistant strains or cultures containing some resistant cells was not ruled out. Buchbinder and Alff (6) state that the so called heat resistant coliforms are of absolutely no practical significance in the coliform test of pasteurized milk.

Watrous, Doan and Josephson (31) collected pasteurized milk samples aseptically from the pasteurizing vat and after 15 days of refrigerated storage the samples still had a negative coliform test. Naylor (22) concluded that heat resistant coliforms occur very, very rarely in raw milk and are of no practical importance.

The coliform test is used to determine post-pasteurization contamination. The Milk Ordinance and Code (30) states that at no time after pasteurization and before delivery shall grade A pasteurized milk have a coliform count exceeding ten per milliliter in more than one of the last four consecutive counts. Frayer (17) states that coliforms rarely survive pasteurization in numbers sufficient to be counted in 1 ml samples and that a standard of not more than ten coliforms per milliliter for pasteurized milk and cream is fair and attainable.

Chilson, Yale and Eglinton (8) did not find coliforms in 1 ml quantities of properly pasteurized milk free from recontamination. Tiedman and Smith (29) believe that well operated plants can produce milk free from coliforms in 1 ml portions.

Elliker (15) states that repeated studies have shown no coliforms immediately after pasteurization and that when they did occur in milk they were picked up from contaminated equipment. His conclusions were that coliforms are associated with faulty cleaning and germicidal treatment or rinsing cleaned equipment with contaminated water.

DeLay (14) concluded that the coliform test is of definite value as an aid in detecting faulty post-pasteurization handling and that well equipped plants can submit bottled milk samples of which less than one per cent will be positive to the coliform test.

In a study of several cities, Dahlberg, Adam and Held (13) found that 96 per cent of all samples complied with the standard of less than ten coliforms per milliliter. This indicated no difficulty in enforcing the standard. Archambault (2) states that it is extremely common in plants

employing careless methods of sanitation to find no coliforms in the milk taken from the pasteurizer but a high coliform content in the bottled milk with no apparent change in the Standard Plate Count.

Fay (16) lists four reasons that coliforms serve well as an index to the care employed in sanitizing equipment. Coliforms are non-spore-forming and are so readily destroyed by pasteurization temperatures that they are rarely, if ever, found in pasteurized milk as it leaves the vat or pasteurizing unit. They are readily identified by rather simple techniques which lend themselves to routine testing. They are widely distributed in nature, and equipment that has been ineffectively washed or sanitized is almost certain to contribute considerable numbers of these organisms to pasteurized milk. When repeatedly observed in the product from a given plant, one may question the effectiveness of sanitization of equipment used after pasteurization.

Barber (5) believes that just conducting the coliform test is not enough but that the proper interpretation of results must be applied or the test will mean little. Roadhouse and Henderson (24) state that high coliform counts in pasteurized milk are due to improper cleaning and sanitization of equipment if the raw milk has a low contamination with coliform and the phosphatase test shows adequate pasteurization.

Archambault (2) states that coliform counts of properly pasteurized and uncontaminated milk taken from pasteurizers are normally so low (probably less than one per liter) that for all practical purposes, the organism may be considered absent from such milk. If they occur in significant numbers at subsequent stages, one must conclude that they

have gained access to, or have multiplied in the milk, as a result of faulty operations or defective equipment.

Ayers and Clemmer (4) found little or no growth of the organisms of the coli-aerogenes group below 50°F for 48 hours. Sherman and Wing (26) found that growth of coli-aerogenes bacteria was stopped in milk cooled below 45°F. Dahlberg (11) held milk at 35-40°F and found little or no change in the number of coliform-positive samples in four days. When stored at 45-50°F, there were definite increases in the numbers of coliforms in three and four days. At 55-60°F there were indications of growth after one day.

In another test Dahlberg (12) found that the majority of quart bottles of pasteurized milk became coliform positive after four days incubation at 55-60°F even though tests of the fresh milk were negative. The growth of coliforms at 45-50°F was slow but significant.

Dahlberg (10) also found that coliforms increased in numbers in pasteurized milk more rapidly than did the total count when held at refrigeration temperatures. One explanation for this more rapid growth is that the coliforms are recontaminants which have not been subjected to the heat treatment and which are actively growing bacteria. Nelson and Baker (23) found no relationship between the rate of development of bacteria in refrigerated fluid dairy products and the initial coliform count.

Burgwald and Johnson (7) noted that the initial coliform counts in summer milks exceeded those of winter milks and that the coliform count in winter milk was little changed during the first seven days

storage at 39-42°F. The summer milk samples began to show an increase after four days' storage at 41-45°F. The most rapid increase came from the sample that was allowed to stand out for two hours prior to storage and was also removed from the refrigerator each day for sampling. Dahlberg (10) observed that the coliforms increased more rapidly in milk bottled in warm weather than in cool weather.

Dahlberg (12) plated 18 samples of freshly pasteurized milk each week for three consecutive weeks and found not more than one quart of milk per week showing presence of coliforms. Holding the freshly pasteurized milk at room temperature to simulate failure to promptly refrigerate delivered milk showed some immediate effects. One to three of the eighteen milk samples were coliform positive on each of three consecutive weeks.

Within the past few years, some work has been done on the effect of temperature change on microorganisms. Application of these studies and techniques to some of the problems in bacterial cytology that have a factual basis may solve some of the controversies (27). Zeuthen and Scherbaum (32) working with Tetrahymena pyriformis confirmed their hypothesis that some phase in the cell cycle has a higher temperature coefficient than any others. Such a phase will be more sensitive to temperature changes than any other. With lowering of the temperature, cells in this phase will tend to drop behind and unite with cells behind them in the cycle, whereas with an increase in temperature they will tend to catch up with cells in front. This would cause a larger number of cells to divide at one time and be detectable at division. In another

experiment Scherbaum and Zeuthen (25) raised the temperature of Tetrahymena pyriformis from optimum to sublethal temperature and then, upon the return to optimum temperature, 85 per cent of the cells could be induced to undergo division simultaneously.

Hotchkiss (19) obtained partial synchronous division by lowering the temperature of pneumococcal organism to 25°C for 15 minutes then returned to 37°C. Maaloe and Lark (21) found that nuclear division is induced in nearly all cells of Salmonella typhimurium when the temperature is raised to 37°C after growing at 25°C for sometime.

Hunter-Szybalska, Szybalski and DeLamater (20) concluded that the most obvious effect of a decrease in temperature is on the rate of some chemical or physical reaction causing the final separation of sister chromosomes. They found that when actively growing cells of Bacillus megaterium were exposed to a suboptimal temperature of 10° to 15°C, the nuclei became highly stainable masses made up of contracted chromosomes. Reincubation at 34°C resulted in resumed multiplication, synchronous for all the cells and nuclei.

