SOME PUBLIC HEALTH ASPECTS OF MARKET MILK PROCESSING METHODS

by

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INTRODUCTION

From the viewpoint of public health, pasteurization of milk is one of the greatest weapons of preventive medicine, since it is only by pasteurization that a safe milk supply can be guaranteed to the consuming public.

From earliest times, mankind has applied heat to food in order to make it palatable, digestible and safe, but it is only in the past two centuries that he has heated it with the additional object of preserving it.

The earlier work was mainly concerned with the canning industry. The bacteriological principles which were revealed by Pasteur's research led to a sounder basis for the application of heat in food preservation.

Pasteurization has expanded considerably beyond its original purpose of infant feeding. Such expansion is, in part, due to such vigorous exponents as Koplick, Soxhlet, Jacobi and Straus.

By 1895 the problems of raw milk contractors were becoming increasingly greater. These dealers were faced with competition from pasteurized milk depots which had been started in New York, Brooklyn, Pittsburgh and Cincinnati. In addition, their sources of supply were being pushed farther and farther away, with consequent deterioration in keeping quality.

Under these conditions, pasteurization, with its freedom from disease germs and the increased keeping quality of the finished product, appealed forcefully to the city milk dealers.

Pasteurization began coming into general use in the United States around the year 1900, the prime object of pasteurization at that time being to increase the keeping quality of the milk.

The dealers generally adopted the flash process of pasteurization, using approximate exposures of 160° - 170° F. for 15 - 30 seconds. This system was misused, depending as it did upon manual operation with no automatic safeguards to ensure proper time and temperature exposure.

This system, with its primary emphasis on the prolongation of keeping quality, was not adequate from the public health standpoint.

From the beginning the need of a system which would give a reasonable assurance of safety as well as better keeping quality was recognized.

This demand resulted in the holder method of pasteurization whereby milk was heated at lower temperatures (143° F.) for longer periods of time (30 minutes). This system through the past forty years has proven to be practical and effective.

It was through a desire for a continuous system which could operate on regenerative principles that a method similar to the earlier flash pasteurization came into vogue. This high-temperature short-time

method of pasteurization makes use of a heat exposure of 161° F. for 16 seconds. It is carefully regulated by automatic devices to ensure proper exposure of the milk to heat treatment and is capable of functioning in a safe, efficient and economical manner.

Pasteurization, then, refers to various processes of heating milk followed by rapid cooling. The heat exposures involved vary from 143° F. for 30 minutes to 161° F. for 16 seconds. A considerable number of pasteurizing units designed to accomplish the heating of milk in various ways are in use, and in actual practice do their work with varying degrees of efficiency.

To be satisfactory, from the standpoint of bacterial efficiency, pasteurization must kill a reasonable percentage of the total bacteria present, including all the pathogenic organisms.

The destruction of pathogens that may be present in the milk is the important point to consider with reference to pasteurization exposures. Since Mycrobacterium tuberculosis is more resistant to heat than the other pathogenic organisms that may be present in milk, its resistance is used most often to establish satisfactory exposures.

Temperature and time cannot be controlled as accurately under plant conditions as they can be in the laboratory. For this reason, exposures established in public health regulations governing commercial pasteurization are generally above those that are necessary for the destruction of M. tuberculosis in laboratory tests.

Since present day methods of laboratory control make use of tests which measure whether milk has been pasteurized in a safe manner, much public health thinking has been turned towards the latent danger of after infection or bacterial contamination of the milk subsequent to pasteurization.

Concern regarding recontamination has been intensified due to the introduction of new processes and equipment. In this regard, perhaps the homogenizer, difficult as it is to sanitize, has added more to the complexity of the problem than has any other single piece of equipment connected with the processing and pasteurization of milk.

While originally the production and distribution of pasteurized and homogenized milk was limited almost entirely to the large urban centres, the consistently increasing demand for this product has brought about the introduction of homogenization of milk in many of the smaller milk plants.

It is from homogenization after pasteurization that the greatest danger of contamination of pasteurized milk arises. Public health regulations have attempted to place limitations on the equipment with which the milk comes in contact after pasteurization. Unfortunately the presence of the lipase enzyme in milk complicates the homogenization process. This enzyme must be inactivated by heat either immediately before or after homogenization to avoid development of rancid flavors. Not all milk plants which are homogenizing milk have the necessary preheating equipment which would enable them to homogenize before pasteurization.

Since many of these plants are working on what is considered a scant profit margin, it has been the desire of public health officials not to work an economic hardship on plant management by insisting on installation of equipment which would enable milk to be homogenized before pasteurization.

In spite of all devices, automatic and otherwise, for pasteurization equipment, the possibility of personal negligence will always be real. In an attempt to measure the public health dangers arising from possible negligence which could lead to contamination of milk subsequent to pastaurization, a study was made of the common processing sequences in commercial use.

This study was made in an attempt to determine the methods of processing and handling of milk and milk products which would be acceptable from a public health standpoint. The main objective was as follows:

1. To determine the effect of the processing sequence on the bacteriological quality of milk.

And incidental to it:

2. To suggest practical treatments for the homogenizer to minimize contamination from this source.
3. To determine the applicability of dye reduction quality tests on a predominately heat resistant bacterial flora.
4. To develop a more objective keeping quality test for pasteurized milk.
5. To study the increases in bacterial counts in homogenized milk

in an attempt to determine what portion is due to breaking up of bacterial clumps.

REVIEW OF LITERATURE

That considerable variation exists in regard to commercial processing methods in the market milk industry is a well noted fact. This situation has developed because of the numerous new pieces of equipment that have been introduced into the processing sequence within the past two or three decades.

The extent of these variations may be realized by reference to surveys. Hood and White (18) found in 1934 that, of 38 plants, 12 were homogenizing before and 26 after pasteurization. These same investigators (19) found in a later survey in 1947 that, of 91 plants homogenizing milk, 60 were homogenizing before and 31 were homogenizing after pasteurization. Layson (22) reported that, of 16 plants, 5 homogenized before and 11 after pasteurization. In 1941, based on a survey of 23 plants in Michigan, Trout and Schied (37) noted that 19 plants homogenized after pasteurization.

Doan and Minster (10) stated that there appeared to be no "best" method of processing homogenized milk which was applicable under all conditions and that only two fundamental rules existed which the operator must consider when homogenizing milk. These rules were: (a) The fat must be in a liquid state at the time of homogenizing and (b) All homogenized milk must be pasteurized either prior to homogenization or immediately afterward. Often a third requisite entered the picture, this being clarification.

Trout (36) stated that several systems or sequences of processing involving homogenization of milk were possible, any of which, with proper control, would yield a satisfactory product. Among the possibilities that he reported were:

a) Clarify, preheat, homogenize, pasteurize, cool.

b) Clarify, preheat, pasteurize, homogenize, cool.

c) Preheat, clarify, homogenize, pasteurize, cool.

d) Preheat, homogenize, clarify, pasteurize, cool.

e) Preheat, clarify, pasteurize, homogenize, cool.

Hood and White (19) added to this list with the following processing methods:

a) Pasteurize, clarify,* homogenize, cool.

b) Clarify, pasteurize, homogenize, cool.

c) Homogenize, preheat, clarify, pasteurize, cool.

d) Pasteurize, homogenize, clarify, cool.

Investigators have long realized that, when milk is homogenized after pasteurization, the likelihood of post-pasteurization contamination is increased. Trout (35) mentioned this possibility but also pointed out other factors which influence the choice of the point at which to homogenize. These considerations included volume and percentage of milk to be homogenized, the facilities for preheating to the temperature suitable for homogenization and for rapid inactivation of the lipase enzyme after homogenization.

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* Clarification and filtration are interchangeable.

There was general agreement among authorities (5,32) that esthetically at least the ideal sequence of processing homogenized milk would be one in which homogenization preceded pasteurization.

However Parfitt (24) noted some commercial instances where such a practice would be impractical. One such instance would be when only part of a vat of milk was to be homogenized and the remainder of the vat was to be bottled as non-homogenized milk. In this case homogenization must be done following pasteurization. He stated further that objections to such a procedure on the basis of possible post-pasteurization contamination were unreasonable in view of the sanitary features of the modern homogenizer. He stated that homogenization after pasteurization was the method most generally used by all smaller distributors and by some of the larger ones that were selling homogenized milk.

In 1935 Tracy (31) stated that the processing sequence should be to preheat to 149° F., homogenize, clarify and pasteurize. Later in 1936 (32) he amended this to the following: clarify, pasteurize, cool in vat to 135° F., homogenize at 2000 to 2500 lbs. per sq.in. pressure.

Brueckner (5) advised that pasteurization should follow homogenization in an attempt to minimize post-pasteurization contamination. This view was in agreement with others (1,2,9,14). Conversely, Ruehe (28), Hood and White (18) and others (6,13) stated that ideally pasteurization should precede homogenization. They gave as their reasons, better flavor control and surer destruction of the lipase enzyme.

According to Dearstyne and Ewing (8) the sanitary significance of the cleanliness of the equipment with which pasteurized milk comes in contact should never be underestimated. Since local health ordinances, e.g. (23) often prohibit the filtration, straining or clarification of milk after pasteurization, it has been assumed (8,39) that the equipment most likely to be in contact with pasteurized milk is the homogenizer, pipe lines, surface or plate coolers, bottling machine or the bottles and caps themselves. That this equipment may have been responsible for post-pasteurization contamination of milk is a matter of record whether it arises from the pipes (7,25,27), surface or plate cooler (15,38), bottling machine (12), bottles and caps (8,20,26) or the homogenizer (10,22,36).

During the 1930's the earlier types of homogenizers were replaced by new, completely demountable, sanitizable machines. Tracy (33) and others (34,40) believed that, with these new machines and if proper procedures were used for washing, sanitizing and processing, the objections to the homogenizer as a source of bacterial contamination of the milk might largely be overcome.

While the use of the homogenizer after pasteurization almost invariably resulted in an increase in the bacterial plate count of the milk being processed (36), this may not always have been due solely to bacterial contamination from the homogenizer itself. Various processes have long been known to break up the clumps of bacteria. Hammer and Hauser (17) noted that clarification breaks up the bacterial clumps as does the ice cream freezer as reported by Hammer and Goss (16) and

Ellenberger (11). Bishop and Murphy (4) were among the first to note that the homogenizer also breaks up the clumps of bacteria resulting in a higher plate count. Tracy (33) stated that this increase was also related to the types of bacteria present in the milk. When large numbers of clumping organisms were present, homogenization would break up the clumps producing an apparent increase in the numbers of bacteria present. James (21) concluded that, even though the machine was sterile, there was in increase in plate count due to breaking up of clumps by pressure and agitation during homogenization. He further stated that this increase varied because of the different numbers and types of bacteria resisting pasteurization.

It should, however, be emphasized that the foregoing authorities did not overlook the possibility of the homogenizer being a source of serious bacterial contamination, especially when cleansing and sanitizing practices tended to be neglected. The conclusion to be drawn might well have been in agreement with that of Tracy (32) who stated that, when the modern type of sanitary homogenizer was used, it was possible to homogenize milk without any appreciable bacterial contamination, provided good sanitation methods were used.

