A BACTERIOLOGICAL SURVEY OF THE PRACTICES USED BY A LOCAL CREAMERY IN HANDLING AND MANUFACTURING DAIRY PRODUCTS

Submitted to the

DAIRY HUSBANDRY DEPARTMENT

VIRGINIA POLYTECHNIC INSTITUTE

BLACKSBURG, VIRGINIA

AS A

MINOR THESIS

FOR THE

PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF MASTER OF SCIENCE

Submitted by:

HORACE E. dward ALPHIN

BLACKSBURG, VIRGINIA

MAY 15, 1935

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INTRODUCTION

Modern dairy practice is subject to many regulations designed to prevent a product being delivered to the consumer in an unsanitary condition. Large numbers of bacteria do not necessarily condemn food products, but usually an excessive number of bacteria is associated with inferior materials and unsanitary practices. Definite legal limits of the number of bacteria permissible in the milk supply have been established by the health authorities, and the burden rests primarily upon the retailer to observe them.

If the distributor is to maintain the highest standards of keeping quality it is essential for him to have some knowledge of the bacterial action going on in the pasteurizing, cooling, and bottling processes. The main factor in protecting the keeping quality of milk becomes one of preventing the entrance of bacteria, of destroying them after they enter, or of keeping them at a low temperature to check their growth. The first interest of the distributor should

be to restrict the number of bacteria getting into the milk, so long as is practical with the expense involved. The problem of plant contamination can be most economically approached by making an extensive survey of the product from the time it enters the creamery until it is ready for delivery.

The survey was made to show the influence that the various manufacturing operations exert on the bacterial content of fluid milk, butter, and ice cream. It is reasonable to suppose that some parts of the processing equipment are greater bacterial contributors than others. If the plant operator had some knowledge of the most abundant sources of contamination, he could make an effort to keep the number of bacteria to a minimum, by exercising greater care in cleaning and sterilizing the utensils.

Any weak or unsatisfactory practices found in the plant will be investigated and a probable means of control will be sought.

REVIEW OF LITERATURE

A review of available literature seems to indicate that little attention has been given to either the absolute or relative importance of any given dairy operation. The surveys that are most frequently made are those conducted to determine the spread of epidemics which are often attributed to the milk supply.

In 1890, Backhaus and Cronheim[1] observed that milk passing over a certain cooler raised its germ content from 11,500 to 33,000 bacteria per cubic centimeter. In 1904, Bergey[2] concluded from his studies on milk contamination that the greater portion of the bacteria with which milk becomes contaminated is derived from the utensils.

Russel and Hoffman[3] found that when milk bottles were washed, steamed and allowed to stand twenty-four hours, the bacteria multiplied in the water resulting from the condensation of the steam. The studies of Prucha and Wetter[4] reveal the fact that it is possible to produce milk with a germ content of less than 10,000 bacteria per cubic centimeter, when the utensils are properly cleaned. Conn[5] stated that the vessels in which milk and cream are to be kept are great sources of bacterial contamination. The small germs gather upon the sides and joints of the vessel and develop in minute

portions of milk which are difficult to destroy by washing.

Harding[6] makes the following statement as to the purpose of a similar investigation: "When health officials, failing to find other means of characterizing sanitary milk, undertook to specify the conditions under which it should be produced, they were confronted with almost total lack of detail information upon this subject."

PRESENT STUDY

The milk and cream selected for this investigation was brought into the V. P. I. Creamery from the college herd and from dairy farmers in the vicinity of Blacksburg. The V. P. I. Creamery was chosen for this work, because to our minds it represents an average up-to-date plant. The output is not as great as that of large plants, nor is the output so small as to exclude piping and other necessary machinery not found in small plants. In order for this survey to represent average conditions every effort was made on the part of the author to collect the samples when normal practices were being followed. All workers in the plant were not aware that any attempt was being made to check the sanitary quality of their work. Samples were collected at

random, so as not to arouse their attention and cause them to take unusual sanitary precautions.

The aim of this study is to follow the milk and cream from the time it arrives on the creamery platform until it is ready for delivery. The survey was divided into four parts and samples were collected throughout the processing of fluid milk, butter, and ice cream to determine the amount of contamination taking place. The cans and bottles used in the creamery were studied to find the number of bacteria left in these utensils after being sterilized. The conditions were entirely different for the different products to be processed. However, the same type of sterilization, pasteurization, and cooling were employed throughout the plant. The 40 quart cans were used exclusively and were always washed and steamed before sending to the farms.

PART I. DETERMINING THE INFLUENCE OF THE MILK CANS AND BOTTLES ON THE BACTERIAL CONTENT

Method of Cleaning the Cans: The cans used in this study were washed in a large vat, containing 60 gallons of a one per cent solution of washing powder. Each can was scrubbed thoroughly with a brush, to deflocculate and remove any filth clinging to the can.

Usually 25 to 35 cans were washed in the same vat of wash water. Immediately following the washing operation the cans were inverted over a steam jet for a period of ten seconds to one-half minute. There was some variation regarding the time that each can was steamed. The period of actual steaming was checked on each can unknown to the operator of the apparatus. As soon as the cans were sterilized they were stood upright with the lids partly on so as to facilitate rapid drying. All cans used in this Dairy are of the 40-quart size.

Counting the Number of Bacteria: The method used to determine the number of bacteria consisted in pouring one liter of sterile water into each can and shaking thoroughly. An aliquot portion of this water was taken to find the number of bacteria. The plate method was used for counting the number of bacteria in these samples. Since the count was always relatively low it was seldom necessary to make more than two dilutions for each sample. The procedure used in counting these samples followed the method outlined by the American Public Health Association for milk analysis.

Results Obtained: In this study 31 cans were used to determine the efficiency of sterilization. When cans were washed and steamed the lowest count was 4 and the highest count was 5,455 bacteria per cubic centimeter. Milk handled in these cans would be affected very little,

since the milk poured in the cans would usually be above 6,000 bacteria per cc.

The highest number of bacteria per cubic centimeter of wash water was 3,693,000 and the lowest was 1,040,000. Such high counts are due to the fact that a small amount of milk remained in the can after pouring and was mixed with the wash water on cleaning.

As indicated in Table 1, the cans that were steamed for 12 seconds only contained higher numbers of bacteria than those steamed for 20 to 25 seconds. The unsteamed cans always had a bacterial count greater than 30,000 bacteria per cubic centimeter.

TABLE 1. Number of Bacteria in Freshly Washed Cans:

Can Number	Treatment of Cans	No. of bact. per cc. of rinse water
1	Cans washed and steamed 12 seconds	5,025
2	" " " " " "	4,100
3	" " " " " "	3,420
4	" " " " " "	5,455
5	" " " " " "	5,178
6	" " " " " "	2,810
7	" " " " " "	4,365
8	" " " " " "	3,600
9	" " " " " "	5,400
10	" " " " " "	5,360
11	" " " " " "	1,900

Table 1 (cont'd)

Can Number	Treatment of Cans	No. of bact. per cc. of rinse water
12	Cans washed and steamed 12 seconds	2,100
13	" " " " " "	4,320
14	" " " " " "	210
15	Cans washed and steamed 17 seconds	380
16	" " " " " "	400
17	" " " " " "	100
18	" " " " " "	50
19	" " " " " "	210
20	Cans washed and steamed 20 seconds	15
21	" " " " " "	50
22	" " " " " "	10
23	" " " " " "	12
24	" " " " " "	30
25	" " " " " "	14
26	" " " " " "	8
27	Cans washed and steamed 25 seconds	16
28	" " " " " "	4
29	" " " " " "	10
30	" " " " " "	5
31	" " " " " "	7

If all the bacteria removed from the 31 ten-gallon cans, listed in table one were added to 310 gallons of milk (the total capacity of the cans), its bacterial content would be increased by 45 bacteria per cubic

centimeter.

