

THE QUALITY OF MARKET MILK AS AFFECTED BY
THE RESAZURIN GRADE OF THE RAW MILK
AND VARIOUS PASTEURIZATION
EXPOSURES



by

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TABLE OF CONTENTS

| | PAGE |
|--|------|
| INTRODUCTION | 1 |
| REVIEW OF THE LITERATURE | 3 |
| The sources and significance of high bacterial counts in pasteurized milk | 3 |
| The effect of increased pasteurization exposures on the bacterial count of milk | 5 |
| The effect of increased pasteurization exposures on the physico-chemical and nutritive properties of milk | 6 |
| Correlation of the resazurin grade of the raw milk and the bacterial counts of the raw and subsequently pasteurized milk | 14 |
| METHODS OF PROCEDURE | 18 |
| Laboratory scale trials | 18 |
| Commercial scale trials | 19 |
| Analysis of samples | 20 |
| RESULTS | 23 |
| Laboratory scale trials | 23 |
| Commercial scale trials | 37 |
| Correlation of the resazurin grade and the bacterial counts of raw and pasteurized milk | 45 |
| DISCUSSION | 52 |
| SUMMARY AND CONCLUSIONS | 56 |
| BIBLIOGRAPHY | 58 |

LIST OF TABLES

| TABLE | PAGE |
|--|------|
| I Bacteria in grade 1 milk; raw, and laboratory pasteurized at 143, 149, and 155°F. for 30 min. | 24 |
| II Bacteria in grade 1 milk laboratory pasteurized at 143, 149, and 155°F. for 45 and 60 min. | 25 |
| III Numbers of 45°C. bacteria in grade 1 milk laboratory pasteurized at 143, 149, and 155°F. for 30, 45, and 60 min. | 27 |
| IV Bacteria in grade 2 milk; raw, and laboratory pasteurized at 143, 149, and 155°F. for 30 min. | 28 |
| V Bacteria in grade 2 milk laboratory pasteurized at 143, 149, and 155°F. for 45 and 60 min. | 29 |
| VI Numbers of 45°C. bacteria in grade 2 milk laboratory pasteurized at 143, 149, and 155°F. for 30, 45, and 60 min. | 30 |
| VII Bacteria in grade 3 milk; raw, and laboratory pasteurized at 143, 149, and 155°F. for 30 min. | 32 |
| VIII Bacteria in grade 3 milk laboratory pasteurized at 143, 149, and 155°F. for 45 and 60 min. | 33 |
| IX Numbers of 45°C. bacteria in grade 3 milk laboratory pasteurized at 143, 149, and 155°F. for 30, 45, and 60 min. | 34 |
| X Bacteria in grade 4 milk; raw, and laboratory pasteurized at 143, 149, and 155°F. for 30 min. | 35 |
| XI Bacteria in grade 4 milk laboratory pasteurized at 143, 149, and 155°F. for 45 and 60 min. | 36 |
| XII Numbers of 45°C. bacteria in grade 4 milk laboratory pasteurized at 143, 149, and 155°F. for 30, 45, and 60 min. | 38 |

| TABLE | PAGE |
|--|------|
| XIII Bacteria in grade 1 milk; raw, and commercially pasteurized at 143, 149, and 155°F. for 30 min. . | 39 |
| XIV Bacteria in grade 2 milk; raw, and commercially pasteurized at 143, 149, and 155°F. for 30 min. . | 40 |
| XV Numbers of 45°C. bacteria in grades 1 and 2 milk commercially pasteurized at 143, 149, and 155°F. for 30 min. | 42 |
| XVI Bacteria in grade 3 milk; raw, and commercially pasteurized at 143, 149, and 155°F. for 30 min. . | 43 |
| XVII Bacteria in grade 4 milk; raw, and commercially pasteurized at 143, 149, and 155°F. for 30 min. . | 44 |
| XVIII Numbers of 45°C. bacteria in grades 3 and 4 milk commercially pasteurized at 143, 149, and 155°F. for 30 min. | 46 |
| XIX Correlation of resazurin and bacterial grades of raw milk | 48 |
| XX Correlation of resazurin grades of raw milk and bacterial grades of pasteurized milk. | 50 |

INTRODUCTION

The resazurin grade of raw milk produced in the Winnipeg milkshed varies considerably during the year.¹ This variation is greatest in the summer months. However, at the present time all milk is subjected to one pasteurization treatment regardless of its grade. This results in variations in the bacterial counts of pasteurized milk.

These variations make it difficult to conform with requirements of many Departments of Health that pasteurized milk contain less than 30,000 bacteria per ml. In addition, the presence of excessive numbers of bacteria in pasteurized milk may cause off-flavors or other defects (48).

Pasteurizing plant operators have considered the practicability of modifying pasteurization temperatures and holding periods to deal with the seasonal variations in the quality of the raw milk. Action in this direction has been hampered by lack of specific information.

The following study was undertaken, (1) to determine the relationship between the resazurin grade of the raw milk, the various pasteurization exposures, and the bacterial count of

¹ Personal communication - Winnipeg Health Department

the pasteurized milk; and (2) to determine the relationship between the resazurin grade of raw milk and the bacterial counts of the same milk before and after pasteurization at 143°F. for 30 min.

REVIEW OF THE LITERATURE

The Sources and Significance of High Bacterial Counts in Pasteurized Milk

Thermoduric bacteria - Corash (9) stated that thermodurics found in properly pasteurized milk are non-pathogenic. Doetsch (12) stated that excessive numbers of thermodurics in milk were significant because they indicated unsanitary conditions somewhere between producer and processor. This view was supported by Macy (34) and Levowitz (32)

Thomas (49) found that sterilization of milking machines and utensils eliminated excessive thermoduric counts in machine drawn milk. Thomas et al. (50) presented evidence that unclean utensils were the chief source of thermoduric organisms. Meanwell (39) found that the bacterial count of pasteurized milk was dependent to a large extent on the cleanliness of the utensils on the farm.

Thermophilic bacteria - Sommer (48) stated that a number of organisms grow in milk at temperatures of 143 - 150°F. He considered such organisms to be significant because they cause high counts and affect milk flavor when present in excessive numbers.

After extensive investigations Hansen (22) concluded that it seemed improbable that ordinary pasteurized milk containing thermophilic bacteria produces harmful effects when used for human consumption, but that such milk cannot be recognized as high grade pasteurized milk.

Cordes (10) claimed that thermophiles were largely an in-plant problem and one of their primary sources was pasteurized milk returned from delivery routes for repasteurization. Charlton (7) traced the presence of large numbers of thermophiles in pasteurized milk to the pasteurized cream used for standardizing the milk.

Meanwell (39) found that most thermophiles in raw milk were dormant and did not multiply extensively until the milk had been held at 145°F. for 4 hr. or longer. He concluded therefore, that pasteurizers should not be run continuously without washing for longer than 4 hr. This is in agreement with earlier work by Martin (37) who did not encounter thermophiles while pasteurizing ice cream mix at 150°F. for periods ranging from 30 - 210 min.

Walts (52) listed the following as additional sources of thermophiles; 1. milkstone, 2. long use of the same filter cloth, 3. foam on milk, 4. dead ends in sanitary piping, 5. hot

milk allowed to remain in preheaters, and 6. condensation from unsterile surfaces dropping into the pasteurized milk.

The Effect of Increased Pasteurization
Exposures on the Bacterial Count of Milk

Ayers and Johnson (3) found that good quality raw milk contained 13,900 and 5,500 bacteria per ml. after pasteurization at 140 and 150°F., respectively. Pasteurization of poor quality raw milk resulted in counts of 124,000 and 28,900.

Gorini (20) isolated 100 lactic cultures from commercially pasteurized milk. Ninety-eight of these survived 145.4°F. for 30 min., but only a small number withstood a temperature greater than 158°F. for 30 min.

Trout (51) reported that the pasteurization of homogenized milk at 158°F. for 30 min. resulted in greater pasteurizing efficiency and no cooked flavor.

Elliker (14) stated that;

"The excessive exposures commonly employed for cream and ice cream mix frequently result in greater bacterial destruction than occurs in the pasteurization of market milk."

The Effect of Increased Pasteurization Exposures on
the Physico-Chemical and Nutritive Properties of Milk

Cream-line destruction - The creaming ability of milk is a physical property which is highly susceptible to injury by heat. Marquard and Dahlberg (36) prepared a graph which illustrated the effects of different time and temperature exposures on creaming ability. Under practical operating conditions these investigators found that exposures of 145°F. for 13 min., 155°F. for one min., and 160°F. for 16 sec. yielded cream-lines comparable to those obtained in milk pasteurized at 142°F. for 30 min.

Holland and Dahlberg (26) measured impairment of creaming ability caused by various pasteurization exposures. These investigators found that an exposure of 143.5°F. for 30 min. caused less than 1 ml. reduction in cream volume per 100 ml. milk. An exposure of 150°F. for 20 min. reduced the cream volume from 21 to 12 ml. per 100 ml. milk. The creaming ability was completely destroyed by 155°F. for 10 min. It is evident from the above work that any pasteurization process which requires an exposure greater than 143.5°F. for 30 min. can only be used for homogenized milk and other products where creaming ability is not important.

