Distribution of Bacteria in a Quart Bottle of Whole Milk Held at 0° C.[1]

J. B. STINE

Kraft-Phenix Cheese Corporation, Wausau, Wisconsin

IT IS a well-known fact that it is necessary to shake a bottle of milk thoroughly before a sample is removed for bacteriological study if the sample is to be representative of all of the milk. This study was made in an effort to determine the relative numbers of organisms at various locations in a standard-shaped, quart bottle of whole milk held for a known period at 0° C. and also to obtain information on the factors influencing the distribution of the organisms.

EFFECT OF HOLDING A STANDARD QUART BOTTLE OF WHOLE MILK AT 0° C. ON THE DISTRIBUTION OF THE BACTERIA

A two-quart sample of whole milk was mixed thoroughly and divided between two sterile quart milk bottles of standard shape. Immediately after filling and before capping with a sterile cap, 1 cc. was removed from each bottle and placed in two 99-cc. water blanks. The quart samples were then placed in ice water at about 0° C. Standard plate counts were made from each of the two water blanks using standard nutrient agar and the plates were incubated at 37° C. for 48 hours. All bacterial counts were made in three dilutions and the plates were poured in duplicate. The results of the counts represented the bacteria per cc. in the original milk.

After the samples had been held at 0° C. for 6 hours, one quart bottle was removed and, without shaking, four 5-cc. samples were taken from different locations in the bottle by means of a slender pipette. The samples were removed from the top of the cream layer, the center of the cream layer, the bottom of the cream layer and the center of the milk below the cream layer. Each of these samples was plated in the same manner as the

[1] Journal Paper No. J423 of the Iowa Agricultural Experiment Station, Ames, Iowa, Project 119.

normal sample taken at the time the milk was placed at 0° C. After the other quart had been held at 0° C. for 30 hours, samples were taken from the same locations as in the sample held for 6 hours and counts were made in the same manner as before.

Thirteen runs were made, 10 using raw milk as it was delivered to the Iowa State College Dairy Department and 3 using pasteurized milk from the College dairy. The data secured are given in table 1.

TABLE 1. *Effect of holding a quart bottle of whole milk at 0° C. on the distribution of the bacteria*

Trial number	Bacterial count on mixed milk	Bacterial count on samples held 6 hours at 0° C.			
		Top of cream	Center of cream	Bottom of cream	Milk below cream
1	285,000	2,330,000	1,875,000	175,000	54,500
2	13,000	201,000	70,000	49,500	1,300
3	475,000	5,800,000	890,000	207,000	10,000
4*	28,500	200,000	172,500	101,500	900
5	98,000	595,000	1,170,000	460,000	7,300
6*	19,000	166,000	32,000	18,000	700
7*	7,700	105,000	69,500	20,300	850
8	1,065,000	14,500,000	6,250,000	1,235,000	203,000
9	130,000	840,000	1,055,000	515,000	53,000
10	93,500	700,000	430,000	199,500	25,500
11	1,570,000	13,000,000	9,800,000	4,525,000	147,500
12	320,000	1,255,000	820,000	117,000	64,500
13	370,000	12,000,000	1,645,000	48,000	19,600
Log. ave.	124,000	1,195,000	500,000	169,800	12,350

TABLE 1—Continued

Trial number	Bacterial count on mixed milk	Bacterial count on samples held 30 hours at 0° C.			
		Top of cream	Center of cream	Bottom of cream	Milk below cream
1	465,000	1,545,000	860,000	74,000	26,500
2	11,650	241,500	115,000	16,000	6,500
3	254,500	4,895,000	3,390,000	248,000	10,150
4*	24,500	205,000	84,000	3,500	880
5	110,500	672,000	1,140,000	96,500	13,400
6*	21,000	105,000	46,500	6,000	350
7*	7,850	97,000	70,000	16,650	4,950
8	1,310,000	12,900,000	8,350,000	925,000	158,500
9	129,000	900,000	1,180,000	101,500	26,000
10	25,000	620,000	370,000	275,000	3,350
11	1,570,000	30,400,000	20,000,000	490,000	85,000
12	206,000	1,470,000	1,275,000	565,000	74,500
13	580,000	1,260,000	660,000	58,000	36,000
Log. ave.	112,000	1,005,000	680,000	78,500	12,050

* Pasteurized milk.

