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To the Graduate Council:

I am submitting herewith a thesis written by Edward Ray Kelly entitled "Some Observations of Preliminary Incubation on Pasteurized Milk." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Dairying.

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**SOME OBSERVATIONS OF PRELIMINARY  
INCUBATION ON PASTEURIZED MILK**

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**A Thesis  
Presented to  
the Graduate Council of  
The University of Tennessee**

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**In Partial Fulfillment  
of the Requirements for the Degree  
Master of Science**

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**by  
Edward Ray Kelly  
December 1961**



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## CHAPTER I

### INTRODUCTION

The dairy industry strives to provide the public with safe and wholesome milk and milk products having good keeping quality. This goal can be accomplished through quality control programs and accepted methods and practices in the production and handling of the milk and its products. Today's longer shelf life requirement, however, makes it necessary for maximum attainment of sanitation through proper processing and handling practices in order to maintain good keeping quality.

The destruction of pathogenic or disease producing microorganisms which may be present in milk and cream is carried out most readily by proper pasteurization. This practice renders milk safe for human consumption unless otherwise recontaminated through careless handling practices. Likewise pasteurization destroys other microorganisms including the psychrophiles. The fact that psychrophiles are commonly found in pasteurized milk of today is an indication that they have been introduced at one or more points during post-pasteurization handling of the product. This suggests an inadequate sanitizing operation.

Rapid cooling of processed milk can temporarily slow the growth rate of bacteria present as a result of post-pasteurization contamination or faulty pasteurization. This would appear to make possible an effective masking of unsanitary processing practices if standard plate counts prepared from these milk samples taken immediately after processing are relied upon wholly as the quality control check.



Johns (16) has shown that short-cuts in dairy farm sanitation programs can be effectively masked if efficient cooling equipment is employed to the extent that bacterial growth rates are temporarily retarded. Bacteria counts made soon after production on rapidly cooled milk from such a farm are inadequate since they may indicate better than actual quality. Preliminary incubation of milk samples from these farms before plating, however, causes growth of these "hidden" bacteria; the extent of which is an indication of the level of sanitation achieved during production.

It would appear that such a treatment would be applicable to pasteurized milk in determining the degree of post-pasteurization contamination or the initial pasteurization efficiency, and to some extent, provide a basis for predicting the keeping quality of the milk.

The purpose of this investigation was to determine and observe the coliform, psychrophilic, and total bacterial counts on freshly pasteurized milk from the Knoxville, Tennessee market area and to compare increases in coliform or total counts brought about by a twenty-four hour preliminary incubation at 15.5° and 18.0° C. with the keeping quality and milk plant inspection records.



## CHAPTER II

### REVIEW OF LITERATURE

The Milk Ordinance and Code (35) emphasizes that the bacterial count of milk and certain milk products is widely accepted as an index to the sanitary quality. A high count does not mean that disease producing organisms are present, but a low count does not necessarily mean that they are absent. However, high counts after pasteurization do mean that the chances of infection have been increased, and there is no question that the keeping quality of milk will be lowered by their presence.

According to Dahlberg (8) and Mummer and Babel (13), proper pasteurization of milk and cream without post-pasteurization contamination is effective in destroying bacteria which belong in the pathogenic or disease producing group. Dahlberg (8) further states that moderate increases in bacterial numbers in pasteurized milk do not constitute a health hazard. It should be pointed out, however, that increases in the coliform group are accompanied by increases in other bacteria. Bacteria of the typhoid group grow readily at 60° F. while none grow significantly in several days at 50° F. It follows that the maximum safe storage temperature for any appreciable length of time should not exceed 50° F. to safeguard against the growth of these pathogenic organisms should they be introduced after pasteurization.



While some few bacteria, referred to as thermoduric organisms, are capable of surviving pasteurization temperatures; other groups such as the coliforms are quite readily destroyed. Watrous et al. (36) found that pasteurized milk samples taken aseptically from the pasteurizer still had a negative coliform test after being held for fifteen days at refrigeration temperatures. Tanner and Windsor (32) showed that most strains of Escherichia coli in milk are destroyed by heating to 62.8° C. for less than thirty minutes. Four cultures which did survive this treatment had been isolated from feces or from water which had been condemned for drinking purposes. All strains isolated from sewage and from commercially pasteurized milk were killed in fifteen minutes or less. Of the twenty-three cultures of Escherichia coli studied, only one was found that had the capacity to withstand a heat treatment of 62.8° C. for thirty minutes in sealed tubes. However, it survived only twenty-one to thirty minutes in open containers. Foster et al. (11) also agree with other workers that proper pasteurization has a lethal effect on coliforms, but they add that a somewhat heat resistant strain may be encountered very uncommonly.

