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THE EFFECT OF FROZEN STORAGE ON THE MICROFLORA OF RAW MILK

BY

JASWANT SINGH

A thesis submitted
in partial fulfillment of the requirements for the
degree Master of Science, Department of
Dairy Science, South Dakota State
College of Agriculture
and Mechanic Arts

January 1964

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THE EFFECT OF FROZEN STORAGE ON THE

MICROFLORA OF RAW MILK

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable as meeting the thesis requirements for this degree, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Adviser Date

Head, Dairy Science Dept. Date

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INTRODUCTION

The proper handling of milk samples prior to bacterial analysis has been somewhat of a problem to dairy processors of South Dakota. There have been, and still are, many queries by these people about the treatment of a milk sample prior to the determination of a bacterial count. Should the milk samples be frozen prior to determining the number of bacteria? If it is feasible, what effect will the freezing have on the number of bacteria found? No tangible information about this problem has been found in the literature.

Standard procedures emphasize mainly the icing of milk samples before analyzing for numbers of bacteria. In this study a comparison has been made of the iced and frozen samples from the bacteriological viewpoint.

The objective of this study is to determine the effect of frozen storage of milk samples on the numbers of bacteria.

REVIEW OF LITERATURE

The effect of refrigeration and freezing on the microflora of milk is of paramount importance in the dairy industry. Some work has been done on the problem concerning how milk should be handled prior to bacteriological analysis.

Pennington (13) in a study of so called "clean" and "market milk" kept at -1.67°C to - 0.55°C found a very significant increase in the number of bacteria despite the fact that milk was semi-solid with ice. In her summary she states,

> Bacterial growth at the end of the week, even in the cleanest milk which contained as low as 300 organisms to the c.c. was pronounced. There was steady increase in the number of organisms for 5 or 6 weeks, and at their maximum they numbered hundreds of millions. Occasionally they passed a billion mark per c.c.

Certain species of bacteria such as Bacillus formosus, B. solitarius and B. raveneli were especially resistant to cold and were the predominating species found.

Ravenel, Hastings and Hammer (16) studied the effect of storage at 0°C and -9°C on the bacterial flora of 2 grades of milk. One was considered the best obtainable and the other was a mixed dairy milk of fair quality. There was no increase of bacteria in the milk held at -9°C for periods of 160 to 203 days. However, in the milks stored at 0°C there was a significant increase in the bacterial content which caused an increase in acidity, an increase in the percentage of soluble nitrogen, and a decrease in total nitrogen content, due probably, to the liberation of free nitrogen. Keith (6) studied the effect of freezing on Bacillus coli in milk. He contends that bacteria are not killed by the low temperature but bacterial longevity is apparently impaired by a destructive metabolism. Frozen foods like milk, cream and eggs favor the survival of bacteria at low temperature, because they maintain physical conditions which protect the bacteria.

Reed and Reynolds (17) investigating the effect of low temperature on the growth and activities of bacteria in milk, discovered that at the temperature of -1°C, Bacillus putidum, B. aerogenes, B. cyanogenes, B. proteus vulgaris, B. coli, B. subtilis, B. fluorescens liquefaciens, B. prodigiosus, Bacterium lactis acidi, Sarcina lutea, Oidium lactis, Microspora tyrogena and M. citricus were all able to grow. Considerable variation was noted in the behaviour of different organisms at this temperature. Some of them as B. lactis acidi, and others belonging to the acid producing group, increased at first but decreased under long and continued storage at this temperature. Others like M. tyrogena grew slowly at first but made considerable growth during the period of continued storage. Milk that was low in acid might have been considered sweet, but had actually undergone marked chemical change which influenced its quality.

Olson et al. (11) studied changes in bacterial counts and flavor of concentrated and recombined milks during storage at low temperature. Results of their studies showed more growth of bacteria in the recombined milk, prepared from the concentrated milk, than in the concentrated milk itself. There was flavor deterioration and poor keeping quality of recombined milk when compared with the concentrated milk. The keeping quality of the concentrate was found to be 2, 3 and 5 weeks after storage at 7°, 4° and 1°C respectively. These periods exceeded those for the commercially prepared concentrated milk. The differences were due to post-pasteurization contamination. There was no semblance of coliform bacteria. During summer months, the temperature of water used for recombination may reach 70°C or higher. This and other conditions of holding may influence the bacterial growth and keeping quality.

Hillard and Davis (4) studied the effect of freezing temperatures upon bacteria and concluded that intermittent freezing temperatures exert a more effective germicidal action upon bacteria than continuous freezing. The decline is much less in milk and cream, as compared with tap water, when freezing temperatures are applied. This is caused by the physical protection of bacteria provided by the colloidal and solid matter suspended in the suspension. As far as destruction of bacteria is concerned, the degree of cold below freezing is not an important factor. There is no critical temperature below freezing where the germicidal effect is greatly enhanced. The death rate of B. coli is much higher in frozen solid media than in that which is not solid and is at a slightly lower temperature. Crystallization results in a mechanical crushing which is a significant germicidal factor in causing the death of bacteria at 0°C or below.

