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A BACTERIOLOGICAL STUDY OF RETAIL ICE CREAM.

By S. Henry Ayers, *Bacteriologist*, and William T. Johnson, Jr., *Scientific Assistant, Dairy Division.*

INTRODUCTION.

A bacteriological examination of ice cream as the consumer receives it from retail sources is, we believe, the first logical step in the study of the bacteriology of ice cream. The results obtained from the examination of ice cream in this last period of its history will show the final bacterial flora. This type of investigation does not show where the organisms are introduced, but it will show the number and kinds of bacteria present in the cream at the time it is consumed. To determine where these organisms are introduced and their significance is another phase of the general problem.

It is well known that commercial retail ice cream contains large numbers of bacteria. This is well illustrated in a summary of the results of examination of ice cream in various cities, presented in Table 1, taken from a paper by B. W. Hammer.[1] From these figures it is evident that the number of bacteria in ice cream throughout the country averages extremely high.

Table 1.—*Summary of Hammer's bacterial investigations of ice cream.*

Source.	Date of investigation.	Number of samples examined.	Bacteria per cubic centimeter.		
			Average.	Highest.	Lowest.
Philadelphia	1905–06	49	17,833,031	79,800,000	70,000
Boston	1906–07	35	23,000,000	150,000,000	1,000,000
Washington	1906–07	263	26,612,371	365,000,000	137,500
Chicago	1909	89	16,662,134	125,000,000	20,000
Do	1910	306	15,401,000	100,000,000	20,000
Do	1911	....	1,800,000	200,000,000	90,000
Milwaukee	1911	26	....	8,000,000,000	200,000
Des Moines	1911	10	19,920,000	39,000,000	4,200,000
Iowa State College	1911–12	11	19,775,000	72,000,000	500,000

[1] See bibliography at end of paper.

Bacterial counts, while of value, only tell us the numbers, and this is not sufficient information. It is necessary for a thorough knowledge of the subject to know the kinds of bacteria which are generally present in ice cream as well as the number.

There is relatively little exact information regarding the bacteriology of ice cream, and in consequence probably many applications of the subject to the industry are neglected. Extensive research work on bacteriological problems connected with the manufacture of ice cream is needed, and it is probable that many investigators will devote themselves to this subject when the value of such work is realized.

Our investigations on the general subject of the bacteria of ice cream will be divided into three main parts.

1. A study of the number and kinds of bacteria in commercial retail ice cream as the consumer receives it.
2. A study of the bacteriology of ice cream during manufacture.
3. A study of the development of bacteria in ice cream during storage.

These studies have been undertaken solely with the idea of securing information of fundamental importance relative to the bacteriology of ice cream. We hope by these investigations to be able to give the manufacturer information which will enable him to produce a product of the highest quality.

We believe that bacteriology will be of great value to the manufacturer in controlling the quality of his raw material and the quality of his final product, and in checking the efficiency of the various operations in the production of ice cream.

OBJECTS OF THIS INVESTIGATION.

The first part of our investigations is presented in this paper, the objects of the work being as follows:

1. To determine the number of bacteria in commercial ice cream during the summer and winter seasons.
2. To determine what groups of bacteria are found in commercial ice cream.
3. To determine the relative value of different methods for the determination of *Bacillus coli* in ice cream.

METHODS OF EXAMINATION.

The ice cream was purchased in one-half-pint paper boxes from various stores throughout the city of Washington, D. C. Throughout this paper reference is made to summer and winter samples. The samples of the former were collected from June 20, 1912, to November 11, 1912. Winter samples were obtained during February and March, 1913, the coldest months of the year in Washington. The samples were taken directly to the laboratory and portions from various parts of each sample were transferred to a sterile Erlenmeyer flask. After the ice cream had melted it was thoroughly shaken to remove as much air as possible and at the same time mix the sample. One cubic centimeter was then plated by the usual methods on plain infusion agar prepared according to standard methods prescribed by the Committee on Standard Methods. The plates were incubated at 30° C. (86° F.) for five days, then counted.

Special methods for the determination of colon bacilli will be discussed later in this paper.

In order to divide the bacteria into groups the "milk-tube method" of differentiation was used. This method, devised by the authors, is fully described in a previous publication.

Briefly described, the milk-tube method consists in picking each colony from an infusion agar plate and inoculating into tubes of litmus milk. The reactions produced in the litmus milk are recorded after 2, 5, and 14 days' incubation at 30° C. (86° F.). Knowing the 14-day reaction produced by bacteria from each colony on the original plate, it is possible to determine the number of bacteria in the original material plated and to divide them into groups, namely, the acid-coagulating, acid-forming, inert, alkali-forming, and peptonizing. An examination of samples collected as mentioned gives the bacterial flora of ice cream just as the consumer would receive it.

THE ACIDITY OF ICE CREAM.

Before entering upon a discussion of the bacteriological results of this work it seems advisable to discuss a few miscellaneous analyses which were made during this study. All the samples of vanilla ice cream were tested for acidity by titration with tenth-normal sodium hydroxid, using phenolphthalein as an indicator. Most of the ice cream of other flavors could not be tested on account of the color. The maximum acidity found was 0.387 per cent and the minimum 0.09 per cent, calculated as lactic acid. The average acidity of 65 samples was 0.206 per cent. Many of the samples were distinctly sour to the taste, and evidently some manufacturers used either old sour cream or else aged the cream in their plants at temperatures sufficiently high to allow lactic-acid bacteria to produce acidity. The acidity did not seem to bear any definite relation to the bacterial count, however, as the samples showing an acidity of 0.387 per cent contained 217,000 bacteria per cubic centimeter, of which 74.51 per cent were acid-forming bacteria, while ice cream with an acidity of 0.09 per cent contained 49,000,000 bacteria per cubic centimeter, of which 89.79 per cent were acid-forming bacteria.

THE NUMBER OF BACTERIA.

Numerous samples of ice cream were purchased from various stores in Washington during the summer and winter season; they represented 24 different manufacturers.[1] Samples representing 11 plants were examined during both seasons. The ice cream from 5 manufacturers was examined during the summer season only, and from 7 plants during the winter season only. In Table 2 are shown the total (average of the two kinds of samples) average counts of ice cream from each plant, together with the average of the samples collected during the summer and winter seasons. It will be seen that only two plants, K_1 and P, out of the 24 showed an average bacterial content of less than 1,000,000 per cubic centimeter. It is possible that if more than one sample had been examined from plant P the average count for that plant would have been more than 1,000,000.