Cooked flavor development - The development of a cooked flavor is another characteristic of milk which prevents the use of an excessive pasteurization exposure. Gould and Sommer (21) found that milk heated at 155 - 161.6°F. for 30 min. developed a cooked flavor.

Josephson and Doan (29) found that cooked flavor became evident when unhomogenized milk was kept at 155°F. for 30 min. A cooked flavor did not develop in homogenized milk.

Bernhard Spur (47) carried out a large number of trials in which milk was homogenized and pasteurized at various temperatures. Six per cent of 119 trials, in which milk was homogenized at 161°F. before pasteurization at 155°F., had a cooked flavor. Twenty-four lots homogenized at 156°F. and pasteurized at 150°F. had no cooked flavor while 3% of 130 lots of milk pasteurized at 143°F. and homogenized at 150°F. had a cooked flavor.

Trout (51) reported that homogenized milk could be pasteurized at 158°F. for 30 min. without danger of a cooked flavor.

The third important factor to be considered in the use of an increased pasteurization exposure is its effect on the

nutritional properties of the milk. According to Marie Harrington (23) milk supplies protein, energy, vitamins A, B, C, D, and G, and minerals, especially calcium and phosphorus.

Proteins - de Freudenreich (11) reported that 15 - 20% of the albumin present in milk was coagulated by heating at 154.4-157°F. for 30 min. Rupp (45) found that albumin did not coagulate as a result of holding the milk at 145°F. for 30 min.; 5.7% was coagulated at 150°F. for 30 min. and 12.75% at 155°F. for 30 min.

Rowland (44) carried out extensive investigations on the heat denaturation of albumin and globulin in milk held at temperatures ranging from 143.4 - 176°F. for periods of $2\frac{1}{2}$ - 60 min. Ten and four tenths per cent of the total soluble protein was denatured at 143.4°F. for 30 min. This was increased to 28% at 158°F. for 30 min. The total protein content remained unchanged until the milk had been held at 176°F. for 30 min.

Shahani and Sommer (46) determined the protein and non-protein nitrogen content of raw milk; milk which was laboratory pasteurized at 143°F. for 30 min.; and milk which was commercially homogenized and pasteurized at 155°F. for 30 min. They found no significant differences in the total and casein nitrogen contents between the raw and either of the processed

milks. There was a slight increase in proteose, peptone, and non-protein nitrogen contents of both types of pasteurized milk.

Henry, Kon, and Watson (25) used Mitchell's method to compare the biological values and true digestibilities of the proteins of raw and pasteurized milks from the same bulk. The milk was pasteurized at 142 - 153°F. for 28 - 44 min. These workers found that pasteurization resulted in a slight, although not statistically significant, increase in the biological value and true digestibility of the proteins.

Vitamins - There was no apparent loss of vitamin A during the sterilization of evaporated milk at 240°F. for 15 min. (48). It is therefore unlikely that a pasteurization exposure of 155°F. for 30 min. would cause any destruction of this vitamin.

Sommer (48) reported that thiamine, of the vitamin B complex, suffered some destruction at 143°F. Niacin and the remaining B complex vitamins were thermo-stable. Houston, Kon and Thompson (28) and Dutcher, Guerrant and McKelvey (13) reported losses of vitamin B₁ (thiamine) during commercial and laboratory pasteurization.

A review of the literature has not revealed any reports of investigations carried out on milk to determine the additional

amounts of thiamine destroyed by a 155°F. pasteurizing temperature. However, it appears to be logical to assume that a certain amount of additional loss will take place. Sommer (48) states that:

"This destruction of ascorbic acid and thiamine is not considered important from a nutritional standpoint. Their content in raw milk is such that it is advisable to supplement it in any case."

King and Waugh (30) reported a significant loss of vitamin C as a result of pasteurization at 143 - 145°F. for 30 min.

Woessner, Weckel, and Schuette (53) found that milk pasteurized at 150°F. lost 21.5% of its total vitamin C. The same milk pasteurized at 145°F. lost only 12.6%.

Krauss and Washburn (31) found a 5 - 15% loss of vitamin C when determinations were made shortly after either boiling or pasteurization. On standing at room temperature after processing this loss increased to 50% in 6 hr.

Gjessing and Trout (18) investigated the effects of high heat treatment and storage periods on the ascorbic acid content of milk. The milk was pasteurized at 145.4 and at 167°F. for 30 min. Ascorbic acid determinations were made before and immediately after pasteurization and on each of the succeeding four days. The raw milk contained 17.1 mg. ascorbic acid per

liter. Immediately after pasteurization at 145.4°F. it contained 15.9 mg. per liter. Pasteurization at 167°F. reduced this to 14.9 mg. per liter.

After three days storage at 41°F. the raw milk contained 11.3 mg. per liter, the 145.4°F. milk contained 11.7 mg. per liter, and the 167°F. milk contained 13.6 mg. per liter. Thus, after three days storage at normal refrigeration temperatures, the milk pasteurized at 167°F. contained more than either the raw or 145.4°F. pasteurized milk. At the end of four days' storage the 167°F. pasteurized milk contained almost twice as much ascorbic acid as raw milk.

Holmes (27) carried out 17 trials to determine the reduced ascorbic acid content of milk at different stages of processing. The raw milk contained 18.6 mg. per liter. Immediately after pasteurization at 143°F. for 30 min. the milk in the vat contained 17.1 mg. per liter. The loss as a direct result of pasteurization was 8.1%. The first bottle to pass over the cooler and through the filler contained only 5.9 mg. per liter. Holmes attributed the large loss in the first bottle to :

- 1.) Contact with air in the equipment, and
- 2.) contact of the first milk with the metal in the equipment.

The work of the above investigators indicates that several

factors may cause greater losses of vitamin C than that caused by an increased pasteurization exposure. In addition to these, Roadhouse and Henderson (42) and Sommer (48) stated that milk is not considered an important source of vitamin C. It is usually supplied in adequate amounts when the diet includes fruits and vegetables. Elvehjem (15) stated that:

"The seasonal differences are far greater than those resulting from pasteurization. 20% of the vitamin C and B₁ are lost during pasteurization. These losses are compensated for by fruits, vegetables and fortified bread in the average diet."

The loss of vitamins B₁ and C in pasteurized milk is offset by the fact that milk is not the best source for these vitamins. The Associates of Rogers (2) state that:

" . . . it must be remembered that it is not usually wise to use milk as the sole article of diet, even for very young animals. The specialists on infant feeding recommend that babies have a small amount of orange juice as a part of their diet from the first few days of life onward, even when they are nursed by their mothers."

Vitamins D and G (Riboflavin) are heat stable and are not affected by temperatures of 240°F. for 15 min. (43) (48).

Calcium, phosphorus and total energy value - Rupp (45) investigated the chemical changes produced in cow's milk by pasteurization. He used a filter constructed from a clay cell of a galvanic element to remove precipitates formed during the pasteurization of milk at 155°F. for 30 min. The precipitate

was analyzed to determine the amount of P_2O_5 , MgO , and CaO present. No changes were found in the MgO , and P_2O_5 between raw and heated milk. There was a slight reduction in the CaO content of pasteurized milk. However, the variations between different samples of raw milk were much larger than the loss caused by pasteurization.

Bell (4) heated milk for 30 min. at temperatures ranging from $140^\circ - 180^\circ F.$ in 10° increments. Insoluble calcium and phosphorus compounds were removed from the milk by a Pasteur-Chamberland filtering tube and also by a continuous supercentrifuge. Measurable amounts of these substances were not removed from solution until the milk had been heated at $170^\circ F.$ for 30 min.

Mattick and Hallett (38) determined the difference in diffusibility between raw and heated milks by means of standardized parchment diffusion capsules. These investigators found no change in the amount of diffusible phosphorus compounds at temperatures below $175^\circ F.$ with a 30 min. holding period. There were marked changes in the diffusibility of calcium salts as a result of heating at $125^\circ F.$ and above for 30 min. The total calcium and phosphorus contents remained the same even after heating at $209^\circ F.$

In a series of experiments carried out by Henry and Kon (24) no significant differences were found in the availability of calcium and phosphorus compounds between raw and pasteurized milk. On the contrary, rats retained 3% more calcium from pasteurized milk than their littermates retained from raw milk. This supports Sommer's (48) statement that:

"The more recent evidence indicates that there is a slight decrease in the soluble calcium and phosphorus of milk on pasteurization. This is not actually lost, and must be expected to redissolve under the acid conditions in the stomach."

Correlation of the Resazurin Grade of the Raw Milk and the Bacterial Counts of the Raw and Subsequently Pasteurized Milk

Ramsdall et al. (41) investigated the use of resazurin dye as an indicator of the sanitary quality of milk. They added one part dye to 200,000 parts milk and incubated the mixture in test-tubes at 37°C. After incubation for 1 hr. the color of the resazurin tubes was compared to a color chart and graded according to the blue color remaining.

Frayer (17) carried out a number of comparative trials between the resazurin test and the bacterial plate count. The resazurin trials were incubated at 37°C. for 1 hr. These were graded according to permanent standards made from painted test

tubes. Frayer found that the resazurin test hardly correlated with the plate count, but that it was sensitive to the presence of cells and to the activity of bacteria.