A study of table 1 shows that, in the milk held 6 and 30 hours, the top of the cream had higher bacterial counts than any other portion of the milk and that the counts obtained from the top of the cream were commonly several times as large as the original counts on the milk. The logarithmic average of all of the runs made shows that there were about 10 times as many bacteria per cc. on the top of the cream as in the original milk, sampled normally. In all of the runs except runs 5 and 9 the cream at the center of the cream layer showed lower counts than the cream on the surface; and, in general, the counts from the center were only about one-half those of the surface. In the milk held both 6 and 30 hours a number of samples obtained from the bottom of the cream layer showed lower counts than those obtained from the original milk.

The counts on the milk below the cream layer were always less than the counts on the original milk sampled normally, as shown by the logarithmic averages. The averages of the counts on the milk below the cream layer were 12,350 and 12,050 when held 6 and 30 hours, respectively, as compared with 124,200 and 112,000 for the counts on the fresh milk, sampled normally.

Whether the sample was held 6 or 30 hours did not seem to affect the counts obtained from the surface of the cream layer to any great extent, although slight variations in counts were noted from other portions of the cream layer. Counts made from the center of the cream layer were commonly lower when the milk was held 6 hours rather than 30 hours, while those obtained from the bottom of the cream layer were usually higher after 6 hours than after 30 hours.

FACTORS AFFECTING THE DISTRIBUTION OF BACTERIA IN A QUART BOTTLE OF WHOLE MILK HELD AT 0° C.

After the distribution of bacteria was found to vary considerably at various locations in the bottles of milk, the question arose as to what caused the distribution found. Were the organisms carried up mechanically by the rising fat globules, or did the motile organisms tend to go to the surface where there was a more abundant supply of oxygen? If the distribution of the organisms was due to the fact that they were carried up mechanically by the rising cream, the larger organisms should be more easily carried up than the smaller ones, leaving a relatively low percentage of the latter left in the milk below the cream layer. Yeasts, therefore, were chosen as the test organisms to determine whether or not size was a factor in determining the distribution. The experiments in which yeasts were employed were conducted in the same manner as those made to determine the distribution of the organisms normally present except that the milk was inoculated with a water suspension of pink yeast before it was divided into the two quart bottles, and the counts were made with Difco malt agar (pH of 3.5) instead of standard nutrient agar; the plates

were incubated at 21° C. for 96 hours. In making the counts only the pink colonies were counted, and the results are expressed as the number of pink yeasts per cc. of milk.

The results of six runs using pink yeast are given in table 2. A study of the table shows that about the same general distribution of yeasts existed in the samples held at 0° C. as was observed in the previous experiments based on bacterial counts. It is significant to note, however, that the milk below the cream line contained a smaller percentage of the original number of yeasts than was observed with the bacteria, which suggests that there may have been a greater tendency for the yeasts to rise than for the bacteria. With the exception of trial 1, the yeast counts on the milk below the cream line were lower in the samples held 30 hours than in those held 6 hours. This fact suggests that the yeasts may have continued to move upward in the milk below the cream layer after the cream layer had formed.