TABLE 2.—*Average number of bacteria per cubic centimeter in ice cream from different manufacturers.*

Manufacturer.	All samples.		Summer series.		Winter series.	
	Total samples.	Average bacteria per cubic centimeter.	Number of samples.	Average bacteria per cubic centimeter.	Number of samples.	Average bacteria per cubic centimeter.
A	32	2,986,187	19	5,008,333	13	416,000
B	27	32,007,500	10	31,226,000	17	47,763,529
C	19	27,779,631	11	47,909,090	8	101,000
D	11	18,836,363	8	26,125,000	3	6,066,666
E	9	44,783,333	3	118,000,000	6	8,175,000
F	8	5,381,500	3	8,966,666	5	3,230,400
G	12	2,580,250	5	6,192,500	7	1,160,428
H	5	4,203,600	4	5,207,500	1	188,000
I	8	15,487,500	5	18,360,000	3	10,700,000
J	3	5,890,000	1	14,100,000	2	1,785,000
K	4	265,750,000	4	265,750,000		
K_1[a]	12	357,583	4	530,000	8	271,375
L	4	32,225,000	4	32,225,000		
M	4	10,910,000	4	10,910,000		
N	3	5,723,333	3	5,723,333		
O	6	46,465,000	6	46,465,000		
P	1	920,000	1	920,000		
Q	2	19,750,000			2	19,750,000
R	1	20,500,000			1	20,500,000
S	3	2,463,333			3	2,463,333
T	4	3,650,000			4	3,650,000
U	2	28,500,000			2	28,500,000
W	3	14,700,000			3	14,700,000
X	3	17,566,666			3	17,566,666

[a] K_1 same plant as K, but there was probably a change in management.

An interesting point is shown in the bacterial averages of ice cream from manufacturers K and K_1. The average bacterial content of ice cream from K was 265,750,000, and from K_1 357,583 per cubic centimeter. These represent the same plant, but in all probability there was a change in management. We are not certain of this point, but after the four samples were taken from K the condition

[1] Throughout this paper reference is made to samples from different manufacturers or plants, but it must be remembered that the samples were obtained from stores and not at the plants.

of the store was much improved as regards cleanliness, the ice cream no longer contained gelatin, and the bacterial count dropped in a remarkable manner.

When the bacterial averages of the two kinds of samples (summer and winter) are compared it may be seen, by reference to Table 2, that of the samples from 11 plants the bacterial counts averaged decidedly lower during the winter months in every case with the exception of plant B. In that case the average winter count was higher than that of the summer season. Assuming cream to be the greatest source of bacteria in ice cream, we should expect to find the bacterial content of ice cream higher during the summer months. This is probably explained by the lack of proper facilities on the farm for keeping cream cool during the summer, and also by the fact that owing to the increased demand for ice cream during the summer season poor grades of cream are utilized. In the case of plant B, we have no explanation for a higher average bacterial count in winter than in summer unless the cream was aged at the plant for a considerable time at temperatures not sufficiently low to prevent bacterial growth.

In order to show more clearly the comparison of the bacteria in ice cream during the summer and winter seasons, the samples have been grouped in classes according to their bacterial content, as shown in Table 3.

TABLE 3.—*Comparison of bacterial content of summer and winter samples of ice cream.*

Bacteria per cubic centimeter.	Summer.		Winter.	
	Number of samples.	Per cent.	Number of samples.	Per cent.
0 to 50,000	0	0	5	5.49
50,001 to 100,000	0	0	8	8.79
100,001 to 500,000	9	9.57	23	25.27
500,001 to 1,000,000	9	9.57	2	2.20
1,000,001 to 5,000,000	16	17.03	21	23.08
5,000,001 to 10,000,000	12	12.77	5	5.49
10,000,001 to 20,000,000	13	13.83	11	12.09
20,000,001 to 30,000,000	10	10.64	5	5.49
30,000,001 to 50,000,000	8	8.51	3	3.30
50,000,001 to 100,000,000	9	9.57	4	4.40
Over 100,000,000	8	8.51	4	4.40
Total	94	100.00	91	100.00

It may be seen that of 94 samples examined during the summer months, none contained fewer than 100,000 bacteria per cubic centimeter, while of the 91 samples examined during the winter season, 14.28 per cent were lower than 100,000. Of the summer samples, 9.57 per cent contained fewer than 500,000 per cubic centimeter, and of the winter samples 39.55 per cent contained fewer than this number. Of the summer samples 19.14 per cent contained fewer than 1,000,000 per cubic centimeter, while 41.75 per cent of the winter samples contained fewer than 1,000,000 per cubic centimeter. Finally, there remain 80.86 per cent of the summer samples and only 58.25 per cent of the winter samples which contained more than 1,000,000 bacteria per cubic centimeter. These results are shown perhaps

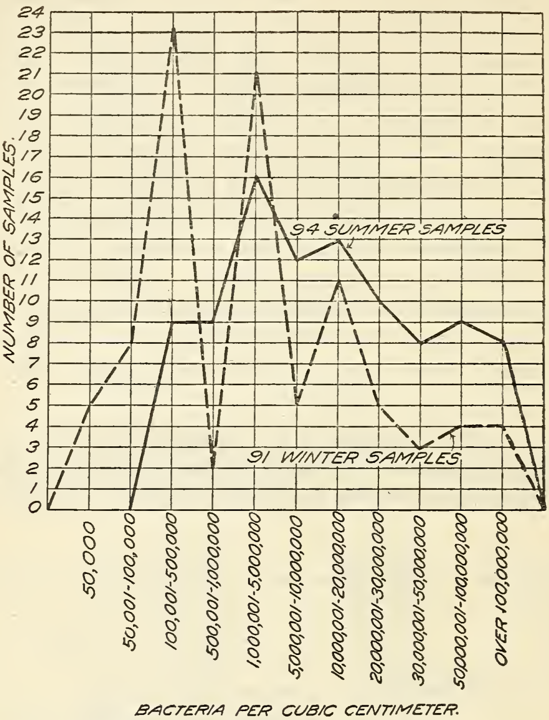

Fig. 1.—Frequency curve showing bacterial content of summer and winter samples of ice cream.

more strikingly in figure 1, where the samples have been plotted in a frequency curve, showing the difference in bacterial content between the summer and winter samples.

The differences in the average counts of ice cream from different manufacturers at different seasons are summarized in Table 4.

TABLE 4.—*Summary of the bacterial counts of ice cream.*

Item.	Summer series (94 samples).	Winter series (91 samples).
Average number of bacteria per cubic centimeter	37,859,907	10,388,222
Maximum number	510,000,000	114,000,000
Minimum number	120,000	13,000

These figures show a wide range in the bacterial content of ice cream during both the summer and winter months. The minimum counts of 120,000 during the summer and 13,000 in the winter series furnish evidence that ice cream can be made commercially, at least under some conditions, with a low bacterial content. If these average counts are compared with the average counts from other cities, as shown in Table 1, it will be seen that, so far as bacteria are concerned, the quality of the ice cream is about the same in different localities.