Since pasteurization temperatures have such a lethal effect on coliforms, their presence in pasteurized milk is of concern in regard to the adequacy of milk plant sanitation. Tanner and Windsor (32) assert that an effectively pasteurized milk should contain no Escherichia coli, and its presence immediately after pasteurization is a valuable index of pasteurization efficiency. It is assumed that Tanner was referring to milk taken aseptically from the pasteurizer. The Milk Ordinance and Code



(35) and Delay (10) are in agreement that the presence of coliform bacteria in pasteurized milk is a usual indication of post-pasteurization contamination. Delay (10) also adds that well equipped plants can, without exceeding the limits of practicability, produce bottled milk of which less than 1% of samples taken will show a positive coliform test.

According to Foster et al. (11) the harm from coliform bacterial activity in milk and milk products is in the form of gassy defects and a number of undesirable flavor changes. The defect, "unclean", is of particular importance. Storage at low temperatures is commonly used to slow bacterial degradation of milk and milk products, however, Dahlberg (8) indicates that some coliforms are able to multiply slowly and to increase their proportion of the total microflora in milk held in the temperature range of 1.6° to 4.4° C. Foster et al. (11) indicated that coliform bacteria can grow slowly at refrigeration temperatures of 3° to 5° C. In an investigation by Schultze and Olson (29), 10.8% of the total microflora consisted of psychrophilic coliforms after one week of storage at 4° C. Each culture of these coliforms had multiplied rapidly enough to attain populations of one million to two hundred fifty million per ml. under these storage conditions. Varying degrees of gas production were common among these organisms which produced the typical rapid acid fermentation of lactose, and most of them showed proteolytic activity which is usually not attributed to coliform bacteria. Dahlberg (9) showed the relationship of coliform growth to growth of all bacteria in pasteurized milk held at three temperature ranges. He concluded that total counts made from pasteurized milk stored at 35° to 40° F. decreased



during four days of storage, but coliform counts on this milk remained constant in cool weather and actually increased slightly in warm weather. When the samples were stored at 45° to 50° F., coliform numbers increased more rapidly than total counts during four days of storage. Finally storage of the samples at 50° to 60° F. resulted in coliform counts exceeding one million per ml. after four days, but bacteria other than coliforms grew more rapidly.

A study of the occurrences of coliform bacteria in the milk market area of Knoxville, Tennessee by Courtney (6) showed that 93% and 80% of pasteurized samples complied with the coliform standard (35) for the periods February 4, 1949 to December 31, 1949 and January 1, 1950 to December 31, 1950 respectively. This information was obtained at a public health laboratory which was doing routine coliform tests. Another investigation by Prow (26) in 1957 revealed that 32% of samples taken from the area were coliform positive, and 13% of these had over ten coliforms per ml.

The importance of psychrophilic bacteria which grow in milk and milk products held at refrigeration temperatures from 0° to 5° C. has been recognized for some time, but increased attention has been given to their detection and study during recent years (33). This rising interest was brought about by longer periods and lower temperatures of storage due to bulk methods of handling. The work of Sherman et al. (30) shows that the attention given to the presence of bacteria in pasteurized milk is essential if good keeping quality is to be maintained. They thought, from observation of other studies involving bacteria, that some



of the chemical changes and the spoilage of milk might have been due to milk enzymes rather than exclusively to bacteria. This, however, did not prove to be true. With the exception of some oxidized flavor, the changes in the milk were apparently due entirely to bacteria. These investigators noted that the eventual bacteriological spoilage of the milk, marked by extensive proteolysis, was in agreement with results of earlier studies.

The results obtained by several investigators show rather conclusively that bacteria belonging to the psychrophilic group are quite readily destroyed by proper pasteurization. Rogick and Burgwald (27) found no psychrophiles in 4.1 ml. of freshly pasteurized milk taken from vat or high-temperature short-time pasteurizers. Kennedy and Weiser (20) report that all but one sample out of fifteen showed a considerable reduction in bacterial numbers after pasteurization. Olson et al. (23) gave an account of samples which were drawn aseptically immediately after laboratory pasteurization which showed even more conclusive results. Of seventy-five samples obtained in this fashion, none showed more than one psychrophile per ml. immediately after pasteurization or after ten and twenty days of storage at 5° C. No evidence of psychrophilic bacteria was found in any sample of pasteurized milk drawn aseptically from the holding vat either when analyzed immediately or after fifteen days of storage at 5° C.