Collins et al. (8) measured the time required for the milk-dye mixture to reach a pronounced pink color, and found that milk which required three or more hours to reach the end point contained less than 400,000 bacteria per ml.

Brannon (6) graded milk according to the time required for the milk-dye mixture to reach a definite end point. The plate count on grade 1 milk varied from 1,000 - 180,000 per ml.; grade 2 milk from 1,000 - 1,800,000; grade 3 milk from 2,100 - 7,600,000 and grade 4 milk from 9,400 - 10,200,000 per ml. A considerable amount of overlapping of counts occurred between the different grades. Brannon considered grade 1 and 2 milk good milk, and grades 3 and 4 poor milk.

Golding and Gorgenson (19) graded milk by means of the Lovibond modification of the resazurin test. Samples were incubated for 10, 30, and 60 min. periods. Color readings were made with a Lovibond comparator. These were compared with the standard plate counts of the raw milk. The resazurin test correlated fairly well with the plate count. The 60 min. incubation period gave the best results.

Okulitch, Millard and Fleming (40) used a mauve pink color standard designated as Munsell P.R.P. 7/8 for the color end point. Readings were made at the end of 1 and 3 hr. incubation. In poor quality milk 80% of the samples were placed in the same grade by the methylene blue and resazurin tests. The standard plate count and the microscopic count supported the resazurin grade in more cases than they did the methylene blue grade. A high percentage of the milks that survived incubation for 3 hr. or longer in the resazurin test had counts below 200,000 per ml.

Boyd and Hansen (5) used the 1 hr. method to compare the resazurin test and plate counts of raw milk. Out of 144 samples graded A by the resazurin test, 79% had plate counts lower than 100,000 per ml. Only 2.7% had counts over 500,000. Of the grade B samples, 76.4% had counts below 500,000 per ml. Seventy-five per cent of the grade C samples had counts exceeding 500,000 and 56% of the grade D samples exceeded 1,000,000 bacteria per ml. However, 4% of the samples placed in grade D contained less than 100,000 bacteria per ml.

Lewton, Markland and Babel (33) obtained an excellent correlation between the resazurin and methylene blue tests when both were carried to their end points. In a comparison of the triple-reading resazurin test using a Munsell P 7/4 color standard end point and the bacterial count, these workers found

that the resazurin test placed 41% of the samples in the same grade as the plate count, 20% one grade lower, 18% two grades lower, and 17% three grades lower. Four per cent were graded higher by the resazurin test. Most of the samples showing extreme differences in classification had plate counts of 50,000 or less. The authors suggested that the reduction of the dye in these samples may have been due to pathological conditions.

METHODS OF PROCEDURE

Laboratory Scale Trials

Forty representative one-half pt. samples of raw milk were obtained from raw milk storage tanks or balance tanks of commercial pasteurizing plants in the Greater Winnipeg area. The plants were selected according to their ability to supply the grade of milk required to carry out investigations on each of the four resazurin grades. In most cases the milk had been held over-night in storage tanks. The samples were taken in the morning immediately prior to pasteurization. In this way a true sample of the type of milk processed was obtained.

The sample, taken in a sterilized bottle, was immediately transported to the dairy laboratory of The Un. of Man. Upon arrival, it was divided into ten sub-samples of approximately 11 ml. each and placed in sterile test tubes. An additional 10 ml. sample was taken for grading by means of the Triple Reading Resazurin Test (1). One of the ten 11 ml. samples was designated as the raw control sample and placed in the refrigerator. The remaining nine samples were laboratory pasteurized at 143, 149, and 155°F. \pm 1° for 30, 45, and 60 min. periods.

One tube of milk pasteurized at each of the three temperatures was removed at the end of each holding period and rapidly cooled to 40^oF. They were placed in the refrigerator until plated.

Commercial Scale Trials

A series of 48 commercial trials, 12 on each of the four grades of milk was carried out to confirm the laboratory trials. On the basis of the laboratory-trial results and after consultation with pasteurizing plant operators, it was decided that it was not commercially feasible to increase the holding time beyond 30 min. Accordingly, only a 30 min. holding period was used in these trials.

Eight-hundred-pound lots of milk were purchased from different pasteurizing plants in Winnipeg. The plants were selected according to the grade of milk that would likely be supplied. Milk produced by The Un. of Man. herd was used for the first seven trials.

The milk was taken directly from the raw-milk-storage tank of the pasteurizing plant just previous to pasteurization. The tank contained milk from at least 10 different shippers before

the 800 lb. lot was removed. The milk was placed in clean 8 gal. cans which were immediately transported to The Un. of Man. pasteurizing plant.

Upon arrival the milk was poured directly into pasteurizing vat No.1. After sufficient agitation three samples were removed. These were used to determine the resazurin grade, bacterial count, and thermoduric count on the raw milk.

Approximately 260 lb. raw milk was then pumped into vat No.2, where it was pasteurized at 155°F. An 11 ml. sample was then removed for bacterial counts. The vat was emptied, washed and sterilized with a 200 p.p.m. chlorine solution.

A second lot of 260 lb. raw milk was pumped into the vat and pasteurized at 149°F. At the same time the milk remaining in vat No.1 was pasteurized at 143°F. Eleven ml. samples were taken from each vat at the end of the 30 min. holding period. These were cooled to 40°F. in a water bath and placed in a refrigerator until plated.

Analysis of Samples

The analytical work was begun approximately 2 hr. after the milk was pasteurized. The raw milk was graded by means of the Triple Reading Resazurin Test (1).

One ml. 0.005% resazurin solution was added to 10 ml. milk in a test tube. After incubation in a water bath at 37°C. for 1 hr., the tube of milk was compared to a Munsell P 7/4 color standard. Milk which was not reduced beyond the standard was inverted once to redistribute the cream and the accompanying bacteria and returned to the water bath. The inspection and inversion was repeated at the end of a second hour. If the milk was reduced during the second hour it was placed in grade 3. If it was not reduced it was returned to the water bath for a third hour. If the milk was reduced during the third hour it was placed in grade 2. If it was not reduced at the three-hour inspection it was placed in grade 1.

The pasteurized milk was plated in three series of duplicate plates in at least two dilutions. All plates were poured with Tryptone-Glucose-Extract-Milk-Agar and incubated for 48 hr. One series was incubated at 35°, a second at 45°, and a third at 55°C. The count at 35°C. will be referred to as the "count"; that at 45°C. as the 45° count, and that at 55°C. as the thermophilic count. The average bacterial count on each grade was determined logarithmically as suggested by Standard Methods (1).

The thermoduric count was obtained by laboratory pasteurizing an 11 ml. sample of raw milk in a test-tube at 143°F.

for 30 min. The milk was plated in suitable dilutions with Tryptone-Glucose-Extract-Milk-Agar and the plates incubated at 35°C. for 48 hr.

The coliform count was determined by plating 1 ml. with Violet Red Bile Agar and incubating at 35°C. for 24 hr.

RESULTS

Coliform organisms were not present in any of the trials carried out. Significant numbers of thermophiles were not present in most of the trials. No further reference will be made to these except in the few trials in which they were present.

Laboratory Scale Trials

Grade 1 milk - The counts on grade 1 milk, raw and pasteurized at 143°, 149°, and 155°F. for 30 min., are given in Table I. The count on the raw milk ranged from 120,000 - 440,000 with an average of 291,300 per ml. After pasteurization at 143°F. two of the five samples had counts higher than 30,000 per ml. At 149°F. only one sample contained more than 30,000 per ml. All five samples had counts below 30,000 after processing at 155°F. The average counts were 36,630; 19,540; and 13,790 per ml., respectively, after pasteurization at 143°, 149°, and 155°F.

TABLE I

Bacteria in grade 1 milk; raw, and laboratory pasteurized at 143, 149, and 155°F. for 30 min.

| Trial No. | Raw Milk | Pasteurization temperature | | |
|-----------|----------|----------------------------|--------|--------|
| | | 143°F.* | 149°F. | 155°F. |
| 1 | 440,000 | 31,000 | 21,000 | 19,000 |
| 2 | 270,000 | 94,000 | 40,000 | 28,000 |
| 3 | 350,000 | 30,000 | 20,000 | 8,700 |
| 4 | 120,000 | 26,000 | 10,000 | 9,800 |
| 5 | 420,000 | 29,000 | 17,000 | 11,000 |
| Ave. | 291,300 | 36,630 | 19,540 | 13,790 |

* The figures in this column also represent thermoduric counts.

Table II contains the counts on five lots of grade 1 milk pasteurized at 143, 149, and 155°F. for 45 and 60 min. After pasteurization at 143°F. for 45 min. two of the five samples contained more than 30,000 bacteria. At 149°F. the counts on all samples were below 30,000. The average counts were 29,240; 16,490; and 9,877, respectively, after pasteurization at 143, 149, and 155°F. for 45 min.

The data for the 60 min. period of heating show that the increased time exposure resulted in a further reduction in counts. After pasteurization at 143°F. the counts ranged

from 5,800 - 43,000 with an average of 15,090 per ml. This average is comparable to that obtained after pasteurization at 155°F. for 30 min. However, after pasteurization at 143°F. for 60 min. one sample contained 43,000 bacteria while the maximum present at 155°F. for 30 min. was 28,000.