THE GROUPS OF BACTERIA.

THE GENERAL GROUPS.

The bacteria in 71 summer samples and 28 winter samples of ice cream were divided into groups by the milk-tube method of differentiation heretofore mentioned. With this method it was possible,

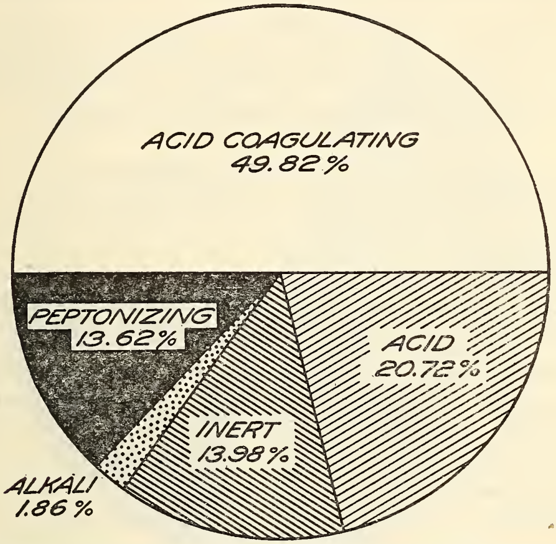

FIG. 2.—Average bacterial groups found in one cubic centimeter of ice cream during the summer months, showing percentages of groups.

Group.	Bacteria per c. c.
Acid-coagulating	18,861,805
Acid-forming	7,844,575
Inert	5,292,815
Alkali-forming	704,195
Peptonizing	5,156,519
Total	37,859,909

on the basis of the 14-day reaction in litmus milk, to divide the bacteria into five groups, namely, acid-coagulating, acid-forming, inert, alkali-forming, and peptonizing. For convenience in obtaining a view of the average groups in an average sample of ice cream, the results are presented graphically. Figure 2 represents 1 c. c. of ice cream and is divided into five sectors, each representing the average percentage of a group of bacteria. The averages are based on the analyses of 71 samples of ice cream examined during the summer season. It may be seen from the figure that the bacteria of 1 c. c. of an average sample of ice cream during the summer season may be divided into five groups in the following proportions:

	Per cent.
Acid-coagulating group	49.82
Acid-forming group	20.72
Inert group	13.98
Alkali-forming group	1.86
Peptonizing group	13.62

The total acid group comprises, therefore, 70.54 per cent of the bacteria in the average ice cream during the summer season. In order to make this picture more nearly complete, we have calculated the number of bacteria of each group which would be found in 1 c. c. of an average sample of summer ice cream. These bacterial numbers were obtained by calculation from the average bacterial count of 1 c. c. of ice cream. Since the average commercial ice cream during the summer months contained 37,859,909 per cubic centimeter, the calculated number of each group would be as follows:

	Bacteria per c. c.
Acid-coagulating bacteria	18, 861, 805
Acid-forming bacteria	7, 844, 575
Inert bacteria	5, 292, 815
Alkali-forming bacteria	704, 195
Peptonizing bacteria	5, 156, 519

Perhaps the most important observation in this connection is the high number of peptonizing bacteria.

In the same manner are shown in figure 3 the bacterial groups in an average sample of winter ice cream. The bacteria may be divided into five groups, as follows:

	Per cent.
Acid-coagulating group	30.84
Acid-forming group	38.03
Inert group	4.81
Alkali-forming group	5.42
Peptonizing group	20.90

A comparison of the total percentage of the acid groups shows 70.54 per cent during the summer and 68.87 per cent during the winter. It will be seen, however, that during the summer 49.82 per cent were acid-coagulating bacteria, while in the winter only 30.84 per cent were of the acid-coagulating group. The increase in the percentage of the alkali and peptonizing groups is particularly noticeable. From the average group percentages and the average bacterial count the calculated bacterial composition of the 10,388,222 in 1 c. c. of winter ice cream would be as follows:

	Bacteria per c. c.
Acid-coagulating bacteria	3, 203, 728
Acid-forming bacteria	3, 950, 641
Inert bacteria	499, 673
Alkali-forming bacteria	563, 042
Peptonizing bacteria	2, 171, 138

A comparison of these figures with those of the average summer samples shows that while the percentages of the alkali and peptonizing groups are higher in winter there were actually smaller numbers of them in ice cream during that season, on account of a lower total number of bacteria during cold weather.

No study was made of the cultural reactions of the bacteria of the various groups, but from our methods of analysis a few points may be brought out.

THE TOTAL-ACID GROUPS.

As we have shown before, of the average bacterial flora of summer ice cream 70.54 per cent is made up of the total group of acid-forming bacteria, and during the winter 68.87 per cent. While using the

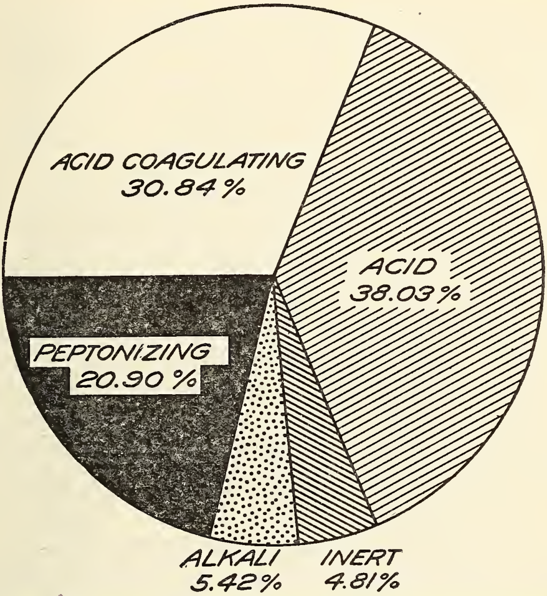

FIG. 3.—Average bacterial groups found in one cubic centimeter of ice cream during the winter months, showing percentages of groups.

Group.	Bacteria per c. c.
Acid-coagulating	3,203,728
Acid-forming	3,950,641
Inert	499,673
Alkali-forming	563,042
Peptonizing	2,171,138
Total	10,388,222

milk-tube method of differentiation the reactions of the litmus milk tubes are recorded after 2, 5, and 14 days, and the total acid-forming group is composed of those bacteria which produce acid in litmus milk during the 14 days of incubation. Those bacteria which form acid and peptonize the milk are included in the peptonizing group. The total-acid group can be further divided into those which produce acid and coagulate the milk and those which simply form acid within 14 days. Since the reaction is recorded after 2, 5, and 14 days, the rapidity of the growth of the acid-forming bacteria can be determined, and this serves as an additional means of separating the group. In Table 5 the percentages of the acid-coagulating and the simple acid-forming groups of bacteria are shown, based on the 2, 5, and 14 day reaction in litmus milk.

