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During the years of the journal's publication, the Butler University Botany Department had an active program of research and student training. 201 bachelor's degrees and 75 master's degrees in Botany were conferred during this period. Thirty-five of these graduates went on to earn doctorates at other institutions.

The Botany Department attracted many notable faculty members and students. Distinguished faculty, in addition to Cain and Friesner , included John E. Potzger, a forest ecologist and palynologist, Willard Nelson Clute, co-founder of the American Fern Society, Marion T. Hall, former director of the Morton Arboretum, C. Mervin Palmer, Rex Webster, and John Pelton. Some of the former undergraduate and master's students who made active contributions to the fields of botany and ecology include Dwight. W. Billings, Fay Kenoyer Daily, William A. Daily, Rexford Daudenmire, Francis Hueber, Frank McCormick, Scott McCoy, Robert Petty, Potzger, Helene Starcs, and Theodore Sperry. Cain, Daubenmire, Potzger, and Billings served as Presidents of the Ecological Society of America.

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A STUDY OF THE EFFECT OF PLANT OPERATIONS UPON THE BACTERIAL COUNT OF MILK[1]

By ALBIN N. SMOLELIS

This study was carried out to determine how the bacterial count of milk is affected by the various operations in a milk plant. Samples were taken at various points throughout the plant during the processing of the milk. The results obtained serve to show where the milk is affected during the various plant operations. The Breed (2) direct microscopic method was used because it enables the investigator to get quickly both a quantitative and a qualitative result. Today this method is accepted as standard and its use is widespread. Nevertheless, objections to results obtained by it have been advanced, and many investigators believe that for research work the plate method should be used.

In 1937 Strynadka and Thornton (8) advanced four objections to the smear method, their summary of objections should be considered, because it is important that the validity of the smear method be established. They maintain that the Breed method is not to be accepted as accurate because. "1. Non-representativeness of the sample of milk being used. 2. The failure of some bacteria to stain. 3. The non-recognition of stained particles as bacteria or foreign matter. 4. The personal factor." These objections do not seem to have a tremendous amount of importance, because the plate method which is advocated by the two authors also has numerous failings and shortcomings. No one can discount either method because of the first objection of the two authors, for neither the plate nor the smear method involves the use of an absolute cross-section sample of milk. In the small sample of milk required by both methods it is hardly possible to get a truly representative sample. The non-recognition of stained particles and the personal factor can be combined into one objection, and a certain amount of practice will enable a microscopist to identify easily the material involved. The personal factor also

[1]A portion of a thesis submitted to the Faculty of the Division of Graduate Instruction in partial fulfillment of the requirements for the degree of Master of Science, in the Department of Botany, Butler University.

manifests itself in the plate method, in that the counting of colonies on plates can be made ineffective by the person who is making the counts. As for the failure of the bacteria to stain, Ward (10), in 1937, reached the conclusion that there is no such difficulty. Ward maintains that, "Pasteurization not merely kills most of the bacteria commonly present in large numbers in raw milk, but renders them invisible when stained with methylene blue." This means that the bacteria which do not stain with methylene blue are the dead bacteria, and those which are stained are live organisms, and thus the unstained bacteria are of no importance.

Hammer (4) points out a few more short comings of the plate method which are not associated with the smear or Breed method. When organisms are grown on nutrient media, there is the possibility of failure to grow because the media are unsuited. The anaerobes will, in all probability, not grow because of the air supply. The time involved in making the dilutions and incubations is a very important factor, and variations occur because of the time involved. Furthermore the plate count does not give a count of individual bacteria, but rather a count of the growing colonies. The colonies might have been started by one organism or by one thousand organisms, and this is a very important part of the data when the results are given as number of bacteria per cc of milk.

It is known that milk when freshly drawn from a healthy udder contains practically no bacterial flora, yet by the time it reaches the consumer the bacterial counts range from a few thousand to millions per cc. This contamination or increase in bacterial content is affected between the time the milk leaves the udder and the time it is delivered to the consumer and indicates that a part of the contamination takes place at the dairy plant where the milk is processed. Just where, within the dairy plant, is the question this study has attempted to answer.