SECTION I

EFFECT OF THE PROCESSING SEQUENCE ON THE BACTERIOLOGICAL QUALITY OF MILK

PROCEDURE

The processing equipment that was used for the experiments on the effect of variations in the sequence of milk processing was that normally used in the operations of the commercial dairy at the University of Manitoba. This equipment consisted of a stainless steel dump tank, a tubular pre-heater, piston type homogenizer, spray type vat pasteurizer, enclosed surface cooler and a power bottle filler and capper. The pipes and fittings used throughout this system were of stainless steel construction.

The milk supply used in the experimental work was that normally obtained from the University dairy herd supplemented from time to time during the length of the project by additional supplies from an independent milk shipper.

When a sequence trial was decided upon, the equipment was placed in position to carry out the particular variant under study and the processing sequence was begun.

Prior to the passage of any milk through the system, chlorine sterilizer of a hypochlorite type (150-200 p.p.m.) was pumped through the system for a five minute period, being brushed over any non-exposed surfaces. The processing equipment had in every case been rinsed and dismantled after the previous day's processing, washed in warm (110 - 120^{o} F.) water containing a balanced general purpose cleanser, rinsed

with hot (170 - 185° F.) water and allowed to dry. The equipment was reassembled and sterilized just prior to use in each test.

Samples were taken at various points in the processing sequence e.g., before and after filtering, preheating, homogenizing, pasteurizing and cooling and bottling. All samples to be correlated were taken as closely together as was possible within the sequence so as to represent a common milk supply. All samples were collected in a manner described by Standard Methods (30) or in the case of the line sampling technique as suggested by Berger and Anderson (3) with the exception that samples were taken from pipe lines by loosening previously disinfected fittings and allowing free flow from connection for 30 seconds before sample was obtained. Sample size was in all cases at least 4 ounces, the 8 ounce size being used where sample was to be later sub-divided.

Samples were immediately placed in an ice-water bath and held under refrigeration until plated. In no instance was this period of refrigeration greater than 2 hours. Samples were plated for total bacteria count on T.G.E.M. agar according to Standard Methods (30). They were plated in duplicate in at least 2 dilutions and were reported as suggested by Standard Methods (30), incubation being at 95° F. for 48 hours. Samples that were analyzed by methylene blue and resazurin tests were carried out according to method outlined in Standard Methods (30). Readings were taken every 15 minutes until reduction proceeded beyond Munsell color standard (P.7/4) in the case of resazurin and at least 4/5 decolorization of visual portion

of contents with the methylene blue test. Hourly inversion of tubes with both tests was carried out.

Laboratory pasteurization was carried out on the raw milk samples as well as those taken from the pasteurizing vat at the end of the pasteurizing period. Laboratory pasteurization was carried out according to methods prescribed by Wilson (39).

To assess keeping quality and influence of post-pasteurization contamination it was decided to re-test the samples after incubation for 24 and 48 hour periods. Consequently the samples from the pasteurizing vat immediately after pasteurization, from the first milk into the bottle filler bowl and from the milk into the filler bowl after ten minutes continuous flow; (Sample points 2,3 and 5 respectively), were subdivided into sterile bottles and incubated at 55° F. They were withdrawn from incubation at 24 and 48 hour periods and analyzed for total bacteria count, resazurin and methylene blue reduction according to Standard Methods (30). This method of assessing keeping quality is closely related to methods proposed by Berger and Anderson (3).

For each sequence bacterial counts are arithmetically shown for each separate trial, they are logarithmically averaged for the sequence and antilogarithms are presented for these logarithmic averages. In all instances bacterial counts as reported represent standard plate counts per cubic centimeter. Unless otherwise stated resazurin and methylene blue reduction times are reported in minutes.

RESULTS

To illustrate the bacteriological "effect" of sequence changes 72 separate pasteurizations were carried out using 6 different processing sequences, twelve pasteurization trials being completed with each sequence.

As may be seen from Table 1 there were three sequences which homogenized before pasteurization and three sequences which homogenized after pasteurization. All the six sequences varied mainly in the position that the various steps within the sequence hold in relation to each other.

Table 1. Variations in processing sequences

Type of seq.	Seq.	Processing steps within sequence
Homo. before past.	A	Filter, preheat, homogenize, pasteurize, cool.
	C	Preheat, homogenize, filter, pasteurize, cool.
	F	Preheat, filter, homogenize, pasteurize, cool.
Homo. after past.	B	Preheat, filter, pasteurize, homogenize, cool.
	D	Pasteurize, filter, homogenize, cool.
	E	Pasteurize, homogenize, filter, cool.

To more closely study the effect of sequence change observation of the results within single sequences is necessary. To illustrate this, Table 2 (a to f) shows the bacterial counts for five separate sampling points within the sequences.

Table 2 (a). Bacterial counts in milk at various stages in processing and resulting pasteurization efficiencies - Sequence A.

Trial No.	Sample Points Raw-(1)*	Raw-(1) lab. Past.+	Past-(2)	Past-(3)	Past-(4)	Past-(5)	Past-(5) Lab Past.	Standard Past. Eff.(a) %	Adjusted Past.Eff. (b) %
1	140,000	150	480	1,800	510	700	230	99.50	99.62
2	31,000	940	400	8.200	860	770	240	97.51	98.54
3	34,000	380	1,100	4,200	4,600	3,800	190	88.82	89.43
4	40,000	1,200	2,200	3,700	1,000	1,300	970	96.75	99.06
5	21,000	1,400	1,900	2,700	1,700	2,000	760	90.47	93.51
6	270,000	360	7,400	8,200	2,000	2,800	200	98.96	99.62
7	93,000	140	1,000	1,500	960	1,100	110	98.81	99.04
8	7,000	310	490	570	440	510	140	94.60	95.21
9	230,000	100	170	510	200	230	70	99.90	99.83
10	88,000	960	1,200	2,700	1,300	3,300	740	96.25	96.91
11	120,000	1,100	1,900	3,300	2,200	2,700	760	97.75	98.42
12	65,000	300	420	3,000	1,300	1,800	360	97.23	98.41
Log.Ave.	4.79	2.63	2.97	3.39	3.03	3.04	2.46	-	-
Antilog.	61,000	430	930	2,500	1,100	1,100	290	-	-
Ave.%	-	-	-	-	-	-	-	96.58	97.30

* Sample Points:
(1) Raw milk from dump tank at beginning of processing run.
(2) Pasteurized milk from vat after 30 mins. at 143° F.
(3) Pasteurized milk - first flow into filler bowl.
(4) Pasteurized milk from vat 10 mins. after no.(2)
(5) Pasteurized milk from filler bowl after 10 mins.flow.

+ Laboratory pasteurized 143° F. - 30 mins.

(a) Standard Pasteurization Efficiency = $\frac{1 - 2}{1} \times 100$

(b) Adjusted Pasteurization efficiency = $\frac{(1-1a) - (2-2a)}{1-1a} 100$

where 1 = Raw milk (1)
2 = Past.milk (5)
1a = Raw milk (1) - lat.past.
2a = Past.milk (5) - lab.past.

Table 2 (b). Bacterial counts in milk at various stages in processing and resulting pasteurization efficiencies - Sequence C.

Trial No.	Sample Points							Standard	Adjusted
	Raw-(1)*	Raw(1) Lab. Past.	Past.(2)	Past-(3)	Past-(4)	Past-(5)	Past-(5) Lab. Past.	Past.Eff. %	Past.Eff. %
13	330,000	2,300	4,000	8,600	3,800	4,700	2,100	98.57	99.25
14	37,000	2,200	3,700	4,600	3,000	3,400	1,600	90.83	95.61
15	34,000	670	2,600	6,300	2,900	3,800	950	85.88	91.13
16	57,000	1,900	2,100	4,600	2,000	2,200	1,000	96.14	97.80
17	48,000	1,300	1,900	3,200	1,700	2,100	1,100	97.70	97.92
18	58,000	220	360	560	320	480	31	99.16	99.82
19	78,000	2,100	4,800	10,000	4,000	5,200	1,600	93.33	95.61
20	22,000	220	440	1,200	620	1,300	110	99.40	99.63
21	60,000	180	220	1,900	340	650	140	98.91	99.61
22	32,000	610	780	2,800	900	1,400	510	98.92	97.20
23	97,000	1,100	2,900	9,000	3,200	4,100	950	95.78	96.80
24	63,000	700	1,400	3,700	1,800	1,900	410	96.98	97.37
Log.Ave.	4.84	2.90	3.15	3.54	3.18	3.39	2.73	-	-
Antilog.	69,000	790	1,500	3,500	1,600	2,500	540	-	-
Ave.%	-	-	-	-	-	-	-	95.96	97.31

* See Table 2(a) foot note.

Table 2(c) Bacterial Counts in milk at various stages in processing and resulting pasteurization efficiencies - Sequence F

Trial No.	Sample Points							Standard Past.Eff. %	Adjusted Past. Eff. %
	Raw-(1)*	Raw-(1) Lab. Past.	Past-(2)	Past.(3)	Past.(4)	Past.(5)	Past.(5) Lab. Past.		
25	35,000	3,800	23,000	28,000	22,000	26,000	16,000	25.71	67.92
26	97,000	340	300	620	340	390	36	99.59	99.87
27	66,000	390	210	420	230	270	86	99.40	98.70
28	74,000	2,700	5,100	14,000	2,000	2,700	1,300	96.35	97.98
29	12,000	110	340	2,300	710	820	69	92.16	93.54
30	24,000	240	390	1,400	410	600	170	97.50	98.62
31	230,000	48,000	15,000	26,000	13,000	17,000	5,600	92.60	94.31
32	51,000	9,200	17,000	25,000	18,000	21,000	15,000	58.82	85.77
33	95,000	400	460	580	400	470	230	99.50	89.91
34	92,000	1,400	1,300	2,100	760	1,300	320	99.67	98.97
35	11,000	200	340	730	400	520	240	95.27	97.35
36	20,000	510	990	3,200	410	490	390	97.55	99.72
Log.Ave.	4.67	2.99	3.15	3.48	3.09	3.21	2.72	-	-
Antilog.	47,000	990	1,500	3,000	1,300	1,700	520	-	-
Ave.%	-	-	-	-	-	-	-	86.16	94.37

* See Table 2(a) Foot note.

Table 2(d). Bacterial counts in milk at various stages in processing and resulting pasteurization efficiencies - Sequence B.

Trial No.	Sample Points							Standard Past. Eff. %	Adjusted Past. Eff. %
	Raw-(1)*	Raw-(1) Lab. Past.	Past.(2)	Past.(3)	Past.(4)	Past.(5)	Past.(5) Lab. Past.		
37	150,000	4,000	6,000	59,000	7,000	66,000	3,000	56.00	56.85
38	99,000	41	2,100	24,000	1,600	17,000	45	82.82	83.00
39	140,000	310	3,300	6,200	2,100	5,700	120	95.92	96.60
40	66,000	8,600	14,000	27,000	20,000	47,000	7,900	28.78	31.85
41	39,000	1,900	4,200	11,000	5,300	7,900	1,200	79.74	81.85
42	43,000	1,100	3,700	7,200	5,400	5,900	760	86.27	87.60
43	47,000	220	2,800	7,900	3,300	9,000	360	80.85	81.70
44	62,000	1,200	2,600	11,000	2,200	6,800	610	89.03	90.13
45	110,000	2,900	4,600	17,000	6,200	13,000	2,100	88.18	90.06
46	73,000	1,200	3,100	14,000	4,200	8,700	960	88.08	89.70
47	72,000	2,700	6,900	17,000	8,400	13,000	1,900	81.94	84.01
48	83,000	4,100	7,200	16,000	10,000	17,000	3,200	79.05	82.80
Log.Ave.	4.87	3.06	3.64	4.18	3.69	4.11	2.95	-	-
Antilog.	74,000	1,200	4,400	15,000	4,900	13,000	890	-	-
Ave.%	-	-	-	-	-	-	-	78.20	79.67

* See Table 2(a) Foot note.