TABLE 5.—*Changes in the percentage of the total-acid group of bacteria in ice cream when determined by litmus-milk reactions after various lengths of incubation.*

Bacterial group.	Per cent reacting after incubation for—		
	2 days.	5 days.	14 days.
	Per cent.	*Per cent.*	*Per cent.*
Average of 71 summer samples:			
Acid-coagulating	26.31	41.52	49.82
Acid-forming	35.43	25.58	20.72
Average of 28 winter samples:			
Acid-coagulating	8.20	25.02	30.84
Acid-forming	44.51	41.30	38.03

An examination of the table shows that among the summer samples 49.82 per cent of the bacteria produced acid and coagulated the milk after 14 days. After 2 days 26.31 per cent produced this reaction. This shows that a little more than half, or 52.81 per cent, of the bacteria which were in the ice cream produced the reaction within 48 hours. The remaining 47.19 per cent coagulated milk more slowly and may represent a different variety of acid-forming bacteria. Turning again to the table and considering the acid-coagulating group of the winter series, it will be seen that of the 30.84 per cent which produced the reaction only 8.20 per cent produced acid and coagulated milk in 2 days. Therefore only 26.69 per cent of the acid-coagulating group of the winter samples were active enough to produce the reaction in 48 hours, while 52.81 per cent of this group in the summer samples brought about the change in 2 days.

There is little to be said regarding the acid-forming bacteria which simply produce acid. Many of them grow slowly and do not show an acid reaction for several days in litmus milk. The milk-tube method furnishes a means of determining the difference in the rapidity with which the bacteria produce acid. As may be seen in Table 5, the percentage of the acid-forming group of bacteria was highest when determined by the 2-day reactions and lowest when based on the 5 and 14 day reactions. This is explained by the fact that many bacteria have simply formed acid after two days in litmus milk and later may coagulate or peptonize the milk, and are therefore thrown into another group.

THE INERT GROUP.

The inert group of bacteria in ice cream comprises those which produce no change in litmus milk during the 14 days' incubation at 30° C. (86° F.). By this method of grouping there are, of course, included in the inert group those cultures which fail to grow in milk and tubes of litmus milk, and which would also be included even though the lack of growth were caused by failure to inoculate the tubes properly. However, this last possibility is small. The inert group is of little interest, on the whole, since the bacteria produce no apparent change in milk, and in all probability the same is true of ice cream.

THE ALKALI GROUP.

The alkali-forming group of bacteria is made up of organisms capable of producing an alkaline reaction and no other apparent change in litmus milk during the 14 days' incubation at 30° C. (86° F.). This group does not include bacteria which produce an alkaline reaction together with visible signs of peptonization. While there are in the literature references which deal with types of alkali-forming bacteria, this group has rarely, if ever, been considered when the flora of milk has been under discussion. The authors in some previous work on bacteria in milk showed that considerable numbers of this group were present in milk. In a later piece of work we have shown the numbers of this type of bacteria in milk, together with some of the cultural reactions of the alkali-forming bacteria. These bacteria, however, give very few positive reactions with the usual cultural media, and it is impossible to give much information regarding this group. A detailed bacteriological and chemical study of these organisms is under way in the research laboratories of the Dairy Division.

It will be seen from Table 6 that during the summer series of ice-cream samples the average sample contained 1.86 per cent of the alkali group of bacteria, and during the winter series 5.42 per cent. In general, the alkaline reaction is not noticeable until after four or five days' incubation in litmus milk. Occasionally, however, the reaction is in evidence in 48 hours. The group percentage for the summer season was 1.86 after 14 days and only 0.15 per cent based on the 2-day reaction. Therefore only 8.06 per cent of the bacteria of the alkali group produce an alkaline reaction within 48 hours. Among the samples collected during the winter season only 3.13 per cent of the bacteria of this group were capable of producing the reaction within two days. Whether this indicates a different variety of organism can not be said with assurance.

TABLE 6.—*Changes in the percentage of the alkali group of bacteria in ice cream when determined by litmus-milk reactions after various lengths of incubation.*

Alkali group.	Per cent reacting after incubation for—		
	2 days.	5 days.	14 days.
	Per cent.	*Per cent.*	*Per cent.*
Average of 71 summer samples	0.15	1.03	1.86
Average of 28 winter samples	.17	4.00	5.42

At present we are unable to state the significance of this group of bacteria in milk and ice cream, but it is evident that they are not present in ice cream in large numbers, as are the bacteria of other groups. The average numbers of alkali-forming bacteria in ice cream from 16 manufacturers during the summer season and from 7 during the winter season are shown in Table 7.

TABLE 7.—*Average number of alkali-forming bacteria per cubic centimeter of ice cream from various manufacturers.*

Manufacturer.	Summer.		Winter.	
	Number of samples.	Average number of alkali bacteria per cubic centimeter.	Number of samples.	Average number of alkali bacteria per cubic centimeter.
A	11	56,945	3	9,192
B	3	1,155,327	5	2,326,068
C	3	1,739,007		
D	1	386,000	3	265,545
E	3	462,871		
F	2	340,820	2	14,146
G	4	136,198	1	31,815
H	1	251,509		
I	1	1,316,800		
J	1	902,400	2	93,867
K_1	4	18,348	1	111,377
L	1	419,240		
M	1	22,464		
N	3	118,219		
O	1	1,044,900		
P	1	47,104		
Grand average	41	526,134	17	407,430
Maximum count		2,998,700		10,400,000
Minimum count		4,681		1,901

Alkali-forming bacteria were not found in each sample examined, but this does not prove that there were none present in the ice cream. Since these organisms are present in small numbers compared to the rest of the bacteria, it is not surprising that none should be found on plates in which the dilution had to be high in order to take care of the large total number of organisms.

THE PEPTONIZING GROUP.

The peptonizing group is probably the most interesting if not the most important group of bacteria in ice cream. This group consists of what are commonly known as the putrefactive bacteria; that is to say, they attack primarily the proteins, decomposing them into less complex organic bodies. Bacteria of this class are usually considered undesirable in articles of food, and it is to them that intestinal troubles are sometimes attributed, perhaps with or without justification. Whatever their true effect is will not be discussed in this paper, but because bacteria of this group are looked upon with suspicion it is therefore of great importance. In Table 8 will be found the averages of the number of peptonizing bacteria in ice cream during the summer and winter seasons from a number of different plants.