HISTORY

In 1929 Leete (7) made a study of the bacterial counts of milk samples taken at various places in the plant. His work was primarily a temperature study and he used the plate count in his work. From Leete's study it is obvious that there is a definite trend of increase and decrease, the question that remains is why this occurs. Leete did not attempt to analyze the causes; he merely made the temperature studies. But this increase in microbial count can be attributed to one or both

of two causes: (1) Contamination from equipment, (2) Normal growth and increase of thermophiles and thermoduric bacteria.

Harding (5) in 1940, showed that thermophiles accounted for many high counts; his study was conducted over a period of two years in two different dairy plants. He also maintains that contaminations are due to milk stone, machines, holding techniques and repasteurization.

Lazurus (6) discusses three different organisms which are associated with pasteurized milk contamination. He has found three different rod forms all of which are thermophilic, and all of which are said, "To grow at pasteurization temperatures and indicate poor plant sanitation, length of pasteurization, and repasteurization." Lazurus also claims that organisms which are killed by pasteurization are often found in pasteurized milk, and these are due to improperly cleansed utensils. Hammer (4) agrees with Lazurus and claims that almost all of the equipment at the milk plant is capable of increasing the bacterial count. According to Hammer, coolers and bottle fillers are two of the most important sources of bacteria, second in importance being the piping line and the pumps.

Contamination in milk can be traced to numerous sources, and Bryan (3), in 1938, developed a key for the identification of milk flora based on the morphology of milk micro-organisms. In this key he indicates which forms are associated with the various causes. This key simplifies the study of the results obtained and is, therefore, included here.

Shape and Type of Organism		Probable Cause
Rods	Short paired (scattered, clumps or in chains)	Utensils wet, unclean surfaces, especially milking machines.
	Short thick Long thick (scattered, clumps or in chains) Long thin Short thin	Dirty cows or barn, manure, dust, wet milking flanks, dirt.
	Very high counts (with high temperatures)	Poor cooling.
Cocci	Short chains (two to five elements)	Poor cooling.
	Clumps or singles (staphyloeocci)	Utensils, scum accumulations in open crevices or open seams.

	Tetrads (scattered or clumps)	Dirty cows or barn.
	Streptococci (more than 6 elements)	Streptococcus mastitis.
	Various types scattered throughout (unsanitary in every respect)	Poor production.
Mold	(Spores or mycelium)	Dust in barn or milk-house.
Cells	Polymorphonuclear Lymphocyte Epithelial	1. Milk used too soon after freshening. 2. Milk used too long at end of lactation period. 3. Injury to the udder (traumatic). 4. Mastitis if streptococci are found in unincubated or incubated samples.

The significance of the presence of body cells in milk was not studied in great detail in this paper, but of interest is the material found by other investigators. Hammer (4) maintains that body cells are normally found in milk and that these come from the linings of the milk ducts, milk cistern, teat canal, teat and udder surfaces. He further states that the significance of these is limited, in that various investigators have not come to any definite conclusions regarding the relationship of body cells to the quality of the milk. The variations in counts of what seems to be normal milk have done much to limit the use of the number of body cells as a criterion for the determination of quality milk. The only definite conclusion reached by the investigations carried out, is that a high body cell count, when associated with large numbers of streptococci, is a definite indication of an udder infection, mastitis. From Hammer's discussion it seems safe to say that an average body cell count is about 300,000 cells per cc of milk.

PROCEDURE

One day each week over a period of five months two sets of milk samples were obtained at a dairy in Indianapolis, Indiana. The first set was taken early in the morning and the second set was obtained about the middle of the morning. The samples were taken from seven places in the plant. The locations of sampling were: (1) The raw milk storage tank, (2) Pre-heater, (3) Clarifier, (4) Pasteurizing tank after pasteurization, (5) Cooler, (6) Bottling machine, (7) Bottle.