According to Sherman (18) there is slow growth of bacteria in pasteurized milk held at 0°C when compared with raw milk. The keeping quality is 2 to 3 times that of raw milk containing the same number of bacteria. The improved keeping quality of pasteurized milk is caused by the entire destruction of certain kinds of bacteria. This is shown when the addition of a small quantity of raw milk decreases the keeping quality of pasteurized milk. The bacteria which cause such spoilage near the freezing point, are gram negative, non spore forming rods belonging to the genus Pseudomonas.

Johns and Berzins (5) in their study on the effect of freezing on the standard plate count of milk, found that freezing was not very effective in killing bacteria. Most of the organisms in pasteurized milk and cream are gram negative and resistant to freezing. There were greater numbers of organisms surviving after 48 hours than after 24 hours, with no reason given. There was a higher rate of surviving bacteria in fast freezing than in slow freezing.

Palmer and McCutcheon (12) studied the effect of variations in the time of plating, on the counts obtained. They studied the effect of holding samples under refrigeration for 24 hours at 42°F before plating. The plates were made 6 hours and 24 hours later than the usual time of plating. A total of 105 samples were plated representing 32 creameries. It was noted that 87% of the samples, after 6 hours refrigeration, showed a decrease in numbers. Similarly there was a decline in counts in 68% of the samples which were refrigerated for 24 hours. In holding the milk samples under refrigeration for 12-20 hours and an additional 6 hours refrigeration time, there is distinct reduction in the bacterial count. When such refrigeration is continued for a total of 48 hours, the results suggest that the bacterial plate count tends to increase.

Dahlberg (3), in his study, collected 108 samples of different lots of pasteurized milk in July and October. These samples were examined for standard plate counts and coliform counts. The milk was stored for 4 days at three different refrigeration temperatures. It was found that coliform bacteria increased more rapidly in numbers than the standard plate count. The coliform types were less than 0.02% of the total bacteria in freshly pasteurized milk. The percentage of coliforms did not increase in October at 35-40°F but an increase of 1.12% in 4 days was observed in July and August. At 45-50°F and 55-60°F the coliform bacteria comprised about 5% of the total count in October, after storage for 4 days. But after 4 days storage at 45-50°F, the coliform count was 68% of the total count in July-August and 50% at 55-60°F. The coliform bacteria grew more rapidly in summer weather than in cool weather.

Murry and Coey (10) investigated the effect of freezing and storage on the bacterial flora of pasteurized milk. They concluded that freezing and limited storage has no effect on the normal flora of pasteurized milk. When contaminants of the coli-aerogenes group were added, their numbers were not reduced by freezing. The changes which occurred in the bacterial content between the 3rd and 4th month dealt with physical changes.

Chaffee (2) studied the bacterial counts in pasteurized milk held in refrigerated storage. There was no appreciable increase in the bacterial content in good quality pasteurized milk, even after 120 hours of storage under proper refrigeration. Occasionally there was a decrease in counts. Poor quality pasteurized milk produced an increase in the bacterial content even under ideal refrigeration storage.

Randall (14) in his study of frozen homogenized milk for army use stated that the duration of time in which milk remained normal depended upon the freezing temperature and storage time. Milk was found normal for 115 days when frozen and stored at -32.8°C. However, the best results were obtained when it was frozen and stored at -40°C. Good quality homogenized milk could be stored up to 120 hours at 1.67°C, before freezing, without any change in keeping quality.

Randall (15) studied the keeping quality of frozen homogenized milk pasteurized at 155°F for 30 minutes. The milk was frozen at -27°F and -40°F and stored at 14°F. Standard plate counts were made on the samples of milk before and after freezing and storing under different conditions. There was a reduction of the plate counts in each case. This was caused by freezing and storage temperatures or a change in storage temperature. There was no growth of cryophilic bacteria. To know whether the bacteria increase faster in the homogenized milk which had been frozen and then thawed, than in fresh homogenized milk, a series of samples of both were kept at different temperatures. The initial plate count was performed before storing the milk and then at the end of the storage period. There was no significant difference in the bacterial count. The samples of homogenized milk were held up to 120 hours at 35°F before freezing and for 89 days in the frozen state at 0°C. There was no appreciable change in the bacterial content in either case. Babcock et al. (1) studied the keeping quality of frozen homogenized milk after thawing. They stored the samples of fresh homogenized milk at 30.5°C, 12.8°C, 7.22°C and 1.67°C. The flavor changes in frozen and thawed homogenized milk were quite akin to fresh homogenized milk. The frozen and thawed homogenized milk could be stored for longer periods than fluid milk before use. Determination by the standard plate count showed that there was no significant difference between the frozen, thawed and fresh homogenized milk from the bacteriological viewpoint. There was no difference in the acid development measured by titratable acidity and pH determinations.

Skean et al. (19) worked on long term preservation of fresh fluid milk and on the keeping quality of frozen homogenized milk. They used milk from three different processors in this study. The samples were placed in a room at -10°F for freezing. The rest of the milk was warmed up to 70°F and subjected to bacteriological analysis. The frozen milk was thawed and examined at the end of 1, 4, 8, 12 and 16 weeks for flavor, appearance, standard plate count and psychrophile count. There was little deterioration in flavor up to 12 weeks but there was a distinct decline in flavor between 12 and 16 weeks. There was little change in the numbers of bacteria during the frozen period but the psychrophiles showed a decline during 16 weeks of storage.