EXPERIMENTAL PROCEDURES

Quart containers of homogenized milk were purchased from Knoxville retail stores representive five processors. Samples II, III, IV and V were purchased at one grocery and Sample I was obtained at another store nearby and an additional sample (VI) was picked up at a milk plant. The age of the milk was not known in any case. The milk was carried in a paper bag to the laboratory and placed in a 5°C refrigerator.

The milk was shaken in the original container, paper cartons in all cases except Sample VI, and plated according to Standard Methods For The Examination of Dairy Products (1) on milk-protein hydrolysate (M-PH) agar for the total count and on violet red bile agar for coliforms. After each sample was plated the milk was poured into sterile flasks and returned to the 5°C refrigerator. Four days later the milk was plated again on M-PH agar and violet red bile agar.

Dilutions 1:10 and 1:100 were made for the total count and direct plating and dilution 1:10 were used for the coliform count. Some difficulty was experienced with the 1 ml and 1/10 ml sticking to the plate or otherwise failing to mix properly in the agar. This was remedied by placing 1 ml of a sterile water blank in the plate prior to adding the milk sample. The milk was then added directly into the water. The plates for the total count were incubated at 32°C for 48 hours and the coliform plates were incubated at 35°C for 24 hours.

Pasteurized milk of a low bacterial count was inoculated with 24 hour coliform cultures in litmus milk to determine the effect of a 5° temperature increase on their growth. This inoculated milk was plated

for coliforms and divided equally into five sterile flasks. Each of these flasks contained about a pint of milk and was stored at 5°C. Sample 1 was plated on the second day and then warmed to 10°C, plated again and returned to the 5°C refrigerator. On the third day, Samples 1 and 2 were plated, warmed to 10°C, plated again and returned to the refrigerator. Samples 1, 2 and 3 were used on the fourth day and Samples 1, 2, 3, 4 and 5 on the fifth day. All samples were warmed by using tap water and the time lapse between plating at 5°C and 10°C was less than five minutes.

The number of plates and amount of media involved in the above method was so great and the extra samples did not seem to contribute any to the results, that after three trials only two samples were used. One of these was warmed up each day, plated and returned to the 5°C refrigerator and the other was left at 5°C during the test period. The milk was inoculated from a 24 hour coliform culture grown in litmus milk. One ml of the litmus milk culture was placed into a 99 ml water blank. One ml from the water blank was used to inoculate one quart of milk. The quart of inoculated milk was plated for coliforms and divided between two sterile flasks. This system of inoculation usually gave 6,000 to 10,000 coliforms per milliliter.

The inoculated milk was stored in a 5°C refrigerator and on the second, third, fourth and fifth days Sample 1 was plated, warmed to 10°C, plated again and returned to the refrigerator. The control sample was plated on the fifth day, warmed to 10°C and plated again. Eight series of tests were run this way with two others running to the sixth day.

Four series were plated on the first, second, third, fourth and fifth days and two were plated on the first, second, third, fourth and fifth days but without a control.

RESULTS AND DISCUSSION

Preliminary tests with coliforms were made to determine if and when an increase occurred after warming to 10°C following storage at 5°C. Plates were made immediately after warming and again after 10 minutes and 20 minutes. Table 1 shows the results of a trial and indicates an increase in coliforms immediately upon reaching 10°C.

In all cases the coliform count dropped considerably from the time of inoculation until the next count was made. This might be explained by the work of Hegarty and Weeks (18) who found that young cells of E. coli are susceptible to cold shock from 37° to 0°C and that mature cells are not affected by an initial cold shock or prolonged holding at 0°C. In most cases after this initial drop the coliform count began to increase. However, in two cases where nine hour cultures were used (Tables 8 and 11) the cells did not begin to show a significant increase up to five days.

Tables 2 through 8 show the growth response of E. coli to an increase of 5°C after refrigerated storage. Tables 2 and 4 show significant growth rates after the initial drop in coliform count with seven increases in each case for eleven trials. Table 3 has five individual increases even though the total coliform count does not increase significantly. Table 5 shows an increase on the third day only while Table 6 shows an increase on the third and sixth days but the trials in Table 5 were only carried five days. An eighteen hour culture of E. coli was used (Table 7) and it compared favorably with the twenty-four hour cultures in response to temperature increase and growth.

TABLE 1

GROWTH RESPONSE OF COLIFORMS IN MILK TO AN INCREASE OF 5°C AFTER REFRIGERATED STORAGE

Milk Sample	Time After Temperature Increase to 10°C			Control[a]
	0	10 Min	20 Min	
7,700	11,000	11,000	11,000	9,000

[a]Control sample is a duplicate of the milk sample that remained at 5°C and was plated again at the end of the twenty minutes.

Only three trials (Tables 9 through 11) were run with Aerobacter aerogenes. All of these showed a gradually decline in the colony count although there were some increases on individual days when warmed up 5°C.

As shown in Table 12 a steady increase occurred each day in the coliform count. Sample 1 which was warmed up to 10°C each day had a coliform count of 190,000 at the end of five days. Whereas Sample 2 which remained at 5°C during the test period had a count of 31,000 at the end of five days. Table 13 shows almost the opposite with Sample 1 having a count of 3,100 at the end of five days and Sample 2 a count of 12,000. An increase in count was realized each time on a 5°C rise in temperature but the coliforms seemed to die off overnight resulting in only a small increase after five days.

Tables 14 through 17 show two increases on each table with at least one of these occurring on the third or fourth day. Sample 1 on Table 18 shows only one increase of 11 per cent on the fifth day while Sample 2 had an increase of 44 per cent on the fifth day. Tables 19 and 20 show a higher coliform count after five days on Sample 1 than on Sample 2.

Of interest is the fact that 54 per cent of the trials with E. coli (Tables 2 through 8) gave increases, 53 per cent of the trials with the coliforms (Tables 12 through 20) while only 35 per cent of the trials with A. aerogenes (Tables 9 through 11) gave an increase. Over half (50.8 per cent) of the 116 trials completed gave an average increase of 52 per cent in coliform count. The per cent of increases varied from 11 to 200 per cent. Since the plate count is only an estimate of the

number of bacteria, any increase of 10 per cent or less was omitted in these averages.

Figure 1 shows that the largest number of increases in coliforms occurred on the third and fourth days after storage. These are important days in many dairy products that are not bottled daily i.e. coffee cream, chocolate milk, whipping cream, etc., as these products may be stored in the plant for several days before reaching the retail outlet.