As soon as the milk was brought into the plant, it was weighed and pumped into a large raw milk storage tank, which was the source of the first sample. The milk was then pumped into a pre-heater; sample number 2 was taken from a pipe line which led from the pre-heater to the clarifier. After the milk left the clarifier another sample was taken, but this sample was taken out of the pasteurizing tank before pasteurization was started, because the pipe line from the clarifier to the pasteurizing tank was a closed unit. After milk was pasteurized at a temperature of 63° C. for 30 minutes, another sample was taken directly from the bottom of the trough of the milk cooler. The next sample came from the bottling machine, while the final sample was taken from a freshly filled and capped bottle. In this way it was possible to follow the same batch of milk through the complete process, and the evolution of the bacterial number was then observed.

The samples were processed according to the directions given by Breed (2) and Standard Methods for the Examination of Dairy Products (1). The samples were taken with a platinum wire loop which was standardized to deliver 0.01 cc of milk. The milk was put on a clean slide and spread over an area of one square cm and then allowed to dry at room temperature. After the samples were brought back to the laboratory, they were first immersed in xylol to remove the fat, allowed to dry, and then put into 95% alcohol to be fixed. The xylol immersion and the alcohol fixation each took about 5 minutes. The slides were then stained in an aqueous-alcoholic solution of methylene blue. The slides were observed with a 1.8 mm oil immersion lens and a 12.5 x ocular, the diameter of the field was 0.146 mm and made visible 1/600,000 cc of the milk per field.

RESULTS

The results obtained were counts of 30 fields of each smear. Each of these 30 fields, being 0.146 mm in diameter, gave a total of 1/20,000 (.00005) cc of milk for a total of 30 fields. The units and organisms counted were: body cells, diplococci, short streptococci, or those with no more than six elements, long streptococci, or those with more than six elements, staphylococci, isolated cocci, isolated rods, rods in clumps and mold fragments. From these individual counts the following figures were obtained: number of bacteria per 30 fields, number of groups per 30 fields, and average number of bacteria per group.

In order that the results be clearly interpreted it is necessary to list here the method used in determining the groups and numbers of baacteria. Diplococci were listed as two individual organisms and as one group of organisms. The streptococci were listed as were the diplococci, the number of elements composing the chain being listed among the number of bacteria counted and the individual chains being listed as groups. Likewise, staphylococci were counted both as individuals and as groups as were also the rods in clumps. Each isolated rod and isolated coccus was designated as an individual organism and as a group.

In Table I, the results are summarized to show the average counts, by month, of the body cells and organisms for each of the seven places in the milk plant. The average for the whole five months during which the experiment was conducted is also given. Figures for six sets of samples, hereafter known as the "X" samples, are omitted from both the monthly and the five-month averages of the "normal" samples. The milk for these six sets had been placed in the raw milk storage tank the day before it was processed. Since the tank was not refrigerated, the bacterial counts from these milk samples were much higher than in the other samples taken. The average bacterial counts for the six high-count samples are listed separately in the table.

To analyze the data which has been accumlated in this study, another chart has been made which shows the averages of all of the counts, including the "X" samples. The counts of each smear were averaged and the percentage increase or decrease from the count for the preceding step was calculated.

Average number of Bacteria per 30 fields (.00005 cc).

		Step by step change	Accumulated change
1. Storage tank	486.1		
2. Pre-heater	423.2	12.9% decrease	
3. Clarifier	458.0	7.6% increase	40.3% decrease
4. Pasteurizer	289.8	36.8% decrease	
5. Cooler	303.7	4.6% increase	
6. Bottling machine	269.3	11.3% decrease	26.2% increase
7. Bottles	365.7	27.1% increase	
Total percentage of decrease		26.8%	

Average number of Groups per 30 fields.