An examination of table 1 shows that nearly every can had a different bacterial content. The closer correlation existed between those cans which were steamed for twenty seconds or more. When cans were steamed less than twenty seconds the number of bacteria was always higher than those cans steamed more than twenty seconds, except for one can.

Method of Cleaning Bottles

The bottles are washed with a small brush type machine. The rotary brush is driven by steam and is mounted about 18 inches above the tank in which the bottles are soaked. This washing vat contains about twenty gallons of a one per cent solution of washing powders. When the dirt or filth was completely removed from the bottles they were inverted in a bottle rack and placed over a water jet, to facilitate thorough rinsing. As soon as the rinsing operation was completed the same bottles were subject to a high pressure of steam, while in the same position. The bottles were then racked and allowed to stand 18 hours before being filled with milk. The quart bottles used in this experiment were always rinsed with 100 cc. of sterile water, and an aliquot portion of this sample was analyzed for the number of bacteria. The procedure is similar to that used in

determining the bacterial content of the milk cans, except 100 cc. of sterile water is used for rinsing instead of 1000 cc. When the bottles were re-rinsed the same amount of rinse water is used, and the number of bacteria are determined by the method outlined by the American Public Health Association for milk analysis.

Results Secured: The following table will serve to show the bacterial content of freshly washed and sterilized bottles.

TABLE 2. The Number of Bacteria Found in Milk Bottles which have been Sterilized

No. of bottle	Capacity of bottle	No. of Bact. removed by rinsing with 100 cc. sterile water		Total No. of bacteria removed
		1st rinsing	2nd rinsing	
1	one quart	30	9	3,900
2	"	80	20	10,000
3	"	105	26	13,100
4	"	15	3	1,800
5	"	12	2	1,400
6	"	28	4	3,200
7	"	70	8	7,800
8	"	110	17	12,700
9	"	8	2	1,000
10	"	3	1	400
11	"	16	4	2,000
12	"	20	5	2,500

Table 2 (cont'd)

No. of bottle	Capacity of bottle	No. of Bact. removed by rinsing with 100 cc. sterile water		Total No. of bacteria removed	Per cc milk
		1st rinsing	2nd rinsing		
13	one pint	12	4	1,600	1.6
14	"	19	5	2,400	2.4
15	"	37	10	4,700	4.7
16	"	10	2	1,200	1
17	"	14	3	1,700	1.7
18	"	9	1	1,000	1
19	"	11	2	1,300	1
20	"	9	0	900	.9
21	"	2	0	200	.2
22	"	0	0	0	0
23	"	8	4	1,200	1.
24	"	4	1	500	.5
25	"	23	3	2,600	2.6

In table 2 we find that the greatest number of bacteria per cubic centimeter was 110 and the lowest number was 0. In every case the second rinse water was lower than the first. On the average, the number of bacteria found in the milk bottles was higher than the number of bacteria found in the 40-quart milk cans. If both cans and bottles were filled with milk of the same bacterial content, it would be found that the number of bacteria per cc. of milk would be higher in the case of the bottles.

PART II. DETERMINING THE SOURCE OF BACTERIA AND THE METHOD OF CONTAMINATION DURING THE PROCESSING OF FLUID MILK

The market milk at the V. P. I. Creamery is processed by two different methods.

The milk going out on the route is produced by the college herd and hereafter designated as milk "A". As soon as this milk arrived at the creamery it was taken to the second floor by an elevator and poured directly into the pasteurizer. After pasteurization the milk is piped to the cooler and from there into the bottling machine.

Part of the milk used by the dining hall was brought to the creamery by farmers from the vicinity of Blacksburg, and will be known hereafter as milk "B". This milk was poured into the receiving vat and pumped to the second floor, into a large holding vat. From there the milk was pumped through a heater and filter into the pasteurizer. After pasteurization, the milk was allowed to run over the cooler and then poured into ten-gallon milk cans which had been thoroughly sterilized.

Collecting Samples: All samples were collected in sterile, 60 cc. bottles, which were stoppered and immediately placed in the anti-hardening room, which had a temperature of 32 degrees Fahrenheit. The samples were

kept at this temperature until they were ready to be tested for the number of bacteria.

Method of Counting Bacteria: In this experiment each sample of milk was tested for the number of bacteria, as far as possible, by three methods of bacterial analysis: the plate count, the microscopic count, and the methylene blue reduction test. The plate count was found to be the most dependable, since it was the only test that could be used during the entire survey. It was not practical to use the microscopic count after pasteurization, as the dead organism would be revealed as well as the active ones. The methylene blue reduction test was not as accurate for testing milk after it had passed over the cooler, because the percentage of dissolved oxygen was appreciably increased.

The plate count was made according to the directions outlined by the A. P. H. A. for the analysis of milk. Dilutions of 1-100 and 1-1000 were used and plated out on nutrient agar. All plates were incubated at 37 degrees Centigrade for 48 hours.

In setting up the methylene blue reduction test, ten cubic centimeters of milk were added to a sterile test tube containing 1 cc. of a 1-20,000 solution of methylene blue. The tubes were incubated in a water bath at a temperature of 37 degrees Centigrade until reduction took place.

The microscopic count was carried out according to the directions given in technical bulletin number 49 of the Geneva, N. Y., Experiment Station.

Processing of Milk "A"

The first sample was taken after the milk was thoroughly mixed in the receiving vat. It was not practical to take a sample of milk from each can before emptying, since the quantity and quality of the milk in each can was quite variable. From the receiving vat the milk was pumped to the second floor by a piston type pump where it emptied into a large, glass-lined holding vat. The second sample was collected as the milk entered the holding vat. The length of time that the milk remained in the holding vat varied from five minutes to one hour. When the milk was ready to be pumped further, a third sample was collected in the vat, after thorough mixing with an electric agitator. By the use of a small centrifugal pump the milk was then forced through the heater and filter, into the pasteurizer. The fourth sample was taken as the milk entered the pasteurizer. From the holding vat to the pasteurizer the milk was kept in pipes and never exposed to the air. The last operation increased the temperature of the milk on an average of 34 degrees Fahrenheit.