In a survey of the Knoxville milk supply conducted with this work, 32 per cent of the samples over a ten month period were coliform positive with 13 per cent over the 10 per milliliter standard. After storage at 5°C for four days 46 per cent of the samples were coliform positive and 23 per cent were over 10 per milliliter. The milk in this survey was purchased from grocery stores and is no doubt older than samples taken by the Public Health Department. Many families buy at least part of their milk at grocery stores and should be assured of getting a good product. Atherton, Doan and Watrous (3) found most of the self-service cabinets employed by stores unsatisfactory for storing milk. Milk was not held at sufficiently low temperatures to prevent deterioration. They found that most store operators have little idea of the efficiency of their milk cabinets and only a vague knowledge of how bottled milk should be handled.

Many investigators (2, 13, 14, 17 and 29) have stated that no difficulty should be found in enforcing the coliform standard. Even though not more than ten coliforms per milliliter is the standard (30) and coliform free samples are attainable goals, not all plants meet these requirements.

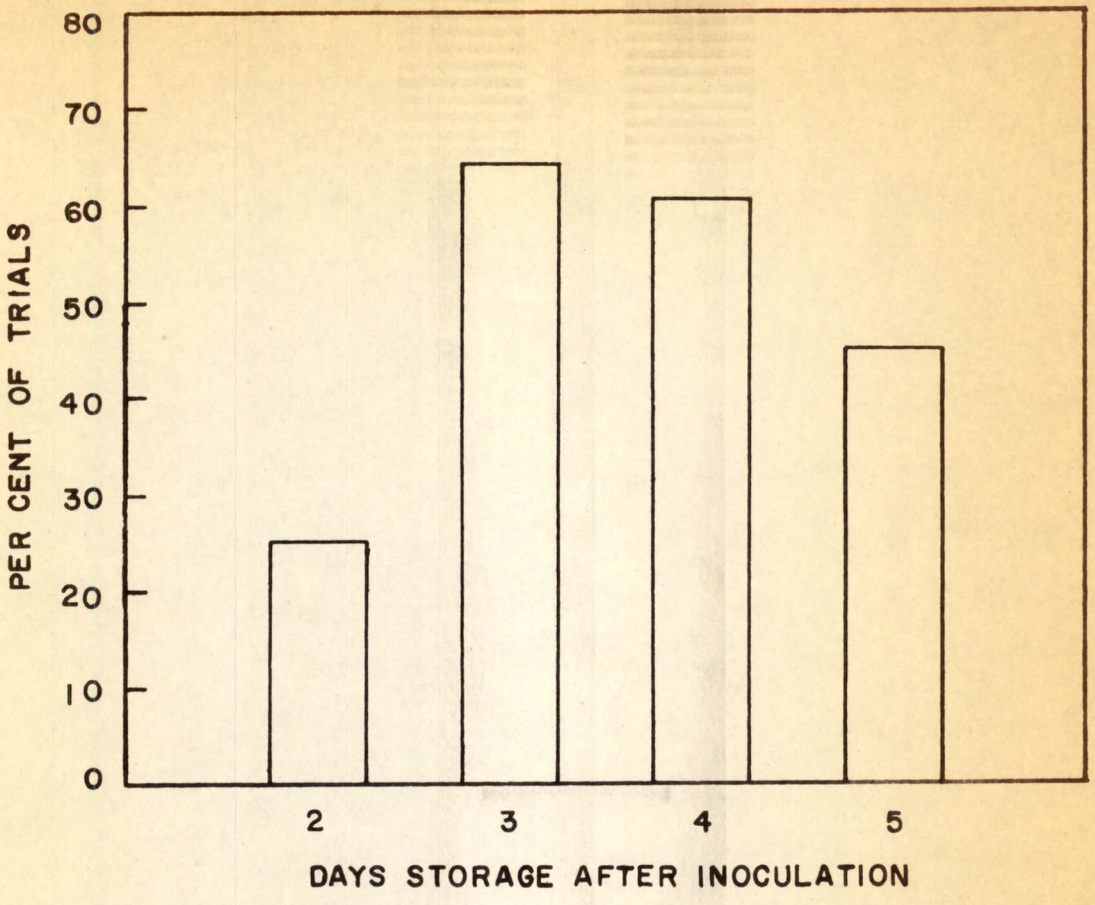

Figure 1. The increase in coliform counts after warming 5°C.

TABLE 2

GROWTH RESPONSE OF *ESCHERICHIA COLI* TO AN INCREASE OF 5°C AFTER REFRIGERATED STORAGE

Sample	*Escherichia coli*	Per cent Increase
Count after Inoculation	18,000	
Sample 1 on Second Day @ 5°C	3,700	
Sample 1 on Second Day @ 10°C	4,900	32
Sample 1 on Third Day @ 5°C	85,000	
Sample 1 on Third Day @ 10°C	96,000	13
Sample 1 on Fourth Day @ 5°C	580,000	
Sample 1 on Fourth Day @ 10°C	520,000	—
Sample 1 on Fifth Day @ 5°C	130,000	
Sample 1 on Fifth Day @ 10°C	390,000	200
Sample 2 on Third Day @ 5°C	140,000	
Sample 2 on Third Day @ 10°C	190,000	36
Sample 2 on Fourth Day @ 5°C	580,000	
Sample 2 on Fourth Day @ 10°C	300,000	—
Sample 2 on Fifth Day @ 5°C	320,000	
Sample 2 on Fifth Day @ 10°C	310,000	—
Sample 3 on Fourth Day @ 5°C	140,000	
Sample 3 on Fourth Day @ 10°C	170,000	21
Sample 3 on Fifth Day @ 5°C	230,000	
Sample 3 on Fifth Day @ 10°C	260,000	11
Sample 4 on Fifth Day @ 5°C	290,000	
Sample 4 on Fifth Day @ 10°C	480,000	65
Sample 5 on Fifth Day @ 5°C	490,000	
Sample 5 on Fifth Day @ 10°C	480,000	—

