UNITED STATES DEPARTMENT OF AGRICULTURE

BULLETIN No. 563

Contribution from Bureau of Animal Industry.
A. D. MELVIN, Chief.

Washington, D. C. PROFESSIONAL PAPER June 19, 1917

THE DETERMINATION OF BACTERIA IN ICE CREAM.

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DIFFICULTY OF MAKING ACCURATE BACTERIOLOGICAL ANALYSES.

Statements have been made that the distribution of bacteria in ice cream is markedly uneven, that there is great variability in the bacterial counts of different portions of the same container, and that this variability is so great that any small sample selected for analysis will not represent the whole mass of the ice cream.

It must be remembered that the accuracy of a bacteriological analysis can never be so great as that of a chemical analysis. In making bacterial counts we are dealing with living organisms which are distributed in the material under examination. The method of analysis follows the assumption that the bacteria, as individual cells, are distributed evenly throughout the sample and that the portion removed for analysis contains a number in exact proportion to the total number in the sample. Having removed a definite part, it must then be placed in a medium suitable for plating in which the individual bacterial cells can multiply and form visible colonies. The inaccuracy of such a method must be evident at once.

We know that some bacteria are in clumps or chains, and many organisms may then develop into one colony which must be counted as a single colony. The removal of a quantity of material which will contain the same number of bacteria in suspension as another like quantity is known to be impossible. Since we are dealing with

living organisms, the bacteriological method of analysis must take into account their distribution and development into colonies on the Petri plates. In this part of the method we encounter the difficulty of separating the bacterial cells and distributing them evenly. Their development is more or less influenced by the growth of different kinds of bacteria, one of which may retard the development of those near it.

All these points are recognized by bacteriologists and are mentioned here merely to call attention to the many difficulties which arise in making accurate bacterial counts and to point out that there must be variations in the result over which the analyst has no definite control. This condition has been recognized, consequently duplicate plates are made and results reported from the average counts of both plates. The variation in bacterial counts is particularly important and must be taken into consideration when a study is made of various samples of any material containing bacteria. If these variations are not considered, mistakes are easily made in the study of the distribution of bacteria.

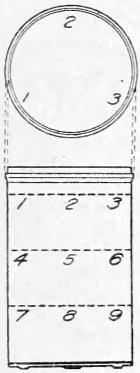

FIG. 1.—Showing location of nine samples taken from 1-gallon can of ice cream.

If the bacteria in ice cream are unevenly distributed, and a bacterial analysis of a sample does not give results which will represent the whole mass of the cream, this fact will greatly complicate any study of the bacteria in the product. Consequently, before starting any further studies on the subject it was considered advisable to carry out some experiments to throw more light on this point.

METHOD OF SAMPLING AND PLATING THE ICE CREAM.

Ice cream from various manufacturers was delivered in 1-gallon cans at the laboratory. As soon as received, the can of cream was removed from the tub, the ice and salt wiped off, the cover removed, and the top layer taken off with a large sterile spoon.

Three samples were taken from the topmost third of the gallon, three from the middle, and three from the bottom third, making a total of nine samples from each gallon, as shown in figure 1. Each sample contained about 30 grams and was removed with a small sterile scoop and placed in a sterile flask. After removing samples from positions 1, 2, and 3, about one-third of the ice cream was removed with a sterile spoon and three more samples taken from positions 4, 5, and 6; similarly the three remaining samples were taken from positions 7, 8, and 9.

The flasks containing the samples were then placed in water at 40° C. (104° F.) for 15 minutes in order to melt the ice cream, the

melting being hastened by frequent shaking with a circular motion. At the end of the 15-minute period each flask containing the melted ice cream was again shaken 30 times, with a circular motion, in order to mix the sample thoroughly and to shake out as much air as possible.

One cubic centimeter of melted ice cream was then removed from each flask and placed in 99 c. c. of sterile water at a temperature of 40° C. (104° F.). The water in all the dilution bottles was at a temperature of 40° C. (104° F.) in order to keep the fat in a melted condition.

The pipettes were so graduated as to deliver 1 cubic centimeter between two marks. This avoids the necessity of blowing out the pipette or immersing the end in the dilution water, and therefore eliminates the introduction of varying quantities of melted cream which adhere to the pipette.

The dilutions were made in the usual way, using 99 c. c. and 9 c. c., respectively, of sterile water. Each dilution bottle or tube was shaken 25 times, and great care was taken to measure the quantity accurately in the pipettes. Standard beef-infusion agar was used, and sufficient medium was prepared to last through the work; consequently no factor of variation was introduced by the plating medium. The plates were incubated at 30° C. (86° F.) for a period of five days, after which the duplicate plates were counted.

VARIATION IN THE BACTERIAL CONTENT OF COMMERCIAL ICE CREAM.

VARIATION IN DUPLICATE SAMPLES FROM VARIOUS PARTS OF THE SAME LOT.