The fifth sample was taken when the pasteurizer was full. The coil in the pasteurizer was started before

collecting the sample, so that the milk would be thoroughly mixed. The milk was pasteurized by heating to a temperature of 145 degrees Fahrenheit, and held at that temperature for twenty minutes. The self-recording thermometer was not used every time the milk was pasteurized. After pasteurization, while the milk was still hot, it was allowed to run over the cooler by gravity. The cooler consisted of two sections, which were intended for water and brine. During this survey, water was the only cooling medium. The milk was cooled down to a temperature of about 65 degrees Fahrenheit; this temperature fluctuated due to the size of the film of milk passing over the cooler. From the cooler the milk was allowed to flow into ten-gallon cans, which had been thoroughly sterilized. The milk was then ready to be delivered to the dining hall.

Cleaning Utensils: After each day's run, the piping and machinery was first rinsed with cold water and then with boiling hot water. Every part of the equipment was thoroughly scrubbed with a brush, using high grade washing powders. The piping was always dissembled so as not to allow any sediment to collect in the crevices where the pipes were fastened together. After scouring, the pipes and machinery were again rinsed with boiling water. All of the piping was thoroughly steamed, then allowed to drain over night, and assembled just prior to using.

Results of Study: This part of the survey consisted in following seven batches of milk from the time it was poured into the receiving vat until it was ready to be delivered. Eight samples were collected at regular intervals during the process, making a total of 56 samples for this part of the survey.

The following tables will serve to indicate the bacterial changes which took place during the various operations in the processing.

TABLE 3. Influence of Processing Market Milk on the Bacterial Content

Sample No.	Place where samples were taken	Plate Count		Microscopic count	Methylene Blue Red. & test
		No. of bact. per cc. milk	Increase in bact. per cc. milk	No. of Bacteria	Hours: Minutes
1 A	Sample taken in emptying vat	10,375		600,000	8:30
1 B	Sample taken from pipe leading into holding tank	23,012	12,637	660,000	8:00
1 C	Sample taken in holding tank	30,794	7,782	850,000	7:30
1 D	Sample taken after going thru heater and filter	41,451	10,637	1,080,000	7:00
1 E	Sample taken in pasteurizer when 1/3 full	47,750	6,319	1,100,000	5:15
1 F	Sample taken in pasteurizer when full	84,300	36,550	1,225,000	2:05
1 G	Sample taken after pasteurization	18,187	-66,113		9:00
1 H	Sample taken after cooling	22,512	4,325		9:00
1 I	Sample taken in cans	31,634	9,122		9:00

TABLE 4. Influence of Processing Market Milk on the Bacterial Content

Sample No.	Place where sample was taken	Plate Count		Microscopic count	Methylene Blue Red. test
		No. Bact.	Increase		
2 A	Sample taken in emptying vat	81,820		310,500	4:00
2 B	Sample taken from pipe leading into holding tank	87,675	5,855	540,000	2:00
2 C	Sample taken in holding tank	135,700	48,025	690,000	1:45
2 D	Sample taken after going thru heater and filter	220,500	84,800	1,050,700	1:20
2 E	Sample taken in pasteurizer when full	227,805	7,305	1,175,000	1:15
2 F	Sample taken after pasteurization	7,600	- 220,205		8:30
2 G	Sample taken after cooling	10,240	2,640		7:45
2 H	Sample taken in cans	12,725	2,485		7:45

TABLE 5. Influence of Processing Market Milk on the Bacterial Content

Sample No.	Place where sample was taken	Plate Count		Microscopic count	Methylene Blue Red. test
		No. of bact. per cc. milk	Increase in bact. per cc. milk	No. of Bacteria	Hours: Minutes
3 A	Sample taken in emptying vat	3,200		900,000	5:45
3 B	Sample taken from pipe leading to holding tank	6,125	2,925	1,095,000	4:40
3 C	Sample taken in holding tank	14,170	8,045	2,400,000	3:30
3 D	Sample taken after going through heater and filter	36,490	22,320	2,700,000	2:30
3 E	Sample taken in pasteurizer when full	43,525	7,035	3,100,000	2:00
3 F	Sample taken after pasteurization	12,250	- 31,275		9:15
3 G	Sample taken after cooling	17,300			8:30
3 H	Sample taken in cans	19,150	1,850		8:15

TABLE 6. Influence of Processing Market Milk on the Bacterial Content

Sample No.	Place where sample was taken	Plate Count		Microscopic count	Methylene Blue Red. test
		No. Bact.	Increase		
4 A	Sample taken in emptying vat	67,575		320,000	3:50
4 B	Sample taken from pipe leading to holding tank	122,970	55,395	495,800	2:45
4 C	Sample taken in holding tank	105,850	- 17,120	503,700	2:30
4 D	Sample taken after going thru heater and filter	131,850	26,000	518,605	2:15
4 E	Sample taken in pasteurizer when full	134,950	3,055	650,500	2:15
4 F	Sample taken after pasteurization	1,180	-133,725		10:30
4 G	Sample taken after cooling	10,550	9,370		10:00
4 H	Sample taken in cans	11,100	550		10:00

TABLE 7. Influence of Processing Market Milk on the Bacterial Content

Sample No.	Place where sample was taken	Plate Count		Microscopic count	Methylene Blue Red. test
		No. of bact. per cc. milk	Increase in bact. per cc. milk	No. of Bacteria	Hours: Minutes
5 A	Sample taken in emptying vat	17,670		240,000	5:30
5 B	Sample taken from pipe leading to holding tank	43,250	25,580	282,000	4:00
5 C	Sample taken in holding tank	62,680	19,350	330,400	3:30
5 D	Sample taken after going through heater and filter	106,060	43,380	440,000	2:30
5 E	Sample taken in pasteurizer when full	116,000	9,940	480,000	2:00
5 F	Sample taken after pasteur'n	1,275	- 114,725		8:45
5 G	Sample taken after cooling	6,200	4,925		7:50
5 H	Sample taken in cans	8,800	2,600		7:40

TABLE 8. Influence of Processing Market Milk on the Bacterial Content

Sample No.	Place where sample was taken	Plate count		Microscopic count	Methylene Blue Red. test
		No. Bact.	Increase		
6 A	Sample taken in emptying vat	10,320		2,400,000	6:40
6 B	Sample taken from pipe leading to holding tank	13,740	3,420	2,520,000	6:10
6 C	Sample taken after going through heater and filter	23,250	9,510	2,640,000	5:00
6 D	Sample taken in pasteurizer when full	36,150	12,900	3,000,000	4:15
6 E	Sample taken after pasteurization	15,725	- 20,325		8:00
6 F	Sample taken after cooling	22,200	6,475		7:30
6 G	Sample taken in cans	32,075	9,875		7:20

TABLE 9. Influence of Processing Market Milk on the Bacterial Content

Sample No.	Place where samples were taken	Plate Count		Microscopic count	Methylene Blue Red. test
		No. of bact. per cc. milk	Increase in bact. per cc. milk	No. of Bacteria	Hours: Minutes
7 A	Sample taken in emptying vat	226,000		2,400,000	3:45
7 B	Sample taken from pipe leading to holding tank	241,000	15,000	2,600,000	3:15
7 C	Sample taken in holding tank	244,050	2,950	& 2,800,000	3:15
7 D	Sample taken after going through heater and filter	253,000	8,950	3,300,000	3:10
7 E	Sample taken in pasteurizer when full	257,700	4,700	3,600,000	3:00
7 F	Sample taken after pasteurization	950	- 256,750		10:15
7 G	Sample taken after cooling	1,125	175		10:10
7 H	Sample taken in cans	1,300	175		10:10

Influence of Pumping on the Bacterial Content: In every case during this survey there was always an increase in the bacterial content, as a result of the first pumping. This increase was quite variable; the smallest increase in the bacterial content per cubic centimeter of milk was 2,925 and the largest increase was 55,395. Since this increase seems unusually large it occurred to the author that a portion of the increase, as measured by the plate count was due, at least in part, to agitation and not altogether to contamination. A study was made to determine the effect of agitation on the number of bacteria, when measured by the plate count. Results of this study will be found on pages 16 to 19, inclusive.