Table 2 (e). Bacterial counts in milk at various stages in processing and resulting pasteurization efficiencies - Sequence D.

Trial No.	Sample Points							Standard Past. Eff. %	Adjusted Past. Eff. %
	Raw-(1)*	Raw-(1) Lab. Past.	Past.(2)	Past.(3)	Past.(4)	Past.(5)	Past.(50 Lab. Past.		
49	49,000	95	2,200	4,100	2,400	3,900	210	92.40	92.73
50	43,000	310	2,300	7,600	5,000	8,000	120	81.39	83.90
51	40,000	360	2,200	7,300	2,600	11,000	140	72.75	72.51
52	38,000	410	4,100	9,500	1,100	4,100	190	89.21	89.46
53	42,000	740	5,600	8,400	3,100	12,000	220	71.42	71.23
54	35,000	850	1,400	7,700	1,700	3,100	720	91.14	92.90
55	97,000	640	960	2,600	1,100	2,400	410	97.52	98.04
56	50,000	480	850	1,900	1,200	2,800	37	94.40	94.51
57	56,000	42	570	900	800	1,200	40	97.93	98.12
58	78,000	4,100	5,100	7,300	4,100	7,000	3,200	91.02	95.30
59	25,000	330	630	1,500	620	1,500	130	94.00	94.96
60	75,000	270	330	2,800	370	1,500	190	98.00	98.62
Log.Ave.	4.69	2.59	3.18	3.60	3.18	3.50	2.20	-	-
Antilog.	49,000	390	1,600	4,000	1,600	3,700	160	-	-
Ave.&	-	-	-	-	-	-	-	89.26	90.19

* See Table 2(a) foot note.

Table 2 (f). Bacterial counts in milk at various stages in processing and resulting pasteurization efficiencies - Sequence E.

Trial No.	Sample Points							Standard Past. Eff. %	Adjusted Past. Eff. %
	Raw-(1)*	Raw-(1) Lab. Past.	Past.(2)	Past.(3)	Past.(4)	Past.(5)	Past.(5) Lab. Past.		
61	23,000	220	1,100	5,900	1,400	4,000	71	82.63	82.16
62	87,000	21,000	25,000	54,000	20,000	40,000	19,000	54.02	68.42
63	13,000	420	600	1,300	580	1,300	370	90.00	92.37
64	5,200	290	480	1,900	600	1,000	74	80.76	81.00
65	11,000	340	760	2,300	810	2,100	110	80.90	80.93
66	12,000	410	430	1,700	1,000	1,800	310	85.00	87.36
67	78,000	2,000	3,600	9,100	2,500	4,900	1,900	93.71	96.21
68	91,000	260	2,300	7,000	2,000	4,100	37	95.49	95.74
69	70,000	1,000	2,100	6,000	1,900	3,700	120	94.71	94.91
70	11,000	510	2,100	14,000	670	1,800	470	83.63	87.16
71	19,000	860	1,900	5,200	1,700	4,100	430	78.42	80.43
72	26,000	720	940	5,000	1,100	3,800	460	93.07	87.16
Log.Ave.	4.42	2.84	3.18	3.71	3.15	3.51	2.49	-	-
Antilog.	26,000	690	1,600	5,200	1,500	3,300	310	-	-
Ave.%	-	-	-	-	-	-	-	84.36	86.15

* See Table 2 (a) foot note.

By reference to Table 2 (a-f) the bacterial counts at various stages in processing and the resulting pasteurization efficiencies may be noted. Each separate table refers to a complete sequence listing the bacterial counts for the five sample points, the bacterial count based on laboratory pasteurization of samples from points 1 and 5 and the pasteurization efficiencies for each trial based upon this information.

Of interest are the antilogarithmic values established for each sequence and for all sample points. This figure follows a definite pattern regardless of the sequence i.e. a high figure for raw milk - sample point 1, a decrease for pasteurized milk - sample point 2, an increase for sample point 3 - first milk into filler bowl, a further decrease for sample point 4 - pasteurized from vat and an increase again for sample point 5 - milk into filler bowl after 10 minutes operation.

A further observation would be that the antilogarithmic figures for the laboratory pasteurized samples were always greater in the case of raw milk laboratory pasteurized than they were for pasteurized milk laboratory re-pasteurized. These ranged from an average figure of 750 for the former to 450 for the latter samples.

It should be noted that with the pasteurization efficiencies the adjusted efficiency was in 64 of the 72 instances higher than the corresponding standard efficiency. The overall average increase was however only 2.43%.

Table 3 summarizes the material from Table 2 (a-f) and averages the antilogarithmic values for the sample points as well as the pasteurization efficiency percentages for the two basic groups of sequences, A.Homogenization before pasteurization and B. Homogenization after pasteurization.

Table 3. Bacterial counts in milk at various stages in processing and resulting pasteurization efficiencies. Summary of Table 2 (a to f.) Expressed as antilogarithms of average counts.

Type of Seq.	Seq.	Sample Points Raw-(1)*	Past.(2)	Past.(3)	Past.(4)	Past.(5)	Standard Past.Eff. %	Adjusted Past. Eff. %
Homo. Before Past.	A	61,000	930	2,500	1,100	1,100	96.58	97.43
	C	69,000	1,500	3,500	1,600	2,500	95.96	97.31
	F	47,000	1,500	3,000	1,300	1,700	86.16	94.37
	Ave.	59,000	1,300	3,000	1,300	1,800	92.90	96.37
Homo. After Past.	B	74,000	4,400	15,000	4,900	13,000	78.20	79.67
	D	49,000	1,600	4,000	1,600	3,700	89.26	90.19
	E	26,000	1,600	5,200	1,500	3,300	84.36	86.15
	Ave.	50,000	2,500	8,100	2,700	6,700	83.94	85.33

* See Table 2 (a) foot note.

It may be seen from Table 3 that with the sequences that homogenize before pasteurization (A.C.F) the antilogarithmic values for the average bacterial counts for each sample point follows the same pattern as noted previously by reference to Table 2. However, it is mainly with the sequences that homogenize after pasteurization (B,D,E) that this pattern is easily noted, the average antilogarithms for the sampled points being: 1 - 50,000, 2 - 2,500, 3 - 8,100, 4 - 2,700 and 5 - 6,700.

The average antilogarithm for sample point 1 (Raw milk) for sequences that homogenize before pasteurization is seen to be 59,000 while the same figure for those sequences that homogenize after pasteurization is 50,000. In spite of this lesser figure the average antilogarithms for sample point 2 (Past. milk) are greater with those sequences that homogenize after pasteurization e.g. 2,500 as compared to 1,300.

That this apparent trend was not caused by heat resistant organisms would seem to be proved by reference to both the standard and adjusted pasteurization efficiencies for the sequences that homogenize before pasteurization. In both cases they are seen to be higher than the corresponding values for those sequences that homogenize after pasteurization e.g. Standard pasteurization efficiency 92.90% as compared to 83.94% and adjusted pasteurization efficiency 96.37% as compared to 85.33%.

Table 4 (a-f_1) presents the results of incubation for 24 and 48 hours at 55° F. upon three samples (sample points 2, 3 and 5) from each trial.

Table 4 (a). Bacterial counts and reduction times of milk incubated at 55° F. for 24 hours - Sequence A.

Trial No.	Past. -(2) * Standard Plate Count	Resaz-urin	Meth. Blue	Past. -(3) Standard Plate Count	Resaz-urin	Meth. Blue	Past. -(5) Standard Plate Count	Resaz-urin	Meth. Blue
1	2,600	270	330	6,000	105	180	15,000	310	300
2	2,100	210	330	450,000	150	300	26,000	240	375
3	23,000	240	360	91,000	165	240	110,000	165	240
4	8,600	555	630	8,600	555	630	8,700	555	630
5	3,600	315	450	7,200	315	450	4,000	315	450
6	19,000	150	225	7,000	310	285	6,400	210	270
7	19,000	315	420	37,000	285	375	24,000	300	360
8	1,100	330	420	7,200	300	405	4,100	330	420
9	27,000	150	240	110,000	105	180	63,000	120	195
10	5,500	345	450	12,000	330	435	4,100	330	435
11	4,800	345	450	17,000	315	420	9,200	330	435
12	7,000	240	300	150,000	150	210	9,600	225	300
Log.Ave.	3.82	-	-	4.34	-	-	4.11	-	-
Antilog.	6,600	-	-	22,000	-	-	13,000	-	-
Ave.	-	289	384	-	249	342		276	368

*See Table 2 (a) foot note.

Table 4 (a_1). Bacterial counts and reduction times of milk incubated at 55° F. for 48 hours. - Sequence A

Trial No.	Past. - (2) Standard Plate Count	Resaz-urin	Meth. Blue	Past.- (3) Standard Plate Count	Resaz-urin	Meth. Blue	Past. - (5) Standard Plate Count	Resaz-urin	Meth. Blue
1	65,000	60	150	3,600,000	30	120	2,900,000	45	150
2	200,000	45	200	24,000,000	15	60	1,000,000	60	105
3	140,000	165	225	390,000	60	120	270,000	90	150
4	42,000	465	555	51,000	405	520	48,000	420	555
5	72,000	300	390	130,000	240	315	91,000	270	330
6	600,000	30	90	720,000	15	120	460,000	45	90
7	110,000	195	300	480,000	120	210	290,000	150	240
8	31,000	300	390	310,000	210	255	97,000	240	300
9	94,000	120	195	330,000	45	75	210,000	90	135
10	67,000	120	180	480,000	45	90	72,000	60	120
11	83,000	180	270	390,000	90	150	120,000	150	210
12	1,100,000	45	105	13,000,000	15	60	1,900,000	30	90
Log.Ave.	5.08	-	-	5.86	-	-	5.43	-	-
Antilog.	120,000	-	-	730,000	-	-	270,000	-	-
Ave.	-	169	254	-	108	175	-	138	206

Table 4 (b) Bacterial counts and reduction times of milk incubated at 55° F. for 24 hours. - Sequence C.