1. Storage tank	228.1		
2. Pre-heater	203.5	10.8% decrease	
3. Clarifier	290.4	29.9% increase	46.0% decrease
4. Pasteurizer	122.3	57.8% decrease	

5. Cooler	130.9	6.6% increase	31.7% increase
6. Bottling machine	112.2	14.3% decrease	
7. Bottles	161.0	30.2% increase	
Total percentage of decrease		29.3%	

DISCUSSION

A very large number of bacteria is found to be present in the milk while it is in the storage tank. This can be explained as being due to handling prior to the time when the milk is brought into the plant. Improper handling on the farm and during shipment can give the milk a very high bacterial count, and that is probably the reason high counts were found here. Also involved is the handling at the plant before the milk reaches the storage tank, and this probably contributes some more to the high numbers. Holding of any sort in the dairy plant also is not advisable and always increases the number of bacteria present. The samples marked "X" all have abnormally high counts, and these high counts can be attributed to the fact that the milk from which these samples were made was kept overnight at room temperature in the storage tank. They had a total bacterial count which averaged twelve times as high as in the normal samples. In order that the number of bacteria be reduced it is imperative that no holding, without refrigeration, be practiced in the storage tank.

After the milk leaves the storage tank it is pumped through the pre-heater. Here the number of bacteria is decreased an average of 22% in the "X" samples where the initial count in the storage tank was high. In the normal samples, it is generally slightly increased, the average increase being 15%. Considering the "X" and the normal samples together, the indications are that no consistent bacterial change is brought about by the pre-heater.

After the milk is pumped through the pre-heater it goes through the clarifier. Here the number of bacteria is increased in the average normal sample by 42% and is decreased in the "X" samples by 16%. This means that the centrifugal clarifier is not capable of decreasing the number of bacteria, but, rather, increases it unless the initial number is high. Body cells in the milk appear to be decreased by the clarifier, on the average about 30-40% in numbers, their larger size probably causing them to be thrown out of the milk. The clarifier appears to increase slightly the number of long and short streptococci and possibly, also, the staphylococci and rod forms.

Upon leaving the clarifier the milk is then pumped into the pasteurizing tanks and kept there for 30 minutes at a temperature of 63° C. It is here, immediately following pasteurization, that the greatest decrease in the number of bacteria is found, for they are decreased an average of 43% in the normal samples and 34% in the "X" samples. Ward (10) maintains that bacteria, killed by pasteurization, are thereby rendered invisible or non-stainable. From this present study, also, it is indicated that a large percentage of the bacteria in milk are rendered invisible by pasteurization. Except for the long and short streptococci in the normal samples, all types of bacteria seem to be reduced in numbers by the pasteurization.

The cooling, following pasteurization, appears to cause no important change in the total number of bacteria nor in any of the types. In the normal samples the average decrease was 8% but this was offset by an average increase in the "X" samples of 19%.

Before the milk reaches the coolers it is held in the pasteurizing tanks and, while it is being held there, the temperature gradually decreases. When more than one pasteurizing tank was in use at this plant, the milk was kept in the tanks for periods of time often approaching one hour after pasteurization. During this holding period the possibilities of increases in bacterial numbers are great. Also to be considered here is the fact that the milk taken for this sample had been pumped to a cooler and subjected to the cooling process. There are three opportunities for the bacteria to multiply, (1) the holding of the milk in the tanks after pasteurization, (2) pumping through long pipe lines and (3) during passage through the cooler. Thus it is possible to see that pasteurization does kill a great many of the bacteria, in fact in this study pasteurization decreased the number of bacteria by as much as 43%. But, after pasteurization took place, conditions again sometimes became such that the numbers of bacteria were increased. To produce a milk low in bacterial count the cooling procedure would have to be revised in such a way as not to present conditions for the growth of bacteria following pasteurization.

After the milk leaves the cooler and goes through the bottling machine another decrease is observed, it being 6% in the normal samples and 26% in the "X." Why this decrease is found, it is impossible to explain, beyond saying that it might be a latent effect of the cooling or pasteurizing. It might be well to note here that a further study of this phase of milk processing should be made, in order that a more complete analysis be presented. However, the fact

that increases are shown in some samples and decreases in others would indicate that the bacterial change is insignificant.