The pump used in this experiment was a piston type pump, driven by a one-half horse power motor.

Collective Influence of Heater and Filter: In every case the filtering and heating operations added materially to the bacterial content. The milk did not flow through the heater and filter by gravity, but was forced through by a centrifugal pump, which probably assisted in breaking up clumps and chains of bacteria. Also, the heater raised the temperature of the milk to about 103 degrees Fahrenheit, which temperature was more favorable to bacterial growth. The cleaning procedure is somewhat more difficult with the heater than any other piece of machinery. Large films of sediment often

collect on the walls of the heater, which act to re-contaminate the subsequent lots of milk. It is thought that more contamination takes place during this operation than any other operation in the processing of milk. Partial proof for this may be found in table 9, where the heater and filter only added 8,950 bacteria per cc. of milk. This improvement in quality resulted in a thorough cleaning of the heater. Since this operation always gave a high increase in the number of bacteria, the plant operator was advised to give special attention to the cleaning of the heater. Because of the change in practice the relatively small increase in bacterial content, given in table 9, was obtained.

During the processing of seven lots of milk, the greatest increase in bacterial content, due to milk passing through the heater and filter, was 84,800, and the smallest increase was 8,950 bacteria per cc. of milk.

Influence of Pasteurization: There was in each of the seven lots of milk a very marked decrease in the bacterial content due to pasteurization. The efficiency of pasteurization varied from 99.6 to 56.2 per cent. This great variation was attributed to the different periods for which the milk was held at the pasteurization temperature. In no case could this increase in bacterial numbers be attributed to thermophylic bacteria. However, there is a possibility that the thermophylic bacteria

which frequented the milk in this survey, varied slightly for the different lots of milk.

The greatest increase in bacterial content per cubic centimeter of milk occurred from the time the milk was poured in the pasteurizer until it was full. Without a doubt, a greater part of the increase came from a poorer quality of milk which passed through the same system subsequent to the first emptying vat full of milk. It took about four vats of the milk to fill the pasteurizer. A small portion of the increase in the number of bacteria was due to the growth which took place from the time the first milk entered the pasteurizer until pasteurization was started.

Influence of the Cooler: The increase in the bacterial content due to the cooler was relatively consistent, since the same cleaning routine was followed daily. At no time during the survey was any cooling medium except water used to cool the milk. However, the cooler was installed with a section connected to a brine tank, but advantage was never taken of this opportunity to cool the milk to a low temperature. Hence, during the period of delivery the milk was at a temperature which was favorable to bacterial growth.

Processing of Milk "B"

The processing of milk "B" did not involve as many operations as milk "A", since the milk was poured directly into the pasteurizer. A reduction in the processing operations was accompanied by a decrease in contamination. By this method, it was possible to process a milk with less contamination because the elimination of extra machinery resulted in maintaining a product with a minimum number of bacteria. All milk used in this process was produced by the college herd. This milk was bottled and delivered to customers in the city of Blacksburg. Usually the milk was bottled and ready for delivery within an hour and a half after the processing was started.

Collecting Samples: In this part of the study only five samples were taken from each lot of milk to determine the influence of the various operations on the bacterial content. The first sample was collected immediately after pouring all of the milk into the pasteurizer. To insure uniformity in mixing, the coils in the pasteurizer were allowed to run for several minutes before the sample was taken.

The second sample was collected directly from the pasteurizer after heating for thirty minutes. In collecting this sample a sterile pipette was always used. As soon as the milk was allowed to pass over the cooler the third sample was taken. This is the same cooler that

was used in cooling the milk processed by method "A". Since milk "B" was the first to be processed, it was allowed to pass over the cooler first.

The fourth sample was taken from the tank or the bottle filler with a sterile pipette. The milk was thoroughly stirred to get a uniform distribution of bacteria as well as butterfat, before the sample was taken. The fifth sample was taken from quart bottles which had been automatically capped. Three samples were taken from each of three bottles and the average number was accepted as the bacterial count for the fifth sample. This was done with the idea of eliminating the possibility of excess contamination due to any one valve on the bottle filler.

Results Obtained: The opportunity for contamination was brought to a minimum when the milk was processed by this method. The amount of equipment was very small and only about fifteen feet of piping was used in carrying the milk from the pasteurizer to the bottling machine.

This part of the survey consisted in collecting samples on five lots of milk. Five samples were taken at various intervals from the time the milk was poured in the pasteurizer until it was bottled and ready for delivery.

All changes which took place during the various steps will be given in the following tables.

TABLE 10. Influence of the Various Operations on the Bacterial Content

Sample No.	Place where sample was taken	Plate Count		Microscopic count	Methylene Blue Red. test
		No. of bact. per cc. milk	Increase in no. of Bact.	No. of Bacteria	Hours: Minutes
8 A	Sample taken in pasteurizer when full	5,850		890,000	8:45
8 B	Sample taken after pasteruization	1,110	- 4,740		8:35
8 C	Sample taken after cooling	6,740	5,630		7:50
8 D	Sample taken in bottling machine	8,395	1,655		7:10
8 E	Sample taken in bottles	10,105	1,710		6:15

TABLE 11. Influence of the Various Operations on the Bacterial Content

Sample No.	Place where sample was taken	Plate Count		Microscopic count	Methylene Blue Red. test
		No. Bact.	Increase		
9 A	Sample taken in pasteurizer when full	5,800		180,000	6:15
9 B	Sample taken after pasteurization	250	- 5,550		9:25
9 C	Sample taken after cooling	1,725	1,475		9:15
9 D	Sample taken in bottling machine	8,770	7,045		9:15
9 E	Sample taken in quart bottles	9,100	330		9:00

TABLE 12, Influence of the Various Operations on the Bacterial Content

Sample No.	Place where sample was taken	Plate Count		Microscopic count	Methylene Blue Red. test
		No. of bact. per cc. milk	Increase in no. of Bact.	No. of Bacteria	Hours: Minutes
10 A	Sample taken in pasteurizer when full	9,110		2,150,000	5:45
10 B	Sample taken after pasteurization	250	- 8,905		6:20
10 C	Sample taken after cooling	9,300	9,095		6:20
10 D	Sample taken in bottling machine	28,150	18,850		6:00
10 E	Sample taken in quart bottles	31,200	3,050		5:15