Trial No.	Past.-(2) Standard Plate Count	Past.-(2) Resazurin	Past.-(2) Meth. Blue.	Past.-(3) Standard Plate Count	Past.-(3) Resazurin	Past.-(3) Meth. Blue	Past.-(5) Standard Plate Count	Past.-(5) Resazurin	Past.-(5) Meth. Blue.
13	6,400	420	600	10,000	375	540	8,300	390	555
14	4,500	450	630	8,500	405	540	7,700	420	570
15	3,400	450	630	8,200	405	555	6,500	420	570
16	5,200	420	600	11,000	405	570	5,900	420	600
17	4,800	450	630	9,600	405	540	8,000	420	570
18	1,000	420	600	890,000	120	195	600,000	210	255
19	6,200	405	540	17,000	390	510	6,900	405	540
20	3,000	420	600	4,200	405	480	3,800	420	510
21	3,600	495	660	8,900	390	570	1,100	420	585
22	1,500	390	465	27,000	300	360	7,800	345	405
23	8,300	375	450	21,000	300	360	16,000	330	390
24	7,200	450	600	21,000	420	570	7,900	450	600
Log.Ave.	3.52	-	-	4.23	-	-	3.96	-	
Antilog.	3,300	-	-	17,000	-	-	9,200	-	
Ave.	-	429	584	-	360	483	-	387	512

Table 4 (b_1). Bacterial counts and reduction times of milk incubated at 55° F. for 48 hours. - Sequence C.

Trial No.	Past. - (2) Standard Plate Count	Resaz-urin	Meth. Blue.	Past. - (3) Standard Plate Count	Resaz-urin.	Meth. Blue	Past. - (5) Standard Plate Count	Resaz-urin	Meth. Blue
13	130,000	240	315	440,000	120	180	320,000	165	240
14	9,000	360	480	23,000	390	435	21,000	390	420
15	27,000	360	450	68,000	195	300	41,000	240	360
16	19,000	420	540	48,000	300	405	28,000	330	435
17	27,000	345	435	53,000	270	360	39,000	300	375
18	4,000	360	480	3,800,000	30	75	2,000.000	60	135
19	41,000	315	420	97,000	225	315	63,000	255	345
20	36,000	210	255	680,000	75	135	460,000	120	195
21	5,000	300	360	11,000	270	330	2,000	300	360
22	2,000	330	420	550,000	240	270	78,000	300	395
23	37,000	375	465	110,000	270	360	58,000	330	420
24	39,000	330	435	110,000	255	360	48,000	300	375
Log.Ave.	4.26	-	-	5.11	-	-	4.76	-	-
Antilog.	18,000	-	-	130,000	-	-	58,000	-	-
Ave.	-	329	421	-	220	294	-	250	338

Table 4 (c), Bacterial counts and reduction times of milk incubated at 55° F. for 24 hours. - Sequence F.

Trial No.	Past. - (2)			Past. - (3)			Past. - (5)		
	Standard Plate Count	Resaz-urin	Meth. Blue	Standard Plate Count	Resaz-urin	Meth. Blue	Standard Plate Count	Resaz-urin	Meth. Blue
25	42,000	195	375	58,000	180	345	57,000	180	345
26	7,100	315	420	16,000	240	315	14,000	255	330
27	12,000	300	405	230,000	180	285	14,000	240	315
28	7,200	330	420	8,900	300	390	7,400	330	420
29	6,600	360	435	420,000	240	330	7,500	360	450
30	700	360	420	3,700	360	420	1,400	360	420
31	480,000	255	360	1,100,000	240	330	910,000	240	345
32	25,000	360	420	110,000	165	195	71,000	240	300
33	400	375	450	1,300	315	420	900	315	420
34	2,200	300	330	33,000	270	330	3,000	300	330
35	900	330	450	9,200	300	375	1,600	330	450
36	1,100	300	435	5,500	300	420	1,100	300	420
Log.Ave.	3.76	-	-	4.49	-	-	3.95	-	-
Antilog.	5,800	-	-	31,000	-	-	9,000	-	-
Ave.	-	315	410	-	258	346	-	288	378

Table 4 (c_1). Bacterial counts and reduction times of milk incubated at 55° F. for 48 hours. - Sequence F.

Trial No.	Past. - (2) Standard Plate Count	Past. - (2) Resaz-urin	Past. - (2) Meth. Blue	Past. - (3) Standard Plate Count	Past. - (3) Resaz-urin	Past. - (3) Meth. Blue	Past. - (5) Standard Plate Count	Past. - (5) Resaz-urin	Past. - (5) Meth. Blue
25	120,000	105	225	590,000	60	195	210,000	75	210
26	1,000,000	60	90	3,900,000	30	45	1,400,000	45	75
27	800,000	105	150	22,000,000	15	45	3,600,000	45	90
28	480,000	150	210	760,000	60	90	510,000	120	180
29	17,000	300	390	8,400,000	105	180	39,000	240	330
30	9,000	360	405	63,000	255	300	21,000	300	375
31	660,000	195	255	2,500,000	75	165	1,300,000	120	195
32	35,000	195	285	4,200,000	30	60	670,000	105	180
33	72,000	180	195	110,000	105	135	77,000	135	180
34	63,000	180	270	190,000	135	210	78,000	165	240
35	6,000	270	330	940,000	90	135	330,000	180	255
36	2,000	300	390	89,000	210	300	51,000	240	330
Log.Ave.	4.92	-	-	5.96	-	-	5.38	-	-
Antilog.	84,000	-	-	920,000	-	-	240,000	-	-
Ave.	-	200	266	-	96	155	-	147	220

Table 4 (d). Bacterial counts and reduction times of milk incubated at 55° F. for 24 hours. Sequence B.

Trial No.	Past. - (2) Standard Plate Count	Resaz-urin	Meth. Blue.	Past. - (3) Standard Plate Count	Resaz-urin	Meth. Blue	Past. - (5) Standard Plate Count	Resaz-urin	Meth. Blue
37	210,000	270	360	290,000	240	300	200,000	240	300
38	61,000	210	300	120,000	195	300	73,000	210	300
39	3,000	375	495	4,500	330	465	17,000	345	480
40	16,000	405	480	71,000	300	360	57,000	300	360
41	16,000	435	600	47,000	405	480	31,000	420	510
42	92,000	420	540	27,000	420	540	16,000	420	540
43	6,000	300	375	19,000	270	300	15,000	285	330
44	14,000	420	600	37,000	390	540	21,000	420	570
45	19,000	450	600	63,000	390	480	48,000	420	495
46	9,700	450	600	39,000	375	450	14,000	420	495
47	23,000	450	600	72,000	360	435	47,000	420	495
48	23,000	450	600	91,000	360	435	60,000	420	495
Log.Ave.	4.32	-	-	4.68	-	-	4.52	-	-
Antilog.	21,000	-	-	48,000	-	-	33,000	-	-
Ave.	-	386	512	-	336	424	-	360	448

Table 4 (d_1). Bacterial counts and reduction times of milk incubated at 55° F. for 48 hours. Sequence B.

Trial No.	Past. - (2) Standard Plate Count	Past. - (2) Resaz-urin	Past. - (2) Meth. Blue	Past. - (3) Standard Plate Count	Past. - (3) Resaz-urin	Past. - (3) Meth. Blue	Past. - (5) Standard Plate Count	Past. - (5) Resaz-urin	Past. - (5) Meth. Blue
37	3,000,000	60	105	59,000,000	15	45	6,600,000	30	75
38	72,000	105	195	1,300,000	75	120	120,000	90	180
39	7,000	300	420	46,000	225	300	22,000	255	330
40	350,000	195	240	590,000	120	180	500,000	150	210
41	73,000	360	435	210,000	255	300	170,000	300	420
42	33,000	390	480	110,000	300	330	61,000	360	420
43	40,000	210	300	150,000	120	180	73,000	150	210
44	62,000	360	420	310,000	150	195	190,000	210	300
45	67,000	345	420	210,000	210	285	190,000	240	315
46	27,000	360	420	230,000	240	315	48,000	255	345
47	97,000	330	420	360,000	180	240	210,000	255	330
48	87,000	360	405	410,000	150	240	230,000	255	300
Log.Ave.	4.89	-	-	5.60	-	-	5.23	-	-
Antilog.	77,000	-	-	400,000	-	-	170,000	-	-
Ave.	-	281	355	-	170	227	-	212	286

Table 4 (e) Bacterial counts and reduction times of milk incubated at 55° F. for 24 hours. Sequence D.

Trial No.	Past. - (2) Standard Plate Count	Past. - (2) Resaz-urin	Past. - (2) Meth. Blue	Past. - (3) Standard Plate Count	Past. - (3) Resaz-urin	Past. - (3) Meth. Blue	Past. - (5) Standard Plate Count	Past. - (5) Resaz-urin	Past. - (5) Meth. Blue
49	2,100	450	600	4,500	405	495	3,700	420	510
50	6,000	435	540	23,000	375	480	17,000	390	510
51	6,000	435	540	17,000	375	480	16,000	375	480
52	6,300	480	600	17,000	405	540	13,000	420	555
53	5,300	375	540	83,000	180	300	71,000	240	390
54	1,600	375	540	750,000	105	180	37,000	210	300
55	3,200	360	450	52,000	300	420	31,000	315	435
56	2,000	480	600	4,700	465	600	3,100	480	600
57	2,600	420	600	2,900	420	600	2,900	420	600
58	7,200	360	420	9,300	300	405	8,700	315	405
59	2,900	360	420	6,000	330	405	3,100	360	420
60	1,100	360	420	4,100	330	405	2,700	360	405
Log.ave.	3.52	-	-	4.20	-	-	3.97	-	-
Antilog.	3,300	-	-	16,000	-	-	9,500	-	-
Ave.	-	407	522	-	332	442	-	358	467

Table 4 (C_1) Bacterial counts and reduction times of milk incubated at 55° F. for 48 hours. Sequence D

Trial No.	Past. - (2) Standard Plate Count	Resaz-urin	Meth. Blue.	Past. - (3) Standard Plate Count	Resaz-urin	Meth. Blue	Past. - (5) Standard Plate Count	Resaz-urin	Meth. Blue
49	27,000	375	450	80,000	225	300	52,000	255	330
50	19,000	390	480	72,000	255	345	41,000	285	345
51	21,000	360	465	50,000	285	375	43,000	300	390
52	27,000	375	465	92,000	210	315	64,000	300	390
53	40,000	360	465	3,100,000	60	105	640,000	150	300
54	5,000	285	360	17,000,000	15	30	1,100,000	90	150
55	34,000	300	420	360,000	150	210	120,000	240	300
56	11,000	375	480	19,000	360	450	14,000	375	480
57	4,000	420	600	110,000	240	300	78,000	405	570
58	290,000	270	360	460,000	210	270	320,000	240	300
59	65,000	180	270	160,000	120	180	84,000	150	210
60	27,000	300	390	99,000	240	300	33,000	300	375
Log.Ave.	4.38	-	-	5.32	-	-	4.89	-	-
Antilog.	24,000	-	-	210,000	-	-	77,000	-	-
Ave.	-	332	434	-	197	265	-	257	345

Table 4 (f). Bacterial counts and reduction times of milk incubated at 55° F. for 24 hours. Sequence E.