TABLE 3

GROWTH RESPONSE OF *ESCHERICHIA COLI* TO AN INCREASE OF 5°C AFTER REFRIGERATED STORAGE

Sample	*Escherichia coli*	Per cent Increase
Count after Inoculation	830	
Sample 1 on Second Day @ 5°C	770	
Sample 1 on Second Day @ 10°C	520	—
Sample 1 on Third Day @ 5°C	270	
Sample 1 on Third Day @ 10°C	240	—
Sample 1 on Fourth Day @ 5°C	220	
Sample 1 on Fourth Day @ 10°C	320	45
Sample 1 on Fifth Day @ 5°C	550	
Sample 1 on Fifth Day @ 10°C	480	—
Sample 2 on Third Day @ 5°C	220	
Sample 2 on Third Day @ 10°C	360	70
Sample 2 on Fourth Day @ 5°C	—	
Sample 2 on Fourth Day @ 10°C	—	
Sample 2 on Fifth Day @ 5°C	390	
Sample 2 on Fifth Day @ 10°C	390	—
Sample 3 on Fourth Day @ 5°C	550	
Sample 3 on Fourth Day @ 10°C	600	—
Sample 3 on Fifth Day @ 5°C	520	
Sample 3 on Fifth Day @ 10°C	690	32
Sample 4 on Fifth Day @ 5°C	270	
Sample 4 on Fifth Day @ 10°C	340	26
Sample 5 on Fifth Day @ 5°C	590	
Sample 5 on Fifth Day @ 10°C	950	61

TABLE 4

GROWTH RESPONSE OF ESCHERICHIA COLI TO AN INCREASE OF 5°C AFTER REFRIGERATED STORAGE

Sample	Escherichia coli	Per cent Increase
Count after Inoculation	17,000	
Sample 1 on Second Day @ 5°C	—	
Sample 1 on Second Day @ 10°C	—	
Sample 1 on Third Day @ 5°C	380	
Sample 1 on Third Day @ 10°C	940	147
Sample 1 on Fourth Day @ 5°C	3,800	
Sample 1 on Fourth Day @ 10°C	6,300	39
Sample 1 on Fifth Day @ 5°C	33,000	
Sample 1 on Fifth Day @ 10°C	32,000	—
Sample 2 on Third Day @ 5°C	1,700	
Sample 2 on Third Day @ 10°C	3,000	43
Sample 2 on Fourth Day @ 5°C	8,000	
Sample 2 on Fourth Day @ 10°C	18,000	125
Sample 2 on Fifth Day @ 5°C	32,000	
Sample 2 on Fifth Day @ 10°C	57,000	78
Sample 3 on Fourth Day @ 5°C	7,500	
Sample 3 on Fourth Day @ 10°C	14,000	86
Sample 3 on Fifth Day @ 5°C	66,000	
Sample 3 on Fifth Day @ 10°C	51,000	—
Sample 4 on Fifth Day @ 5°C	5,800	
Sample 4 on Fifth Day @ 10°C	11,000	89
Sample 5 on Fifth Day @ 5°C	36,000	
Sample 5 on Fifth Day @ 10°C	21,000	—

TABLE 5

GROWTH RESPONSE OF ESCHERICHIA COLI TO AN INCREASE OF 5°C AFTER REFRIGERATED STORAGE

Sample	Escherichia coli	Per cent Increase
Count after Inoculation	4,900	
Sample 1 on Second Day @ 5°C	1,200	
Sample 1 on Second Day @ 10°C	1,300	--
Sample 1 on Third Day @ 5°C	1,800	
Sample 1 on Third Day @ 10°C	2,000	11
Sample 1 on Fourth Day @ 5°C	12,000	
Sample 1 on Fourth Day @ 10°C	12,000	--
Sample 1 on Fifth Day @ 5°C	50,000	
Sample 1 on Fifth Day @ 10°C	49,000	--
Sample 2 on Fifth Day @ 5°C	27,000	
Sample 2 on Fifth Day @ 10°C	15,000	--

TABLE 6

GROWTH RESPONSE OF *ESCHERICHIA COLI* TO AN INCREASE OF 5°C AFTER REFRIGERATED STORAGE

Sample	*Escherichia coli*	Per cent Increase
Count after Inoculation	18,000	
Sample 1 on Second Day @ 5°C	2,200	
Sample 1 on Second Day @ 10°C	1,700	—
Sample 1 on Third Day @ 5°C	2,900	
Sample 1 on Third Day @ 10°C	4,800	65
Sample 1 on Fourth Day @ 5°C	9,400	
Sample 1 on Fourth Day @ 10°C	9,200	—
Sample 1 on Fifth Day @ 5°C	39,000	
Sample 1 on Fifth Day @ 10°C	33,000	—
Sample 1 on Sixth Day @ 5°C	100,000	
Sample 1 on Sixth Day @ 10°C	140,000	40
Sample 2 on Sixth Day @ 5°C	19,000	
Sample 2 on Sixth Day @ 10°C	24,000	26

TABLE 7

GROWTH RESPONSE OF ESCHERICHIA COLI TO AN INCREASE OF 5°C AFTER REFRIGERATED STORAGE

Sample	Escherichia coli	Per cent Increase
Count after Inoculation	13,000	
Sample 1 on First Day @ 5°C	3,100	
Sample 1 on First Day @ 10°C	5,800	87
Sample 1 on Second Day @ 5°C	—	
Sample 1 on Second Day @ 10°C	—	
Sample 1 on Third Day @ 5°C	3,400	
Sample 1 on Third Day @ 10°C	7,400	117
Sample 1 on Fourth Day @ 5°C	29,000	
Sample 1 on Fourth Day @ 10°C	31,000	—
Sample 1 on Fifth Day @ 5°C	93,000	
Sample 1 on Fifth Day @ 10°C	110,000	18
Sample 2 on Fifth Day @ 5°C	54,000	
Sample 2 on Fifth Day @ 10°C	59,000	—

TABLE 8

GROWTH RESPONSE OF *ESCHERICHIA COLI* TO AN INCREASE OF 5°C AFTER REFRIGERATED STORAGE

Sample	*Escherichia coli*	Per cent Increase
Count after Inoculation	2,200	
Sample 1 on First Day @ 5°C	1,800	
Sample 1 on First Day @ 10°C	2,000	11
Sample 1 on Second Day @ 5°C	1,400	
Sample 1 on Second Day @ 10°C	1,500	—
Sample 1 on Third Day @ 5°C	970	
Sample 1 on Third Day @ 10°C	1,000	—
Sample 1 on Fourth Day @ 5°C	790	
Sample 1 on Fourth Day @ 10°C	780	—
Sample 1 on Fifth Day @ 5°C	1,300	
Sample 1 on Fifth Day @ 10°C	1,700	30