TABLE II

Bacteria in grade 1 milk laboratory pasteurized at 143, 149, and 155°F. for 45 and 60 min.

| Trial No. | Forty-five minutes | | | Sixty minutes | | |
|-----------|--------------------|--------|--------|---------------|--------|--------|
| | 143°F. | 149°F. | 155°F. | 143°F. | 149°F. | 155°F. |
| 1 | 27,000 | 23,000 | 13,000 | 19,000 | 15,000 | 6,300 |
| 2 | 93,000 | L.A.* | 16,000 | 43,000 | 28,000 | 7,000 |
| 3 | 32,000 | 15,000 | 6,800 | 15,000 | 12,000 | 5,900 |
| 4 | 14,000 | L.A. | 8,100 | 11,000 | 23,000 | 8,300 |
| 5 | 19,000 | 13,000 | 8,200 | 5,800 | 10,000 | 5,500 |
| Ave. | 29,240 | 16,490 | 9,877 | 15,090 | 16,320 | 6,531 |

* Laboratory accident - no data.

The counts on milk pasteurized at 149°F. ranged from 10,000 - 28,000 per ml. The average was 16,320. Although this average count was slightly higher than that obtained at 143°F., there was less variation between counts on individual samples.

Pasteurization at 155°F. reduced the average count to 6,531 , with a minimum of 5,500 and maximum of 8,300 per ml.

Table III contains the 45°C. counts on five lots of Grade 1 milk pasteurized at 143, 149, and 155°F. for 30, 45, and 60 min. The counts on every sample were reduced with an increase in either the temperature or holding time. After 30 min. exposures the average counts were 10,400; 2,033; and 132 at 143, 149, and 155°F., respectively. The 45 min. exposures reduced the counts to 5,180; 2,040; and 85. The 60 min. exposure resulted in average counts of 1,521 , 1,391 , and 25.

Grade 2 milk - Table IV contains the counts on grade 2 milk, raw and pasteurized at 143, 149, and 155°F. for 30 min. The counts on the raw milk ranged from 180,000 - 810,000 , with an average of 322,200 per ml. This was a slight increase over the count on grade 1 milk.

The counts after pasteurization at 143°F. ranged from 7,700 - 93,000 , with an average of 26,590 per ml. Two of the seven samples contained more than 30,000 per ml. At 149°F. the average count was reduced to 21,900 , with a minimum of 15,000 and a maximum of 44,000 per ml. The average count at 155°F. was 13,670 per ml., with only one sample yielding a count higher than 30,000 per ml.

TABLE III

Numbers of 45°C. bacteria in grade 1
milk laboratory pasteurized at 143,
149, and 155°F. for 30, 45, and 60 min.

| Trial No. | 30 Minutes | | | 45 Minutes | | | 60 Minutes | | |
|--------------|------------|--------|--------|------------|--------|--------|------------|--------|--------|
| | 143°F. | 149°F. | 155°F. | 143°F. | 149°F. | 155°F. | 143°F. | 149°F. | 155°F. |
| 1 | 3,400 | 2,000 | 0* | 1,600 | 500 | 0 | 600 | 730 | 0 |
| 2 | 51,000 | 31,000 | 16,000 | 60,000 | 38,000 | 6,600 | 32,000 | 23,000 | 1,900 |
| 3 | 8,000 | 7,700 | 4,100 | 9,300 | 8,600 | 1,300 | 5,300 | 6,200 | 5,500 |
| 4 | 3,500 | 450 | 0 | 2,200 | 1,800 | 0 | 400 | 100 | 0 |
| 5 | 25,000 | 170 | 610 | 1,900 | 1,200 | 520 | 200 | 500 | 0 |
| Ave. | 10,400 | 2,033 | 132 | 5,180 | 2,040 | 85 | 1,521 | 1,391 | 25 |

* No significant count on any of the plates.

TABLE IV

Bacteria in grade 2 milk; raw, and laboratory pasteurized at 143, 149, and 155°F. for 30 min.

| Trial No. | Raw Milk | Pasteurization temperature | | |
|-----------|----------|----------------------------|--------|--------|
| | | 143°F.* | 149°F. | 155°F. |
| 6 | 260,000 | 8,500 | 22,000 | 9,900 |
| 7 | 180,000 | 29,000 | 20,000 | 15,000 |
| 8 | 280,000 | 7,700 | 16,000 | 7,900 |
| 9 | 810,000 | 30,000 | 20,000 | 12,000 |
| 10 | 450,000 | 25,000 | 26,000 | 8,300 |
| 11 | 180,000 | 93,000 | 44,000 | 38,000 |
| 12 | 420,000 | 71,000 | 15,000 | 20,000 |
| Ave. | 322,200 | 26,590 | 21,900 | 13,670 |

* The figures in this column also represent thermoduric counts.

The counts on grade 2 milk pasteurized at 143, 149, and 155°F. for 45 and 60 min. are given in Table V. A noticeable decrease in count occurred when the holding period was increased from 30 - 45 min. The average counts were 24,130; 20,180; and 8,890, respectively, at 143, 149, and 155°F.

When the holding period was lengthened to 60 min. the average counts were 20,610; 16,500; and 6,455, respectively, at 143, 149, and 155°F. At 60 min. four of the seven samples contained fewer than 10,000 and all of the samples contained

fewer than 30,000 bacteria per ml.

TABLE V

Bacteria in grade 2 milk laboratory pasteurized at 143, 149, and 155°F. for 45 and 60 min.

| Trial No. | Forty-five minutes | | | Sixty minutes | | |
|-----------|--------------------|--------|--------|---------------|--------|--------|
| | 143°F. | 149°F. | 155°F. | 143°F. | 149°F. | 155°F. |
| 6 | 18,000 | 23,000 | 8,300 | 29,000 | 12,000 | 14,000 |
| 7 | 25,000 | 18,000 | 12,000 | 16,000 | 14,000 | 9,500 |
| 8 | 17,000 | 15,000 | 4,200 | 14,000 | 14,000 | 2,300 |
| 9 | 22,000 | 16,000 | 10,000 | 13,000 | 13,000 | 12,000 |
| 10 | 22,000 | 18,000 | 2,300 | 32,000 | 16,000 | 2,400 |
| 11 | 99,000 | 38,000 | 38,000 | 45,000 | 40,000 | 27,000 |
| 12 | 13,000 | 20,000 | 12,000 | 13,000 | 17,000 | 7,400 |
| Ave. | 24,130 | 20,180 | 8,890 | 20,610 | 16,500 | 6,445 |

Table VI contains the number of 45°C. bacteria in grade 2 milk pasteurized at 143, 149, and 155°F. for 30, 45, and 60 min. The 45°C. count decreased from 10,150 at 143°F. to 5,738 at 149°F. and 4,556 at 155°F. with a 30 min exposure. There was a slight increase in the count at 149°F. for 45 min. The 60 min. exposure caused a further reduction in the 45°C. count. At 143°F. the average count was 3,651. This was increased to

TABLE VI

Numbers of 45°C. bacteria in grade 2
milk laboratory pasteurized at 143,
149, and 155°F. for 30, 45, and 60 min.

| Trial No. | 30 Minutes | | | 45 Minutes | | | 60 Minutes | | |
|--------------|------------|--------|--------|------------|--------|--------|------------|--------|--------|
| | 143°F. | 149°F. | 155°F. | 143°F. | 149°F. | 155°F. | 143°F. | 149°F. | 155°F. |
| 6 | 1,200 | 570 | 320 | 3,100 | 610 | 410 | 1,300 | 490 | 260 |
| 7 | 20,000 | 16,000 | 12,000 | 14,000 | 16,000 | 8,400 | 8,400 | 11,000 | 5,200 |
| 8 | 5,700 | 11,000 | 5,200 | 10,000 | 12,000 | 1,100 | 7,400 | 8,900 | 380 |
| 9 | 2,900 | 540 | 440 | 800 | 300 | 610 | 500 | 500 | 390 |
| 10 | 20,000 | 14,000 | 6,100 | 17,000 | 15,000 | 760 | 13,000 | 11,000 | 300 |
| 11 | 61,000 | 15,000 | 38,000 | 76,000 | 42,000 | 35,000 | L.A.* | 35,000 | 16,000 |
| 12 | 23,000 | 18,000 | 20,000 | 8,200 | 18,000 | 8,900 | 4,500 | 22,000 | 5,200 |
| Ave. | 10,150 | 5,738 | 4,556 | 8,668 | 6,310 | 2,461 | 3,651 | 5,732 | 1,259 |

* Laboratory accident - no count.

5,732 at 149°F., and lowered to 1,259 bacteria per ml. at 155°F.

Sample 12 was the only sample to contain thermophiles (incubated at 55°C.). It contained 2,200 and 6,000 thermophiles per ml. after pasteurization at 143, and 149°F. for 45 min. These increased to 16,000 and 28,000 at 143, and 149°F. for 60 min. The thermophilic count was reduced to 720 and 500 per ml. at 155°F. for 30 and 45 min. A count at 60 min. was not recorded because of mold contamination on plates.

Grade 3 milk - Table VII contains the counts on eight trials on grade 3 milk, raw, and pasteurized at 143, 149, and 155°F. for 30 min. The count on this grade of raw milk ranged from 710,000 - 32,000,000 with an average of 2,031,000 per ml. This was more than double the count on grade 2 milk.