TABLE 8.—*Average number of peptonizing bacteria per cubic centimeter of ice cream from various manufacturers.*

Manufacturer.	Summer.		Winter.	
	Number of samples.	Average number of peptonizing bacteria per cubic centimeter.	Number of samples.	Average number of peptonizing bacteria per cubic centimeter.
A	15	909,132	3	30,358
B	7	3,579,261	7	1,159,888
C	8	2,752,681	2	2,097
D	6	2,587,978	3	247,944
E	3	1,064,463		
F	3	1,018,440	3	104,008
G	5	567,409	3	197,314
H	1	328,119		
I	1	406,400		
J	1	1,128,000	2	290,623
K_1	4	415,018	2	117,316
L	4	4,370,497		
M	4	874,754		
N	2	72,253		
O	3	2,879,467		
P	1	247,656		
Grand average	68	1,449,533	25	268,693
Maximum count		21,000,000		2,974,400
Minimum count		36,063		1,194

The bacterial averages were much higher in the summer than during the winter. It is evident from these figures that the average commercial ice cream contains a large number of peptonizing bacteria. In order to show the range in the percentage of peptonizers, the entire lot of summer and winter samples has been plotted in a frequency curve, as shown in figure 4. It will be seen that the majority of samples contained from 0.1 to 5 per cent of peptonizing bacteria. A large proportion contained, however, as high as 25 to 30 per cent, and in a few samples they were present to the extent of from 90 to 95 per cent of all the bacteria.

Among this group there are a large number of different types of organisms. Many rapidly peptonize the casein of milk and render milk alkaline or slightly acid, while others first attack the lactose and only produce a slight peptonization after several days' growth. From the milk-tube method of differentiation of the bacterial groups it was possible to gain some information as to the extent of these different classes of peptonizers. In Table 9 are shown the average percentages of the peptonizing group in summer and winter samples of ice cream. Based on the 14-day reaction among the summer

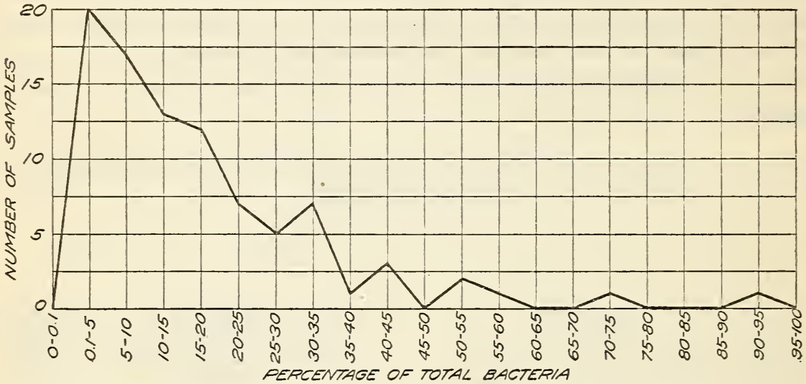

FIG. 4.—Frequency curve showing percentage of peptonizing bacteria per cubic centimeter of ice cream, summer and winter series combined.

samples, 13.62 per cent of the bacteria belonged to the peptonizing group. According to the 2-day reaction, there were 5.93 per cent. Therefore 43.54 per cent of the peptonizing bacteria were sufficiently active to produce a peptonization within two days. Among the winter samples 34.06 per cent of the peptonizing bacteria were sufficiently active to peptonize milk within 48 hours. These active peptonizing bacteria are more important than the slower-acting varieties, since their peptonizing action is usually more complete than that of the latter-named varieties, and if any harm is produced by this group, they are most likely to be the organisms concerned.

TABLE 9.—*Changes in the percentage of the peptonizing group of bacteria in ice cream when determined by litmus-milk reactions after various lengths of incubation.*

Peptonizing group.	Per cent reacting after incubation for—		
	2 days.	5 days.	14 days.
	Per cent.	*Per cent.*	*Per cent.*
Average of 71 summer samples	5.93	9.76	13.62
Average of 28 winter samples	7.12	13.58	20.90

COLON BACILLI IN ICE CREAM.

Since the presence of colon bacilli has been understood in water analysis to indicate fecal contamination, many investigators and boards of health apply the same tests to milk, and naturally then to ice cream with the same idea.

In water analysis lactose-bile fermentation tubes are used for the examination for colon bacilli. By using different dilutions the minimum number of gas-forming bacteria in a given amount of water may be determined. This preliminary test has to be followed by confirmatory tests in which cultures are isolated and their characteristics studied in order to prove the presence of colon bacilli. In our work we have used this method to some extent, but have endeavored to prepare a synthetic medium which would restrict the growth of the majority of bacteria found in ice cream and at the same time would allow colon bacilli to develop and produce characteristic reactions. During the experiments 53 different combinations were used, as shown in Table 10.

Asparagin was used as a source of nitrogen in almost all media. Throughout our work we used medium No. 1, which was made as follows: Agar, 1.5 per cent; asparagin, 0.3 per cent; sodium dibasic phosphate, 0.1 per cent; lactose, 1 per cent; and 2 per cent of a saturated neutral solution of litmus. This medium proved the most satisfactory of all, and when tested with 10 different strains of *Bacillus coli* it was found that they all grew well. Medium No. 13, recommended by Dolt, did not prove satisfactory, as several of the strains of coli did not develop well on this medium. Medium No. 53, composed of agar 1.5 per cent, lactose 1 per cent, ammonium malate 0.2 per cent, sodium acid phosphate 0.02 per cent, and 2 per cent saturated neutral solution of litmus, proved very satisfactory in a few examinations, but as this medium was developed late in the work it has not been thoroughly tested. The value of the other media will not be discussed here but will be reserved for a later continued study of the subject.

When ice cream was plated on litmus-lactose-asparagin agar, colon bacilli proved to be about the only organisms which grew and formed acid. The colonies were quite distinctive in their appearance and with little practice could readily be counted. Occasionally there developed on the plate a few very small acid colonies which were not gas formers, and sometimes a few nonacid-forming colonies were observed. The majority of bacteria in ice cream did not grow on this medium, at least not in 48 hours at 37° C. (97.5° F.), and it was possible to plate as low as one-tenth of a cubic centimeter when $\frac{1}{1000000}$ dilution had to be used with infusion agar.

TABLE 10.—*Composition of synthetic media in an attempt to produce a medium suitable for the determination of Bacillus coli in ice cream.*

[Amounts expressed as percentages.]