TABLE 13. Influence of the Various Operations on the Bacterial Content

Sample No.	Place where sample was taken	Plate Count		Microscopic count	Methylene Blue Red. test
		No. Bact.	Increase		
11 A	Sample taken in pasteurizer when full	5,850		890,000	8:45
11 B	Sample taken after pasteurization	1,110	- 4,740		8:55
11 C	Sample taken after cooling	6,740	5,630		7:50
11 D	Sample taken in bottling machine	8,395	1,655		7:10
11 E	Sample taken in quart bottles	10,105	1,710		6:15

TABLE 14. Influence of the Various Operations on the Bacterial Content

Sample No.	Place where sample was taken	Plate Count		Microscopic count	Methylene Blue Red. test
		No. of bact. per cc. milk	Increase in no. of Bact.	No. of Bacteria	Hours: Minutes
12 A	Sample taken in pasteurizer when full	34,500		210,000	5:40
12 B	Sample taken after	175	- 34,325		9:00
12 C	Sample taken after cooling	5,260	5,085		9:00
12 D	Sample taken in bottling machine	7,570	2,310		9:00
12 E	Sample taken in quart bottles	7,800	230		9:00

Influence of the Pasteurizer: By studying the figures presented in tables 10 to 14, it is found that in every case the bacterial content was considerably reduced by pasteurization. This pasteurizer was not equipped with a self-recording, dial thermometer, and the temperature was controlled by close observation, using a floating type thermometer. After pasteurization, the milk was never allowed to cool down, but passed over the cooler immediately. In the five lots of milk the highest number of bacteria which escaped pasteurization was 1,110 and the lowest was 175 bacteria per cc. of milk. Usually the poorest quality of raw milk showed the most complete destruction of bacterial growth, during the heating process.

Influence of Cooler: The milk started over the cooler at a temperature of 140 degrees Fahrenheit and was cooled to about 65 degrees Fahrenheit before being bottled. In every case the cooler increased the bacterial content. The greatest increase was 9,095 and the smallest increase was 1,100 bacteria per cc. of milk. It is thought that a small portion of the increase came from the atmosphere in which the cooler was operating.

Influence of the Bottle Filler: The bottle filler used in the plant was of the rotary type and

and could be adjusted for any size bottles. It consisted of a tank and six valves, each valve having a stem, a sleeve, an air tube, a wire coil spring, and a rubber washer.

The tank and all parts were scrubbed thoroughly with a brush and washing powders (tri-sodium phosphate), then rinsed with a hose. The bottle filler was not steamed but was always washed with boiling hot water and allowed to stand sixteen hours before use.

There was always a slight contribution of bacteria due to contamination in the bottle filler. It is believed that the greatest contamination took place in the valves since they were the hardest to clean, and are more likely to collect filth. The largest number of bacteria added to the milk by the bottle filler, in any case, was 18,850 and the smallest number of bacteria was 1,655 per cc. of milk.

Collective Influence of the Utensils: The milk was processed, bottled and ready for delivery within two and a half hours after it was brought to the creamery, in the raw state.

In table 9 it will be noted that the bacterial contamination was much reduced. This improvement is a result of a thorough cleaning of all utensils, which was followed by a thorough steaming of all utensils where feasible. These results show that when the utensils

are thoroughly cleaned, milk of high quality can be produced without difficulty.

After making a comprehensive study of the results of this experiment, it was found that the collective influence of the heater and filter are the greatest source of contamination in the plant. The increase in the number of bacteria during this operation is probably due to a breaking up of the clumps of bacteria and also the fostering of bacterial growth, by a favorable temperature.

Effect of Agitation

There was always a distinct increase in the bacterial content of milk from the time it left the weighing vat until it reached the pasteurizer. An effort to find this variation resulted in an inspection of the various operations. It is thought that this increase is not entirely due to contamination but rather it is due to a breaking up of the clumps of bacteria. The segregation of such clumps give rise to smaller clumps, each of which produce a colony on the agar plate.

With this thought in mind an investigation was made to see if a ratio could be found whereby it would give the degree of breaking up of bacteria which usually frequent market milk. This ratio was ascertained by comparing shaken and unshaken samples of milk.

The plan of approach to determine the effect

of agitation consisted in taking a sample of market milk produced in the normal way, and pouring 35 cc. of this sample into a dilution bottle. This sample of milk was then plated out after shaking a definite number of strokes. Each stroke consisted in shaking the milk back and forth, through a distance of eighteen inches. After each interval of shaking, one cc. of the milk was pipetted into a dilution bottle containing 99 cc. of sterile water, thus making a 1-100 dilution. The dilution bottles were always shaken the same number of strokes as the original one cc. of milk was shaken. Each dilution resulting from a definite number of strokes was plated out in triplicate to find the number of colonies per cc. of milk.

The number of colonies developing from four samples of milk, with increasing amounts of agitation, are listed in tables 15 to 18, inclusive.

Results Secured:

TABLE 15. Effect of Agitation on the Bacterial Content of Milk

Amount of Shaking	PLATE COUNT No. of bact. per cc. of milk	Change in Bact. Content
Circular motion to distribute clumps uniformly	23,125	
Six strokes	28,555	+ 5,430
Ten strokes	30,050	+ 1,495
Fourteen strokes	29,020	- 1,030
Eighteen strokes	28,975	- 45
Per cent increase due to agitation..... 25%		

TABLE 16. Effect of Agitation on the Bacterial Content of Milk

Amount of Shaking	PLATE COUNT No. of bact. per cc. of milk	Change in Bact. Content
Circular motion to distribute clumps	98,450	
Six strokes	112,200	+ 13,750
Ten strokes	119,760	+ 7,560
Fourteen strokes	125,150	+ 5,390
Per cent increase due to agitation..... 27%		

TABLE 17. Effect of Agitation on the Bacterial Content of Milk

Amount of Shaking	PLATE COUNT No. of bact. per cc. of milk	Change in Bact. Content
Circular motion to distribute clumps uniformly	11,930	
Five strokes	12,620	+ 690
Ten strokes	13,000	+ 370
Fifteen strokes	14,150	+ 850
Twenty strokes	14,500	+ 350
Thirty strokes	14,890	+ 390
Fifty strokes	14,320	- 570
Per cent increase due to agitation..... 25%		

TABLE 18. Effect of Agitation on the Bacterial Content of Milk

Amount of Shaking	PLATE COUNT No.of bact. per cc. of milk	Change in Bact. Content
Five strokes	10,050	
Ten strokes	14,250	4,200
Fifteen strokes	16,820	2,570
Twenty strokes	17,015	195
Thirty Strokes	16,400	615
Fifty strokes	14,920	1,480
Per cent increase due to agitation..... 69%		

The results secured with the four samples of milk seem to indicate that agitation materially affects the number of bacteria, when measured by the agar plate method. When milk is thoroughly agitated the clump and chains of bacteria break up and form separate colonies. When samples of milk were shaken twenty to thirty times there was an increase from 25 to 69 per cent. Judging from these results it is very obvious that a large percentage of the increase in bacterial content which took place in the processing of milk was due to agitation and not entirely to contamination. The percentage of variation was always greater when the flora of the milk was dominated by Streptococcus.

After making a study of the results given in tables 15 to 18, inclusive, it is concluded that the milk samples and the dilution bottles should receive twenty-five strokes each, in order to insure thorough agitation and uniformity of results.