Marth and Frazier (8) in their study of the bacteriology of milk held at farm bulk cooling tank temperatures and the effect of the number of bacteria in the original milk, took raw milk samples of two bacteriological groups (below 50,000 and over 50,000 bacteria per ml). They stored the milk samples at 36°F, 38°F, 38°F with periodic raises to 45°F and to 50°F. At 36°F storage the milk samples with less than 50,000 bacteria per ml showed little growth after 3 days but a rapid growth after 4 days in the same sample. At 38°F there was a similarity in results as compared with at 38°F with periodic raises to 45°F and 50°F. The smaller number of samples with low counts showed a more appreciable increase in bacterial growth than high count milk at 38°F. However, some samples did not show any increase in numbers of bacteria after 2, 3 and 4 days storage at 38°F irrespective of their low or high count. At 45°F there was more rapid growth of bacteria in low count milk than in the high count milk. At all storage temperatures, according to these authors, there is more likelihood of an increase in bacterial numbers in samples with 50,000 bacteria per ml than those containing lower counts. After two days storage there was more rapid growth of psychrophiles in low count milk than in high count milk.

LaCrosse and Piraux (7) studied the behaviour of milk during storage at temperatures of 4°C and 7°C and its apparent behaviour when using "bulk collection." It was found that the multiplication rate depends upon the initial number of bacteria in milk. At 7°C the milk is more prone to organoleptic spoilage. As far as the possible behaviour of milk when using bulk collection is concerned, the rate of multiplication of the total number of bacteria and of psychrophilic bacteria is relatively low unless the milk contained a high initial psychrophilic count.

EXPERIMENTAL PROCEDURE

Preparation of Samples: In this study raw milk from different milk producers was used. The milk was kept cold in bulk tanks on the farm and was thoroughly agitated before removing the samples. Samples of milk, each representing a different producer were taken and brought to the laboratory in clean, sterile 4 oz. bottles. These sample bottles were kept cold in a container filled with ice until ready to be used. The bottles were shaken 25 times and approximately 5 to 10 ml of milk were transferred with sterile pipettes to clean, sterile screw capped test tubes. The test tubes were tightly closed after the sampling procedure was completed. This procedure was repeated each time before storage.

Storage Conditions: The raw milk samples were plated for standard plate count, coliform count and psychrophilic count each time before storage. The screw capped test tubes containing the samples mentioned above were placed in the freezing compartment of a household type refrigerator at the temperature of -15°C for one week.

The other two storage temperatures utilized were a household deep freeze at -23°C and a laboratory ultrafreeze at -45°C. The duration of time in these cases was also one week. The purpose of storage of the milk samples at the three different temperatures was to check the temperature effects on the microflora of the milk.

Experiments were conducted to determine the effect of frozen storage, at intervals of 24 hours, on the bacterial content of the milk.

In this case seven sets of identical milk samples were placed in clean, sterile screw capped test tubes. All of the milk samples were stored in the freezing compartment of the household refrigerator at -15°C, the household deep freeze at -23°C or the laboratory ultrafreeze at -45°C until ready to use. A set of milk samples was removed from frozen storage each 24 hours and analyzed bacteriologically.

Bacteriological Procedure: At the end of each storage period, the tubes were removed from the freezing chamber, thawed in a water bath at a temperature of 45°C, well shaken and plated for standard plate count, coliform count and psychrophilic count.

Preparation of the plates for determining the individual numbers of organisms of each type was done as follows:

1. Standard Plate Count: Since this group of organisms implicates a large number of organisms, dilutions of 1:1,000, 1:10,000 and 1:100,000 were used. The dilutions were plated and poured with Standard Method Agar (20). The plates were incubated at 32°C for 48 hours.

2. Coliform Count: Dilutions of 1:100 and 1:1,000 were utilized for this group of organisms. These dilutions were plated and poured with Violet Red Bile Agar (20) which is specific for these organisms. The plates were incubated at 37°C for 24 hours.

3. Psychrophilic Count: The dilutions employed in this case were 1:100, 1:1,000 and 1:10,000. These were plated and poured with Standard Method Agar (20). The plates were incubated at 7°C for 7 days.

Counting: A dark-field Quebec colony counter was used for counting the colonies of bacteria. A hand tally, a mechanical counting device, was utilized in the process of counting the colonies (20).

The count per milliliter in each case was determined by multiplying the number of colonies counted by the dilution of the individual plate.

Reduction Tests: These tests were performed for determining the bacterial activity in the raw milk. Raw milk samples from different producers were transferred to clean, sterile glass test tubes and closed with a rubber stopper. The quantity of milk used was 10 ml each time. The tubes were stored in the freezing compartment of the household refrigerator at -15°C and in the household deep freeze at -23°C. After 24 or 48 hour intervals, the tubes were removed from frozen storage, thawed in a water bath at 45°C and subjected to the methylene blue reduction test (20).