In our first experiment twenty-two 1-gallon lots of ice cream were obtained from seven different manufacturers. This cream, as intended, was of different flavors, was made in different ways, and included products containing different ingredients and varying percentages of butterfat.

The complete results obtained from a study of these samples are shown in Table I, in which is recorded the percentage of fat in the ice cream from each manufacturer, also the presence or absence of gelatin, the flavor of each lot, the dilution used in plating, the number of colonies found on each of the duplicate plates, and the calculated average number of bacteria in a cubic centimeter of melted ice cream. Where there is a blank space in the number of colonies on duplicate plates no count could be made on account of "spreaders," which entirely obscured the colonies. Every count that could be obtained is included in this table of results, and no count was left out as being a "freak" result.

A study of the table shows that the bacterial counts of the nine samples from as many different positions in each gallon lot of ice cream check remarkably well with one another.

TABLE I.—*Bacteria per cubic centimeter in 198 samples from twenty-two 1-gallon lots of commercial ice cream obtained from different manufacturers.*

Plant No.	Lot.	Sample No.	Flavor.	Dilution.	Number of colonies on duplicate plates.		Average count per c. c.
1	A (fat 9.5 per cent, gelatin +)...	1	Vanilla.....	1/100000	243	229	2,360,000
		2			219	198	2,085,000
		3			242	243	2,425,000
		4			231	245	2,380,000
		5			250	.....	2,500,000
		6			264	233	2,485,000
		7			230	.....	2,300,000
		8			243	245	2,440,000
		9			271	235	2,530,000
	B.....	1	Chocolate...	1/100000	45	46	455,000
		2			40	49	445,000
		3			50	47	485,000
		4			67	40	535,000
		5			52	47	495,000
		6			60	43	515,000
		7			42	48	450,000
		8			49	45	470,000
		9			57	60	585,000
	C.....	1	Peach.....	1/100000	146	153	1,495,000
		2			160	150	1,550,000
		3			137	155	1,460,000
		4			148	145	1,465,000
		5			140	156	1,480,000
		6			170	151	1,605,000
		7			153	155	1,540,000
		8			154	148	1,510,000
		9			154	166	1,600,000
	D.....	1	Vanilla.....	1/10000	24	26	25,000
		2			23	25	24,000
		3			80	.....	80,000
		4			20	32	26,000
		5			20	20	20,000
		6			30	22	26,000
		7			40	.....	40,000
		8			25	26	25,500
		9			25	27	26,000
	E.....	1	Vanilla.....	1/100	64	79	7,150
		2			117	.....	11,700
		3			120	96	10,800
		4			109	118	11,350
		5			95	118	10,650
		6			102	.....	10,200
		7			138	97	11,750
		8			77	.....	7,700
		9			131	129	13,000
2	A.....	1	Vanilla.....	1/1000000	25	62	43,500,000
		2			44	54	49,000,000
		3			54	46	50,000,000
		4			53	47	50,000,000
		5			59	.....	59,000,000
		6			58	54	56,000,000
		7			67	55	61,000,000
		8			50	59	54,500,000
		9			59	55	57,000,000
	B.....	1	Chocolate...	1/1000000	217	218	217,500,000
		2			212	193	201,500,000
		3			186	.....	186,000,000
		4			233	217	225,000,000
		5			204	195	199,500,000
		6			204	184	194,000,000
		7			187	174	180,500,000
		8			203	200	201,500,000
		9			192	184	188,000,000

TABLE I.—*Bacteria per cubic centimeter in 198 samples from twenty-two 1-gallon lots of commercial ice cream obtained from different manufacturers*—Continued.

Plant No.	Lot.	Sample No.	Flavor.	Dilution.	Number of colonies on duplicate plates.		Average count per c. c.
2	C (fat 9.6 per cent, gelatin +)...	1	Vanilla.....	1/100000	60	64	62,000,000
		2			63	65	64,000,000
		3			73	62	67,500,000
		4			70	68	69,000,000
		5			64	70	67,000,000
		6			73	77	75,000,000
		7			63	57	60,000,000
		8			63	72	67,500,000
		9			57	72	64,500,000
3	A (fat 9.6 per cent, gelatin +)...	1	Vanilla	1/10000	286	290	288,000
		2			360	254	307,000
		3			270	289	279,500
		4			250	270	260,000
		5			314	250	282,000
		6			251	252	251,500
		7			268	250	259,000
		8			311	287	299,000
		9			261	306	283,500
	B.....	1	Peach.....	1/1000	219	186	202,500
		2			214	.....	214,000
		3			222	190	206,000
		4			192	.....	192,000
		5			212	194	203,000
		6			197	200	198,500
		7			212	208	210,000
		8			231	205	218,000
		9			218	224	221,000
	C.....	1	Chocolate...	1/100000	107	104	1,055,000
		2			118	99	1,085,000
		3			96	99	975,000
		4			106	96	1,010,000
		5			108	119	1,135,000
		6			123	124	1,235,000
		7			102	97	995,000
		8			124	123	1,235,000
		9			103	121	1,120,000
4	A (fat, 22.5 per cent, gelatin−)...	1	Vanilla.....	1/100000	341	334	33,750,000
		2			274	266	27,000,000
		3			284	.....	28,400,000
		4			294	302	29,800,000
		5			284	270	27,700,000
		6			301	303	30,200,000
		7			325	331	32,800,000
		8			321	329	32,500,000
		9			314	.....	31,400,000
	B.....	1	Peach.....	1/100000	321	310	31,500,000
		2			270	305	28,750,000
		3			386	372	37,900,000
		4			278	305	29,150,000
		5			367	356	36,150,000
		6			354	357	35,550,000
		7			431	466	44,850,000
		8			470	.....	47,000,000
		9			434	.....	43,400,000
	C.....	1	Chocolate...	1/100000	310	298	30,400,000
		2			286	.....	28,600,000
		3			330	.....	33,000,000
		4			.....	.....	.....
		5			293	288	29,050,000
		6			284	288	28,600,000
		7			328	292	31,000,000
		8			286	278	28,200,000
		9			275	285	28,000,000