All samples pasteurized at 143°F. for 30 min. contained more than 30,000 bacteria per ml. The average count was 74,540. This was reduced to 33,310 at 149°F., and to 18,590 at 155°F. Two of the eight samples pasteurized at 155°F. contained more than 30,000 bacteria per ml.

TABLE VII

Bacteria in grade 3 milk; raw, and laboratory pasteurized at 143, 149, and 155°F. for 30 min.

| Trial No. | Raw Milk | Pasteurization temperature | | |
|-----------|------------|----------------------------|---------|--------|
| | | 143°F.* | 149°F. | 155°F. |
| 13 | 2,300,000 | 110,000 | 39,000 | 24,000 |
| 14 | 710,000 | 92,000 | 20,000 | 15,000 |
| 15 | 32,000,000 | 32,000 | 14,000 | 11,000 |
| 16 | 1,200,000 | 160,000 | 100,000 | 41,000 |
| 17 | 4,700,000 | 71,000 | 40,000 | 12,000 |
| 18 | 1,200,000 | 77,000 | 37,000 | 26,000 |
| 19 | 890,000 | 37,000 | 23,000 | 6,400 |
| 20 | 920,000 | 91,000 | 32,000 | 44,000 |
| Ave. | 2,031,000 | 74,540 | 33,310 | 18,590 |

* The figures in this column also represent thermoduric counts.

Table VIII contains the counts on grade 3 milk pasteurized at 143, 149, and 155°F. for 45 and 60 min. With the 45 min. holding period the average counts were 42,110; 29,210; and 8,191 at 143, 149, and 155°F., respectively. The 60 min. holding period reduced these to 38,930; 26,090; and 4,688 per ml. Seven of the eight samples contained less than 10,000 bacteria after pasteurization at 155°F. for 60 min.

TABLE VIII

Bacteria in grade 3 milk laboratory pasteurized at 143, 149, and 155°F. for 45 and 60 min.

| Trial No. | Forty-five minutes | | | Sixty minutes | | |
|-----------|--------------------|--------|--------|---------------|--------|--------|
| | 143°F. | 149°F. | 155°F. | 143°F. | 149°F. | 155°F. |
| 13 | 29,000 | 29,000 | 11,000 | 27,000 | 22,000 | 7,900 |
| 14 | 62,000 | 21,000 | 13,000 | 16,000 | 17,000 | 9,200 |
| 15 | 13,000 | 17,000 | 5,600 | 19,000 | 13,000 | 4,100 |
| 16 | 69,000 | 71,000 | 11,000 | 61,000 | 77,000 | 3,300 |
| 17 | 86,000 | 39,000 | 3,100 | 68,000 | 34,000 | 3,500 |
| 18 | 75,000 | 34,000 | 19,000 | 75,000 | 36,000 | 13,000 |
| 19 | 19,000 | 16,000 | 3,900 | 22,000 | 16,000 | 1,800 |
| 20 | 50,000 | 34,000 | 10,000 | 94,000 | 29,000 | 2,900 |
| Ave. | 42,110 | 29,210 | 8,191 | 38,930 | 26,090 | 4,688 |

Table IX contains the 45°C. counts on grade 3 milk pasteurized at 143, 149, and 155°F. for 30, 45 and 60 min. With the 30 min. holding period the counts decreased steadily as the temperature increased from 143 - 155°F. The average counts at 149°F. for 45 and 60 min. increased slightly from that at 143°F. It was lowest at 155°F. for all holding periods. Only one of the eight samples contained more than 30,000 45°C. bacteria after pasteurization at 155°F. for 30 min.

TABLE IX

Numbers of 45°C. bacteria in grade 3
milk laboratory pasteurized at 143,
149, and 155°F. for 30, 45, and 60 min.

| Trial No. | 30 Minutes | | | 45 Minutes | | | 60 Minutes | | |
|--------------|------------|--------|--------|------------|--------|--------|----------------|--------|--------|
| | 143°F. | 149°F. | 155°F. | 143°F. | 149°F. | 155°F. | 143°F. | 149°F. | 155°F. |
| 13 | 39,000 | 14,000 | 80 | 9,900 | 13,000 | 3,300 | 9,500 | 13,000 | 850 |
| 14 | L.A.* | 1,800 | 2,000 | 2,500 | 2,900 | 1,500 | 0 ^x | 0 | 800 |
| 15 | 11,000 | 6,400 | 2,200 | 3,100 | 5,600 | 900 | 4,900 | 6,600 | 220 |
| 16 | 110,000 | 62,000 | 33,000 | 48,000 | 65,000 | 8,700 | 49,000 | 68,000 | 1,200 |
| 17 | 57,000 | 38,000 | 9,600 | 39,000 | 29,000 | 500 | 43,000 | 32,000 | 1,800 |
| 18 | 26,000 | 25,000 | 17,000 | 26,000 | 24,000 | 8,100 | 27,000 | 20,000 | 2,300 |
| 19 | 19,000 | 14,000 | 4,300 | 14,000 | 13,000 | 930 | 13,000 | 10,000 | 0 |
| 20 | 34,000 | 13,000 | 800 | 29,000 | 25,000 | 460 | 6,100 | 24,000 | 1,300 |
| Ave. | 33,290 | 14,280 | 2,913 | 14,050 | 15,370 | 1,692 | 4,627 | 5,546 | 407 |

^x No significant count on any of the plates.

* Laboratory accident - no count.

Grade 4 milk - Table X contains the counts on 20 samples of grade 4 milk, raw, and pasteurized at 143, 149, and 155°F. for 30 min. The average count on the raw milk was 11,780,000 per ml. After pasteurization at 143°F. the average was 71,410 per ml. This was reduced to 27,000 at 149°F., and to 18,710 at 155°F.

TABLE X

Bacteria in grade 4 milk; raw, and laboratory pasteurized at 143, 149, and 155°F. for 30 min.

| Trial No. | Raw Milk | Pasteurization temperature | | |
|-----------|------------|----------------------------|--------|--------|
| | | 143°F.* | 149°F. | 155°F. |
| 21 | 87,000,000 | 120,000 | 24,000 | 12,000 |
| 22 | 43,000,000 | 58,000 | 28,000 | 15,000 |
| 23 | 13,000,000 | 35,000 | 22,000 | 17,000 |
| 24 | 32,000,000 | 180,000 | 39,000 | 29,000 |
| 25 | 12,000,000 | 40,000 | 25,000 | 19,000 |
| 26 | 21,000,000 | 56,000 | 20,000 | 24,000 |
| 27 | 19,000,000 | 43,000 | 15,000 | 31,000 |
| 28 | 61,000,000 | 68,000 | 43,000 | 24,000 |
| 29 | 19,000,000 | 180,000 | 43,000 | 26,000 |
| 30 | 2,300,000 | 97,000 | 25,000 | 19,000 |
| 31 | 7,400,000 | 36,000 | 17,000 | 19,000 |
| 32 | 4,100,000 | 78,000 | 39,000 | 29,000 |
| 33 | 13,000,000 | 96,000 | 33,000 | 27,000 |
| 34 | 16,000,000 | 140,000 | 35,000 | 19,000 |
| 35 | 1,400,000 | 120,000 | 29,000 | 22,000 |
| 36 | 11,000,000 | 71,000 | 32,000 | 16,000 |
| 37 | 4,300,000 | 30,000 | 16,000 | 8,600 |
| 38 | 9,200,000 | 63,000 | 20,000 | 8,600 |
| 39 | 4,300,000 | 68,000 | 29,000 | 14,000 |
| 40 | 8,000,000 | 57,000 | 32,000 | 18,000 |
| Ave. | 11,780,000 | 71,410 | 27,000 | 18,710 |

* The figures in this column also represent thermoduric counts.

Table XI contains the counts on 20 samples of grade 4 milk pasteurized at 143, 149, and 155°F. for 45 and 60 min. Pasteurization at 143°F. for 45 min. resulted in an average count of 39,040 per ml. This was reduced to 21,720 and 13,390 at 149 and 155°F., respectively.