TABLE 9

GROWTH RESPONSE OF AEROBACTER AEROGENES TO AN INCREASE OF 5°C AFTER REFRIGERATED STORAGE

Sample	Aerobacter aerogenes	Per cent Increase
Count after Inoculation	6,400	
Sample 1 on Second Day @ 5°C	8,900	
Sample 1 on Second Day @ 10°C	8,700	—
Sample 1 on Third Day @ 5°C	8,700	
Sample 1 on Third Day @ 10°C	8,700	—
Sample 1 on Fourth Day @ 5°C	8,300	
Sample 1 on Fourth Day @ 10°C	7,900	—
Sample 1 on Fifth Day @ 5°C	6,500	
Sample 1 on Fifth Day @ 10°C	5,800	—
Sample 1 on Sixth Day @ 5°C	5,100	
Sample 1 on Sixth Day @ 10°C	4,200	—
Sample 2 on Sixth Day @ 5°C	4,500	
Sample 2 on Sixth Day @ 10°C	4,300	—

TABLE 10

GROWTH RESPONSE OF AEROBACTER AEROGENES TO AN INCREASE OF 5°C AFTER REFRIGERATED STORAGE

Sample	Aerobacter aerogenes	Per cent Increase
Count after Inoculation	11,000	
Sample 1 on First Day @ 5°C	7,600	
Sample 1 on First Day @ 10°C	11,000	44
Sample 1 on Second Day @ 5°C	7,800	
Sample 1 on Second Day @ 10°C	7,500	—
Sample 1 on Third Day @ 5°C	8,200	
Sample 1 on Third Day @ 10°C	8,300	—
Sample 1 on Fourth Day @ 5°C	5,300	
Sample 1 on Fourth Day @ 10°C	6,500	22
Sample 1 on Fifth Day @ 5°C	3,300	
Sample 1 on Fifth Day @ 10°C	4,100	24
Sample 2 on Fifth Day @ 5°C	4,300	
Sample 2 on Fifth Day @ 10°C	4,200	—

TABLE 11

GROWTH RESPONSE OF AEROBACTER AEROGENES TO AN INCREASE OF 5°C AFTER REFRIGERATED STORAGE

Sample	Aerobacter aerogenes	Per cent Increase
Count after Inoculation	5,700	
Sample 1 on First Day @ 5°C	3,400	
Sample 1 on First Day @ 10°C	5,000	47
Sample 1 on Second Day @ 5°C	3,100	
Sample 1 on Second Day @ 10°C	3,200	—
Sample 1 on Third Day @ 5°C	2,100	
Sample 1 on Third Day @ 10°C	2,400	14
Sample 1 on Fourth Day @ 5°C	1,600	
Sample 1 on Fourth Day @ 10°C	1,800	12
Sample 1 on Fifth Day @ 5°C	760	
Sample 1 on Fifth Day @ 10°C	760	—

TABLE 12

GROWTH RESPONSE OF COLIFORMS TO AN INCREASE OF 5°C
AFTER REFRIGERATED STORAGE

Sample	Coliforms	Per cent Increase
Count after Inoculation	6,300	
Sample 1 on First Day @ 5°C	2,100	
Sample 1 on First Day @ 10°C	900	—
Sample 1 on Second Day @ 5°C	1,400	
Sample 1 on Second Day @ 10°C	1,600	14
Sample 1 on Third Day @ 5°C	11,000	
Sample 1 on Third Day @ 10°C	12,000	—
Sample 1 on Fourth Day @ 5°C	62,000	
Sample 1 on Fourth Day @ 10°C	71,000	14
Sample 1 on Fifth Day @ 5°C	190,000	
Sample 1 on Fifth Day @ 10°C	310,000	63
Sample 2 on Fifth Day @ 5°C	31,000	
Sample 2 on Fifth Day @ 10°C	29,000	—

TABLE 13

GROWTH RESPONSE OF COLIFORMS TO AN INCREASE OF 5°C
AFTER REFRIGERATED STORAGE

Sample	Coliforms	Per cent Increase
Count after Inoculation	5,500	
Sample 1 on First Day @ 5°C	900	
Sample 1 on First Day @ 10°C	1,800	100
Sample 1 on Second Day @ 5°C	2,100	
Sample 1 on Second Day @ 10°C	5,200	147
Sample 1 on Third Day @ 5°C	1,200	
Sample 1 on Third Day @ 10°C	1,600	33
Sample 1 on Fourth Day @ 5°C	3,100	
Sample 1 on Fourth Day @ 10°C	6,400	106
Sample 1 on Fifth Day @ 5°C	3,100	
Sample 1 on Fifth Day @ 10°C	3,800	19
Sample 2 on Fifth Day @ 5°C	12,000	
Sample 2 on Fifth Day @ 10°C	20,000	66

TABLE 14

GROWTH RESPONSE OF COLIFORMS TO AN INCREASE OF 5°C
AFTER REFRIGERATED STORAGE

Sample	Coliforms	Per cent Increase
Count after Inoculation	6,300	
Sample 1 on Second Day @ 5°C	950	
Sample 1 on Second Day @ 10°C	870	—
Sample 1 on Third Day @ 5°C	1,800	
Sample 1 on Third Day @ 10°C	3,400	88
Sample 1 on Fourth Day @ 5°C	15,000	
Sample 1 on Fourth Day @ 10°C	20,000	33
Sample 1 on Fifth Day @ 5°C	62,000	
Sample 1 on Fifth Day @ 10°C	56,000	—
Sample 2 on Fifth Day @ 5°C	36,000	
Sample 2 on Fifth Day @ 10°C	37,000	—

TABLE 15

GROWTH RESPONSE OF COLIFORMS TO AN INCREASE OF 5°C
AFTER REFRIGERATED STORAGE

Sample	Coliforms	Per cent Increase
Count after Inoculation	9,300	
Sample 1 on Second Day @ 5°C	4,200	
Sample 1 on Second Day @ 10°C	3,600	—
Sample 1 on Third Day @ 5°C	8,100	
Sample 1 on Third Day @ 10°C	17,000	109
Sample 1 on Fourth Day @ 5°C	120,000	
Sample 1 on Fourth Day @ 10°C	140,000	16
Sample 1 on Fifth Day @ 5°C	690,000	
Sample 1 on Fifth Day @ 10°C	460,000	—
Sample 2 on Fifth Day @ 5°C	950,000	
Sample 2 on Fifth Day @ 10°C	380,000	—