Trial No.	Past. - (2) Standard Plate Count	Past. - (2) Resaz-urin	Past. - (2) Meth. Blue	Past. - (3) Standard Plate Count	Past. - (3) Resaz-urin	Past. - (3) Meth. Blue	Past. - (5) Standard Plate Count	Past. - (5) Resaz-urin	Past. - (5) Meth. Blue
61	2,000	450	570	5,000	360	465	3,700	420	540
62	39,000	300	420	120,000	270	360	76,000	300	390
63	1,200	360	420	3,100	300	360	2,200	330	405
64	1,200	360	420	37,000	300	390	6,200	360	405
65	1,700	360	420	37,000	300	390	28,000	360	420
66	1,100	360	420	2,700	360	420	2,000	360	420
67	9,000	420	585	32,000	360	435	21,000	390	450
68	6,000	420	600	24,000	390	540	16,000	405	570
69	9,000	435	600	24,000	360	450	19,000	390	480
70	5,000	420	600	170,000	300	360	26,000	405	570
71	3,100	450	615	78,000	375	435	6,700	420	570
72	1,600	420	600	31,000	360	435	17,000	390	450
Log.Ave.	3.53	-	-	4.40	-	-	4.04	-	-
Antilog.	3,400	-	-	25,000	-	-	11,000	-	-
Ave.	-	396	522	-	336	420	-	377	472

Table 4 (f_1). Bacterial counts and reduction times of milk incubated at 55° F. for 48 hours. Sequence E.

Trial No.	Past. - (2) Standard Plate Count	Past. - (2) Resazurin	Past. - (2) Meth. Blue	Past. - (3) Standard Plate Count	Past. - (3) Resazurin	Past. - (3) Meth. Blue	Past. - (5) Standard Plate Count	Past. - (5) Resazurin	Past. - (5) Meth. Blue
61	10,000	315	360	140,000	180	255	100,000	240	300
62	320,000	150	270	2,700,000	30	45	940,000	60	90
63	40,000	270	330	170,000	180	240	120,000	240	300
64	19,000	330	420	480,000	180	240	110,000	270	330
65	19,000	300	420	230,000	210	270	190,000	240	330
66	9,000	360	420	29,000	270	360	22,000	285	375
67	41,000	345	420	110,000	150	255	91,000	180	285
68	33,000	360	435	110,000	165	240	67,000	195	270
69	24,000	360	435	63,000	285	360	47,000	300	375
70	32,000	390	450	580,000	210	255	190,000	270	330
71	16,000	390	435	390,000	270	390	84,000	360	420
72	22,000	360	435	160,000	270	330	91,000	315	390
Log.Ave.	4.43	-	-	5.30	-	-	5.04	-	-
Antilog.	27,000	-	-	200,000	-	-	110,000	-	-
Ave.	-	325	402	-	200	270	-	246	316

Table 4 (a - f_1) shows the bacterial counts and reduction times for each sequence, twelve trials per sequence, on samples from sample points 2, 3 and 5, incubated for 24 and 48 hour periods at 55° F.

It may be noted that trends which were apparent with non-incubated samples from these sampling points tended to be magnified by incubation. This magnification however did not destroy the pattern of behavior as may be noted from reference to Table 4 (a) and comparing it to Table 2 (a). In the case of sample point 2 the antilogarithm of non-incubated samples as compared to 24 hour incubated samples at 55° F. was 930 and 6,600 respectively. For sample point 3 similar figures reveal counts of 2,500 and 22,000 and for sample point 5 the respective antilogarithms were 1,100 and 13,000. Similar comparisons may be noted with either the 24 or 48 hour incubated samples at 55° F., related to the same samples of a non-incubated nature. This holds true for individual trial results as well as for average figures based upon an entire sequence.

The reduction times for both the resazurin and methylene blue tests follow a similar pattern as might be expected. For samples that were incubated for 24 hours at 55° F. the ratio of resazurin reduction times to methylene blue reduction times was noted to be 1:1.32. For 48 hour incubation of samples the ratio was 1:1.35.

An interesting observation with regard to the reduction time averages of samples from sequences that homogenize before pasteurization Table 4 (a - c_1) as compared to sequences that homogenize after pasteurization, Table 4 (d - f_1) was that in relation to the antilogarithmic

figures the reduction times in some cases failed to follow the rough inverse relationship that might be expected.

Table 5 (a). Bacterial counts and reduction times of milk incubated at 55° F. for 24 hours. Summary of Table 5 (a-f). Bacterial counts expressed as antilogarithms of average counts.

Type of Sequence	Seq.	Past.-(2) * Antilog.	Resaz-urin	Meth. Blue	Past. -(3) Antilog.	Resaz-urin	Meth. Blue	Past. -(5) Antilog.	Resaz-urin	Meth. Blue
	A	6,600	289	384	22,000	249	342	13,000	276	368
Homo.	C	3,300	429	584	17,000	360	483	9,200	387	512
Before	F	5,800	315	410	31,000	258	346	9,000	288	378
Past.	Ave.	5,200	344	459	23,000	289	387	10,000	317	419
	B	21,000	386	512	48,000	336	424	33,000	360	448
Homo.	D	3,300	407	522	16,000	332	442	9,500	358	467
After	E	3,400	396	522	25,000	336	420	11,000	377	472
Past.	Ave.	9,200	397	519	30,000	334	429	18,000	365	462

* See Table 2 (a) footnote.

Table 5 (a_1). Bacterial counts and reduction times of milk incubated at 55° F. for 48 hours. Summary of Table 5(a_1 - f_1) Bacterial counts expressed as antilogarithms of average counts.

Type of Sequence	Seg.	Past. - (2) * Antilog.	Resaz-urin	Meth. Blue	Past. - (3) Antilog.	Resaz-urin	Meth. Blue	Past. - (5) Antilog.	Resaz-urin	Meth. Blue
	A	120,000	169	254	730,000	108	175	270,000	138	206
Homo Before Past.	C	18,000	329	421	130,000	220	294	58,000	258	338
	F	84,000	200	266	920,000	96	155	240,000	147	220
	Ave.	74,000	233	314	590,000	131	208	190,000	181	255
	B	77,000	281	355	400,000	170	227	170,000	212	286
Home After Past.	D	24,000	332	434	210,000	197	265	77,000	257	345
	E	27,000	325	402	205,000	200	270	107,000	246	316
	Ave.	43,000	313	394	270,000	189	254	120,000	238	316

* See Table 2 (a) foot note.

Table 5 (a & a_1) presents consolidated bacterial counts and reduction time averages for the six sequences, the samples (sample points 2, 3 and 5) being incubated at 55° F. for 24 hour (Table 5a) and 48 hour (Table $5a_1$) periods. The sequences (A, C, F) which homogenize before pasteurization are treated as a single entity, being averaged as such; similarly the sequences (B, D, E) that homogenize after pasteurization are also averaged.

It may be noted that the average of the antilogarithms for sequences A, C and F at incubation of 55° F. for 24 hours are respectively 5,200, 23,000 and 10,000 for sample points 2, 3 and 5. At incubation of 55° F. for 48 hours the average values are seen to be 74,000, 590,000 and 190,000. Similar averages for sequences B, D and E are 9,200, 30,000 and 18,000 for the 24 hour incubation period and 43,000, 270,000 and 120,000 for the 48 hour incubation period. From this it may be observed that after 24 hour incubation at 55° F. the average antilogarithmic values for sample points 2, 3 and 5 of the sequences that homogenize before pasteurization are lesser than those sequences that homogenize after pasteurization. However after 48 hour incubation at 55° F. this picture is reversed i.e., those sequences that homogenize before pasteurization have higher antilogarithmic values than do the sequences that homogenize after pasteurization.

Of further interest is the fact that the reduction time relationship with antilogarithmic value fails to follow the expected pattern after 24 hour incubation at 55° F., i.e., inverse relationship was not apparent however at 55° F. for 48 hours it was noted that this expected inverse relationship did develop.

SECTION 1A

STATISTICAL DATA RELATED TO

SECTION 1

RESULTS

The figures presented in the foregoing tables illustrate the various bacteriological changes undergone by the milk during processing which might be attributed to variations within the processing sequences. However since no absolute duplication of any processing trial could be carried out in the absence of a second pasteurizing vat, comparisons of results should be made if possible by ignoring or omitting the variable influence of the raw milk. To this end the data were reviewed on a statistical basis.

Analysis of variance of Y without X

Source of Variation	D.F.	Sums of Squares S.S.	Mean Square M.S.
Within lots	66	6,351,646,217	96,237,064
Series	5	2,099,054,577	419,810,915
Total	71	8,450,700,794	F=4.38 F.01= 3.31

Where X = raw milk (sample point 1)

Y = pasteurized milk (sample point 5).

From the foregoing it may be seen there are significant differences between the Y values of each sequence when the differences in X are not taken into consideration. Since there is a considerable difference in the X values (raw milk) it was desirable to study the Y values after the effect of the X values had been removed. This was done by the method of covariance (29).

Data on bacterial count of raw milk as compared to pasteurized milk in -

A. Homogenization before pasteurization
B. Homogenization after pasteurization

Level	Sx	Sy	Sx^2	Sxy	Sy^2
A	2,862,600	123,800	424,242,760,000	11,437,446,000	1,578,067,600
B	2,105,000	348,500	184,503,000,000	29,091,300,000	9,970,790,000
Total	4,967,600	472,300	598,745,760,000	40,528,746,000	11,548,857,600

Analysis of covariance and test of significance of adjusted lot means.

Source of Variation	D.F.	Sums of squares and products Sx^2	Sxy	Sy^2
Total	71	246,008,957,778	7,942,669,889	8,450,700,794
Series	5	23,486,711,111	464,556,889	2,099,054,577
Within lots (error)	66	222,522,246,667	7,478,113,000	6,351,646,217

Source of Variation	D.F.	Errors of Estimate S.S.	D.F.	M.S.
Total	71	8,194,262,958	70	
Sequences	5			
Within lots (error)	66	6,100,335,731	65	93,851,319
		2,093,927,227	5	418,785,445

$$F = \frac{418,785,445}{93,851,319} = 4.46.$$

D.F. = 5.65
F.01 = 3.31

It may then be noted that there is a significant difference between sequences. This difference exists even after the effect of the original bacterial counts (x) are removed. It is then desirable to examine the sequences to see which is most effective from the standpoint of bacterial destruction. This is best accomplished by calculation of the adjusted mean for 6 sequences.

By reference to the data on the calculation of the adjusted mean it will be noted that the order or rank as calculated on the basis of bacterial destruction is unchanged from the mean gain column to the adjusted mean gain column. Further it may be seen that the sequences are ranked in order with the greatest bacterial destruction (lowest count) being considered to be the highest in order of rank.

Calculation of adjusted mean for 6 sequences

Lot	mean raw milk count X	Deviation from expt. mean (x)	Product bx	mean gain Y	Adjusted mean gain Y-bx	Order	Seq.
1	94,967	-25,973	872.69	1,750.8	878.1	1	A
2	76,333	- 7,339	246.59	2,602.5	2,355.9	2	C
3	67,250	- 1,744	-58.60	5,963.3	6,021.9	4	F
4	82,000	13,006	437.00	18,083.3	17,646.3	6	B
5	52,333	-16,661	-559.81	4,908.3	5,468.1	3	D
6	41,083	-27,911	-937.81	6,050.0	6,987.8	5	E

Test of significance between regression coefficients of series A (homogenization before pasteurization) and series B (homogenization after pasteurization).