Direct Microscopic Count: Raw milk samples from different producers were kept in the freezing compartment of a household refrigerator at -15°C and in a household deep freeze at -23°C. After 7 days storage, the samples were thawed in a water bath at 45°C, shaken and 0.01 ml of each was put on a glass slide and spread over an area of 1 cm^2. After drying, the milk films were stained using North's aniline oil methylene blue stain (20) and counted with a microscope. The Direct Microscopic count was conducted to determine any alteration of

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the size of bacterial clumps in raw milk after frozen storage at two temperatures.

RESULTS AND DISCUSSION

The contents of Table I are a summation of five trials, which are shown in Tables VIII, IX, X, XI, and XII in the Appendix. The information in Table II represents the data shown in Tables XIII, XIV and XV in the Appendix.

The logarithmic averages of the standard bacteriological counts of raw milk kept in the freezing compartment of a household refrigerator, a household deep freeze and a laboratory ultrafreeze for a period of 7 days are shown in Table I. It is apparent from the results that there was an increase in the standard plate count of bacteria in all of the experimental trials except trial 1. This increase in count usually occurred when the raw milk was stored in the household deep freeze and the laboratory ultrafreeze. The coliform count tended to decrease in the milk during frozen storage in the freezing compartment of the household refrigerator and the household deep freeze. This is evident from trials 2, 3, 4 and 5. It was also quite clear from the results that the number of coliform bacteria was approximately the same or showed a slight increase over the original count, when the milk was placed in the laboratory ultrafreeze. There was an increase in the psychrophile count in most trials when the milk was stored in the freezing compartment of a household refrigerator, the household deep freeze and the laboratory ultrafreeze for seven days.

The logarithmic averages of the standard bacteriological counts of raw milk kept at three different freezing temperatures for intervals

Table I. Logarithmic averages of standard bacteriological counts of raw milk kept at three different freezing temperatures for seven days

Trial	Original count	Household (-15°C)	Deep freeze (-23°C)	Ultrafreeze (-45°C)
		Standard plate count		
1	620,000	640,000	390,000	195,000
2	1,400,000	1,700,000	2,000,000	2,200,000
3	600,000	1,100,000	1,100,000	1,200,000
4	490,000	740,000	990,000	1,000,000
5	580,000	700,000	730,000	820,000
		Coliform count		
1	1,800	2,400	3,400	1,200
2	3,100	1,900	2,400	3,000
3	3,200	1,200	830	3,500
4	1,200	540	990	1,300
5	1,700	700	740	1,900
		Psychrophile count		
1	360,000	490,000	450,000	690,000
2	370,000	800,000	1,300,000	1,400,000
3	260,000	180,000	290,000	390,000
4	100,000	150,000	260,000	280,000
5	100,000	120,000	130,000	140,000

Table II. Logarithmic averages of standard bacteriological counts of raw milk kept at three different freezing temperatures and sampled at intervals of 24 hours
(Count expressed in thousands)

Type of count	Original count	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs	144 hrs	168 hrs
Freezing compartment of household refrigerator (-15°C)								
Standard plate	2,900	2,100	2,200	2,100	2,000	2,200	1,800	1,800
Coliform	11	6.1	4	2.4	3	3	3.1	2.1
Psychrophile	300	280	310	320	240	330	330	260
Household deep freeze (-23°C)								
Standard plate	1,300	1,500	1,200	1,400	1,000	1,000	970	1,400
Coliform	3	1.3	1.7	.65	1.2	1	.75	1.3
Psychrophile	120	270	150	200	160	180	130	180
Laboratory ultrafreeze (-45°C)								
Standard plate	1,100	1,300	1,100	1,300	1,100	1,100	1,100	1,200
Coliform	1.8	2	2	2.1	2.4	2.1	2.7	1.9
Psychrophile	470	620	490	460	480	410	410	380

of 24 hours are presented in Table II. Storage in the household refrigerator indicated that the standard plate count declined in comparison to the original count. The coliform count showed a considerable reduction while the psychrophiles remained approximately the same when compared to the original count. In the case of the household deep freeze storage the standard plate count increased slightly after 24, 72 and 168 hours. The coliform count decreased appreciably more than the original count, but there was very little change in the psychrophile count. In laboratory ultrafreeze storage, there was not much alteration in the standard plate count. The coliform count increased slightly after 96 and 144 hours, but it again dropped and approximated the original count. The psychrophile count showed very little change.

The methylene blue reduction test results for raw milk kept in the household deep freeze and in the freezing compartment of the household refrigerator are given in Tables III, IV and V. It is seen from the results in these tables that the reduction time of raw milk is prolonged after frozen storage. The reduction time is also lengthened by the duration of the freezing time. There is a greater increase in the reduction time of the raw milk during its storage in the freezing compartment of the household refrigerator than that shown following storage in the household deep freeze for the same period of time.