TABLE I.—*Bacteria per cubic centimeter in 198 samples from twenty-two 1-gallon lots of commercial ice cream obtained from different manufacturers*—Continued.

Plant No.	Lot.	Sample No.	Flavor.	Dilution.	Number of colonies on duplicate plates.		Average count per c. c.
5	A (fat, 17 per cent, gelatin —)...	1	Vanila.....	1/100000	44	.....	440,000
		2			45	48	465,000
		3			46	49	475,000
		4			36	.....	360,000
		5			49	.....	490,000
		6			35	44	395,000
		7			47	43	450,000
		8			49	41	450,000
		9			26	41	335,000
	B.....	1	Peach.....	1/100000	44	53	485,000
		2			46	38	420,000
		3			44	.....	440,000
		4			50	.....	500,000
		5			48	.....	480,000
		6			42	44	430,000
		7			41	46	435,000
		8			48	.....	480,000
		9			41	.....	410,000
	C.....	1	Chocolate...	1/1000000	142	.....	142,000,000
		2			123	.....	123,000,000
		3			137	107	122,000,000
		4			156	152	154,000,000
		5			120	123	126,500,000
		6			138	135	136,500,000
		7			142	130	136,000,000
		8			143	130	136,500,000
		9			145	176	165,000,000
6	A (fat, 9.2 per cent, gelatin+)...	1	Vanilla.....	1/1000000	144	178	16,100,000
		2			128	136	13,200,000
		3			179	161	17,000,000
		4			200	.....	20,000,000
		5			235	190	21,250,000
		6			152	.....	15,200,000
		7			130	133	13,150,000
		8			270	.....	27,000,000
		9			222	.....	22,200,000
	B.....	1	Peach.....	1/1000000	102	.....	102,000,000
		2			86	108	97,000,000
		3			109	111	110,000,000
		4			107	107	107,000,000
		5			111	105	108,000,000
		6			117	115	115,000,000
		7			117	115	116,000,000
		8			90	108	99,000,000
		9			103	98	100,500,000
	C.....	1	Chocolate...	1/1000000	45	43	44,000,000
		2			41	39	40,000,000
		3			37	48	42,500,000
		4			.....	.....	.....
		5			39	43	41,000,000
		6			41	44	41,000,000
		7			43	42	42,500,000
		8			37	.....	37,000,000
		9			52	41	46,500,000
	D.....	1	Vanilla.....	1/100000	121	102	1,115,000
		2			114	98	1,060,000
		3			120	125	1,225,000
		4			126	106	1,160,000
		5			105	113	1,090,000
		6			87	124	1,055,000
		7			134	149	1,415,000
		8			122	125	1,235,000
		9			137	137	1,370,000
7	A.....	1	Chocolate...	1/100000	170	176	1,730,000
		2			161	.....	1,610,000
		3			161	183	1,720,000
		4			179	165	1,720,000
		5			162	192	1,770,000
		6			174	.....	1,740,000
		7			131	.....	1,310,000
		8			184	143	1,635,000
		9			157	.....	1,570,000

The maximum and minimum bacterial count of the samples from each gallon of ice cream, together with the per cent of variation, is shown in Table II.

In the samples from Plant No. 1 there was a variation of 300 per cent among the samples from lot D, which showed a minimum count of 20,000 and a maximum of 80,000 per cubic centimeter. This case, as may be noted, is extreme and may be accounted for by the fact that the 80,000 count was obtained from one plate only, the duplicate being covered with spreaders. Reference to the complete results in Table I for this lot shows that among the other samples the colony counts were very nearly alike. A similar explanation holds for lot E from Plant No. 1, in which there was a variation of 81.81 per cent, with a maximum and minimum count of 13,000 and 7,150.

Throughout the rest of the samples the only high percentage of variation was among the samples taken from lot A, Plant No. 6, a variation of 105.32 per cent. In this case the melted cream was a thick, viscous mass, which made it difficult to measure accurately in a pipette. Special care was taken in the remaining determinations of bacteria in the ice cream from this manufacturer, with the result that the percentage of variation in bacterial counts was very low.