Source of Variation	D.F.	Errors of estimate S.S.	M.S.
Average within levels	69	7,692,891,385	
Deviation from indicated regressions	68	5,904,510,940	86,831,043
Between regression coefficients	1	1,788,480,445	1,788,480,445

F = 20.597 D.F. = 1,69

F.01 = 7.02

From the foregoing it may be seen that there is a significant difference between series A and series B. It is then desirable to examine the two series to observe which is best in terms of bacterial destruction.

Adjusted mean of two main groups.

Series	Mean X	Deviation from mean (x)	Product bx	Mean gain y	Adjusted means	Order
A	79,517	10,524	455.7	3,439	2,983.3	1
B	58,472	10,522	-455.6	-9,681	10,136.6	2

From the above it may be observed that Series A (Homogenization before pasteurization) has a considerably lower adjusted mean and is ranked higher in terms of bacterial destruction than is Series B (Homogenization after pasteurization).

DISCUSSION

An observation of the results of the work embodied in Section I could well lead to a query concerning the choice of sampling points upon which the samples, their bacteriological characteristics and the whole basis of the research was founded. It is not difficult to accept the contention that a choice of alternate sampling points could have a bearing on the results which could alter any conclusions appreciably.

Sample points 1 and 2 were chosen as an evaluation of both raw and pasteurized milks must be made. Sample point 3 (pasteurized milk - first flow into filler bowl) was chosen as an indicator of effect of residual contamination on equipment with which milk came in contact and it resembles the water rinse technique. The results based on such a sample point could have commercial application, especially with any "in-plant" sanitation program. Sample points 4 and 5 were chosen to compare pasteurized and pasteurized homogenized milk of as common an origin as was possible. It was felt that the sample points chosen would enable conclusions to be made based on the results arising from bacteriological analysis of the samples.

Table 3 presents a summary of the effect on the bacterial count of the various phases within the processing sequence for the six different sequences. A study of the results tabled under sample point 3 and a comparison of these results with those of sample point 5 would tend to show the value of taking initial milk flow samples. These results (3) are consistently higher than those from a supposedly comparable source (5) and would indicate that almost invariably there remains a residue of bacterial contamination on the surface cooler, pipes, etc., which would likely be largely washed away after some 10 minutes' continuous milk flow.

Again the choice of sample points is reflected by reference to results gained from incubation and subsequent bacteriological analysis of samples. That such incubation tends to "magnify" relatively insignificant differences between samples based on immediate analysis is a matter of record and Table 4 lists the results of such incubation, for each of the 72 trials. Table 5 ($a_1\bar{a}_1$) summarizes these results on a sequence level for the 55° F, 24 and 48 hour incubation respectively.

These latter tables again show more strikingly the difference between initial milk flow (3) and flow after 10 minutes operation (5), largely because of the incubation period with its effect on the geometric increase in bacterial population.

Of considerable interest are the results of the incubation for 24 hours at 55° F. compared to those for the 48 hour 55° F. incubation period. The results from the former are as would be expected from the individual trial results, i.e., the antilogarithms of the samples from

sample points 3 and 5 are lower with sequences that homogenize before pasteurization. That this was the normal trend was apparent early in the work and was to be expected due to the influence of the homogenizer.

However, the results of the 48 hour incubation period reveal antilogarithms that are higher for the samples from sample points 3 and 5 with sequences that homogenize before pasteurization. The reason for this apparent reversal of form lies in the fact that these are results based upon averages and a perusal of the individual results fails to show this characteristic in many of the cases.

Table 3 shows the raw and pasteurized antilogarithms of average counts and the resulting pasteurization efficiencies. It will be noted that there are two types of pasteurization efficiencies: a) standard - usual type of efficiency calculation and b) adjusted.

The adjusted pasteurization efficiency involves laboratory pasteurization of the raw and repasteurization of the pasteurized milk and is designed to illustrate the effectiveness of the system against those bacteria capable of destruction by normal heat treatment methods. It was felt that, by taking into consideration the possible presence of heat-resistant bacteria in the raw milk, unattainable pasteurization efficiency standards could be avoided. Further, in any close comparison between different sequences, such as was embodied in this report, any formula which could eliminate uncontrollable discrepancies between results of pasteurization efficiency should be considered.

Too often it is noted that certain plants are failing to

meet the local pasteurized milk bacterial count standard and, by applying the formula for the adjusted pasteurization efficiency (Table 2a), a more accurate picture could be gained of the plants ability to effect maximum bacterial destruction.

CONCLUSIONS

1. While the initial raw milk bacterial count of sequences A, C and F (homogenization before pasteurization) exceeded the raw milk bacterial count of sequences B, D and E (homogenization after pasteurization) the bacterial counts for the pasteurized milk (sample points 2, 3, 4 and 5) with sequences B, D and E were higher than those of sequences A, C and F.

2. Higher pasteurization efficiencies were obtained with sequences that homogenize before pasteurization as compared to sequences that homogenize after pasteurization.

3. With all six sequences the adjusted pasteurization efficiency (based only on those bacteria capable of destruction at pasteurization temperatures) was higher than the standard pasteurization efficiency.

4. Incubation of samples from sample points 2, 3 and 5 at 55° F. for 24 and 48 hour periods exhibited a magnification of bacteriological differences scarcely apparent when non-incubated samples were bacteriologically analysed.

Statistical analysis of the results of Section 1 revealed the following:

5. There were significant differences between the bacterial counts of the pasteurized samples (sample point 5) from each sequence when the bacterial count of the raw samples (sample point 1) was eliminated from consideration.

6. Comparing sequences A, C and F (homogenization before pasteurization) with sequences B, D and E (homogenization after pasteurization) there was a difference between the two types of sequence which was significant at the 1% level. This difference existed after the effect of the original raw bacterial count was removed.

7. By calculation of the adjusted mean for the six sequences the order was calculated on the basis of bacterial destruction. The greatest bacterial destruction was considered to be highest in order of rank.

Sequence	A-1	F-4
	C-2	E-5
	D-3	B-6

8. Calculation of the adjusted mean for the two main groups showed that sequences A, C and F (homogenization before pasteurization) exhibited greater bacterial destruction than did sequences B, D and E (homogenization after pasteurization).

SECTION II

SANITATION OF THE HOMOGENIZER

PROCEDURE

During the period that data for Section 1 were being compiled the question of practical, efficient sanitization treatments for the homogenizer was frequently raised. It was decided to apply 4 different sanitizing methods to an otherwise regularly washed homogenizer subsequent to the day's processing and determine, if possible, which method would be suitable for average plant conditions.

Fourteen separate sanitization trials were run using four different types of sterilization. 1.) Quaternary ammonium compound, 2.) Chlorine compound (hypochlorite). 3.) Hot water. 4.) Flowing wet steam.

In all instances the homogenizer which had been used to process about 1200 pounds of milk was washed as follows: The homogenizer was rinsed with cold water by allowing it to operate at 2000 lbs./sq./in. pressure for a period of 5 minutes. It was then dismantled, all parts were washed in warm, general purpose balanced cleanser solution, parts were then rinsed in water at 150° F., allowed to dry and re-assembled.

The milk used in the trials was autoclaved in a three gallon cream can which was fitted with a special pipe and coupling attachment which would allow it to be joined in an airtight manner to the inlet of the homogenizer. Bacterial plate counts, made in duplicate, on this autoclaved milk were negative in every trial.

RESULTS

Table 6. Comparative effectiveness of different methods of sanitizing the homogenizer. Samples from homogenizer outlet and expressed as standard plate count per ml.

Sanitizing Agent	1st milk thru homo.	15 secs. after start.	30 secs. after start	45 secs. after start	60 secs. after start	Average all Samples
80 lbs.	161	140	127	121	140	137
200 p.p.m.	111	100	72	71	79	86
Quaternary	92	116	48	41	12	77
Ammonium cmpd.100°F.	43	29	24	17	17	26
Ave.	102	96	68	63	62	82
80 lbs.	41	29	31	16	11	26
200 p.p.m.	16	16	12	0	6	10
Chlorine	23	Sp.	14	19	7	16
(hypochlorite)	27	20	17	17	4	19
Cmpd. 100° F.						
Ave.	27	22	19	13	7	18
Hot water	140	129	114	106	102	118
for 5 mins.	197	214	163	127	128	166
180° F.	84	116	41	9	16	53
Ave.	140	153	106	81	82	112
Wet Steam	27	16	7	2	4	11
flowing	19	14	6	0	0	8
for 10 min.	102	67	61	23	29	56
Ave.	49	32	25	8	10	25

It may be observed from Table 6 that samples were taken as the first milk came from the homogenizer outlet and at four 15 second intervals thereafter. These samples were plated, the results as reported in Table 6 being indicative of the effectiveness of the four different methods used to sanitize the homogenizer.

It may be noted that the bacterial count of the previously sterilized milk after it had passed through the homogenizer was, in all instances, fairly low. The highest bacterial count reported was 214.

Each group of samples was cross averaged, i.e., an individual trial average was made as well as a group average for each sample point with each sanitizing agent.

The final vertical volumn in Table 6 gives the trial averages for the combined sample points and furnishes comparative information.

It may be seen that, by using water at 180° F. as the sanitizing agent an average bacterial count (all five trials) of 112 was obtained. With the quaternary ammonium compound this figure was seen to be 82. By using wet steam a further decrease was noted, in this case the figure being 25. Finally maximum destruction efficiency was noted by use of the chlorine sanitizing agent, the bacterial count in this case being 18.

DISCUSSION

Observation of the results, showing the comparative effective-

ness of the four different sanitizing agents on the homogenizer produced results which, while relatively inconclusive, might well be expected. It should be noticed that, with each of the trials, regardless of the sanitizing agent used, the homogenizer was washed in a recommended, complete and careful manner. In this regard actually lies any reason for the apparent success of any sanitizing agent. To attempt to approximate comparable contamination from trial to trial would, it was realized, have been difficult. That the results showing the initial bacterial contamination (1st, milk through homogenizer) were so comparable was due more probably to coincidence and the small number of trials than to any other factor.

The results do, however, emphasize the oft repeated statement that cleaning and sanitizing are so closely inter-related that the success of one is dependent upon the other. With the modern easily dismantled homogenizer and proper cleaning materials and methods the choice of a recognized sanitizing agent depends mainly upon ease of handling and comparative costs.

Since it is recognized that sanitization by any means must be thorough and careful to be successful these results do not warrant any statements on the relative merits of the agents used.

CONCLUSIONS

1. Based on 14 separate trials using four different sanitizing agents for the homogenizer, the results would seem

to indicate that success with any sanitizing agent depends upon proper cleaning and washing of the homogenizer.

2. Using sterile whole milk as the rinse medium, chlorine (hypochlorite) sanitization led to slightly lower baterial counts than did the other three sanitizing agents.

3. Samples taken initially from the homogenizer outlet and at 15 second intervals showed progressively decreasing bacterial counts due probably to washing away of residual bacterial contamination by the milk.

SECTION III

DYE REDUCTION TESTS ON HEAT RESISTANT BACTERIA

PROCEDURE

Throughout the entire project the pasteurized milk from the sequence trials was tested for potential keeping quality by means of bacterial plate counts as well as dye reduction tests. Generally speaking, dye reduction tests, i.e., methylene blue and resazurin, are not used on non-incubated pasteurized milk samples because of their several limitations. (3,30.)