TABLE 16

GROWTH RESPONSE OF COLIFORMS TO AN INCREASE OF 5°C
AFTER REFRIGERATED STORAGE

Sample	Coliforms	Per cent Increase
Count after Inoculation	8,100	
Sample 1 on Second Day @ 5°C	3,000	
Sample 1 on Second Day @ 10°C	590	—
Sample 1 on Third Day @ 5°C	3,500	
Sample 1 on Third Day @ 10°C	3,400	—
Sample 1 on Fourth Day @ 5°C	16,000	
Sample 1 on Fourth Day @ 10°C	21,000	31
Sample 1 on Fifth Day @ 5°C	80,000	
Sample 1 on Fifth Day @ 10°C	110,000	37
Sample 2 on Fifth Day @ 5°C	26,000	
Sample 2 on Fifth Day @ 10°C	23,000	—

TABLE 17

GROWTH RESPONSE OF COLIFORMS TO AN INCREASE OF 5°C
AFTER REFRIGERATED STORAGE

Sample	Coliforms	Per cent Increase
Count after Inoculation	9,900	
Sample 1 on Second Day @ 5°C	380	
Sample 1 on Second Day @ 10°C	600	58
Sample 1 on Third Day @ 5°C	4,700	
Sample 1 on Third Day @ 10°C	5,500	27
Sample 1 on Fourth Day @ 5°C	18,000	
Sample 1 on Fourth Day @ 10°C	17,000	--
Sample 1 on Fifth Day @ 5°C	70,000	
Sample 1 on Fifth Day @ 10°C	62,000	--
Sample 2 on Fifth Day @ 5°C	27,000	
Sample 2 on Fifth Day @ 10°C	24,000	--

TABLE 18

GROWTH RESPONSE OF COLIFORMS TO AN INCREASE OF 5°C
AFTER REFRIGERATED STORAGE

Sample	Coliforms	Per cent Increase
Count after Inoculation	8,600	
Sample 1 on Second Day @ 5°C	910	
Sample 1 on Second Day @ 10°C	890	--
Sample 1 on Third Day @ 5°C	3,500	
Sample 1 on Third Day @ 10°C	3,400	--
Sample 1 on Fourth Day @ 5°C	6,200	
Sample 1 on Fourth Day @ 10°C	4,400	--
Sample 1 on Fifth Day @ 5°C	27,000	
Sample 1 on Fifth Day @ 10°C	30,000	11
Sample 2 on Fifth Day @ 5°C	34,000	
Sample 2 on Fifth Day @ 10°C	49,000	44

TABLE 19

GROWTH RESPONSE OF COLIFORMS TO AN INCREASE OF 5°C
AFTER REFRIGERATED STORAGE

Sample	Coliforms	Per cent Increase
Count after Inoculation	12,000	
Sample 1 on Second Day @ 5°C	4,100	
Sample 1 on Second Day @ 10°C	3,700	--
Sample 1 on Third Day @ 5°C	50,000	
Sample 1 on Third Day @ 10°C	31,000	--
Sample 1 on Fourth Day @ 5°C	280,000	
Sample 1 on Fourth Day @ 10°C	310,000	11
Sample 1 on Fifth Day @ 5°C	420,000	
Sample 1 on Fifth Day @ 10°C	510,000	21
Sample 2 on Fifth Day @ 5°C	260,000	
Sample 2 on Fifth Day @ 10°C	310,000	19

TABLE 20

GROWTH RESPONSE OF COLIFORMS TO AN INCREASE OF 5°C
AFTER REFRIGERATED STORAGE

Sample	Coliforms	Per cent Increase
Count after Inoculation	9,000	
Sample 1 on Second Day @ 5°C	1,500	
Sample 1 on Second Day @ 10°C	1,000	---
Sample 1 on Third Day @ 5°C	1,600	
Sample 1 on Third Day @ 10°C	2,800	75
Sample 1 on Fourth Day @ 5°C	12,000	
Sample 1 on Fourth Day @ 10°C	20,000	66
Sample 1 on Fifth Day @ 5°C	87,000	
Sample 1 on Fifth Day @ 10°C	76,000	---
Sample 2 on Fifth Day @ 5°C	12,000	
Sample 2 on Fifth Day @ 10°C	22,000	83

On June 14 (Table 21) three samples of milk were coliform positive when purchased and after four days there were four samples well over the standard. Tables 22 and 25 show the largest number of coliform positive samples for any sampling period. In both cases Samples I and III increased several times after storage while the other positive samples remained at low numbers. Sample III was well over the maximum on several trials, especially after four days storage at 5°C (Tables 21 through 25 and Tables 26 through 31. Sample I followed closely by being over the standard nine times after storage (Tables 21, 22, 24 through 27, 29, 32 and 33). Four of these nine were below the standard when purchased. The only other sample that was ever over the standard when purchased was Sample V. This coliform count of 24 per milliliter decreased during storage to 6 per milliliter. Each source sampled had at least one coliform positive sample during the ten month period but Samples II, IV, V and VI showed no difficulty in meeting the present standard. Since all samples had coliforms at one time or another in the market, the importance of coliform response to temperature increases is emphasized.

A much larger per cent (Figure 2) of the milk samples were coliform positive during the months of hot weather (Tables 21 through 27) than milk sampled during cooler weather (Tables 28 through 33). However, the per cent of samples over 10 per milliliter at the time of purchase was only slightly higher during hot weather as compared to the cooler months (Figure 3). The months of hot weather included June, July, August, September and October while the cooler months included March, April, November and December.

After four days storage at 5°C during the hot weather, the coliform positive samples had increased to 59 per cent (Figure 2) and the per cent over 10 per milliliter had more than doubled to 39 per cent (Figure 3). During the cool weather the samples over 10 per milliliter only increased from 11 to 13 per cent (Figure 3) while the number of coliform positive samples increased from 17 to 30 per cent (Figure 2).