The results secured in this experiment confirm the rules given by the American Public Health Association for milk analyses, which state that "Each sample of milk and the corresponding dilution bottle should be shaken twenty-five times.

PART III. DETERMINING THE YEAST, MOLD, AND BACTERIAL CONTENT OF CREAM DURING THE VARIOUS OPERATIONS IN BUTTER-MAKING

Many buttermakers are unaware of the bacteriological changes which take place during the various operations in buttermaking. This investigation will consist in following the bacterial changes that take place in the buttermaking process at the V. P. I. Creamery. By using raw cream of good, medium, and poor quality, it is possible to find the efficiency of pasteurization and the effect of neutralization on the number of bacteria. More attention will be given to the yeast and mold content, since they are a more reliable index of keeping quality than the actual number of bacteria. Large numbers of flavor and acid-producing bacteria do not jeapardize the quality of the butter, but are considered very beneficial. It is thought that the cream used in this work would be similar to that found on the platform of any buttermaking plant.

Since there was not enough surplus cream available to allow but one survey of the buttermaking process, it was necessary to follow the changes which took place in the yeast and mold content of cream and butter when pasteurized and churned under a pressure of carbon dioxide.

Historical: In 1920 Lund[8] made a study of the microorganisms in butter and concluded that a large number of the molds in pasteurized creamery butter is due to contamination following pasteurization; this especially occurs in the churn. Lund[9] also found that yeast and mold are killed by pasteurizing at ordinary temperatures. Bouska and Brown[10] reported that the churn may be an important source of bacteria unless it is carefully treated. They also found that more organisms are added to the butter during the working than during the churning, because of the organisms being forced out of the working parts of the churn. Gregory[11] shows that the ordinary cleaning methods do not eliminate churn contamination. His studies reveal the fact that the bacteria in the churn are far more numerous than the acid-producing organisms, which are largely yeast and molds. Stiritz[12] suggested that yeast and mold counts should be used as an index to the whole buttermaking process, rather than to pasteurization only.

In 1931 Libbert[13] isolated 57 yeast and mold cultures from various churns, and all except three of the yeast cultures grew on a medium containing water, agar, and ground wood fiber.

Method of Study: In making a bacteriological study of the butter-making process it was deemed

unnecessary to make counts on the actual number of bacteria since most acid-producing organisms are beneficial and are essential to high quality. The number of yeasts and molds was determined by plating out on wort agar and incubating the plates at room temperature. The butter and cream to be tested was melted and pipetted into sterile petri dishes. This was held in suspension by having about one cc. of sterile water in each dish. Samples were plated out in duplicate of one and two drops each. The medium used in making these counts had the following composition:

Agar shreads..... 20 grams
Malt syrup..... 40 cc.
Sucrose..... 20 grams
Distilled water.....1000 cc.

The reaction of this medium was not adjusted, since a a relatively acid medium is essential to inhibit the growth of bacteria. All plates were incubated for a period of five days or longer.

The number of proteolytic bacteria in the butter and cream was determined by adding one cc. of sterile skim milk to fifty cc. of sterile agar and plating out in appropriate dilutions. All plates were incubated for a period of 48 hours, at a temperature of 37 degrees Centigrade. Proteolysis was indicated by a clearing around the colony.

Lipolytic bacteria were detected by adding one cc. of sterile butter to fifty cc. of sterile agar, and pouring into petri dishes containing an aliquot portion of the sample to be tested. The lipolytic colonies were detected by flooding the plate with a ten per cent solution of copper sulphate. The colonies retaining the stain were recorded as lipolytic bacteria.

Collecting Samples: The bacteriological survey made on the large batch of cream consisted in collecting four samples. Each sample was collected in a sterile bottle and the test was set up immediately. The first sample was taken after pouring the cans of cream into the pasteurizer. After neutralization, the second sample was taken and the number of yeast and mold determined in the usual way. After the cream had been pasteurized at a temperature of 190 degrees Fahrenheit, for 30 minutes, the third sample was taken from the pasteurizer, with a sterile pipette. When the cream had cooled to a temperature of 37 degrees Centigrade it was inoculated with a pure culture of streptococcus Lactis. The churning process was carried on in the normal way and after salting the fourth sample was taken directly from the churn.

Results obtained from a determination of the yeast and mold content of the samples are given in table 19.

TABLE 19. Number of Yeast and Mold found in Samples of Butter and Cream

Sample No	Treatment of Sample	No. of Mold	No. of Yeast	Incubation period
1	Before pasteurization	44	18,585	6 days
2	After neutralization	28	7,560	7 "
3	After pasteurization	0	0	7 "
4	Butter, after salting	61	4	7 "

After neutralization there was a considerable decrease in the number of yeast and a slight decrease in the number of molds per cc. of cream. Judging from these results the molds are apparently more resistant to an alkaline medium than the yeast. When the pasteurizing process was carried on at a temperature of 190 degrees F. it proved to be very effective in destroying yeast and mold growth. Since the number of molds in the butter are much higher, it appears that the churn is a more prolific source of mold than yeast.

These results tend to confirm the conclusions of Coulter[14], in which he concluded that the churn is a greater source of molds than yeast.

Determining the Effect of Carbon Dioxide on the Number of Microorganisms Found in Butter and Cream

The author was very fortunate in being able to secure samples of butter and cream from an experiment carried on by a graduate student[15] at the Virginia Polytechnic Institute, who was trying to find the effect of carbon dioxide on the keeping quality of butter. The cream was churned and pasteurized under a high pressure of carbon dioxide. Usually nine or ten gallons of cream was processed at the time by this method.

When the cream was delivered to the creamery it was thoroughly mixed and a sample taken in a sterile bottle, then analyzed for the number of yeast and mold. After neutralization the cream was poured into a special ten-gallon can, which was equipped with a pipe leading to a tank of carbon dioxide. Another pipe was connected to the churn in a similar manner. The cream was continuously subject to a carbon dioxide atmosphere while it was being pasteurized. The cream was pasteurized by heating in a large vat of water for 45 minutes at a temperature of 185 degrees Fahrenheit. When the pressure of carbon dioxide was released the second sample was taken. After the cream had cooled to a temperature of 37 degrees Centigrade it was inoculated with a viable culture of streptococcus lactus. The cream was then

incubated for twenty-four hours and churned under a pressure of carbon dioxide. Immediately after churning the third sample was taken to find the effect of carbon dioxide on the finished product.

This same procedure was followed on other batches of cream, with and without the use of carbon dioxide. This was done in order to obtain results on untreated samples, for comparison.

Results Obtained after Treatment with Carbon Dioxide: Three batches of cream were churned in the presence of carbon dioxide. In every case there was considerable variation in the quality of the raw cream used in the experiment. The effect of carbon dioxide on the number of yeast and mold will be found in tables 20, 21 and 22. The number of proteolytic and lipolytic bacteria were determined on one sample of butter.

TABLE 20. Number of Microorganisms Found in Cream and Butter when Treated with Carbon Dioxide

Sample No.	Treatment of Sample	No. of Molds	No. of Yeast	No. of bact. per cc. Lipo.	Proto	Incubation period
1 a	Raw cream (sour)	1,890	13,160	-	-	5 days
1 b	Cream past. with carbon dioxide	4	0	-	-	6 "
1 c	Butter churned with carbon dioxide	123	9	-	-	5 "
1 d	Butter			27	245	48 Hrs.