However in an attempt to compare the sequences on the basis of post-pasteurization contamination, it was decided to make fairly extensive use of these dye reduction tests, particularly on incubated samples. The method followed was generally similar to that used by Berger and Anderson (3). Early in the project it was realized that the pasteurized samples contained a bacterial flavor of essentially a heat resistant nature. These samples often were contaminated by means of non-sterile equipment (surface cooler, pipe lines, etc.) and were incubated at 55° F. for varying periods of time, thus probably restoring a more normal bacterial flora. It was felt however that it was desirable, particularly in the absence of much reported research, to investigate by means of reduction trials some cultures of heat resistant organisms.

Seven heat resistant cultures were chosen because they had repeatedly survived time and temperature exposures approximating those of holder pasteurization. These cultures were prepared and carried by Mr. A. Myhr and were used by him in a study on the Thermal resistance of Micrococci in milk.

RESULTS

Table 7. Reduction of sterile milk inoculated with pure cultures of heat resistant bacteria and incubated at three temperatures. Reduction times expressed in hours.

Culture	Initial bacterial count at start of reduction	68° F		98.6° F.			113° F.	
		Res.	M.B.	Res.	M.B.	M.B.*	Res.	M.B.
P.43	1,900,000	1	2	0.25	1	0.5	0.25	1
P.39	62,000	3	33	1	9	8	1	24
P.46	7,000,000	1	1	0.25	1	0.5	0.25	1
P.23	3,000,000	1	6	1	5	1	1	3
P.11	880,000	8	27	3	10	9	1	11
P.6	700,000	1	42	1	17	11	1	27
P.42	1,200,000	1	40	1	11	10	1	16

* Methylene Blue test on milk enriched with 1 gm. dextrose per 100 ml. sterile milk.

From Table 7 it may be noted that, with the exception of culture P11 and P39 at the 68° F. incubation temperature, none of the cultures took longer than 1 hour to reduce resazurin while the same cultures took from 1 to 42 hours to reduce methylene blue. At the 98.6° F. incubation temperature the resazurin reduction times ranged from 0.25 hours to 3 hours while with methylene blue the divergence was greater, ranging from 1 hour to 17 hours. The enriched methylene blue was slightly more susceptible to bacterial reduction, the results ranging from 0.5 hour to 11hour. At 113° F. incubation temperature reduction times with resazurin ranged from 0.25 hour to 1 hour and with methylene blue from 1 hour to 27 hours.

Observation of the initial bacterial counts of the seven cultures at the start of reduction revealed counts ranging from 62,000 to 7,000,000. The antilogarithm of logarithmically averaged bacterial counts was 1,100,000. This was of interest since it was attempted to have a bacterial count of approximately 1,000,000 at the start of reduction with each culture.

DISCUSSION

The work done with dye reduction tests on heat resistant flora in this report has considerable significance in any consideration of the results of the incubated samples.

It was felt that, if the dye reduction tests were sensitive with heat resistant cultures, then their use could be advocated in

bacteriological analyses of pasteurized milks. Samples taken from the pasteurizing vat after pasteurization could be considered to contain heat resistant bacteria.

The use of seven different cultures provided a rather rigid test since each had repeatedly survived temperature exposures approximating that of pasteurization. The transfer procedure also was designed in such a manner so as to produce a culture with a bacterial count of approximately 1,000,000 young, fairly actively growing bacterial cells.

The results do not support the contention of Hammer (15) and others (30,39) who stated that heat resisting organisms were poor reducers and that the resazurin and methylene blue test were unsuitable for detecting them.

The results would seem to indicate that, with resazurin at least and with the cultures under study, the reduction times at 68° F. and 98.6° F. were of a sufficiently short duration as to indicate fairly prompt and definite powers of reduction. At 113° F. the results were in even more close agreement, no culture taking longer than 1 hour to reduce resazurin. With methylene blue on the other hand the results do not seem to follow a pattern and, while the 98.6° F. incubation temperature did result in lowered reduction times, the results lack consistency and range for the three incubation temperatures from 1 hour to 42 hours. Enrichment of the methylene blue test at 98.6° F. failed to produce significant lowering of the reduction times.

The reason for the increased response to resazurin as compared

to methylene blue might be explained as follows:

The theory that the exhaustion of an undetermined part of the oxygen dissolved in milk accompanies reduction of methylene blue has received quite widespread support. This being assumed, there is every reason to believe that, with reduction of resazurin, one is dealing with a slightly more electro-positive dye. Slightly less oxygen need be exhausted in order to bring about complete reduction and even less to upset the oxygen balance so as to induce the color changes observed during the conduct of the test. The difference between the conduct of resazurin and methylene blue could be then traced perhaps to reduced activity of heat resistant organisms insufficient in the case of methylene blue reduction to reduce to oxygen threshold but sufficiently active with resazurin to bring about complete reduction.

CONCLUSIONS

1. With the seven heat resistant bacterial cultures under study at incubation temperatures of 68° F., 98.6° F. and 113° F. reduction times of resazurin dye were seen to be considerably shorter than that of methylene blue. A ratio of 1:10 existed with the respective reduction times.

2. A noticeable lack of uniformity of reduction time was noted with the methylene blue dye as compared to the resazurin.

3. Incubation at 113° F. with resazurin and at 98.6° F. with methylene blue resulted in shorter respective reduction times.

4. The resazurin test at 113° F. produced results indicative of the metabolic activity of heat resistant bacteria.

SECTION IV

DEVELOPMENT OF A KEEPING QUALITY TEST FOR PASTEURIZED MILK

PROCEDURE

The work of Berger and Anderson (3) reported that incubation of live run samples with subsequent plating and methylene blue reduction determinations gave reliable indication of the keeping quality of pasteurized milk. It was decided that a somewhat similar method of assessing post-pasteurization contamination and keeping quality would be followed in an attempt to develop a more objective keeping quality test which might have worthwhile application in the dairy plant laboratory.

With each of the processing sequences and with every trial within a sequence samples were gathered at three sample points (2, 3 and 5). These samples were analysed immediately for bacterial count and after incubation at 55° F. for 24 and 48 hour periods were again analysed for bacterial count and also for resazurin and methylene blue reduction.

It was felt that complete disregard of flavor evaluation would be unwise and to this end the samples (from sample point 5) that were incubated at 55° F. for 24 and 48 hour periods were scored for off-flavor development at the same time as bacteriological analysis were carried out. Due to subjective drawbacks reported by Berger and Anderson (3) and Wilson (39) the particular types of off-flavor development were not noted but rather the scoring method was designed to list quantitatively the ascending development of objectionable flavor.

Sample point 5 was chosen because it was felt that this would give a fairer picture bacteriologically than would other sample points in that post-pasteurization would be largely minimized due to the rinsing action of continuous milk flow.

RESULTS

Table 8. Bacterial counts and resazurin reduction times of milk incubated at 55° F. for 48 hours. Averages for 3 sample points from 6 sequences.

No. of antilogs. of ave. counts in excess of 50,000	Ave. antilog. of counts in excess of 50,000	Average resazurin reduction times corresponding to antilogs. in excess of 50,000									
		Over 250 Mins.		Between 200-250 Mins.		Between 150-200 mins.		Between 100-150 mins.		Under 100 mins.	
		No.	%	No.	%	No.	%	No.	%	No.	%
15	180,000	3	20	5	33.3	3	20	3	20	1	6.7

As may be noted from Table 8 there were 15 antilogarithms out of a total of 18 which exceeded 50,000. The average antilogarithm was seen to be 180,000. The resazurin reduction times of the samples corresponding to those that exceeded 50,000 were mainly (73.3%) in the range from 100 to 250 minutes.

Table 9. Bacterial counts and methylene blue reduction times of milk incubated at 55° F. for 48 hours, Averages for 3 sample points from 6 sequences.

No. of antilogs. of ave. counts in excess of 50,000	Ave. Antilog. of counts in excess of 50,000	Average methylene blue reduction times corresponding to antilogs. in excess of 50,000									
		Over 350 mins.		Between 300-350 mins.		Between 250-300 mins.		Between 200-250 mins.		Under 200 mins.	
		No.	%	No.	%	No.	%	No.	%	No.	%
15	180,000	1	6.7	3	20	6	40	3	20	2	13.3

From Table 9 as with Table 8 it may be seen that there were 15 antilogarithms (average of 180,000) out of a possible 18 that exceeded counts of 50,000. The methylene blue reduction times that correspond to these antilogarithms fall largely (80%) in the range from 200 to 350 minutes.

Tables 8 and 9 made no attempt to show the corresponding flavor scores on such incubated samples as this is better shown on an individual trial basis. Table 10 presents such data.

Table 10. Bacterial counts, reduction times and flavor of milk incubated at 55° F. for 48 hours. Based on samples with bacterial counts in excess of 50,000.

Bacterial count	Res. Red. min.	M.B. red. mins.	Flavor	Bacterial count	Res. red. mins.	M.B. red. mins.	Flavor
2,900,000	45	150	+ + +	500,000	150	210	0
1,000,000	60	105	+ +	170,000	300	420	0
270,000	90	150	+ +	61,000	360	420	0
91,000	270	330	0	73,000	150	210	0
460,000	45	90	+ + +	210,000	255	330	0
97,000	240	300	0	230,000	255	300	0
210,000	90	135	0	52,000	255	330	0
72,000	60	120	+ +	64,000	300	390	0
120,000	150	210	0	640,000	150	300	0
1,900,000	30	90	+ + + +	1,100,000	90	150	+ +
320,000	165	240	0	120,000	240	300	0
2,000,000	60	135	+ +	320,000	240	300	0
63,000	255	345	0	84,000	150	210	0
460,000	120	195	0	100,000	240	300	0
78,000	300	395	0	940,000	60	90	+
210,000	75	210	+	120,000	240	300	0
1,400,000	45	75	+ + +	110,000	270	330	0
3,600,000	45	90	+ +	190,000	240	330	0
510,000	120	180	0	91,000	180	285	0
1,300,000	120	195	0	67,000	195	270	0
670,000	105	180	0	190,000	270	330	0
78,000	165	240	0	84,000	360	420	0
330,000	180	255	0	91,000	315	390	0
51,000	240	330	0	Average	Ave.	Ave.	
6,600,000	30	75	+ +	(antilog.)			
120,000	90	180	+	250,000	172	241	

Flavor key: 0 = no objectionable flavor
+ = very slight objectionable flavor
+ + = slight objectionable flavor
+ + + = pronounced objectionable flavor
+ + + + = very pronounced objectionable flavor.

From Table 10 it may be seen that after incubation at 55° F. for 48 hours the bacterial count of the 49 samples that exceeded a count of 50,000 ranged from a low of 51,000 to a high of 6,600,000, with an average antilogarithm of 250,000. The resazurin reduction times ranged from a low of 30 minutes to a high of 360 minutes or an average of 172 minutes. The methylene blue reduction times ranged from a low of 75 minutes to a high of 420 minutes averaging 241 minutes. There were 14 samples that showed some objectionable flavor development, ranging from very slight to very pronounced in intensity. It is of interest to note that none of the resazurin reduction times of those samples that showed objectionable flavor development exceeded 90 minutes, the average being 59 minutes. The corresponding methylene blue reduction times show a slightly larger range, from 75 to 216 minutes an average of 122 minutes.