TABLE XI

Bacteria in grade 4 milk laboratory pasteurized at 143, 149, and 155°F. for 45 and 60 min.

| Trial No. | Forty-five minutes | | | Sixty Minutes | | |
|-----------|--------------------|--------|--------|---------------|--------|--------|
| | 143°F. | 149°F. | 155°F. | 143°F. | 149°F. | 155°F. |
| 21 | 49,000 | 5,200 | 7,800 | 28,000 | 14,000 | 6,400 |
| 22 | 26,000 | 19,000 | 10,000 | 17,000 | 17,000 | 5,100 |
| 23 | 28,000 | 21,000 | 16,000 | 20,000 | 20,000 | 10,000 |
| 24 | 54,000 | 32,000 | 17,000 | 34,000 | 29,000 | 20,000 |
| 25 | 32,000 | 25,000 | 19,000 | 21,000 | 17,000 | 16,000 |
| 26 | 13,000 | 24,000 | 19,000 | 15,000 | 23,000 | 13,000 |
| 27 | 31,000 | 13,000 | 24,000 | 27,000 | 6,800 | 15,000 |
| 28 | 55,000 | 30,000 | 16,000 | 46,000 | 26,000 | 8,100 |
| 29 | 150,000 | 58,000 | 23,000 | 77,000 | 35,000 | 26,000 |
| 30 | 14,000 | 18,000 | 12,000 | 25,000 | 25,000 | 9,400 |
| 31 | 25,000 | 19,000 | 12,000 | 21,000 | 23,000 | 12,000 |
| 32 | 73,000 | 19,000 | 21,000 | 27,000 | 29,000 | 12,000 |
| 33 | 62,000 | 25,000 | 16,000 | 30,000 | 25,000 | 14,000 |
| 34 | 90,000 | 47,000 | 20,000 | 52,000 | 22,000 | 22,000 |
| 35 | 62,000 | 22,000 | 8,200 | 65,000 | 16,000 | 3,100 |
| 36 | 40,000 | 25,000 | 15,000 | 25,000 | 19,000 | 11,000 |
| 37 | 32,000 | 14,000 | 7,300 | 24,000 | 10,000 | 2,800 |
| 38 | 55,000 | 18,000 | 3,300 | 49,000 | 16,000 | 4,600 |
| 39 | 38,000 | 25,000 | 12,000 | 15,000 | 27,000 | 5,800 |
| 40 | 14,000 | 23,000 | 14,000 | 7,000 | 18,000 | 17,000 |
| Ave. | 39,040 | 21,720 | 13,390 | 27,030 | 19,630 | 9,872 |

The 60 min. holding period further reduced the counts to 27,030; 19,630; and 9,872 at 143, 149, and 155°F. respectively.

Table XII contains the 45°C. counts on 20 samples of grade 4 milk pasteurized at 143, 149, and 155°F. for 30, 45, and 60 min. The 45°C. counts were progressively lower at 149° and 155° than at 143°F.; and also at 45 and 60 min. exposures as compared to the 30 min. exposure.

Commercial Scale Trials

Grade 1 milk - Table XIII contains the counts on grade 1 milk, raw and pasteurized at 143, 149, and 155°F. for 30 min. The average count on the raw milk was 60,060. This low average reflected the superior quality milk, produced by the University dairy herd, used in the first seven trials. The average thermoduric count was 2,351, with a maximum of 24,000 per ml.

The average count after pasteurization at 143°F. was 3,436. Counts on two of the twelve samples were higher than 30,000. The average counts were 2,127 and 693 at 149 and 155°F. respectively. Pasteurizing at 155°F. reduced the count on every sample to less than 30,000.

TABLE XII

Numbers of 45°C. bacteria in grade 4 milk laboratory pasteurized at 143, 149, and 155°F. for 30, 45, and 60 min.

| Trial No. | 30 Minutes | | | 45 Minutes | | | 60 Minutes | | |
|-----------|------------|--------|----------------|------------|--------|--------|------------|--------|--------|
| | 143°F. | 149°F. | 155°F. | 143°F. | 149°F. | 155°F. | 143°F. | 149°F. | 155°F. |
| 21 | 700 | 180 | 0 ^x | 300 | 0 | 0 | 3,500 | 180 | 0 |
| 22 | 5,000 | 7,600 | 2,300 | 6,200 | 4,000 | 1,000 | 4,100 | 310 | 690 |
| 23 | 7,900 | 70 | 570 | 300 | 760 | 0 | 320 | 700 | 260 |
| 24 | 2,600 | 460 | 0 | 700 | 470 | 110 | 1,100 | 380 | 0 |
| 25 | 100 | 530 | 340 | 2,000 | 560 | 310 | 500 | 230 | 120 |
| 26 | 5,600 | 920 | 1,500 | 2,800 | 1,100 | 570 | 2,700 | 1,200 | 290 |
| 27 | 9,900 | 910 | 4,100 | 12,000 | 1,400 | 950 | 9,200 | 220 | 570 |
| 28 | 6,600 | 690 | 0 | 6,400 | 1,100 | 680 | 3,100 | 0 | 380 |
| 29 | 120,000 | 5,400 | 110 | 56,000 | 2,300 | 390 | 30,000 | 140 | 0 |
| 30 | 16,000 | 16,000 | 13,000 | 6,800 | 14,000 | 8,000 | 11,000 | 14,000 | 4,100 |
| 31 | 17,000 | 23,000 | L.A.* | 400 | 10,000 | 8,000 | 300 | L.A. | L.A. |
| 32 | 12,000 | 5,100 | 4,200 | 9,600 | 6,400 | 1,300 | 5,800 | 5,600 | 340 |
| 33 | 14,000 | 300 | 6,500 | 800 | 8,700 | 2,800 | 500 | 12,000 | 160 |
| 34 | 90,000 | 11,000 | 330 | 15,000 | 15,000 | 1,100 | 13,000 | 11,000 | 1,800 |
| 35 | 43,000 | 79,000 | 4,500 | 14,000 | 7,500 | 1,200 | 13,000 | 7,900 | 440 |
| 36 | 32,000 | 1,000 | 3,000 | 14,000 | 4,600 | 930 | 3,600 | 5,600 | 440 |
| 37 | 8,600 | 5,200 | 2,800 | 3,700 | 5,200 | 700 | 2,900 | 3,400 | 190 |
| 38 | 5,300 | 12,000 | 4,200 | 15,000 | 10,000 | 610 | 9,600 | 12,000 | 440 |
| 39 | 21,000 | 17,000 | 10,000 | 12,000 | 15,000 | 5,400 | 0 | 20,000 | 440 |
| 40 | 800 | 11,000 | 9,100 | 200 | 9,900 | 8,000 | 2,000 | 7,800 | 7,400 |
| Ave. | 8,069 | 2,698 | 671 | 3,500 | 2,508 | 590 | 2,060 | 1,365 | 194 |

^x No count on any of the plates.

* Laboratory Accident - no count.

TABLE XIII

Bacteria in grade 1 milk; raw, and commercially pasteurized at 143, 149, and 155°F. for 30 min.

| Trial No. | Raw Milk | Thermoduric | Pasteurization temperature | | |
|-----------|----------|-------------|----------------------------|--------|--------|
| | | | 143°F. | 149°F. | 155°F. |
| 1 | 12,000 | 6,700 | 6,600 | 6,200 | 8,000 |
| 2 | 260,000 | 910 | 30,000 | 990 | 1,000 |
| 3 | 15,000 | 570 | 2,400 | 1,200 | 80 |
| 4 | 3,900 | 420 | 160 | 120 | 35 |
| 5 | 63,000 | 3,300 | 3,300 | 1,800 | 0* |
| 6 | 2,100 | 420 | 70 | 100 | 60 |
| 7 | 75,000 | 560 | 550 | 650 | 570 |
| 8 | 280,000 | 23,000 | 58,000 | 45,000 | 28,000 |
| 9 | 430,000 | 12,000 | 23,000 | 15,000 | 12,000 |
| 10 | 210,000 | 21,000 | 42,000 | 25,000 | 16,000 |
| 11 | 370,000 | 24,000 | 25,000 | 29,000 | 27,000 |
| 12 | 130,000 | 180 | 200 | 170 | 110 |
| Ave. | 60,060 | 2,351 | 3,436 | 2,127 | 693 |

* No count on any of the plates.

Grade 2 milk - Table XIV contains the counts on 12 samples of grade 2 milk, raw, and pasteurized at 143, 149, and 155°F. for 30 min. The average count on the raw milk was 385,800 per ml. The thermoduric average was 11,580, with three of the twelve samples containing more than 30,000 per ml.

TABLE XIV

Bacteria in grade 2 milk; raw, and commercially pasteurized at 143, 149, and 155°F. for 30 min.

| Trial No. | Raw Milk | Thermoduric | Pasteurization temperature | | |
|-----------|-----------|-------------|----------------------------|--------|--------|
| | | | 143°F. | 149°F. | 155°F. |
| 13 | 8,900 | 1,200 | 1,200 | 1,500 | 1,200 |
| 14 | 320,000 | 580 | 1,700 | 920 | 300 |
| 15 | 700,000 | 730 | 120 | 130 | 95 |
| 16 | 390,000 | 65,000 | 61,000 | 46,000 | 28,000 |
| 17 | 310,000 | 30,000 | 38,000 | 30,000 | 12,000 |
| 18 | 640,000 | 21,000 | 24,000 | 18,000 | 17,000 |
| 19 | 830,000 | 46,000 | 47,000 | 32,000 | 27,000 |
| 20 | 1,900,000 | 23,000 | 21,000 | 13,000 | 14,000 |
| 21 | 1,400,000 | 14,000 | 21,000 | 7,000 | 4,100 |
| 22 | 500,000 | 25,000 | 57,000 | 23,000 | 7,100 |
| 23 | 290,000 | 93,000 | 58,000 | 19,000 | 11,000 |
| 24 | 220,000 | 8,100 | 19,000 | 9,500 | 3,710 |
| Ave. | 385,800 | 11,580 | 12,870 | 7,839 | 4,574 |

The average count after pasteurization at 143°F. was 12,870 with five of the twelve samples containing more than 30,000 per ml. Two samples contained more than 30,000 per ml. at 149°F. and at 155°F. the counts on all samples were below 30,000. The average counts at 149 and 155°F., respectively were 7,839 and 4,574.