The results of the microscopic examination of the raw milk samples stored in the freezing compartment of a household refrigerator and in a household deep freeze for seven days are shown in the Tables

Table III. Methylene blue reduction test results for raw milk stored in a household deep freeze (-23°C)

Reduction time (hrs.)	Reduction times after frozen storage				
	0 hrs	48 hrs	96 hrs	144 hrs	192 hrs
1/2	-	-	-	-	-
1 1/2	3*	-	-	-	-
2 1/2	-	3	3	3	3
3 1/2	-	-	-	-	-
4 1/2	7	-	-	-	-
5 1/2	-	7	7	7	7
6 1/2	2,5	5	5	5	5
7 1/2	-	-	-	-	-
> 7 1/2	1,4	1,2,4	1,2,4	1,2,4	1,2,4

*Sample identification numbers

Table IV. Methylene blue reduction test results for raw milk kept in the freezing compartment of a household refrigerator (-15°C)

Reduction time (hrs)	Reduction times after frozen storage							
	0 hrs	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs	144 hrs	168 hrs
1/2	-	-	-	-	-	-	-	-
1 1/2	7*	-	-	-	-	-	-	-
2 1/2	-	7	7	7	7	7	7	-
3 1/2	1	-	-	-	-	-	-	7
4 1/2	-	-	-	-	-	-	-	-
5 1/2	22,24	-	-	-	-	-	-	-
6 1/2	5,17	1,22,24	22,24	-	-	-	-	-
7 1/2	14	5,17	5,17	5,17 22,24	5,17,22	5,22	-	-
> 7 1/2	2,3,4,8, 9,10,11, 18,21,23	2,3,4,8, 9,10,11, 14,18,21, 23	1,2,3,4, 8,9,10, 11,14,18, 21,23	1,2,3,4, 8,9,10, 11,14,18, 21,23	1,2,3,4, 8,9,10, 11,14,18, 21,23,24	1,2,3,4, 8,9,10, 11,14,17, 18,21,23, 24	1,2,3,4, 5,8,9,10, 11,14,17, 18,21,22, 23,24	1,2,3,4, 5,8,9,10, 11,14,17, 18,21,22, 23,24

*Sample identification numbers

Table V. Methylene blue reduction test results for raw milk kept in a household deep freeze (-23°C)

Reduction time (hrs)	Reduction times after frozen storage							
	0 hrs	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs	144 hrs	168 hrs
1/2	-	-	-	-	-	-	-	-
1 1/2	-	-	-	-	-	-	-	-
2 1/2	-	-	-	-	-	-	-	-
3 1/2	-	-	-	-	-	-	-	-
4 1/2	-	13*	-	-	-	-	-	-
5 1/2	2,13,16	2,16	2,16	2,16	2,16	16	16	-
6 1/2	7,22	7,22	13,7,22	13,7,22	7,13	7,13,2	2	2,16,7
7 1/2	5,17	14,11,5	11,5,14	5,14	5,14,22	5,22	7,13 5,22	22,13,5
> 7 1/2	1,3,4,8 9,10,11 14,18,21 23,24	1,3,4,8 9,10,17 18,21,23 24	1,3,4,8 9,10,17 18,21,23 24	1,3,4,8 9,10,11 17,18,21 23,24	1,3,4,8 9,10,11 17,18,21 23,24	1,3,4,8 9,10,11 14,17,18 21,23,24	1,3,4,8 9,10,11 14,17,18 21,23,24	1,3,4,8 9,10,11 14,17,18 21,23,24

*Sample identification numbers

Table VI. Microscopic examination of raw milk samples stored in the freezing compartment of a household refrigerator (-15°C) for seven days

Sample	Before freezing Direct clump count per ml	After freezing Direct clump count per ml
1	3,500,000	3,200,000
2	1,200,000	1,600,000
3	1,000,000	1,300,000
4	1,800,000	1,300,000
5	1,500,000	370,000
6	5,500,000	5,300,000
7	1,100,000	780,000
8	480,000	600,000
9	780,000	1,200,000
10	670,000	1,000,000
11	1,100,000	560,000
12	1,600,000	410,000
13	700,000	330,000
14	1,100,000	480,000
15	1,800,000	890,000
16	300,000	220,000
17	850,000	1,700,000
Log average	1,150,000	881,000

Table VII. Microscopic examination of raw milk samples stored in a household deep freeze (-23°C) for seven days

Sample	Before freezing Direct clump count per ml	After freezing Direct clump count per ml
1	2,600,000	3,400,000
2	1,200,000	1,700,000
3	1,100,000	1,700,000
4	1,200,000	1,400,000
5	290,000	680,000
6	570,000	860,000
7	290,000	400,000
8	860,000	460,000
9	3,100,000	5,000,000
10	1,600,000	680,000
11	2,000,000	1,800,000
12	1,600,000	1,800,000
13	1,800,000	1,400,000
14	860,000	1,100,000
15	1,000,000	970,000
16	2,400,000	2,500,000
17	340,000	680,000
18	680,000	910,000
19	460,000	1,400,000
Log average	1,010,000	1,250,000

VI and VII. It is quite evident in Table VI that the direct clump count was less after freezing than before, when the raw milk was placed in the freezing compartment of the household refrigerator. After storage of the raw milk in the household deep freeze, the direct clump count increased over the numbers found before freezing, as shown in Table VII.