DISCUSSION

Bacteriological control of pasteurized milk is generally carried out on samples taken the same day the milk leaves the dairy. Since the keeping quality of the milk is largely of consumer interest, it was decided to apply the consumer's point of view as far as possible in an attemptto establish a more objective keeping quality test on pasteurized milk.

To this end, samples were stored at 55° F. (average summer ice refrigeration temperature) for 24 and 48 hour periods and the bacteriological changes in the milk during this time were observed.

The results would seem to bear out the contention of others (3,39) that the correlation between the first day's bacterial count and the keeping quality of the milk is slight and that in consequence very little can be prognosticated with safety on the basis of the bacterial count regarding the quality of the milk at the time of consumption.

Since it was felt that off flavor development was the end result of bacterial metabolism and that the dye reduction tests reported such metabolic activity and further, that good correlation could be expected between keeping quality of pasteurized milk and dye reduction tests on incubated samples (39), a keeping quality test might be evolved using these tests as a standard. It is logical to assume that essentially a keeping quality test with commercial applications must be relatively simple to operate. It should give information as to acceptability or non-acceptability of the product from a keeping quality standpoint as well as pointing out which different part of the processing sequence is responsible for any non-acceptability of the product.

Accepting this assumption, it would be necessary to take line samples of the pasteurized product and analyse these to fulfil the above objectives of an adequate keeping quality test.

The results of such analyses carried out throughout this study have revealed possible application towards establishment of a brood standard which would be applicable in assessing keeping quality.

It was noted from Table 8 that the bulk (73.3%) of the resazurin reduction times fall within the range from 100 to 250 minutes. The

methylene blue reduction times (Table 9) for the same set of samples fall largely (80%) in the range from 200 to 350 minutes. The average antilogarithm for the same samples was 180,000.

At 55° F. 24 hour incubation period there were no antilogarithms in excess of 50,000 and none showed objectionable flavor development.

The samples from sample point 5 were checked for flavor at the end of 48 hour incubation at 55° F. Forty-nine of the 72 samples had bacteria counts in excess of 50,000. Of the 14 samples that showed some objectionable flavor development the bacterial counts ranged from 72,000 to 6,600,000. The relation of intensity of flavor development to bacterial count showed no definite pattern. However none of the 14 samples had a resazurin reduction time in excess of 90 minutes and no methylene blue reduction time exceeded 210 minutes. The relationship of off-flavor intensity to reduction times was considerably more definite than was the off-flavor-bacterial count relationship.

From these results a broad pattern seems evident and tentative establishment of keeping quality standards seems possible as follows:

Samples could be taken from the pasteurizing vat after pasteurization, from the first milk flow into bottling machine (to show contamination from pipe-lines, etc.) and a similar sample into bottling machine after 10 minutes continuous milk flow. These samples should be secured in as simple a manner as is possible, perhaps by means of petcocks on the equipment. They should be incubated for 48 hours at 55° F. and subsequently analysed by the resazurin test. The results of such analysis could be

interpreted on the basis of keeping quality as either acceptable or non-acceptable. Numerically this standard could be arrived at as follows:

Flavor likely not affected - A) Acceptable - resazurin reduction time in excess of 4 hours, probable bacterial count not exceeding 200,000.

B) Acceptable - resazurin reduction time between 2 and 4 hours, probable range of bacterial count 200,000 to 1,000,000.

Flavor likely affected - A) Non-acceptable - resazurin reduction time less than 2 hours, probable bacterial count in excess of 1,000,000.

CONCLUSIONS

1. There does not seem to be any definite relationship between the intensity of undesirable flavor development and the bacterial count of pasteurized milk incubated at 55° F. for 48 hours on the basis of the results obtained.

2. Twenty four hour incubation at 55° F. revealed no undesirable flavor development with samples taken from the filler bowl after 10 minutes of continuous milk flow.

3. Of the 14 samples (out of a possible 72) that showed some undesirable flavor development after 48 hours at 55° F., none of the corresponding resazurin reduction times exceeded 90 minutes.

SECTION V

EFFECT OF BACTERIAL CLUMB BREAKUP ON BACTERIAL COUNTS OF HOMOGENIZED MILK

PROCEDURE

In an attempt to show by variations in the plating procedure the breakup of bacterial clumps by an homogenizer samples were taken on six different trial days using pasteurized milk. The samples were taken after 10 minutes continuous operation of the homogenizer at approximately 1700 lbs./sq./in. pressure. The temperature of the pasteurized milk being homogenized was between 120° - 130° F. The samples were taken as close together as was possible so as to represent a comparative milk supply, the sample points being a) inlet pipe to homogenizer and b) outlet pipe from homogenizer, the samples being secured by loosening sanitary pipe connections.

The specific variation in plating procedure was as follows: Several 99 ml. water dilution bottles (sterile) were prepared with the addition of glass beads, approximately 25 beads per bottle. The samples were subdivided and suitable dilutions made using both types of dilution bottles i.e. containing beads and containing no beads. The samples were shaken 25 times with each bottle and a portion removed and plated. The samples in dilution bottles containing glass beads were then shaken 75 more times, portions being removed at intervals of 50, 75 and 100 times, and plated. The standard dilution bottles (no beads) were shaken another 75 times, a sample being removed and plated after shaking a total of 100 times. Samples were plated in duplicate and in at least two dilutions on T.G.E.M. agar incubation being at 95° F. for 48 hours.

Table 11. Influence of plating procedure on bacterial count of homogenized milk.

No.	Source of sample from homogenizer*	Bact. count shaken 25X	Bact. count shaken 100X	Bact. count shaken 25X beads	Bact. count shaken 50X beads	Bact. count shaken 75X beads	Bact. count shaken 100X Beads	% increase beads to no beads shaken 25X
1	inlet	49,000	51,000	59,000	63,000	67,000	67,000	20.4
	outlet	73,000	74,000	77,000	76,000	72,000	79,000	5.4
2	inlet	21,000	29,000	42,000	44,000	50,000	57,000	100.0
	outlet	54,000	54,000	58,000	51,000	63,000	58,000	7.4
3	inlet	13,000	17,000	22,000	22,000	20,000	27,000	69.1
	outlet	29,000	31,000	34,000	30,000	30,000	36,000	17.2
4	inlet	4,200	4,700	6,300	6,900	7,400	7,500	50.0
	outlet	8,100	8,000	8,400	8,300	8,700	8,700	3.7
5	inlet	7,000	7,200	11,000	11,000	11,000	12,000	57.1
	outlet	13,000	13,000	14,000	13,000	14,000	17,000	7.6
6	inlet	1,300	1,500	2,900	3,100	3,300	3,000	123.0
	outlet	3,400	3,400	3,700	4,000	4,100	3,900	8.8

* All samples from homogenizer taken after 10 minutes continuous operation.

It may be seen from Table 11 that dilution bottle shaking with glass beads tends to increase the bacterial count of a sample. Such increase is greater with samples from the inlet of homogenizer (non homogenized). Comparing samples shaken 25 times with and without beads if may be noted that the increases of those samples shaken with beads as compared to those shaken without beads ranges from 20.4 to 123%, an average of 69.9% with non homogenized samples (inlet) while with homogenized samples the range is from 3.7% to 17.2%, an average increase of 8.3%.

The results are more easily noted by reference to Table 12 which presents antilogarithms of averaged bacteria counts for the six trials.

Table 12. Influence of plating procedure on bacterial count of homogenized milk. Summary of material from Table 11. Expressed as antilogarithms of average counts.

Source of sample from homo.	Bact. count shaken 25X no beads	Bact. count shaken 100X no beads	Bact. count shaken 25 X beads	Bact. count shaken 50X beads	Bact. count shaken 75X beads	Bact. count shaken 100X beads
Inlet	9,000	10,000	15,000	16,000	16,000	18,000
Outlet	18,000	21,000	20,000	20,000	20,000	21,000

From Table 12 comparing the antilogarithms of inlet and outlet samples for the six different plating procedures, the following observations may be made. Using the standard procedure i.e. shaking 25 times with no glass beads the average increase due to the homogenizer was 9,000. Prolonged shaking i.e. 100 times with no beads resulted in an average increase

of 11,000. However using beads the increase between inlet and outlet samples was reduced as follows: 25 times = 5,000, 50 times = 4,000, 75 times = 4,000 and 100 times = 3,000.

From the foregoing it may be seen that both homogenization and shaking with glass beads tends to increase the apparent bacterial count but that the increase between samples from inlet and outlet of the homogenizer is not as great when both samples are shaken with glass beads as when standard procedures are used. It is of interest to note that identical antilogarithms resulted from shaking a non homogenized (inlet) sample 100 times with glass beads as when an homogenized (outlet) sample was shaken in the standard manner. In both cases the antilogarithm was 18,000.

DISCUSSION

It is felt that the results from Table 11 illustrate quite effectively the fact that a large proportion of the apparent bacterial count increase due to homogenization is in actuality caused by disruption and breaking up of the bacterial clumps.

Table 12 which summarizes the material from Table 11 shows that, with inlet or non homogenized samples of milk with undisrupted bacterial clumps, the percentage increase in bacterial count from shaking 25 times with no beads to shaking 25 times with beads was 66.6%. With the outlet or homogenized samples of milk with disrupted bacterial clumps the comparative increase was 11.1%. The ratio of increase between inlet and outlet

samples was therefore 6:1 with 25 times shaking with beads compared to 25 times shaking with no beads.

With samples from inlet shaken 100 times with beads the percentage increase in bacterial count compared to the same sample shaken 100 times without beads was 80%. With outlet samples there was no comparative increase (0%).

While it is realized that the bacterial flora of milk is rarely if ever the same from one pasteurization to the next and that consequently the clumping characteristics would tend to differ, the results do seem to indicate quite definitely one or two major premises.

Since it is a matter of record (4, 21, 33) that homogenization breaks up the bacterial clumps of milk resulting in an apparently higher bacterial count and further it has been shown that, by a variation in plating technique (shaking of dilution bottles with glass beads), this apparent bacterial count increase can be simulated in the laboratory, certain assumptions may be made on the basis of this evidence.

The basic assumption might well be that it would be logical to minimize the apparent significance of such bacterial count increases due to homogenization, particularly when samples are obtained after a period (10 minutes) of continuous homogenizer operation when the residual bacterial contamination of the homogenizer could be said to be largely washed away. The reasons for such action being that present evidence seems to point to the fact that bacterial count increases are probably largely due to bacterial clump breakup.

CONCLUSIONS

1. Shaking with glass beads tends to increase the bacterial count of milk, the increase being greater with non-homogenized than with homogenized milk.

2. Homogenization generally increases the bacterial count of milk.

3. The possibility exists that a considerable part of any bacterial count increase due to homogenization is caused by disruption or breaking up of bacterial clumps rather than by actual bacterial infection from the homogenizer.

4. The antilogarithm of the average of 6 trials shows that with a non-homogenized sample shaken 100 times with glass beads, about the same effect in breaking the bacterial clumps is achieved as with an homogenized sample shaken in a standard manner.

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