The general variation among the samples from each gallon of ice cream was from 20 to 30 per cent, which is decidedly low, although at first thought it may seem high.

In Reprint 295 of the Public Health Reports[1] it is stated that in analyzing duplicate samples of milk the general average variation in each of four laboratories ranged from about 110 to 380 per cent. Just what percentage of variation in duplicate counts is normal to the method of bacterial analysis we shall not attempt to say, but a variation of 20 per cent means only the difference between 100 and 120 colonies on a Petri plate.

The small variation in our results indicates that in the ice cream examined the bacteria were rather evenly distributed and that an analysis of one sample taken in the manner described would show for all practical purposes the bacterial content of any other sample in the 1-gallon lot.

[1] Conn, H. W. Standards for determining the purity of milk.

TABLE II.—*Variation in bacterial content of 9 duplicate samples from each of twenty-two 1-gallon lots of commercial ice cream.*

Plant No.	Lot.	Flavor.	Bacteria per cubic centimeter.		Variation in bacterial count.
			Minimum.	Maximum.	
					Per cent.
1	A (fat, 9.5 per cent)	Vanilla	2,085,000	2,530,000	21.34
	B	Chocolate	445,000	585,000	31.46
	C	Peach	1,460,000	1,600,000	9.59
	D	Vanilla	20,000	80,000	300.00
	E	Vanilla	7,150	13,000	81.81
2	A	Vanilla	43,500,000	61,000,000	40.23
	B	Chocolate	180,500,000	225,000,000	24.65
	C (fat, 9.6 per cent)	Vanilla	60,000,000	75,000,000	25.00
3	A (fat, 9.6 per cent)	Vanilla	251,500	307,000	22.31
	B	Peach	192,000	221,000	15.10
	C	Chocolate	975,000	1,235,000	26.66
4	A (fat, 22.5 per cent)	Vanilla	27,000,000	33,750,000	25.00
	B	Peach	28,750,000	47,000,000	63.47
	C	Chocolate	28,000,000	33,000,000	17.85
5	A (fat, 17 per cent)	Vanilla	335,000	490,000	46.27
	B	Peach	410,000	500,000	21.95
	C	Chocolate	122,000,000	160,500,000	31.55
6	A (fat, 9.2 per cent)	Vanilla	13,150,000	27,000,000	105.32
	B	Peach	97,000,000	116,000,000	19.59
	C	Chocolate	37,000,000	46,500,000	25.67
	D	Vanilla	1,055,000	1,415,000	34.12
7	A	Chocolate	1,310,000	1,770,000	35.11

VARIATION WHEN HELD IN AN ICE-CREAM CABINET.

It was thought that there might be an uneven distribution of bacteria in ice cream held in an ice-cream cabinet where it is allowed to soften, then is repacked with ice and salt and again hardened. To determine this point, three 1-gallon lots of ice cream were purchased from three different manufacturers and held for 11 days in a commercial ice-cream cabinet, such as is used in stores.

The ice cream was packed with ice and salt once a day in the regular way. From day to day it softened and again hardened. In Table III it may be seen that even under this extreme condition the highest variation among the samples from each lot was only 37.03 per cent. In this case the lowest count was 1,080,000 and the highest 1,420,000 per cubic centimeter.

TABLE III.—*Variation in bacterial counts of 9 samples of ice cream taken from each of three 1-gallon lots which had been held in a cabinet for 11 days.*

Plant No.	Sample No.	Dilution.	Number of colonies on duplicate plates.		Average count per c. c.	Variation between lowest and highest counts.
1	1	1/1000	44	48	46,000	Samples 5 and 7, 34.21 per cent.
	2		52	43	47,500	
	3		46	.....	46,000	
	4		48	.....	48,000	
	5		38	.....	38,000	
	6		43	46	44,500	
	7		51	.....	51,000	
	8		37	50	43,500	
	9		43	.....	43,000	
2	1	1/10000	108	.....	1,080,000	Samples 1 and 8, 37.03 per cent.
	2		111	125	1,180,000	
	3		112	.....	1,120,000	
	4		142	134	1,380,000	
	5		132	152	1,420,000	
	6		127	113	1,200,000	
	7		136	149	1,425,000	
	8		130	166	1,480,000	
	9		138	149	1,435,000	
5	1	1/10000	62	76	690,000	Samples 2 and 5, 14.73 per cent.
	2		64	65	645,000	
	3		76	61	685,000	
	4		65	.....	650,000	
	5		74	.....	740,000	
	6		65	77	710,000	
	7		66	71	685,000	
	8		66	.....	660,000	
	9		70	68	690,000	

Keeping in mind the normal variation in bacterial counts, as indicated in Table II, we do not believe that the results warrant the conclusion that there is any great uneven distribution of bacteria in ice cream, even when held under the extreme conditions of this experiment.

VARIATION WHEN HELD IN STORAGE.