TABLE 21. Number of Yeast and Mold Found in Cream and Butter when Treated With and Without Carbon Dioxide

Sample No.	Treatment Received	No. of Molds	No. of Yeast	Incubation Period
2 a	Raw cream (sour)	4,130	3,010	5 days
2 b	Cream past. without carbon dioxide	17	0	5 "
2 c	Butter churned without CO_2	4	1,690	6 "
2 d	Butter churned with CO_2	21	0	5 "

TABLE 22. Number of Yeast and Mold Found in Butter when Treated with Carbon Dioxide

Sample No.	Treatment Received	No. of Molds	No. of Yeast	Incubation Period
3 a	Sweet cream	4	120	5 days
3 b	Pasteurized	Sample bottle was contaminated		
3 c	Butter churned with carbon dioxide	9	0	5 days
3 d	Butter churned without carbon dioxide	29	6	5 "

In every case the cream that was pasteurized with carbon dioxide was always higher in the number of molds than the yeast. From this observation it appears that the molds are more resistant to heat as well as carbon dioxide. The yeast were killed in every batch of cream that was pasteurized. There was a decrease of 99 per

cent in the number of molds in each sample of pasteurized cream.

PART IV. DETERMINING THE INFLUENCE OF VARIOUS OPERATIONS ON THE BACTERIAL CONTENT OF ICE CREAM

The increasing popularity of ice cream in every state is bringing it under closer inspection by the health officers. During the last few years enough data has been accumulated to prove, beyond a doubt, that freezing can not be depended upon to kill either pathogenic or non-pathogenic bacteria. Large numbers of bacteria do not necessarily condemn ice cream any more than small numbers necessarily insure its sanitary quality. However, we have come to associate an excessive number of bacteria in most food products with inferior materials and unsanitary practices. This is particularly true in the dairy industry where total numbers of bacteria have been used as an index to the sanitary quality. In order to supply the public with a wholesome product, bacterial standards for ice cream have been established. Such standards, plus intelligent inspection supplemented by careful plant operation, will aid considerably in manufacturing ice cream with a low bacterial count. If the manager of an ice cream plant had a knowledge of the operations which

materially add to the bacterial content, definite sanitary steps could be taken to avoid such contamination.

Many times during the past few years sanitary inspectors, health officers and plant managers have asked the question "Which operation or operations in the manufacture of ice cream are mainly responsible for high bacterial counts". The present work was undertaken partly to answer the above question.

Previous Studies: Several investigations have been made, from a bacteriological standpoint, of each step in the process of ice cream manufacture, but most of the investigations have been made on individual operations. Hammer[15] reported that ice cream with a low bacterial count can be produced by pasteurizing and homogenizing the mix. He also found the homogenizer to be a gross source of contamination. Pennington and Walker[16] were among the first investigators to study the influence of manufacturing operations on the bacterial content of ice cream. Their work reveals the fact that after pasteurization the ice cream contains a relatively large number of streptococci.

Hammer[17] made an investigation on the number of bacteria found at various intervals during the manufacture of ice cream, and concluded that it is possible to produce ice cream with a low bacterial count without the use of expensive methods. The studies of Hammer and

Goss[17], on the influence of hardening and storage, show that there is no increase in the number of organisms contained in ice cream under proper storage, while there is commonly a decrease.

The influence of storage on the number of bacteria in ice cream has been studied by several workers. Gordon[18], Esten and Mason[19], and Ellenbarger[20] have covered this phase of the problem very adequately.

Methods Used: Bacterial counts were made on samples of ice cream taken after various operations in the manufacture of ice cream in the ice cream laboratory at this college. Usually the batches of ice cream were rather small, but they were manufactured with equipment operated on a commercial basis and under practical conditions. The samples of various batches were taken at random from January 2 to April 3. The arrangement of the machinery is comparable to that found in an up-to-date plant.

All strainers, cans and other utensils were thoroughly steamed and the freezer carefully scalded. The pasteurizer was scrubbed with a brush and cleaning powders, followed by a rinsing with hot water. Hot water was run through the homogenizer with considerable force.

Each sample was collected in a sterile bottle and cooled immediately. The samples were taken at the following points during the process of ice cream

manufacture: After pasteurization, after homogenization, after cooling, after aging, and after freezing. All samples were melted slowly and plated out on the cc. basis. The method of plating, diluting, and kind of medium was similar to that outlined by the A. P. H. A. for analysis of milk. Every dilution was plated out in duplicate, and the average count for the two plates represented the final count. All samples studied, with the exception of one, were vanilla ice cream. The flavoring was added at the time of freezing.

The machinery consisted of one tubular cooler, one homogenizer, one Miller ice cream freezer, and a 75-gallon pasteurizer. In addition to the above equipment, there were about ten 10-gallon cans and about twenty feet of connecting pipe used in the manufacturing process.

After the mix had been partly pasteurized it was started through the homogenizer. This resulted in a mix that was not uniformly pasteurized. For this reason the first portion of the mix was higher in bacterial content than the last few cans. In view of this procedure the last few cans were always used in this investigation, so as to insure uniformity of results.

Most Frequent Sources of Contamination: There are three principal sources of bacteria in ice cream. First, the materials from which the ice cream is made; this can be controlled by thorough pasteurization.

Second, the machinery equipment; this is the most difficult source of contamination to control. However, some parts of the equipment can be thoroughly cleaned so as to maintain a product of low bacteria count. Third, the person handling the product; oftentimes this becomes a great source of contamination, especially if the operator is careless. The contamination arising from this source can be kept to a minimum by keeping clean hands and wearing white clothes.

Results Secured: The results secured after following four batches of ice cream mix are presented in tables 23 to 26, inclusive.

TABLE 23. Effect of the Various Operations on the Bacterial Content of Ice Cream Mix

Sample No.	Treatment of Sample	No. of bact. per cc. of mix	Change in bact. content
20 a	Past. for 25 min. at 135° F.	15,000	
20 b	Homog. at 2500# pressure	24,000	9,000
20 c	Cooled to 58° F.	32,235	8,235
20 d	In cans before aging	33,000	765
20 e	In cans after aging	45,120	12,120
20 f	Vanilla ice cream, 60% overrun	34,960	-10,160
20 g	Vanilla ice cream, 95% overrun	15,765	-29,355
20 h	Chocolate ice cream, 95% overrun	17,630	-27,470

TABLE 24. Effect of the Various Operations on the Bacterial Content of Ice Cream Mix

Sample No.	Treatment of Sample	No. of bact. per cc. of mix	Change in bact. content
21 a	Past. for 25 min. at 140° F.	370	
21 b	Homog. at 2600# pressure	13,820	13,450
21 c	Cooled at 60° F.	16,475	2,655
21 d	In cans before aging	18,000	1,535
21 e	In cans after aging	19,300	1,290
21 f	Vanilla ice cream, 95% overrun	10,500	-8,800

TABLE 25. Effect of the Various Operations on the Bacterial Content of an Ice Cream Mix

Sample No.	Treatment of Sample	No. of bact. per cc. of mix	Change in bact. content
22 a	Past. for 25 min. at 146°F.	3,010	
22 b	Homog. at 2300# pressure	7,250	4,240
22 c	Cooled to 60° F.	8,200	950
22 d	In cans after aging for 32 hours	11,850	3,650
22 e	Vanilla ice cream*	14,100	2,250
22 f	Vanilla ice cream 95% overrun	8,850	- 5,250

*The ice cream was thoroughly shaken to expel air.