TABLE 2. *Effect of holding a quart bottle of whole milk at 0° C. on the distribution of yeast cells*

Trial number	Yeast count on mixed milk	Yeast count on samples held 6 hours at 0° C.			
		Top of cream	Center of cream	Bottom of cream	Milk below cream
1	12,850	250,000	101,500	27,500	275
2	12,250	133,500	71,000	26,500	555
3	30,500	188,000	149,000	75,000	3,500
4	20,700	146,500	100,000	78,000	1,500
5	16,850	135,000	91,000	7,500	1,350
6	6,500	255,000	36,000	1,650	580
Log. ave.	15,200	177,500	83,800	19,370	895

TABLE 2—Continued

Trial number	Yeast count on mixed milk	Yeast count on samples held 30 hours at 0° C.			
		Top of cream	Center of cream	Bottom of cream	Milk below cream
1	12,450	180,000	105,000	17,000	1,450
2	14,850	180,000	145,000	19,850	385
3	33,500	280,000	202,000	127,500	790
4	19,350	236,000	188,000	15,000	490
5	16,350	136,000	121,500	94,000	500
6	6,950	125,000	52,000	1,100	135
Log. ave.	15,200	177,500	83,800	19,370	895

Studies to determine the effect of motility on the distribution of organisms were carried out in the same manner as the previous experiments except that the milk was inoculated with motile organisms. Standard nutrient agar was employed and the plates were incubated at 21° C. for 96 hours. Two trials were made using *Pseudomonas fluorescens,* and two using *Serratia marcescens.* Only the greenish colonies were counted in the experiments in which *Ps. fluorescens* was used, while the red or pink colonies were counted when *S. marcescens* was employed. The data on these experiments are given in table 3 and the results are recorded as the number of *Ps. fluorescens* or *S. marcescens* organisms per cc. of milk.

TABLE 3. *Effect of holding a quart bottle of whole milk at 0° C. on the distribution of Pseudomonas fluorescens and Serratia marcescens*

Trial number	Count on mixed milk	Count on samples held 6 hours at 0° C.			
		Top of cream	Center of cream	Bottom of cream	Milk below cream
Ps. fluorescens					
1	14,600	68,000	29,500	11,600	1,090
2	55,000	215,000	195,000	146,000	5,300
S. marcescens					
3	103,000	494,000	380,000	260,000	15,500
4	72,000	347,500	225,000	170,000	9,900

TABLE 3—Continued

Trial number	Count on mixed milk	Count on samples held 30 hours at 0° C.			
		Top of cream	Center of cream	Bottom of cream	Milk below cream
Ps. fluorescens					
1	13,250	71,000	35,000	11,300	1,230
2	58,000	450,000	260,000	68,000	5,400
S. marcescens					
3	154,500	560,000	420,000	124,000	4,900
4	62,500	1,000,000	790,000	410,000	6,850

The four trials using the test organisms yielded about the same results as were reported in table 1. The top of the cream contained several times as many organisms per cc. as the original mixed milk. In both runs the center of the cream layer regularly showed lower counts than the top of the cream, and the counts from the bottom of the cream were, in all cases, lower than those from the center. The milk below the cream contained, in general, only about one-tenth as many organisms as the freshly inoculated milk, sampled normally. In the trials where *Ps. fluorescens* was used (trials 1 and 2) the counts obtained on the milk below the cream layer were about the same after 30 hours as they were after 6 hours, whereas in the trials where *S. marcescens* was employed (trials 3 and 4), the counts after 30 hours were considerably lower than those after 6 hours. These results suggest that *Ps. fluorescens* did not continue to rise toward the cream layer during extended holding, while with *S. marcescens* there was apparently a tendency for the organisms to move upward. These data, however, are not sufficient to warrant specific conclusions.

CONCLUSIONS

1. Bacterial counts obtained from the cream layer of standard quart bottles of milk held at 0° C. for 6 and 30 hours were several times larger than counts from the milk below the cream layer.

2. Counts from the extreme top of the cream layer were higher than those found at other locations, and the counts appeared to decrease progressively at greater depths in the bottle.

3. Apparently there was a tendency for the organisms to move upward in the milk after extended holding.

4. The distribution of the organisms appeared to be caused largely by the mechanical filtering action of the fat as it rose in the milk. This view is supported by the observation that yeast cells showed a greater tendency to accumulate at the top of the bottles than did bacterial cells.

5. The presence of motile bacteria may have some effect on the distribution of the organisms.