In order to determine the effect of cold storage upon the distribution of bacteria in ice cream, three 1-gallon cans were filled with ice cream from the same freezer. Of these, one was examined while fresh, one was held in cold storage in a hardening room at a plant for one month, and the third was similarly held for two months.

The results of this experiment, recorded in Table IV, show that there was no increase in the variation among the samples from each gallon lot, even after two months' storage. The samples as a whole checked remarkably well, showing nothing to indicate any marked uneven distribution of bacteria.

TABLE IV.—*Variation in the bacterial content of samples of ice cream taken from gallon lots held in cold storage.*

Age of ice cream.	Sample No.	Number of colonies on duplicate plates.		Average count per c. c.	Variation between lowest and highest counts.
Fresh.....	1	64	79	7,150	Samples 1 and 9, 81.81 per cent.
	2	117	.....	11,700	
	3	120	96	10,800	
	4	109	118	10,350	
	5	95	118	10,650	
	6	102	.....	10,200	
	7	138	97	11,750	
	8	77	.....	7,700	
	9	131	129	13,000	
One month.....	1	118	.....	11,800	Samples 7 and 8, 15.68 per cent.
	2	106	.....	10,600	
	3	112	.....	11,200	
	4	111	117	11,400	
	5	110	112	11,100	
	6	104	.....	10,400	
	7	118	.....	11,800	
	8	102	.....	10,200	
	9	117	106	11,150	
Two months.....	1	76	77	7,650	Samples 2 and 9, 18.31 per cent.
	2	66	76	7,100	
	3	76	76	7,600	
	4	92	68	8,000	
	5	79	82	8,050	
	6	73	72	7,250	
	7	70	80	7,500	
	8	75	76	7,500	
	9	76	92	8,400	

VARIATION IN SAMPLES TAKEN DIRECTLY FROM FREEZER.

The question of whether a sample of ice cream taken from a freezer represents in bacterial content the entire contents of the freezer is one of considerable importance. To obtain information on this point 10 series of from 6 to 10 samples were taken from the large commercial freezers in an ice-cream plant. The samples were taken in the following manner: As soon as the cream was frozen and ready to flow into the final containers for hardening, the gate was opened and about 1 pint allowed to flow out. The first sample of about 50 grams was then taken by allowing the partially frozen cream to flow into a sterile salt-mouth bottle. About 1 gallon of ice cream was then allowed to flow out and a second sample taken. The remaining samples were secured in a similar way, the last one being taken from the very last portion. All were immediately iced and taken to the laboratory, where they were plated in the manner previously described. The results are shown in Table V.

TABLE V.—*Variation in bacterial content of samples of ice cream taken from the same freezer.*

Date samples taken.	Sample No.	Number of colonies on duplicate plates (1/1000 c. c. plated).		Average counts per c. c.	Variation between lowest and highest counts.
9-14-14.....	1	48	56	52,000	Samples 7 and 9, 31.39 per cent.
	2	66	47	56,500	
	3	48	46	47,000	
	4	60	43	51,500	
	5	46	56	51,000	
	6	51	50	50,500	
	7	61	52	56,500	
	8	52	48	50,000	
	9	42	44	43,000	
9-15-14.....	1	22	.....	22,000	Samples 4 and 5, 29.54 per cent.
	2	25	24	24,500	
	3	28	22	25,000	
	4	29	28	28,500	
	5	22	.....	22,000	
	6	23	26	24,500	
9-16-14.....	1	107	.....	107,000	Samples 6 and 7, 7.84 per cent.
	2	102	.....	102,000	
	3	110	100	105,000	
	4	118	.....	118,000	
	5	104	.....	104,000	
	6	102	.....	102,000	
	7	110	.....	110,000	
9-17-14 (A).....	1	58	55	56,500	Samples 1 and 3, 28.41 per cent.
	2	53	.....	53,000	
	3	44	.....	44,000	
	4	52	.....	52,000	
	5	53	52	52,500	
	6	56	46	51,000	
	7	53	.....	53,000	
	8	58	53	55,500	
	9	53	.....	53,000	
	10	53	49	51,000	
9-17-14 (A).....	1	52	.....	52,000	Samples 3 and 6, 25 per cent.
	2	45	52	48,500	
	3	53	57	55,000	
	4	45	.....	45,000	
	5	55	39	47,000	
	6	40	48	44,000	
	7	44	51	47,500	
	8	48	50	49,000	
	9	42	47	44,500	
	10	47	.....	47,000	
9-18-14 (B).....	1	159	155	157,000	Samples 3 and 10, 35.87 per cent.
	2	156	157	156,500	
	3	167	189	178,000	
	4	164	162	163,000	
	5	126	173	149,500	
	6	149	144	146,500	
	7	157	160	158,500	
	8	157	154	155,500	
	9	130	160	145,000	
	10	131	.....	131,000	
9-18-14 (B).....	1	186	160	173,000	Samples 3 and 9, 30.07 per cent.
	2	157	.....	157,000	
	3	158	227	192,500	
	4	179	.....	179,000	
	5	166	.....	166,000	
	6	185	.....	185,000	
	7	178	.....	178,000	
	8	165	.....	165,000	
	9	148	.....	148,000	
	10	168	.....	168,000	
9-19-14.....	1	82	77	79,500	Samples 3 and 5, 40.47 per cent.
	2	83	.....	83,000	
	3	94	83	88,500	
	4	85	84	84,500	
	5	55	71	63,000	
	6	98	68	83,000	
	7	82	.....	82,000	
	8	83	75	79,000	