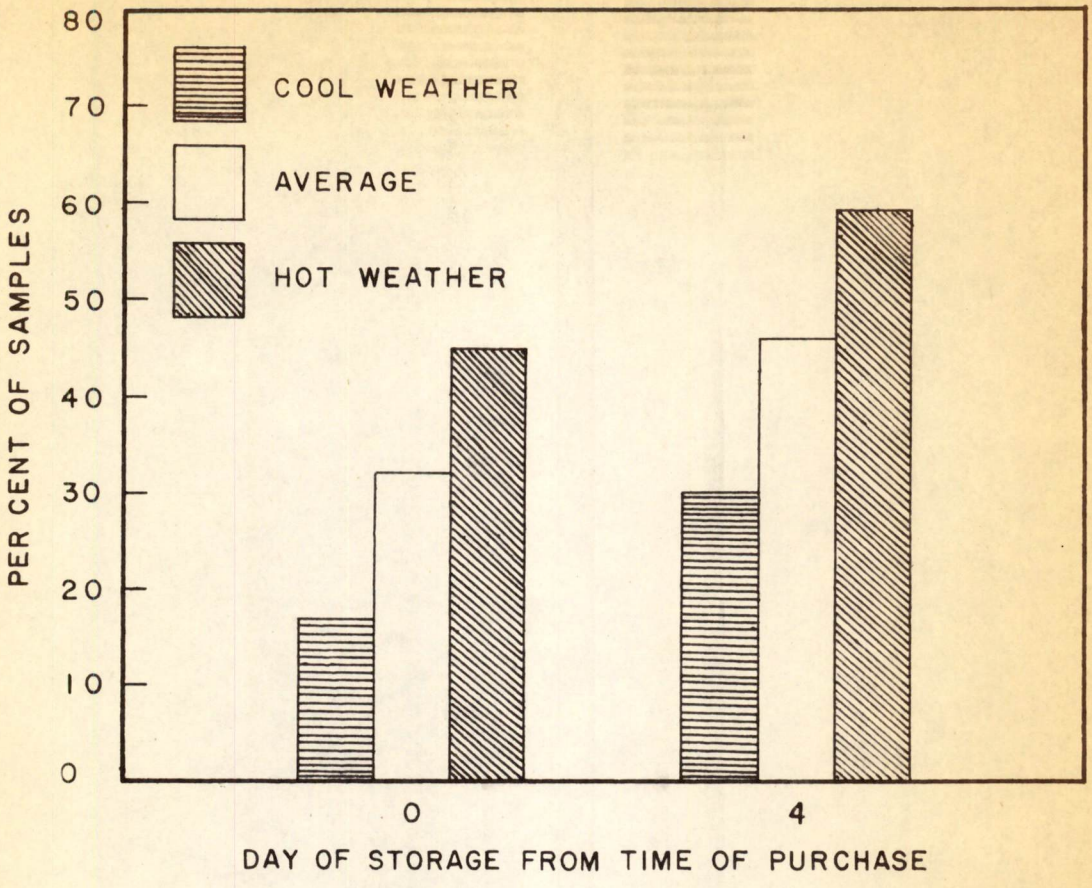

Figure 2. The increase in coliform positive samples after storage at 5°C.

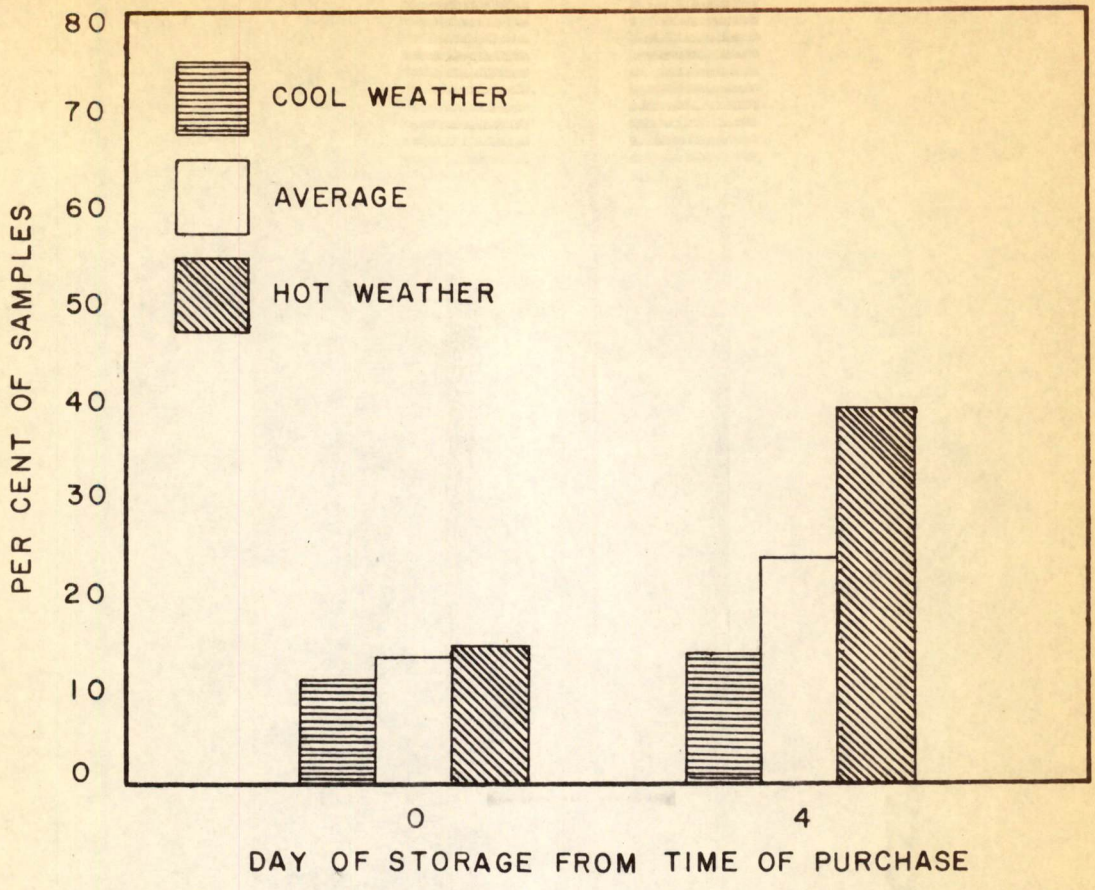

Figure 3. Influence of seasonal temperatures on the number of milk samples that developed ten or more coliforms per milliliter after storage at 5°C.

TABLE 21

BACTERIAL COUNT OF MARKET MILK WHEN PURCHASED AND AFTER FOUR DAYS OF REFRIGERATED STORAGE, JUNE 14, 1956

Sample	Coliform	Total Count	After 4 Days Storage at 5°C	
			Coliform	Total Count
I	1	5,200	38	17,000,000
II	8	19,000	1,170	390,000
III	0	29,000	6,500	3,900,000
IV	4	14,000	146	20,000
V	0	51,000	0	68,000
VI	0	300	0	1,300,000

TABLE 22

BACTERIAL COUNT OF MARKET MILK WHEN PURCHASED AND AFTER FOUR DAYS OF REFRIGERATED STORAGE, JULY 5, 1956

Sample	Coliform	Total Count	After 4 Days Storage at 5°C	
			Coliform	Total Count
I	12	12,000	1,850	4,200,000
II	5	30,000	4	380,000
III	0	4,100	72	410,000
IV	2	24,000	1	22,000
V	0	14,000	0	14,000
VI	2	300	3	7,100