Table XV contains the 45°C. counts on grades 1 and 2 milk pasteurized at 143, 149, and 155°F. The grade 1 milk contained less than 10,000 bacteria after every exposure. The counts were steadily reduced as the temperature increased. The average counts were 190; 122; and 25 at 143, 149, and 155°F., respectively.

One sample of grade 2 milk contained more than 30,000 bacteria at 143°F. All samples contained less than 30,000 at 149 and at 155°F. The average counts at 143, 149, and 155°F. were 2,806; 109; and 62, respectively.

Grade 3 milk - The counts on grade 3 milk, raw, and pasteurized at 143, 149, and 155°F. are presented in Table XVI. The raw milk counts ranged from 320,000 - 4,300,000 with an average of 1,299,000. Two of 12 lots contained less than 1,000,000 bacteria. The average thermoduric count on this milk was 28,850 with six of 12 samples containing more than 30,000 per ml.

The average count after processing at 143°F. was 40,100 per ml. Nine of 12 samples contained more than 30,000 bacteria. At 149°F. five samples contained more than 30,000 bacteria; and at 155°F. two samples exceeded this count. Average counts at 149° and 155°F. were 12,900 and 6,996, respectively.



TABLE XV

Numbers of 45°C. bacteria in grades 1 and 2 milk commercially pasteurized at 143, 149, and 155°F. for 30 min.

| Grade 1 milk | | | | Grade 2 milk | | | |
|--------------|----------------------------|----------------|--------|--------------|----------------------------|--------|--------|
| Trial No. | Pasteurization temperature | | | Trial No. | Pasteurization temperature | | |
| | 143°F. | 149°F. | 155°F. | | 143°F. | 149°F. | 155°F. |
| 1 | 1,500 | 1,300 | 1,200 | 13 | 320 | 260 | 280 |
| 2 | 330 | 480 | 670 | 14 | 60 | 0 | 35 |
| 3 | 45 | 0 ^x | 0 | 15 | 0 | 0 | 0 |
| 4 | 0 | 0 | 0 | 16 | 4,100 | 0 | 0 |
| 5 | 0 | 0 | 0 | 17 | 1,800 | 0 | 0 |
| 6 | 0 | 0 | 65 | 18 | 4,100 | 380 | 0 |
| 7 | 350 | 360 | 790 | 19 | 4,500 | 560 | 350 |
| 8 | 3,400 | 710 | 0 | 20 | 6,700 | 810 | 280 |
| 9 | 2,200 | 330 | 0 | 21 | 11,000 | 750 | 620 |
| 10 | 6,800 | 1,400 | 1,200 | 22 | 36,000 | 5,200 | 1,400 |
| 11 | 5,700 | 630 | 85 | 23 | 28,000 | 9,800 | 6,900 |
| 12 | 40 | 40 | 0 | 24 | 6,200 | 1,700 | 510 |
| Ave. | 190 | 122 | 25 | Ave. | 2,806 | 109 | 62 |

^x No count on any of the plates.

TABLE XVI

Bacteria in grade 3 milk; raw, and commercially pasteurized at 143, 149, and 155°F. for 30 min.

| Trial No. | Raw Milk | Thermoduric | Pasteurization temperature | | |
|-----------|-----------|-------------|----------------------------|---------|--------|
| | | | 143°F. | 149°F. | 155°F. |
| 25 | 930,000 | 55,000 | 67,000 | 30,000 | 17,000 |
| 26 | 1,700,000 | 320,000 | 330,000 | 87,000 | 44,000 |
| 27 | 1,100,000 | 8,300 | 13,000 | 7,600 | 3,900 |
| 28 | 4,300,000 | 170,000 | 220,000 | 140,000 | 85,000 |
| 29 | 1,700,000 | 26,000 | 52,000 | 34,000 | 14,000 |
| 30 | 320,000 | 300 | 400 | 0* | 0 |
| 31 | 1,000,000 | 66,000 | 70,000 | 44,000 | 16,000 |
| 32 | 1,900,000 | 50,000 | 63,000 | 31,000 | 22,000 |
| 33 | 1,900,000 | 18,000 | 22,000 | 14,000 | 7,200 |
| 34 | 1,300,000 | 24,000 | 44,000 | 17,000 | 8,600 |
| 35 | 1,100,000 | 43,000 | 57,000 | 24,000 | 13,000 |
| 36 | 1,100,000 | 28,000 | 54,000 | 29,000 | 14,000 |
| Ave. | 1,299,000 | 28,850 | 40,100 | 12,900 | 6,996 |

* No count on any of the plates.

Grade 4 milk - Table XVII contains the counts on grade 4 milk, raw, and pasteurized at 143, 149, and 155°F. for 30 min. The count on the raw milk ranged from 1,900,000 - 28,000,000 with an average of 12,890,000 per ml. The thermoduric count was 64,850 with nine out of 12 samples containing more than 30,000 bacteria.

TABLE XVII

Bacteria in grade 4 milk; raw, and commercially pasteurized at 143, 149, and 155°F. for 30 min.

| Trial No. | Raw milk | Thermoduric | Pasteurization temperature | | |
|-----------|------------|-------------|----------------------------|---------|---------|
| | | | 143°F. | 149°F. | 155°F. |
| 37 | 25,000,000 | 75,000 | 90,000 | 50,000 | 53,000 |
| 38 | 25,000,000 | 58,000 | 210,000 | 21,000 | 16,000 |
| 39 | 8,700,000 | 100,000 | 170,000 | 70,000 | 61,000 |
| 40 | 13,000,000 | 67,000 | 85,000 | 39,000 | 25,000 |
| 41 | 26,000,000 | 290,000 | 410,000 | 290,000 | 15,000 |
| 42 | 28,000,000 | 7,300 | 450,000 | 3,500 | 650 |
| 43 | 22,000,000 | 890,000 | 450,000 | 360,000 | 100,000 |
| 44 | 22,000,000 | 38,000 | 69,000 | 27,000 | 15,000 |
| 45 | 10,000,000 | 86,000 | 170,000 | 40,000 | 19,000 |
| 46 | 8,100,000 | 27,000 | 63,000 | 30,000 | 14,000 |
| 47 | 1,900,000 | 57,000 | 64,000 | 44,000 | 35,000 |
| 48 | 5,500,000 | 20,000 | 32,000 | 14,000 | 8,400 |
| Ave. | 12,890,000 | 64,850 | 134,300 | 40,740 | 19,300 |

Pasteurization at 143°F. resulted in an average count of 134,300 per ml. Every sample contained more than 30,000 bacteria. When the temperature was increased to 149°F. the average was reduced to 40,740. A further increase to 155°F. reduced the count to 19,300 per ml. with four of 12 samples containing more than 30,000 bacteria.

The 45°C. counts on grades 3 and 4 milk pasteurized at 143, 149, and 155°F. are given in Table XVIII. These counts showed progressive reduction as the temperature was increased. The average counts on the grade 3 milk were 14,240; 2,686; and 808 after pasteurization at 143, 149, and 155°F., respectively. The grade 4 counts followed a similar trend of reduction except that they were higher in every case. The average counts at 143, 149, and 155°F. were 44,000; 18,400; and 2,221 per ml., respectively. At 155°F. only one sample contained more than 30,000 bacteria per ml.

Correlation of the Resazurin Grade and the
Bacterial Counts on Raw and Pasteurized Milk

Grades 1, 2, 3, and 4 refer to the resazurin grades. Grades A, B, and C refer to the grades based on bacterial counts as described in the United States Public Health Service Standard Milk Ordinance (16). These are:

1. Raw milk:

Grade A - contains less than 200,000 bacteria per ml.

Grade B - contains more than 200,000 and less than 1,000,000 bacteria per ml.

Grade C - contains more than 1,000,000 bacteria per ml.

TABLE XVIII

Numbers of 45°C. bacteria in grades 3 and 4 milk commercially pasteurized at 143, 149, and 155°F. for 30 min.

| Grade 3 milk | | | | Grade 4 milk | | | |
|----------------------------|--------|----------------|--------|----------------------------|---------|---------|--------|
| Pasteurization temperature | | | | Pasteurization temperature | | | |
| Trial No. | 143°F. | 149°F. | 155°F. | Trial No. | 143°F. | 149°F. | 155°F. |
| 25 | 8,900 | 5,600 | 1,500 | 37 | 36,000 | 29,000 | 28,000 |
| 26 | 25,000 | 3,200 | 3,000 | 38 | 39,000 | 17,000 | 13,000 |
| 27 | 5,000 | 2,300 | 1,700 | 39 | 69,000 | 39,000 | 35,000 |
| 28 | 39,000 | 20,000 | 8,200 | 40 | 48,000 | 23,000 | 18,000 |
| 29 | 19,000 | 8,200 | 3,900 | 41 | 450,000 | 320,000 | L.A.* |
| 30 | 1,800 | 0 ^x | 0 | 42 | 170,000 | 2,200 | L.A. |
| 31 | 5,900 | 4,800 | 0 | 43 | 380,000 | 220,000 | 5,200 |
| 32 | 28,000 | 13,000 | 14,000 | 44 | 6,100 | 4,800 | 3,800 |
| 33 | 10,000 | 4,300 | 2,600 | 45 | 19,000 | 11,000 | 5,200 |
| 34 | 28,000 | 4,400 | 2,100 | 46 | 8,600 | 4,000 | 9,500 |
| 35 | 35,000 | 5,200 | 2,300 | 47 | 23,000 | 26,000 | 23,000 |
| 36 | 29,000 | 3,400 | 1,800 | 48 | 17,000 | 4,000 | 2,800 |
| Ave. | 14,240 | 2,686 | 808 | Ave. | 44,000 | 18,400 | 2,221 |

^x No counts on any of the plates.