It will be seen from the table that the highest variation among the samples taken from any freezer was 40.47 per cent, the minimum count in that case being 63,000 and the maximum 88,500 bacteria per cubic centimeter. The lowest variation was 7.84 per cent, the counts in this instance ranging from 102,000 to 118,000 per cubic centimeter.

It is interesting to note that the two sets of samples marked A were taken on the same day from two different freezers, which were probably filled with the same "mix." The same is true of the sets marked B. In each of these cases the samples check well with each other. From these results it seems evident that the bacterial content of one sample from a freezer may be said to represent for practical purposes the bacterial content of the rest of the ice cream in the freezer.

COMPARISON OF INCUBATION OF PLATES AT 37° C. FOR TWO DAYS AND 30° C. FOR FIVE DAYS.

The plates in our experiments were incubated at 30° C. (86° F.) for five days. Since in general practice plates are incubated at 37° C. (98.6° F.) and counts made after 48 hours, it was considered advisable to make a comparison between these two methods of incubation.

A gallon of ice cream was obtained and nine samples taken from nine different positions in the usual manner. Two sets of duplicate plates were made; one set was incubated at 37° C. (98.6° F.) and counted after 48 hours. The other set was incubated at 30° C. (86° F.) and counted after five days. The results of this experiment (see Table VI) show that the variation among the samples from the same lot of ice cream was 16.89 per cent after the 48-hour count at 37° C. (98.6° F.) and 35.11 per cent after incubation at 30° C. for five days.

From this experiment it seems evident that incubation at 37° C. (98.6° F.) for 48 hours does not give counts which show any greater variation than those obtained by incubation at 30° C. (86° F.) for five days. It is interesting to observe, however, that the count obtained by incubation for five days at 30° C. (86° F.) is practically double that obtained by incubation at 37° C. (98.6° F.) for 48 hours.

TABLE VI.—*Variation in counts obtained by incubation of plates at 37° C. for two days and 30° C. for five days.*

Sample No.	Incubated at 37° C. for 48 hours.				Incubated at 30° C. for 5 days.			
	Number of bacteria on duplicate plates.		Average count per c. c.	Variation between lowest and highest counts.	Number of bacteria on duplicate plates.		Average count per c. c.	Variation between lowest and highest counts.
1	77	80	785,000	Samples 2 and 9, 16.89 per cent.	170	176	1,730,000	Samples 5 and 7, 35.11 per cent.
2	74	.....	740,000		161	.....	1,610,000	
3	77	.....	770,000		161	183	1,720,000	
4	76	79	775,000		179	165	1,720,000	
5	81	83	820,000		162	192	1,770,000	
6	75	82	785,000		174	.....	1,740,000	
7	73	80	765,000		131	.....	1,310,000	
8	80	.....	800,000		184	143	1,635,000	
9	83	90	865,000		157	.....	1,570,000	

THE NUMBER OF COLONIES MOST DESIRABLE ON PETRI PLATES.

When a sample of ice cream is plated it is of course necessary to make several dilutions, since the bacterial content is unknown. It is sometimes a question as to which dilution will give the most accurate count.

Some interesting facts regarding this point are shown by reference to Table VII. Seven 1-gallon lots of ice cream were obtained, and nine samples from each were plated in the usual manner. Three dilutions were made, but only two are recorded in the table. The aim was to obtain plates with about 200 colonies, as in bacteriological work that number is believed to give the most nearly accurate count. The two recorded, therefore, are the counts obtained from the dilution which gave about 200 colonies per plate and the counts from the next highest dilution.

An examination of the table shows that the variation between samples taken from the same lot of ice cream ranged from 15.10 to 105.32 per cent when the dilution was such that from 100 to 300 colonies were on the plates. The same samples at the next higher dilution, in which the number of colonies was less than 50, varied from 35 to 1,014.28 per cent. In every case in which there was a small number of colonies on the plates the variation between the samples from each gallon lot was decidedly greater than when a lower dilution was used. This fact is by no means new, but it should be kept in mind when interpreting the results obtained from a bacterial analysis of duplicate samples of ice cream.