TABLE 23

BACTERIAL COUNT OF MARKET MILK WHEN PURCHASED AND AFTER FOUR DAYS OF REFRIGERATED STORAGE, JULY 31, 1956

Sample	Coliform	Total Count	After 4 Days Storage at 5°C	
			Coliform	Total Count
I	0	3,400	0	130,000
II	0	7,400	0	13,000
III	340	360,000	1660	4,600,000
IV	8	30,000	8	16,000
V	24	19,000	6	18,000
VI	0	170	0	3,000

TABLE 24

BACTERIAL COUNT OF MARKET MILK WHEN PURCHASED AND AFTER FOUR DAYS OF REFRIGERATED STORAGE, AUGUST 23, 1956

Sample	Coliform	Total Count	After 4 Days Storage at 5°C	
			Coliform	Total Count
I	0	17,000	39	25,000
II	0	22,000	1	29,000
III	4,290	30,000	8,600	67,000,000
IV	0	28,000	0	20,000
V	0	7,500	0	50,000
VI	0	300	0	3,000

TABLE 25

BACTERIAL COUNT OF MARKET MILK WHEN PURCHASED AND AFTER FOUR DAYS OF REFRIGERATED STORAGE, AUGUST 30, 1956

Sample	Coliform	Total Count	After 4 Days Storage at 5°C Coliform	After 4 Days Storage at 5°C Total Count
I	14	19,000	26,000	510,000
II	1	23,000	4	140,000
III	10	4,400	430	1,300,000
IV	1	12,000	6	14,000
V	0	16,000	0	210,000
VI	0	1,300	1	3,000

TABLE 26

BACTERIAL COUNT OF MARKET MILK WHEN PURCHASED AND AFTER FOUR DAYS OF REFRIGERATED STORAGE, SEPTEMBER 20, 1956

Sample	Coliform	Total Count	After 4 Days Storage at 5°C Coliform	After 4 Days Storage at 5°C Total Count
I	0	2,400	62	72,000
II	0	13,000	0	12,000
III	0	26,000	0	65,000,000
IV	0	8,900	0	5,200
V	0	23,000	0	21,000
VI	0	360	0	7,800

TABLE 27

BACTERIAL COUNT OF MARKET MILK WHEN PURCHASED AND AFTER FOUR DAYS OF REFRIGERATED STORAGE, OCTOBER 18, 1956

Sample	Coliform	Total Count	After 4 Days Storage at 5°C Coliform	After 4 Days Storage at 5°C Total Count
I	2,900	160,000	67,000	7,300,000
II	3	41,000	4	140,000
III	1	16,000	1	260,000
IV	0	20,000	1	30,000
V	0	2,400	0	30,000
VI	1	1,100	0	30,000

TABLE 28

BACTERIAL COUNT OF MARKET MILK WHEN PURCHASED AND AFTER FOUR DAYS OF REFRIGERATED STORAGE, MARCH 22, 1956

Sample	Coliform	Total Count	After 4 Days Storage at 5°C Coliform	After 4 Days Storage at 5°C Total Count
I	0	1,700	0	880,000
II	0	6,500	4	6,500
III	1	2,900	0	19,000
IV	0	26,000	5	280,000
V	0	28,000	9	4,200,000
VI	0	2,900	0	3,100

TABLE 29

BACTERIAL COUNT OF MARKET MILK WHEN PURCHASED AND AFTER FOUR DAYS OF REFRIGERATED STORAGE, APRIL 5, 1956

Sample	Coliform	Total Count	After 4 Days Storage at 5°C	
			Coliform	Total Count
I	0	4,500	37	4,500
II	0	15,000	1	10,000
III	0	1,400	0	1,300
IV	0	16,000	0	170,000
V	1	13,000	0	38,000
VI	0	2,100	0	870,000

TABLE 30

BACTERIAL COUNT OF MARKET MILK WHEN PURCHASED AND AFTER FOUR DAYS OF REFRIGERATED STORAGE, APRIL 12, 1956

Sample	Coliform	Total Count	After 4 Days Storage at 5°C	
			Coliform	Total Count
I	0	4,600	0	330,000
II	0	21,000	0	33,000
III	1,056	5,000	53,000	75,000
IV	0	11,000	0	27,000
V	0	15,000	1	48,000
VI	0	800	0	10,000

TABLE 31

BACTERIAL COUNT OF MARKET MILK WHEN PURCHASED AND AFTER FOUR DAYS OF REFRIGERATED STORAGE, APRIL 19, 1956

Sample	Coliform	Total Count	After 4 Days Storage at 5°C	
			Coliform	Total Count
I	0	3,900	0	11,000,000
II	0	20,000	0	16,000
III	106	1,600	5,800	6,500
IV	0	1,100	0	5,000
V	0	13,000	0	15,000
VI	0	5,600	0	8,500

TABLE 32

BACTERIAL COUNT OF MARKET MILK WHEN PURCHASED AND AFTER FOUR DAYS OF REFRIGERATED STORAGE, NOVEMBER 8, 1956

Sample	Coliform	Total Count	After 4 Days Storage at 5°C Coliform	After 4 Days Storage at 5°C Total Count
I	13	21,000	14	200,000
II	0	56,000	2	55,000
III	0	3,200	0	2,600,000
IV	0	9,500	0	6,600
V	0	12,000	0	26,000
VI	0	2,500	0	16,000

TABLE 33

BACTERIAL COUNT OF MARKET MILK WHEN PURCHASED AND AFTER FOUR DAYS OF REFRIGERATED STORAGE, DECEMBER 13, 1956

Sample	Coliform	Total Count	After 4 Days Storage at 5°C Coliform	After 4 Days Storage at 5°C Total Count
I	220	18,000	430	3,300,000
II	0	4,900	0	2,800
III	0	110,000	0	300,000
IV	0	2,600	0	8,200
V	0	3,100	0	3,400
VI	0	300	0	3,000

SUMMARY AND CONCLUSION

Coliforms will grow in milk refrigerated at 5°C. An increase in coliforms may occur in milk warmed to 10°C after storage at 5°C. In this study 50.8 per cent of the trials gave an average increase in coliform count of 52 per cent when warmed 5°C.

A study of the market milk in Knoxville revealed that 32 per cent of the milk samples were coliform positive and 13 per cent of the samples had over ten coliforms per milliliter. During hot weather 45 per cent of the samples were coliform positive while only 17 per cent were coliform positive during cooler weather. The per cent of samples over ten coliforms per milliliter was about the same for both seasons when purchased at grocery stores but the hot weather samples increased from 14 to 39 per cent whereas the cool weather samples increased only from 11 to 13 per cent.

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