After the milk reaches the bottles and is capped another increase in numbers of bacteria is found. It seems evident that the bottles are responsible for a residual contamination, or, to be more explicit, the rinse water left in the bottles after washing possibly has a large number of bacteria, which would account for the increase following bottling. Also involved here is the cap; it might be the source of some bacteria. This coupled with the rinse water might be the reason for the increase in numbers of bacteria immediately following bottling. The average increase in the number of total bacteria in milk in the bottle over the previous number is 8% in normal samples and 69% in the "X" samples. Part of this may merely be an apparent increase due to the settling out of minute air bubbles in the milk, since there is a slight increase, also, in the average number of body cells.

The total percentage of decrease in the average number of bacteria from the beginning to the end of the process is 24.6%, the decrease in the average number of groups is 29.3%. The average decrease from the time the milk is in the storage tank to the time the pasteurization process is complete is 40.3%. Between pasteurization and bottling there is an increase of 20.9%. The increase which follows pasteurization, or rather the increase between pasteurization and bottling is due to the machinery and the handling of the milk. If there were no more handling of the milk following pasteurization, low count milk would be a simpler matter, but the handling and processing following pasteurization offsets, in part, the beneficial effect of pasteurization from the standpoint of numbers of bacteria. Processing appears to have decidedly different effects on high compared to low count samples. These differences are made evident in the following columns.

Average percentage of increase or decrease of total bacterial count for each procedure in the processing of the milk.

	All Samples	Low count "normal" samples	High count "X" samples
Pre-heater	—13	+15	—22
Clarifier	+ 8	+12	—16
Pasteurizer	—37	—43	—34
Cooler	+ 5	— 8	+19
Bottler	—11	— 6	—26
Bottle	+27	+ 8	+69

The effects of processing on the form types of bacteria are probably not as marked as on the total bacterial count. Diplococci, rods and bacterial "groups" behave essentially in the same manner as does the total bacterial count. Streptococci are increased in number in the clarifier, are not reduced in the pasteurizer, but are decreased slightly by the cooler. Staphylococci vary in numbers throughout the processing but, in general, are not changed significantly as the milk moves through the plant. Isolated cocci are increased in number by the pre-heater after which they decrease. Mold fragments were not found in the milk. Due to the predominance of diplococci, the number of bacteria per group remained much the same, being 2.4 in the "normal" samples and 2.1 in the "X" samples.

Body cells were reduced an average of 39% in normal samples and 33% in the "X" samples by the clarifier. Except for another average decrease of 33% by the pre-heater in the high count samples, the body cells were not greatly affected by the other steps in the milk processing.

CONCLUSIONS

1. The effect of each piece of apparatus used at a certain dairy plant was studied, using the Breed (direct microscopic) method.

2. The results for each sampling date, and even as monthly averages, show considerable variation in the number of organisms present in the milk at the seven sampling points in the dairy plant.

3. Processing in general decreases the number of bacteria found in milk about 27%; the decrease in the number is due primarily to pasteurization.

4. The pre-heater, clarifier, cooler and bottle frequently cause an increase in the number of bacteria in milk.

5. The pre-heater, pasteurizer and particularly the clarifier tend to cause a decrease in the numbers of body cells in milk.

6. Milk with an initial high bacterial count is affected often in an opposite manner from milk with a normal, low initial count.

7. The final bacterial count could be reduced if: (a) Cleaner milk were produced at the farm, and the clarification process at the dairy plant were eliminated; (b) The holding time between the filling of the storage tank and the pre-heating were reduced to a minimum; (c) The holding time between pasteurization and cooling were eliminated; (d) Greater care in the cleaning and rinsing of bottles were exercised.

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TABLE I

Monthly averages of body cells and organisms per .00005 cc for seven locations in the dairy