TABLE VII.—*Comparison of bacterial counts obtained from the same samples of ice cream but with different dilutions.*

Lot.	Sample No.	Dilution.	Number of colonies on duplicate plates.		Average count per c. c.	Variation between lowest and highest counts.	Dilution.	Number of colonies on duplicate plates.		Average count per c. c.	Variation between lowest and highest counts.
						Per ct.					*Per ct.*
A	1	1/10,000	243	229	2,360,000	21.34	1/100,000	27	30	2,850,000	36.36
	2		219	198	2,085,000			22	43	3,250,000	
	3		242	243	2,425,000			24	40	3,200,000	
	4		231	245	2,380,000			36	24	3,000,000	
	5		250	.....	2,500,000			36	39	3,750,000	
	6		264	233	2,485,000			30	25	2,750,000	
	7		230	.....	2,300,000			37	28	3,250,000	
	8		243	245	2,440,000			36	22	2,900,000	
	9		271	235	2,530,000			34	25	2,950,000	
B	1	1/100,000	341	334	33,750,000	25.00	1/1,000,000	41	31	36,000,000	35.00
	2		274	266	27,000,000			31	29	30,000,000	
	3		284	.....	28,400,000			30	....	30,000,000	
	4		294	302	29,800,000			30	....	30,000,000	
	5		284	270	27,700,000			33	36	34,500,000	
	6		301	303	30,200,000			38	29	33,500,000	
	7		325	331	32,800,000			38	44	41,000,000	
	8		321	329	32,500,000			35	43	39,000,000	
	9		314	.....	31,400,000			35	46	40,500,000	
C	1	1/1,000	286	290	288,000	22.31	1/10,000	42	53	475,000	50.79
	2		360	254	307,000			41	....	410,000	
	3		270	289	279,500			40	33	365,000	
	4		250	270	260,000			31	42	365,000	
	5		314	250	282,000			38	39	385,000	
	6		251	252	251,500			30	33	315,000	
	7		268	250	259,000			32	38	350,000	
	8		311	287	299,000			32	44	380,000	
	9		261	306	283,500			35	52	435,000	

TABLE VII.—*Comparison of bacterial counts obtained from the same samples of ice cream but with different dilutions*—Continued.

Lot.	Sample No.	Dilution.	Number of colonies on duplicate plates.		Average count per c. c.	Variation between lowest and highest counts.	Dilution.	Number of colonies on duplicate plates.		Average count per c. c.	Variation between lowest and highest counts.
						Per ct.					*Per ct.*
D	1	1:100,000	321	310	31,500,000	63.47	1:1,000,000	22	31	26,500,000	88.69
	2		270	305	28,750,000			35	36	35,500,000	
	3		386	372	37,900,000			24	37	30,500,000	
	4		278	305	29,150,000			33	25	29,000,000	
	5		367	356	36,150,000			35	34	34,500,000	
	6		354	357	35,550,000			35	....	35,000,000	
	7		431	466	44,850,000			43	27	35,000,000	
	8		470	.....	47,000,000			52	39	45,500,000	
	9		434	.....	43,400,000			55	45	50,000,000	
E	1	1:1,000	219	186	202,500	15.10	1:10,000	24	20	220,000	105.71
	2		214	.....	214,000			25	....	250,000	
	3		222	190	206,000			23	24	235,000	
	4		192	.....	192,000			13	22	175,000	
	5		212	194	203,000			26	16	210,000	
	6		197	200	198,500			36	....	360,000	
	7		212	208	210,000			20	24	220,000	
	8		231	205	218,000			31	25	280,000	
	9		218	224	221,000			36	22	290,000	
F	1	1:100,000	310	298	30,400,000	17.85	1:1,000,000	19	13	16,000,000	131.25
	2		286	.....	28,600,000			27	29	28,000,000	
	3		330	.....	33,000,000			21	22	21,500,000	
	4		293	288	29,050,000			29	22	25,500,000	
	5		284	288	28,600,000			31	28	29,500,000	
	6		328	292	31,000,000			36	31	33,500,000	
	7		286	278	28,200,000			35	39	37,000,000	
	8		275	285	28,000,000			27	32	29,500,000	
G	1	1:100,000	144	178	16,100,000	105.22	1:1,000,000	22	20	21,000,000	1,014.28
	2		128	136	13,200,000			17	47	32,000,000	
	3		179	161	17,000,000			7	14	10,500,000	
	4		200	.....	20,000,000			30	14	22,000,000	
	5		235	190	21,250,000			6	1	3,500,000	
	6		152	.....	15,200,000			15	15	15,000,000	
	7		130	133	13,150,000			15	9	12,000,000	
	8		270	.....	27,000,000			38	....	38,000,000	
	9		222	.....	22,200,000			33	45	39,000,000	

VARIATION BETWEEN DUPLICATE COUNTS FROM SAME SAMPLE AND SAME DILUTION.

As stated, we do not intend to say just what variation should be allowed between duplicate counts or a series of samples of ice cream from the same lot. Table VIII, however, shows some colony counts obtained by making a series of from five to eight plates from the same dilution. In five different samples a variation of from 7 to 26.6 per cent was found. Among the duplicate plates in the examination of other samples of ice cream a variation as high as 41 per cent was found. From figures given by other investigators of the results of bacterial counts of ice cream, variations in counts between duplicate plates as high as 88 per cent have been observed. It is obvious that the factor of variation among plates from the same dilution of ice cream must be taken into consideration when interpreting the results of the bacterial analysis of duplicate samples or a series of samples from a given mass of ice cream. To this factor of variation must be also added that caused by the difficulty of removing exactly equal quantities of different samples of ice cream in order to make the first dilution.