* Laboratory accident - no count.

2. Pasteurized milk:

Grade A - contains less than 30,000 bacteria per ml.

Grade B - contains more than 30,000 and less than 50,000 bacteria per ml.

Grade C - contains more than 50,000 bacteria per ml.

Table XIX contains a correlation of the resazurin and the bacterial grades of raw milk. The counts on grade 1 milk ranged from 2,100 - 440,000, with an average of 95,570 per ml. These are in close agreement with those obtained by Collins (8) who found that grade 1 milk contained less than 400,000 bacteria per ml. However, they are substantially higher than the counts obtained by Brannon (6). Although the average count of this grade met the requirements for grade A milk, only eight of 17 samples had a count low enough for grade A. The remainder were grade B. Thus grade 1 milk is equivalent to grade A or B.

Three of 19 samples of grade 2 milk were equivalent to grade A, 14 to grade B, and three to grade C. The average count was 361,100, with a minimum of 8,900 and a maximum of 1,900,000 bacteria per ml. These results were in close agreement with those of Brannon (6).

The bacterial counts on the grade 3 milk ranged from 320,000 - 32,000,000, with an average of 1,553,000 per ml. These were considerably higher than the counts reported by

TABLE XIX

Correlation of resazurin and bacterial grades of raw milk

| Resazurin grade | Total No. of samples | Range of counts | Average bacterial count | Bacterial grade | | |
|-----------------|----------------------|------------------------|-------------------------|--------------------|---------------------------|---------------------|
| | | | | A Below 200,000 | B 200,000 to 1,000,000 | C Over 1,000,000 |
| 1 | 17 | 2,100 - 440,000 | 95,570 | 8 | 9 | 0 |
| 2 | 19 | 8,900 - 1,900,000 | 361,100 | 3 | 14 | 2 |
| 3 | 20 | 320,000 - 32,000,000 | 1,553,000 | 0 | 5 | 15 |
| 4 | 32 | 1,400,000 - 87,000,000 | 12,180,000 | 0 | 0 | 32 |

other investigators (5) (6). Five of 20 samples were grade B and 15 were grade C. This was a considerable decrease in grade from that of the grade 2 milk.

All of the grade 4 milks were equivalent to grade C. The average count was 12,180,000 , with a minimum of 1,400,000 and a maximum of 87,000,000. These were substantially higher than the counts reported by other investigators (5) (6).

Table XX contains a correlation of the resazurin grades of raw milk with the bacterial grades of pasteurized milk. Fourteen of 17 samples of grade 1 milk were grade A, two were grade B, and one was grade C. The counts ranged from 580 - 93,000 , with an average of 5,271 per ml.

Twelve of 19 samples of grade 2 milk were grade A, three were grade B, and four were grade C. The counts ranged from 420 - 94,000 , with an average of 15,730. Pasteurization converted a large percentage of grade B raw milk into grade A pasteurized milk.

The counts on grade 3 pasteurized milk were considerably higher than the grade 2 counts. They ranged from 300 - 320,000 , with an average of 42,180. Six of 20 samples were grade A, four grade B, and 10 grade C.

TABLE XX

Correlation of resazurin grades of raw milk
and bacterial grades of pasteurized milk.

| Resazurin grade | Total No. of samples | Range of counts | Average bacterial count | Bacterial grade | | |
|-----------------|----------------------|-----------------|-------------------------|----------------------|-----------------------------|---------------------|
| | | | | A Below 30,000 | B 30,000 to 50,000 | C Over 50,000 |
| 1 | 17 | 580 - 93,000 | 5,271 | 14 | 2 | 1 |
| 2 | 19 | 420 - 94,000 | 15,730 | 12 | 3 | 4 |
| 3 | 20 | 300 - 320,000 | 42,180 | 6 | 4 | 10 |
| 4 | 32 | 7,300 - 890,000 | 68,870 | 3 | 7 | 22 |

The average count on grade 4 pasteurized milk was 68,870 per ml. Individual counts ranged from 7,300 - 890,000. Three of 32 samples were grade A, seven grade B, and 22 grade C.

These results indicate general agreement, with a great deal of overlapping, between the resazurin grades of raw milk and the bacterial counts of raw and pasteurized milks.

DISCUSSION

The quality of the raw milk pasteurized by two of the three largest plants in Winnipeg during the summer and autumn of 1951 was very poor. Twenty samples taken during this period were grade 4, and had an average count of 11,780,000 per ml. The third plant was processing grade 3 milk which had an average count of 2,031,000 per ml. Comparatively few trials were carried out on grade 1 and 2 milk during this period because of difficulty in obtaining samples.

The results obtained in this work indicate that the pasteurization requirements of the Manitoba Public Health Act (35) (143°F. for 30 min.) are too lenient for the quality of raw milk received for processing. The results show that a pasteurization exposure of 155°F. for 60 min. yielded the lowest counts with every grade of milk. However, several investigators (20) (29) (51) have proven that this exposure causes a cooked flavor, whereas 155°F. for 30 min. does not cause cooked flavor in homogenized milk. The slight decrease in counts between the 30 and 60 min. holding periods does not justify the 60 min. exposure. For practical purposes an exposure of 155°F. for 30 min. gives the best results.

The destruction of creaming ability at this exposure (26) (36) is not important in the greater Winnipeg area because approximately 80% of the milk processed is homogenized.

An exposure more severe than 143°F. for 30 min. caused a greater bacterial reduction in grades 3 and 4 milk than it did in the grades 1 and 2 milk. Thus, if cream-line pasteurized milk is required, grade 1 and 2 raw milk could be pasteurized at 143°F. for 30 min. The average count of this milk would probably be less than 30,000 per ml. Grades 3 and 4 milk should be used only in homogenized products pasteurized at 155°F. for 30 min.

The additional bacterial destruction caused by an increased pasteurization temperature raises a few other controversial points. These findings are important from the commercial aspect because they indicate that there is a method of reducing the count of poor quality raw milk to less than 30,000 per ml. in the pasteurized product. This would permit pasteurizing plants to process poor quality raw milk and sell it as grade A pasteurized milk.

From a public health aspect, the objection which may be raised against the use of the higher temperature is that a high bacterial count in pasteurized milk indicates the use of unsanitary practices somewhere in the production or processing

of the milk. The increased pasteurization temperature would destroy these bacteria and thus hide the unsanitary practices.

Before the use of an increased pasteurization temperature can be recommended by health departments, the advantage of a lower bacterial count pasteurized milk regardless of the quality of the raw milk, must be carefully weighed against the disadvantage of hiding poor quality milk and unsanitary practices.

The dairy industry and health departments should carry out additional research to find out if poor grade raw milk pasteurized at 155°F. for 30 min. is equivalent in nutritional value and in keeping quality to grade 1 milk pasteurized at 143°F. for 30 min.

The results of this study indicate that there is general agreement with a considerable amount of overlapping, between the resazurin grades and the bacterial counts of raw and pasteurized milk. The bacterial counts of grades 1 and 2 milk were almost identical; while the counts of grades 3 and 4 milk were substantially higher. This confirms Brannon's statement (8) that grades 1 and 2 are good quality milks and grades 3 and 4 are poor quality milks.

It is evident from the variations in the bacterial counts of the different grades of milk that factors other than the bacterial count affect the resazurin grade. It is unfortunate that the scope of this work did not permit an investigation into the causes of this phenomenon.

SUMMARY AND CONCLUSIONS

Eighty-eight trials were carried out to determine the effect of various pasteurization exposures on the bacterial counts of the four resazurin grades of raw milk. Forty of these trials were on a laboratory scale. Samples of the different grades of raw milk were obtained just prior to pasteurization from pasteurizing plants in Winnipeg. Portions of these were laboratory pasteurized at 143, 149, and 155°F. for 30, 45, and 60 min. periods. The bacterial counts, 45°C. counts, thermophilic counts and coliform counts on each sample were then determined.

The experiment was repeated in forty-eight commercial scale trials. Twelve trials were carried out on each grade of the milk. On the basis of the laboratory trial results and consultations with pasteurizing plant operators, only a 30 min. exposure was used in the commercial trials.

The laboratory and commercial trials indicated that a pasteurization exposure of 143°F. for 30 min. was insufficient to yield acceptable bacterial counts from grades 3 and 4 raw milk. Acceptable counts were obtained when this milk was processed at 155°F. for 30 min. This higher temperature also

reduced the 45°C. and thermophilic counts on all samples of milk below those obtained at 143°F. On the basis of these results it is recommended that grades 1 and 2 raw milks be processed at 143°F. for 30 min. only when cream-line milk is required. All other milk, regardless of grade, should be processed at 155°F. for 30 min. to ensure acceptable bacterial counts.

There was general correlation, with a considerable amount of overlapping, between the resazurin grades of raw milk and the bacterial counts of the raw and pasteurized milks.

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