TABLE 26. Effect of the Various Operations on the Bacterial Content of An Ice Cream Mix

Sample No.	Treatment of Sample	No. of bact. per cc. of mix	Change in bact. content
23 a	Past. for 30 min. at 148°F.	4,225	
23 b	Homog. at 2600# pressure	11,275	7,055
23 c	Cooled to 58° F.	11,400	125
23 d	In cans after aging 52 hours	32,700	21,300
23 e	Vanilla ice cream*	33,900	1,200
23 f	Vanilla ice cream, 95% overrun	19,100	-14,800

*The ice cream sample was thoroughly shaken to expel all air.

The influence of pasteurization on the bacterial content of an ice cream mix: Because of the diverse sources of solid and liquid materials contained in the ice cream mix, a bacterial count was not made of the ingredients before pasteurization. However, none of the samples were very high in bacterial numbers, nor was any sample completely sterile after pasteurization. The efficiency of pasteurization varied from 15,000 in one sample to a minimum of 370 bacteria per cc. of mix. According to the studies of Fabian[21], an ice cream mix should be pasteurized at a temperature of 150 degrees Fahrenheit for 30 minutes. Because of the nature of the materials, an ice cream mix is not pasteurized as readily as milk. Even though the bacterial content of the mix is relatively low it is thought

that better results could have been secured if the mix was thoroughly pasteurized before homogenization was started.

The influence of Homogenization upon the bacterial content of an ice cream mix: After the mix had been heated to a temperature of 135 - 145 degrees Fahrenheit for about 15 minutes, it was started through the homogenizer where it was under a pressure of 2500 to 2600 pounds.

An inspection of the figures given in tables 23 to 26 shows a maximum increase of 13,450 and a minimum increase of 4,240 bacteria per cc. of the mix. A slight increase in the bacterial content can always be expected, since this machine is hard to clean and has a tendency to break up the clumps of bacteria. As the process was continued, the presumption was that the contamination would become less due to the washing effect. However, most of the increase found in the tables is presumably due to agitation, since most of the samples were not caught until most of the mix had been homogenized. It is thought that most of the bacteria had been washed out before the samples were collected.

The influence of cooling the ice cream mix on the bacterial content: After each mix had passed through the homogenizer it was forced to the cooler, where it was cooled to 68 degrees Fahrenheit. The first part of the mix passing over the cooler was higher in bacterial count,

because of the slight washing effect. In this experiment the samples were not taken until most of the mix had passed over the cooler. By cooling the mix from a temperature of 125 degrees to 68 degrees Fahrenheit it is thought that this sudden change would have had some effect on the number of bacteria. In every case there was an increase in the number of bacteria per cc. of the mix. The smallest increase in any of the samples was one per cent and the greatest increase was nineteen per cent. There is a possibility that a few of the bacteria were killed in the sudden change of temperature, but if such a decrease existed it was overcome by the contamination received by the cooler. Despite the fact that this operation increases the bacterial content, it is indispensible to keep bacterial activity to a minimum.

The influence of aging on the bacterial content of an ice cream mix: The mix was aged at a temperature of 32 to 35 degrees Fahrenheit. At this temperature some increase in bacterial growth took place. There was a slight variation in temperature, but not more than two or three degrees. It has been found by many workers that aging an ice cream mix is very beneficial in increasing viscosity, and this influences the yield of the finished product. The influence of this process can be found in tables 25 to 26, inclusive. Every batch showed an increase in the number of bacteria per cc. of the mix.

The samples showed an increase ranging from 7 per cent to as high as 187 per cent. This increase shows conclusively that the temperature was not low enough to inhibit the growth of all organisms. However, the bacteria commonly found in milk do not grow very rapidly at this temperature. According to the studies of Marshall[22], it is very obvious that certain of the spore-bearing non-acid bacteria will develop rapidly at a temperature of 10 degrees Centigrade.

The influence of freezing on the bacterial content of an ice cream mix: The mix was usually frozen about two days before it was made. This period would vary according to the demand for the finished product. The temperature of the brine at which the mix was frozen ranged from -10 to -5 degrees Fahrenheit. All samples were collected just as the mix left the freezer. There was a variation in the Bacterial content corresponding to the overrun percentage. As it would be expected, the higher the overrun the fewer the number of bacteria per cc. of ice cream. When the overrun percentage was considered the ice cream samples were not shaken before making the dilutions. All other ice cream samples were thoroughly shaken, to exclude the air incorporated during the freezing process, before dilutions were made. It is thought that the breaking up of clumps of bacteria during the freezing process is much less than that due

to homogenization. When the overrun was not considered, the greatest increase was 16 per cent and the greatest decrease was 68 per cent. Most of the increase is probably due to contamination from the freezer.

The influence of storage on the bacterial content was not studied with these samples.

CONCLUSIONS

Results reported show that milk, butter and ice cream can be produced with a low bacterial count by the methods employed in this plant. The effect of each step in the process on the bacterial count has been dealt with separately and the most significant conclusions found are as follows:

1. This work confirms the conclusions of other investigators, that:- the utensils and equipment in which milk is handled are prolific sources of bacteria when not thoroughly cleaned and sterilized.
2. The bacteria in freshly washed ten-gallon cans are destroyed when they are steamed for twenty seconds or more. When the cans are steamed less than twenty seconds, considerable growth takes place.

3. More contamination took place during the heating and filtering operation than with any other operations in the plant.

4. When the number of bacteria in milk are measured by the plate method, the count is much greater after pumping the milk than before.

5. The cooler was found to be a consistent source of contamination. In no case was the increase in the number of bacteria per cc. of milk exceptionally high nor exceptionally low when it was allowed to pass over the cooler.

6. The results given in this experiment tend to confirm the results secured by the American Public Health Association for analyses of milk, which states that "Each sample of milk and the corresponding dilution bottles should be shaken twenty-five times." When the milk is thoroughly agitated the clumps and chains of bacteria break up and form separate colonies on the agar plate.

7. Molds found in market cream appear to be more resistant to an alkaline medium than the yeast.

8. Since the number of molds in the butter are much higher, it seems that the churn is a more prolific source of molds than yeasts.

9. Carbon dioxide was more effective in destroying yeasts than it was in destroying molds which frequent

market cream and butter.

10. When the ice cream mix was homogenized there was always a distinct increase in the number of bacteria when measured by the plate method. The increase may be apparent rather than real.

11. When the ice cream mix was aged there was an increase in the number of bacteria ranging from 7 to 187 per cent.

12. After the mix is pasteurized every subsequent operation has a tendency to increase the plate count.

13. The number of bacteria was found to be directly proportional to the overrun percentage.

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