Medium No.	Agar.	Asparagin.	Lactose.	Sodium dibasic phosphate.	Potassium dibasic phosphate.	Potassium acid phosphate.	Saturated neutral litmus solution.	Cubic centimeter of 10 per cent alcoholic fuchsin in 1 liter of medium.	Cubic centimeter of 2½ per cent solution of anhydrous sodium sulphite in 1 liter of medium.	Sodium chlorid.	Aesculin.	Iron citrate.	Sodium citrate.	Ammonium citrate.	Sodium sulphite.	Ammonium lactate.	Ammonium sulphate.	Urea.	Phenol.	Casein.	Sodium carbonate.	Pepton.	Ox bile.	Cubic centimeter NaOH N solution.	½ per cent solution neutral red.	1 per cent solution Dahlia.	Reaction corrected to—	Remarks. (Reaction not changed unless noted.)
1	1.5	0.3	1	0.1			2																					
2	1	.3	1	.1			2			0.5																		
3	1	.3	1	.1			2			.5												0.5						
4	1	.3	1	.1			2			.5				0.5														
5	1	.3	1	.1			2			.5						0.5												
6	1	.3	1	.1			2			.5								0.5										
7	1	.3	1	.1			2			.5										0.5								
8	1	.3	1	.1			2			.5				.2														
9	1	.3	1	.1			2			.5			0.2															
10	1	.3	1	.1			2			.5				.2														
11	1	.3	1	.1			2			.5				.1														
12	1	.3	1	.1			2			.5				.2						.5								
13	1.5		1	.1			2									.5											0.0	
14	1.5	.3	1	.1			2							.2													+ .2	
15	1.5	.3	1	.1			2							.2													+ .5	
16	1.5	.3	1	.1			2										0.2											
17	1	.3	1		0.1		2																					
18	1	.3	1		.1		2			.5																		
19	1	.3	1		.1		1																					
20	1	.3	1		.1																					0.2		
21	1	.3	1		.1																							1 per cent azolitmin.
22	1	.3	1		.1		2			.5																		
23	1	.3	1		.1		2			.5															1			
24	1	.3	1		.1		3																					
25	1	.3	1		.1		2								0.1													
26	1	.3	1		.1		2								.5													
27	1	.3	1		.1		2								1.0													
28	1.5	.3	1		.1		2												0.10									
29	1.5	.1	1				2												4.25									
30	1.5	.1	1		.1		2												.25									
31	1.5	.1	1		.1		2												.25								+1.5	
32	1	.3	1				2																					
33	1	.3			.1						0.1	0.05																
34	1	.1	1			0.5					.1	.05																

35	1	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	[1]1	...	...	...	...	
36	1.5	.3	1	.1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	.5	...	
37	1.5	.3	1	.1	...	...	...	...	...	...	...	...	...	.2	...	...	...	...	...	...	...	...	...	...	...	.5	...	
38	1	.3	1	...	.1	...	...	5	25	...	...	...	...	...	...	...	...	...	...	...	0.1	...	1.6	...	...	...	...	
39	1	.3	1	...	.1	...	...	5	25	...	...	...	...	...	...	...	...	...	...	...	.5	...	1.6	...	...	...	...	
40	1	.3	1	.1	...	...	...	5	25	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
41	1	.3	1	.5	...	...	...	5	25	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
42	1	.3	...	.1	...	...	...	5	25	...	...	...	...	.5	...	...	...	...	...	...	...	...	...	...	...	...	...	
43	1	.3	1	.1	...	...	...	1	5	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
44	1	.3	1	.1	...	...	...	.5	2.5	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
45	1	.3	1	...	...	...	...	5	25	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
46	1	.3	1	.1	...	...	...	2.5	12.5	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
47	1	.3	1	.1	...	...	...	5	50	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
48	1	.3	1	.1	...	...	...	5	25	...	...	...	...	.2	...	...	...	...	...	...	...	...	...	...	...	...	...	
49	1	.3	1	.1	...	...	...	5	25	...	...	...	...	.5	...	...	...	...	...	...	...	...	...	...	...	...	...	
50	1	.3	1	.1	...	...	...	5	25	...	...	...	...	.1	...	...	...	...	...	...	...	...	...	...	...	...	...	
51	1.5	.3	...	.1	...	...	...	5	25	...	...	...	.2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
52	1.5	.3	...	.1	...	...	...	5	25	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
53	1.5	...	1	...	...	...	2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0.2 ammonium malate and 0.02 sodium acid phosphate.

[1] Liter.

In order to test the value of this medium as compared with the bile tube method, 43 samples of ice cream were examined. The results are shown in Table 11. It may be seen that the colon count as determined on litmus-lactose-asparagin agar was higher than the number estimated from bile tubes in 41 of 43 samples. In most samples the number determined from the asparagin plates was much higher; in one sample it was 47 times as high as the number calculated from bile tubes.

TABLE 11.—*Comparison of the number of Bacillus coli in ice cream determined by the bile-tube method and on litmus-lactose-asparagin-agar plates.*

Sample No.	Bile medium.	Litmus-lactose-asparagin-agar plates.				Sample No.	Bile medium.	Litmus-lactose-asparagin-agar plates.			
	B. coli.	B. coli.	Cultures picked.	Cultures picked which form gas in lactose broth.	Per cent gas formers.		B. coli.	B. coli.	Cultures picked.	Cultures picked which form gas in lactose broth.	Per cent gas formers.
1	....	15,400	154	146	94.80	23	5,000	108,000	....	....	....
2	....	86,000	80	78	97.50	24	50,000	120,000	....	....	....
3	50	200	....	....	....	25	5,000	75,000	....	....	....
4	([1]) 0	100	1	1	100.00	26	100,000	300,000	....	....	....
5	500	8,000	8	8	100.00	27	100,000	600,000	....	....	....
6	500	900	9	9	100.00	28	1,000	47,000	....	....	....
7	1,000	2,500	22	22	100.00	29	250	5,600	....	....	....
8	5,000	13,000	12	12	100.00	30	1,000	6,500	....	....	....
9	750	2,900	29	29	100.00	31	100	1,200	....	....	....
10	2,500	5,600	53	51	98.01	32	2,500	15,000	....	....	....
11	1,000	7,200	62	57	91.93	33	500	3,000	....	....	....
12	250	400	4	4	100.00	34	500	3,000	....	....	....
13	1,000	12,000	12	11	91.61	35	250	1,730	....	....	....
14	2,500	11,900	86	80	93.02	36	75	550	....	....	....
15	7,500	123,000	91	85	93.29	37	1,000	2,530	....	....	....
16	75	3,000	3	3	100.00	38	250	2,350	....	....	....
17	50	3,900	39	34	87.17	39	75	20	....	....	....
18	750	39,000	38	38	100.00	40	50	([2]) 0	....	....	....
19	250	5,000	5	5	100.00	41	75	100	....	....	....
20	750	2,400	....	....	....	42	100	230	....	....	....
21	1,000	7,700	....	....	....	43	750	820	....	....	....
22	250	2,400	....	....	....						