Location	Body cells	Diplococci	Short Streptococci	Long Streptococci	Staphylococci	Isolated cocci	Isolated rods	Rods in clumps	Mold Fragments	Number of Bacteria	Number of groups	Average number of Bacteria per group
October												
1	63	140	16	8	57	31	14	9	2	275	128	2.2
2	62	230	11	23	56	124	7	3	0	449	255	2.0
3	36	146	30	30	45	61	24	2	0	327	176	2.1
4	30	82	21	12	22	48	7	2	0	202	107	2.2
5	38	88	11	7	41	31	7	8	0	190	90	2.3
6	18	24	7	0	10	10	6	0	0	55	30	2.4
7	31	58	9	13	13	5	5	2	0	99	49	2.0
November												
1	63	132	12	10	51	3	3	3	1	203	74	2.7
2	62	176	12	9	52	1	3	2	0	251	96	2.5
3	45	156	20	16	65	2	3	2	0	251	93	2.2
4	28	134	36	13	134	3	2	0	0	325	84	3.4
5	37	132	22	13	66	3	4	9	0	240	78	2.9
6	29	118	26	14	147	11	2	1	0	319	84	3.8
7	30	146	30	11	144	16	3	2	0	349	105	3.2
December												
1	48	236	12	3	1	3	2	2	0	258	126	2.2
2	59	154	5	9	4	0	0	9	0	180	82	2.2
3	35	158	7	4	9	0	1	0	0	179	82	2.4
4	33	96	9	5	7	0	2	2	0	120	53	2.2
5	34	74	6	12	0	0	1	1	0	92	40	2.4
6	32	86	4	1	5	0	1	0	0	96	45	2.1
7	36	46	9	2	4	0	1	0	0	62	26	2.4
January												
1	95	94	1	3	9	1	0	0	0	109	49	2.3
2	70	156	1	2	0	0	1	0	0	158	79	2.3
3	38	260	5	3	0	0	1	0	0	270	132	2.0
4	43	104	1	2	0	0	0	0	0	107	51	2.0
5	41	90	5	3	17	0	1	0	0	114	48	2.4
6	42	132	2	3	3	0	0	0	0	141	67	2.1
7	41	136	2	16	0	0	0	0	0	154	70	2.2

TABLE I—(Continued)

Monthly averages of body cells and organisms per .00005 cc for seven locations in the dairy

Location	Body cells	Diplococci	Short Streptococci	Long Streptococci	Staphylococci	Isolated cocci	Isolated rods	Rods in clumps	Mold Fragments	Number of Bacteria	Number of groups	Average number of Bacteria per group
February												
1	73	162	1	0	16	1	1	15	0	194	85	2.7
2	60	124	1	3	0	0	1	0	0	128	63	2.1
3	35	676	3	4	25	1	3	23	1	733	346	2.1
4	30	148	5	23	7	0	1	5	0	190	79	2.5
5	34	170	3	16	10	3	0	1	0	203	91	2.1
6	45	154	1	2	13	2	0	0	0	172	81	2.1
7	35	136	2	4	0	0	2	0	0	143	71	2.0
March												
1	55	44	2	0	3	0	0	4	0	54	24	2.2
2	52	72	1	2	3	0	0	1	0	80	36	2.2
3	34	72	2	0	3	0	2	1	0	82	40	2.0
4	25	60	3	5	0	0	0	0	0	68	31	2.2
5	33	80	2	2	6	1	2	1	0	92	44	2.2
6	27	80	2	6	0	0	1	0	0	90	43	2.1
7	32	114	4	3	18	0	1	1	0	139	60	2.3
October-March Averages												
1	66	134	7	4	23	7	3	6	1	182	64	2.4
2	61	152	5	8	19	21	2	3	0	208	102	2.2
3	37	244	11	10	25	11	6	5	0	307	145	2.1
4	32	102	13	10	28	9	2	2	0	169	68	2.4
5	36	106	8	9	23	6	3	3	0	155	65	2.4
6	32	100	7	4	30	4	2	0	0	146	58	2.4
7	34	106	9	8	30	4	2	1	0	158	64	2.4
Averages of "X" Samples												
1	94	2082	5	16	33	2	2	28	0	2168	1051	2.1
2	63	1596	12	22	32	3	8	17	0	1688	817	2.1
3	42	1294	15	28	35	2	6	29	0	1413	671	2.1
4	36	868	13	12	27	1	1	5	0	927	442	2.1
5	35	1010	5	22	63	1	4	1	0	1101	514	2.2
6	35	736	8	24	26	2	1	4	0	811	376	2.2
7	42	1302	11	24	27	0	1	17	0	1381	658	2.1