The increase in the standard plate count of the raw milk when stored in a household deep freeze and a laboratory ultrafreeze may be explained by the fact that the breaking of bacterial clumps occurs during freezing. This has been confirmed by the results of experimental work shown in Table VII. There was an increase in the clump count after one week of storage of raw milk in the household deep freeze. Pennington (13) reported that there was an increase in the number of bacteria during the storage of milk at a temperature of -1.67°C and -0.55°C. According to her, milk was semi-solid with ice at these temperatures. Some authors (16) have reported that there was considerable increase in the bacterial content when the milk was stored at 0°C. Reed and Reynolds (17) were of the opinion that some species of bacteria grew slowly at first, but made a remarkable growth during continued storage of milk at -1°C.

There seems to be no major change in the psychrophile count during the storage of milk at the three different temperatures for intervals of 24 hours. Similarly the standard plate count was the same as the original count. There may be several explanations for the survival of these organisms at such freezing temperatures. The low temperatures favor the bacterial longevity by diminishing destructive metabolism. It seems that watery foods like milk, freeze in such a way that most of the bacteria are pushed out of the water crystals with other nonaqueous suspended material and remain in this material during freezing without being crushed. On the contrary, in pure water in which the whole mass becomes solidly crystalline, the bacteria have no such protection and are mechanically destroyed between the crystals (6). The faster freezing also aids in the survival of bacteria. The reason for this is unknown (5). At low temperatures, as -20°C, there is no precipitation of coagulable proteins of the bacteria and hence they survive (9). According to some authors (21) the intracellular ice does not form in bacteria because a more lethal effect would be expected at temperatures at which ice would be formed in the cells.

Finally there is a possibility of bacteria becoming dormant during freezing. The methylene blue reduction tests justify this assumption. The results in Tables III, IV and V indicate that certain raw milk samples have longer reduction times after freezing than before. The slower rate of freezing has a more protracting effect on the reduction time of raw milk than the faster rate of freezing. This is indicated by the exposures in the freezing compartment of a household refrigerator and the household deep freeze. Perhaps this is caused by the bacteria entering a dormancy stage during freezing.

The standard plate count remained unaltered, the coliform count decreased and the psychrophile count was not changed, when raw milk was stored in the freezing compartment of the household refrigerator. This can be explained because slower freezing had a more destructive influence on the bacteria than faster freezing. The reduction tests also elucidate this point. When milk was stored in the freezing compartment of the household refrigerator and this storage was continued, bacteria were killed and the reduction time was prolonged. The results in Table IV clarify this contention.

SUMMARY AND CONCLUSIONS

Raw milk samples from different producing farms were used in this study. The samples were transferred to clean, sterile screw capped test tubes which were stored in the freezing compartment of a household refrigerator at -15°C, a household deep freeze at -23°C or a laboratory ultrafreeze at -45°C for seven days. After varying times, the samples were removed, thawed in a water bath at 45°C and plated for the standard plate count, the coliform count and the psychrophile count.

The results indicated that the standard plate count increased during storage of the raw milk in a household deep freeze and a laboratory ultrafreeze. There was no change in the standard plate count when the milk was stored in the freezing compartment of household refrigerator. This rise in count was attributed to the breaking of bacterial clumps when the milk was frozen. The clump breakage was shown by the results of the direct microscopic examination of the milk after storage in the household deep freeze.

The coliform count decreased when the raw milk was stored in the freezing compartment of a household refrigerator and household deep freeze. This was caused by the destructive action of slower freezing on the bacteria in milk. In laboratory ultrafreeze storage, the coliform count was the same as the original count.

The psychrophile count showed a slight rise when the raw milk was placed at the three freezing temperatures. The increase in count was due to the same cause mentioned for the standard plate count.

The methylene blue reduction tests for raw milk samples before and after freezing indicated that frozen storage prolonged the reduction time. Slower freezing in the freezing compartment of a household refrigerator had a greater effect on lengthening the reduction time than the faster freezing in a household deep freeze.

The freezing of milk samples prior to bacteriological analysis is not satisfactory. No doubt there is reduction in bacterial content during the storage of milk in the freezing compartment of a household refrigerator. There is also a subsequent increase in the bacteriological counts when the milk is kept in a household deep freeze and laboratory ultrafreeze. Because of this variation in counts, the freezing of milk samples before bacteriological analysis cannot be recommended.

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APPENDIX

Table VIII. Effect of frozen storage for seven days on the standard bacteriological counts of raw milk (Trial 1)

Sample	Original count	A	B	C
		Standard plate count		
1	230,000	240,000	160,000	210,000
2	2,000,000	380,000	340,000	310,000
3	820,000	600,000	640,000	1,200,000
4	6,000,000	4,000,000	2,000,000	2,100,000
5	10,000	130,000	83,000	1,000
6	2,600,000	2,300,000	560,000	240,000
		Coliform count		
1	3,900	200	200	21,000
2	19,000	15,000	16,000	16,000
3	< 100	1,000	1,500	< 100
4	2,000	37,000	27,000	300
5	< 100	< 100	400	< 100
6	24,000	18,000	28,000	3,300
		Psychrophile count		
1	120,000	370,000	300,000	230,000
2	3,000,000	2,000,000	7,800,000	8,500,000
3	350,000	370,000	480,000	2,900,000
4	1,400,000	2,900,000	700,000	1,300,000
5	5,200	5,600	3,700	4,800
6	2,500,000	3,000,000	2,800,000	3,100,000