[1] In $\frac{1}{100}$ dilution.
[2] In $\frac{1}{10}$ dilution.

TABLE 12.—*Bacteria of the colon group found in ice cream as determined on litmus-lactose-asparagin agar.*

Sample No.	Manufacturer.	B. coli per cubic centimeter.	Sample No.	Manufacturer.	B. coli per cubic centimeter.	Sample No.	Manufacturer.	B. coli per cubic centimeter.
32	A	120	160	C	10	49	K	120,000
33	A	86,000	161	C	30	50	K	75,000
37	A	100	186	C	140	51	K	300,000
38	A	100	187	C	700	79	K[1]	150
39	A	8,000	188	C	50	80	K[1]	40
40	A	900	44	D	2,400	81	K[1]	30
83	A	420	45	D	7,700	82	K[1]	40
124	A	80	89	D	4,600	134	K[1]	[1]0
125	A	10	52	E	600,000	135	K[1]	20
138	A	[1]0	53	E	47,000	136	K[1]	[1]0
139	A	[1]0	54	E	5,600	137	K[1]	[1]0
143	A	[1]0	140	E	30	70	M	1,730
151	A	10	141	E	[1]0	71	M	550
152	A	40	142	E	400	72	M	2,530
153	A	10	67	F	3,900	73	M	2,350
168	A	50	68	F	3,900	84	O	7,600
169	A	30	69	F	5,000	87	O	12,200
31	B	15,400	146	F	100	62	L	11,900
41	B	2,500	147	F	[1]0	63	L	3,000
42	B	17,100	74	G	20	64	L	3,000
43	B	2,800	75	G	[1]0	65	L	123,000
126	B	300	76	G	100	66	P	3,000
127	B	100	77	G	230	144	Q	20
128	B	200	78	G	820	145	Q	20
133	B	1,000	148	G	120	132	R	[1]0
158	B	2,900	149	G	50	175	S	300
162	B	[1]0	150	G	200	176	S	800
163	B	400	59	H	6,500	177	S	600
164	B	700	60	H	1,200	171	T	90
165	B	[1]0	61	H	15,000	172	T	800
166	B	1,400	157	H	[1]0	173	T	1,200
167	B	1,800	55	I	5,600	174	T	100
170	B	14,000	56	I	7,200	178	U	1,400
46	C	700	57	I	400	179	U	4,000
47	C	2,400	58	I	12,000	180	W	2,700
85	C	13,300	154	I	[1]0	181	W	300
86	C	13,500	155	I	900	182	W	700
90	C	6,000	156	I	300	183	X	4,100
91	C	8,300	88	J	3,100	184	X	30
159	C	40	48	K	108,000	185	X	300

[1] In $\frac{1}{10}$ dilution.

During this series of examinations the suspected colon colonies on the asparagin-agar plates from 19 samples were picked off and inoculated into lactose-broth fermentation tubes. After incubation for 48 hours at 37° C. (97.5° F.) the tubes were examined for the presence of gas. The number of cultures picked off and the number showing gas, together with the percentage of the cultures suspected of being gas formers from the examination of the plate, are shown in Table 11. An examination of the results shows that in 10 of the 19 plates studied all the colonies considered gas formers developed gas in fermentation tubes. In other words, 100 per cent were gas formers. Among the other 9 samples the percentage ranged from 87.17 to 100 per cent. This shows that it is possible to detect quite accurately any colonies of gas-forming bacteria on litmus-lactose-asparagin agar.

One hundred and twenty samples of ice cream were examined during the summer and winter seasons by the use of the above-mentioned medium. The results are tabulated in Table 12. Gas-forming bacteria were present in 106, or 88.33 per cent, of the samples, and absent in one-tenth of a cubic centimeter in 14, or 11.67 per cent, of the samples. Of the 14 negative samples, 13 were of the winter series and the other one was examined during October. The number of gas formers ranged from 10 to 600,000 per cubic centimeter. Among the entire number of samples the average number of gas-forming bacteria was 16,298 per cubic centimeter. Of the 57 summer samples, the average was 29,544. The winter samples showed an average of 889 gas formers per cubic centimeter. Apparently ice cream contains a far greater number of colon bacilli during the summer months than in the winter season.

The question naturally arises, Does this asparagin agar bring out all the gas-forming or colon bacilli? To gain some information on this point a comparison of this medium was made with Endo's medium, which is highly recommended by some authorities for the determination of the colon bacillus. The Endo medium was prepared according to the directions given by Kinyoun and Dieter.

Table 13.—*Comparison of colon counts on litmus-lactose-asparagin agar and on Endo's medium.*

Sample No.	Number of B. coli.		Sample No.	Number of B. coli.	
	Litmus-lactose-asparagin agar.	Endo's medium.		Litmus-lactose-asparagin agar.	Endo's medium.
1	90	125,000	10	2,700	11,000
2	800	75,000	11	300	10,000
3	1,200	98,000	12	700	12,000
4	100	25,000	13	30	5,700
5	300	92,000	14	300	11,400
6	800	3,000	15	140	600
7	600	92,000	16	700	2,200
8	4,000	39,000	17	50	1,200

As shown in Table 13, the number of colon bacilli determined in litmus-lactose-asparagin agar was decidedly lower than when Endo's medium was used. It was then a question of what percentage of the colonies on Endo plates called colon bacilli were generally gas formers. To determine this percentage typical colonies were picked off from six Endo plates. Inoculations were made in lactose-broth fermentation tubes and the presence of gas recorded after 48 hours' incubation at 37° C. (97.5° F.). The percentage of the gas-forming cultures from colonies which would be called colon colonies is shown in Table 14.