TABLE VIII.—*Colony counts on duplicate plates from the same dilution of a sample of ice cream.*

Sample No.	Number of colonies on duplicate plates.								Variation.
	1	2	3	4	5	6	7	8	
									Per cent.
1	243	229	225	208	232	218	.....	.....	16.8
2	450	460	510	470	480	470	500	570	26.6
3	321	302	307	300	310	.....	.....	.....	7.0
4	319	310	267	298	280	.....	.....	.....	19.4
5	144	152	153	178	178	.....	.....	.....	23.6

INTERPRETING DIFFERENCES IN BACTERIAL COUNTS.

When expressing bacterial counts the mere statement of the difference in the number of bacteria between two samples is not sufficient; in fact, it is impossible to interpret such differences properly without taking into consideration the total number of bacteria found in each sample. If, for example, it is stated that 1 cubic centimeter of ice cream contains 75,000,000 more bacteria than another sample, the difference of so many millions conveys to the mind a marked disparity in the two samples. If, on the other hand, there is said to be a difference of only 750 or 7,500 bacteria per cubic centimeter, we should immediately think of the samples as being practically the same.

TABLE IX.—*Hypothetical statement showing variable differences in bacterial counts of two samples of ice cream having a fixed ratio between colony counts and a fixed per cent of variation.*

Colony	Dilution.	Bacteria per c. c.	Difference in count.	Variation between counts.
				Per cent.
375	1 to 10.....	3,750	750	25.00
300		3,000		
375	1 to 100.....	37,500	7,500	25.00
300		30,000		
375	1 to 1,000.....	375,000	75,000	25.00
300		300,000		
375	1 to 10,000.....	3,750,000	750,000	25.00
300		3,000,000		
375	1 to 100,000.....	37,500,000	7,500,000	25.00
300		30,000,000		
375	1 to 1,000,000...	375,000,000	75,000,000	25.00
300		300,000,000		
375	1 to 10,000,000..	3,750,000,000	750,000,000	25.00
300		3,000,000,000		

Let us consider a hypothetical case, as shown in Table IX. We will assume that one sample of ice cream shows an average colony count of 375 and another 300. With a dilution of from 1 to 10, there would be a total count of 3,750 in one sample and 3,000 in the other, a difference of 750 bacteria per cubic centimeter, and a variation of 25 per cent between counts. If the same colony count were from a dilution of from 1 to 100 the difference in the bacteria in the samples would be 7,500, l the variation would be still the

same, or 25 per cent. As the total count becomes still higher, necessitating higher dilutions, the difference in bacteria per cubic centimeter increases until at a dilution of from 1 to 1,000,000 the difference would be 75,000,000 per cubic centimeter.

A study of this assumed case merely serves to show that even though there is a difference of 75,000,000 between two samples of ice cream, there is no greater percentage of variation between the samples than when the difference was only 750 per cubic centimeter. It also shows, however, that a difference between counts expressed in bacteria per cubic centimeter should never be considered by itself but should be interpreted in relation to the total number of bacteria per cubic centimeter in each sample.

SUMMARY AND CONCLUSIONS.

The method of collecting samples and making bacterial counts used in our experiments gives results which indicate that bacteria in commercial ice cream are distributed quite evenly and that an analysis of one sample from a gallon of ice cream gives results which will hold for any other similar sample from the same gallon.

Storage of ice cream for 11 days in a commercial ice-cream cabinet or in a hardening room for a period of two months did not seem to cause an uneven distribution of bacteria.

In a series of from 5 to 10 samples taken directly from a large commercial freezer the bacterial counts on each sample checked within the usual limits of error of bacterial analyses.

No greater variation in bacterial counts between samples was observed when the plates were incubated at 37° C. (98.6° F.) for 48 hours than when incubated at 30° C. (86° F.) for a period of five days.

When dilutions were such that about 200 colonies were present on the plates a lower variation between counts of samples of ice cream was found than when there were 50 or fewer colonies per plate.

The variation between a series of plates made from the same sample and dilution was found to range from 7 to 26.6 per cent. Among duplicate plates a variation as high as 41 per cent was observed. This must be remembered in connection with the fact that the variation found in our experiments between average counts of different samples of ice cream from the same gallon lot ranged, generally speaking, between 20 and 30 per cent. To this variation between duplicate plates or a series of plates from the same dilution must be added the error introduced in removing 1 c. c. portions of ice cream from different samples.

When interpreting bacterial counts, differences in the number of bacteria per cubic centimeter should never be considered except in relation to the total count of each sample.

WASHINGTON : GOVERNMENT PRINTING OFFICE : 1917