A - Freezing compartment of household refrigerator (-15°C)
B - Household deep freeze (-23°C)
C - Laboratory ultrafreeze (-45°C)

Table IX. Effect of frozen storage for seven days on the standard bacteriological counts of raw milk (Trial 2)

Sample	Original count	A	B	C
		Standard plate count		
1	980,000	1,200,000	1,900,000	2,000,000
2	3,900,000	5,400,000	29,000,000	21,000,000
3	3,800,000	6,500,000	5,400,000	5,000,000
4	4,000,000	6,100,000	10,000,000	9,500,000
5	380,000	590,000	540,000	970,000
6	2,500,000	1,700,000	1,900,000	1,700,000
7	230,000	360,000	130,000	220,000
8	130,000	290,000	180,000	200,000
9	820,000	660,000	700,000	900,000
10	17,000,000	8,800,000	21,000,000	26,000,000
		Coliform count		
1	800	2,800	1,900	5,400
2	70,000	14,000	15,000	16,000
3	100	100	< 100	< 100
4	1,700	400	1,400	23,000
5	100	< 100	< 100	< 100
6	9,000	3,700	6,200	6,300
7	< 100	< 100	< 100	< 100
8	70,000	12,000	21,000	9,800
9	1,300	1,900	2,500	12,000
10	100,000	430,000	470,000	320,000
		Psychrophile count		
1	140,000	690,000	1,100,000	1,800,000
2	> 3,000,000	> 3,000,000	> 3,000,000	> 3,000,000
3	2,500,000	14,000,000	12,000,000	15,000,000
4	600,000	2,200,000	16,000,000	16,000,000
5	67,000	122,000	170,000	180,000
6	180,000	154,000	180,000	240,000
7	140,000	420,000	430,000	480,000
8	170,000	250,000	1,000,000	720,000
9	83,000	273,000	460,000	500,000
10	> 3,000,000	> 3,000,000	> 3,000,000	> 3,000,000

A - Freezing compartment of household refrigerator (-15°C)
B - Household deep freeze (-23°C)
C - Laboratory ultrafreeze (-45°C)

Table X. Effect of frozen storage for seven days on the standard bacteriological counts of raw milk (Trial 3)

Sample	Original count	A	B	C
		Standard plate count		
1	840,000	1,200,000	960,000	1,400,000
2	190,000	290,000	210,000	230,000
3	310,000	270,000	200,000	340,000
4	710,000	3,800,000	6,500,000	3,800,000
5	640,000	450,000	490,000	410,000
6	14,000,000	11,000,000	12,000,000	15,000,000
		Coliform count		
1	3,500	3,200	1,500	4,200
2	7,600	1,400	1,000	9,200
3	< 100	< 100	< 100	100
4	14,000	700	1,100	7,200
5	100	< 100	< 100	100
6	300,000	110,000	200,000	220,000
		Psychrophile count		
1	1,000,000	780,000	420,000	1,600,000
2	96,000	14,000	190,000	230,000
3	1,000,000	1,300,000	700,000	2,500,000
4	310,000	240,000	570,000	420,000
5	3,700	5,500	9,300	4,300
6	2,300,000	2,000,000	2,300,000	2,300,000

A - Freezing compartment of household refrigerator (-15°C)
B - Household deep freeze (-23°C)
C - Laboratory ultrafreeze (-45°C)

Table XI. Effect of frozen storage for seven days on the standard bacteriological counts of raw milk (Trial 4)

Sample	Original count	A	B	C
		Standard plate count		
1	970,000	910,000	940,000	1,300,000
2	190,000	730,000	920,000	870,000
3	290,000	1,200,000	1,800,000	1,400,000
4	3,700,000	4,000,000	7,300,000	9,000,000
5	360,000	430,000	540,000	530,000
6	190,000	110,000	160,000	160,000
		Coliform count		
1	1,000	2,900	1,800	9,100
2	27,000	8,100	13,000	13,000
3	$<$ 100	$<$ 100	400	100
4	7,900	1,100	11,000	7,000
5	1,400	100	100	600
6	100	$<$ 100	$<$ 100	100
		Psychrophile count		
1	70,000	120,000	120,000	130,000
2	410,000	1,800,000	3,300,000	4,100,000
3	420,000	2,000,000	1,900,000	2,300,000
4	650,000	400,000	930,000	1,300,000
5	6,000	3,800	6,300	7,000
6	23,000	20,000	76,000	38,000

A - Freezing compartment of household refrigerator (-15°C)
B - Household deep freeze (-23°C)
C - Laboratory ultrafreeze (-45°C)

Table XII. Effect of frozen storage for seven days on the standard bacteriological counts of raw milk (Trial 5)