TABLE 14.—*Cultures picked from Endo's plates showing the number of gas formers.*

Endo plate.	Colonies picked.	Cultures showing gas in lactose broth.		
		Gas+	Gas−	Gas+
	Number.	*Number.*	*Number.*	*Per cent.*
1	160	91	69	56.87
2	32	7	25	21.87
3	73	69	4	94.52
4	9	1	8	11.11
5	10	0	10	.00
6	21	16	5	76.19

From one plate, of the 10 colonies picked off, none produced gas in lactose broth. Of 160 cultures from suspected colon colonies from sample 1 only 56.87 per cent were gas formers. In sample 3 a high percentage—that is, 94.52 per cent of cultures—were gas formers. It is evident that in many cases it is impossible to count the typical colonies on Endo plates and say that they represent gas formers or colon bacilli. Typical colon bacilli are supposed to form on Endo's agar medium-sized colonies which are distinctly red and have a metallic sheen, but doubtless the appearance of colon colonies is quite variable. In a study of the colonies of 10 different strains of *Bacillus coli* on Endo's plates it was found that many of the colonies showed no metallic sheen. Some were pinkish instead of red; there was a red coloration of the medium around some colonies and none around others, and many of the colonies were very small. We also found that a number of nongas-forming bacteria of the acid group and some peptonizers would grow readily on Endo's medium, and the colonies had all the appearance of those of some of the 10 strains of *B. coli* used during these experiments. It must be conceded, however, that a much larger number of gas-forming bacteria can be found in ice cream when using Endo's medium than when plating on litmus-lactose-asparagin agar, and the asparagin plates showed a much higher number than the lactose-bile tubes. The use of Endo's medium for the determination of colon bacilli in ice cream is complicated by the fact that bacteria other than gas formers may give the typical reaction of the colon bacilli; also because when a dilution low enough to determine the number of *B. coli* is used the plate is sometimes overgrown by other bacteria which are present in large numbers.

It is not the purpose of this paper to discuss the best method for the determination of *B. coli* in ice cream, for the reason, which is obvious, that an entirely satisfactory method has not yet been perfected. Each process has its merits and objectionable features, but in view of our present knowledge of the colon group of bacteria it is impossible to say that any one method is the best.

SUMMARY AND CONCLUSIONS.

Samples of ice cream as sold at retail were examined in Washington during the summer season from June 20 to November 11, 1912, and also during the winter season in February and March of 1913.

1. The average acidity of 65 samples of vanilla ice cream examined during the summer and winter season was 0.206 per cent, calculated as lactic acid. The maximum acidity was 0.387 per cent, the minimum 0.09 per cent. No definite relation was found between acidity and the bacterial count.

2. The average number of bacteria found in 94 samples of ice cream examined during the summer months was 37,859,907 per cubic centimeter. The maximum count was 510,000,000, the minimum 120,000. Of the 91 samples examined during the winter months the average count was 10,388,222, the maximum 114,000,000, and the minimum 13,000 bacteria per cubic centimeter. Among the 94 summer samples none contained fewer than 100,000 bacteria per cubic centimeter, while 14.28 per cent of the 91 winter samples were lower than 100,000. Of the summer samples 9.57 per cent contained fewer than 500,000 per cubic centimeter, and of the winter 39.55 per cent contained less than that number. Of the summer samples, 19.14 per cent contained fewer than 1,000,000 per cubic centimeter, while 41.75 per cent of the winter samples contained fewer than that number and 80.86 per cent of the summer samples and 58.25 per cent of the winter samples contained more than 1,000,000 bacteria per cubic centimeter. There was a wide range in the bacterial content during the summer and winter seasons, and with one exception, the average number of bacteria in ice cream from each manufacturer was distinctly lower during the winter months.

3. The bacteria in 71 summer samples and 28 winter samples were divided into groups by the milk-tube method. The percentages of the various groups of bacteria, together with the calculated number of bacteria in each group in an average sample of ice cream examined during the summer and winter seasons, are summarized in the following table:

TABLE 15.—*Summary of bacteria in ice cream.*[1]

Bacterial groups.	Summer samples.		Winter samples.	
	Average number of bacteria per cubic centimeter.	Average group percentage.	Average number of bacteria per cubic centimeter.	Average group percentage.
Acid-coagulating	18,861,805	49.82	3,203,728	30.84
Acid-forming	7,844,575	20.72	3,950,641	38.03
Inert	5,292,815	13.98	499,673	4.81
Alkali-forming	704,195	1.86	563,042	5.42
Peptonizing	5,156,519	13.62	2,171,138	20.90
Total	37,859,909	100.00	10,388,222	100.00

[1] The counts were made from the average percentage of each group and the average total count.

The bacterial groups bore much the same relation to each other in the average summer and winter samples. There was, however, in the summer samples a higher percentage of the acid-coagulating group of bacteria and a lower percentage of the alkali and peptonizing groups than in the winter samples. In spite of a higher percentage of the two groups last named in the average winter sample, the number of bacteria of these was much lower, owing to a lower average total bacterial count.

4. Among the summer samples of ice cream 52.81 per cent of the bacteria of the acid-coagulating group were active enough to coagulate milk in 48 hours when incubated at 30° C. (86° F.). The remaining 47.19 per cent coagulated milk more slowly and may represent a different variety of acid-forming bacteria. Only 26.69 per cent of the acid-coagulating group of bacteria in the winter samples coagulated in 48 hours. There is therefore a higher percentage of rapid acid-coagulating bacteria in ice cream during the summer months.

5. The average number of peptonizing bacteria found in the summer samples of ice cream was 1,449,533. The maximum count was 21,000,000, the minimum 36,063, peptonizing bacteria per cubic centimeter. During the winter season the average number was 268,693, which is about one-fifth the average summer count. The maximum number was 2,974,400 and the minimum 1,194 bacteria per cubic centimeter. The majority of the samples during the entire investigation contained from 0.1 to 5 per cent of peptonizers. A large proportion contained as high as 25 to 30 per cent, and in a few samples they were present to the extent of from 90 to 95 per cent of the total bacteria. Among the summer samples 43.54 per cent of the peptonizing bacteria were sufficiently active to produce a peptonization in milk within 48 hours at 30° C. (86° F.), while 34.06 per cent of these bacteria in the winter samples were able to produce this change in the same time. It is probable that these active peptonizers are more important than the slower-acting varieties, since they usually produce a greater decomposition of the milk.

6. Gas-forming bacteria of the colon-aerogenes group when determined on litmus-lactose-asparagin agar were found present in one-tenth of a cubic centimeter in 106, or 88.33 per cent, of the 120 samples examined, and absent in one-tenth of a cubic centimeter in 14, or 11.67 per cent, of the samples. Of the 14 negative samples, 13 were of the winter series and 1 was examined during October. The average number of gas formers in the entire series of samples was 16,298 per cubic centimeter. Fifty-seven samples examined during the summer averaged 29,544 per cubic centimeter. The 49 winter samples contained an average of 889 per cubic centimeter. Ice cream contained a much larger number of gas-forming organisms during the summer season. A large number of media were used in an attempt

to devise a suitable medium for the detection of *Bacillus coli* in ice cream, and our results show that there is no entirely satisfactory method known at present.

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