Sample	Original count	A	B	C
		Standard plate count		
1	1,300,000	880,000	680,000	1,100,000
2	230,000	270,000	230,000	320,000
3	430,000	360,000	440,000	480,000
4	5,500,000	6,500,000	8,100,000	7,600,000
5	110,000	360,000	390,000	410,000
6	530,000	580,000	670,000	560,000
		Coliform count		
1	6,500	4,000	7,300	4,700
2	5,200	3,500	1,100	6,400
3	200	200	< 100	< 100
4	7,500	2,900	3,000	12,000
5	2,300	1,400	700	5,600
6	200	< 100	< 100	200
		Psychrophile count		
1	350,000	150,000	210,000	320,000
2	23,000	97,000	79,000	61,000
3	130,000	210,000	240,000	370,000
4	630,000	470,000	520,000	460,000
5	97,000	100,000	120,000	100,000
6	19,000	17,000	19,000	19,000

A - Freezing compartment of household refrigerator (-15°C)
B - Household deep freeze (-23°C)
C - Laboratory ultrafreeze (-45°C)

Table XIII. Effect of frozen storage at intervals of 24 hours on the standard bacteriological counts of raw milk kept in the freezing compartment of a household refrigerator (-15°C)
(Count expressed in thousands)

Sample	Original count	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs	144 hrs	168 hrs
				Standard plate count				
1	510	480	460	480	370	410	260	440
2	550	1,100	1,000	1,500	1,500	1,400	1,000	1,400
3	> 30,000	> 30,000	> 30,000	> 30,000	> 30,000	> 30,000	> 30,000	> 30,000
4	5,700	4,100	5,300	4,100	2,800	3,500	3,200	1,800
5	3,500	1,400	1,500	1,400	1,400	1,600	1,400	1,300
6	1,200	930	920	780	990	1,200	960	860
				Coliform count				
1	68	39	34	7	15	21	25	9.4
2	19	12	11	5.7	9.2	9.2	9.6	9.5
3	430	370	310	130	390	300	340	130
4	15	8	4.1	4	1.2	1.3	1.1	.7
5	< .1	.2	.1	.1	< .1	.1	< .1	< .1
6	2.5	.2	< .1	< .1	< .1	< .1	.1	< .1
				Psychrophile count				
1	1,500	1,900	1,200	2,000	690	1,400	550	960
2	26	36	42	50	51	96	130	49
3	> 3,000	> 3,000	> 3,000	> 3,000	> 3,000	> 3,000	> 3,000	> 3,000
4	490	770	650	460	400	730	1,200	460
5	35	32	49	42	46	48	51	51
6	370	97	190	180	110	90	99	100

Table XIV. Effect of frozen storage at intervals of 24 hours on the standard bacteriological counts of raw milk in a household deep freeze (-23°C) (Count expressed in thousands)

Sample	Original count	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs	144 hrs	168 hrs
				Standard plate count				
1	1,700	2,500	1,500	1,900	1,100	1,800	1,300	1,900
2	740	720	780	740	420	640	530	690
3	650	890	620	640	630	610	510	620
4	6,800	7,300	4,100	5,800	5,100	5,600	5,800	7,200
5	1,000	1,200	1,100	1,100	1,000	490	900	1,200
6	950	780	1,000	1,000	1,000	700	460	910
				Coliform count				
1	9.7	8.4	8.9	1.8	6.2	4.3	4.2	4.8
2	8	4.2	4.4	2.6	4.2	3.2	3.6	5.2
3	.5	.4	.4	.2	.5	.3	.3	.2
4	10	8.3	8.9	4	8.5	7.7	9.9	9.8
5	.8	.3	.8	< .1	< .1	.2	< .1	.5
6	2.5	.2	.2	.2	.3	.2	.4	.2
				Psychrophile count				
1	290	580	400	260	230	450	240	510
2	22	45	32	50	44	37	38	47
3	100	420	110	160	110	200	93	150
4	340	1,200	540	1,200	1,000	720	780	750
5	340	370	330	440	300	270	270	270
6	52	82	43	59	52	51	25	43

Table XV. Effect of frozen storage at intervals of 24 hours on the standard bacteriological counts of raw milk in a laboratory ultrafreeze (-45°C) (Count expressed in thousands)

Sample	Original count	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs	144 hrs	168 hrs
				Standard plate count				
1	680	800	530	900	810	630	680	1,400
2	1,500	1,800	1,700	1,600	1,400	1,800	1,300	1,700
3	820	1,100	1,100	1,300	840	1,000	870	1,200
4	340	410	200	370	300	240	240	180
5	1,700	2,400	2,300	2,000	1,800	2,300	2,100	1,800
6	3,700	2,900	4,000	4,200	3,000	3,300	4,400	3,800
				Coliform count				
1	> 30	> 30	> 30	> 30	> 30	> 30	> 30	> 30
2	21	29	27	29	28	29	25	25
3	.6	2.7	3	2.1	1.8	2.5	2.4	1.1
4	8.1	2.6	1.6	5	5.6	3.6	5.6	5
5	< .1	.1	.2	.1	.1	.1	.4	.1
6	.1	< .1	< .1	< .1	.2	< .1	< .1	< .1
				Psychrophile count				
1	> 3,000	> 3,000	> 3,000	> 3,000	> 3,000	> 3,000	> 3,000	> 3,000
2	170	160	100	120	130	120	96	110
3	2,400	3,300	2,500	3,200	1,300	1,800	1,700	1,100
4	180	470	230	280	440	260	280	180
5	460	800	850	300	610	420	370	490
6	100	91	86	92	84	72	92	89