Such studies show quite conclusively that proper pasteurization destroys the psychrophilic bacteria in raw milk, at least to the extent that they would not cause flavor deterioration over an extended period of storage. Kaufmann and Andrews (19) add that an average initial



psychrophilic count of less than ten per ml. can be achieved through good practical plant operating conditions. Olson et al. (23) indicate that psychrophiles are commonly found in pasteurized milk, and their presence is an indication that they have been introduced at one or more points during post-pasteurization handling of the product. This in turn suggests the lack of adequate sanitation programs. Thomas (34) agrees entirely with Olson et al. (23) in this respect. Dirty equipment with which pasteurized milk comes into contact is an important source or re-contamination with psychrophiles, and Rogick and Burgwald (28) have shown the milk bottle to be a factor in introducing them into pasteurized milk. Their examination of rinses of washed bottles showed that fifteen of thirty-four freshly washed half-pint bottles contained psychrophilic bacteria with numbers ranging from zero to seventy per ml. The average number per bottle was sixteen. The bottles containing the rinse solution were then held for seven days at 4° to 7° C. Colony counts per ml. then ranged from 10 to 1900 with an average of 292. Olson et al. (22) emphasize that pasteurized milk must be extremely low in psychrophilic population in order to assure good keeping quality. Kaufmann and Andrews (19) add that the growth rate of contaminants may be more important in determining the storage period than the initial degree of contamination. Atherton et al. (2) report common occurrences of fermented flavors when there is excessive psychrophilic bacteria multiplication in pasteurized milk and the production of flat or chalky flavors in samples remaining low in these counts during storage. Thomas (34) stated that fermented or stale flavors of a proteolytic character were most commonly encount-



ered in aged pasteurized milk held at 4.4° C. Parker et al. (25) stated more specifically that various bacteria belonging to the genus Pseudomonas were responsible for rancid, fruity, fishy, and a group of other defects, and Pseudomonas fluorescens and Pseudomonas fragi were particularly noted for production of rancidity.

Lawton and Nelson (21) isolated strains of psychrophilic bacteria from commercial samples of pasteurized milk and inoculated sterile skim-milk with them. These inoculated skimmilk samples were then held at 10° C. or below to see if these temperatures, commonly used by the dairy industry, would be sufficient in controlling bacterial growth. It was found that this treatment only controlled the growth of some bacteria. An initial contamination of a few thousand organisms per ml. in other cases resulted in psychrophilic counts of ten million after three or four days of storage at 5° C.

The dairy plant operator must be concerned about the bacteria which are capable of growing at low storage temperatures in milk and milk products because he also has problems arising during handling of his products by retail distributors and ultimate consumers. Overcast and Slean (24) have shown that a temperature rise is most likely to occur if properly refrigerated milk is placed in a number of display cabinets found in grocery stores in the Knoxville, Tennessee area. Their study of temperature conditions in twenty-two display cabinets showed a range from 34° to 65° F. Temperatures were even found to vary as much as 17° F. within the same cabinet from bottom to top. Only two of the twenty-two cabinets had temperatures of 40° F. or less; a temperature normally



considered necessary for storing milk. Josephson et al. (18) made a study of thirteen retail market milk delivery routes in Columbus and Cleveland, Ohio, St. Louis, Missouri, and Boston, Massachusetts to learn how quickly consumers removed bottled milk from their doorstep. It was learned that 70% of milk deliveries were removed within five minutes, but 3.3% of these customers left milk exposed for more than two and one-half hours. Examples such as these involving inadequate refrigeration can be more serious in milk quality deterioration than the initial number of bacteria present according to Burgwald and Josephson (5). These investigators, in arriving at this decision, exposed 184 pasteurized milk samples from five processing plants to room temperature for varying lengths of time before storing them at 39.4° F. They noted that longer exposure time which consequently allowed the samples to become warmer before storage resulted in quicker bacterial degradation than that occurring when these samples were stored at 39.4° F. without being allowed a temperature rise. Other investigators support the theory of Burgwald and Josephson (5) by comparing the initial bacteria counts of pasteurized milk with keeping quality. Atherton et al. (2) found no correlation between plate counts and off-flavor development and suggested that psychrophilic types and not numbers are of primary significance in flavor changes. Bertelsen et al. (4) tested thirty-eight market milk samples and found no significant difference in keeping quality for a total of up to one million bacteria per ml. Only when the counts exceeded this figure did a definite deterioration in keeping quality become apparent.

The conclusions of those workers which are accounted for in the pre-



ceeding material indicate that there is no excuse for pasteurized milk having bacterial populations the size and nature of which will constitute a health hazard or cause rapid deterioration in quality. Nevertheless the United States Public Health Service has adopted legal maximums of bacterial populations for specific grades of pasteurized milk as one means for the control of milkborne diseases (35). One method used by health authorities for checking pasteurized milk for compliance with these regulations is the Standard Plate Count (1), but this method may not show actual milk quality if the test is conducted on milk which has been rapidly cooled soon after pasteurization. Some studies have been conducted which show that rapid cooling can mask unsanitary practices in milk handling if plate counts made soon after production are relied upon as a quality control check.

Johns (16) using a preliminary incubation before plating has shown that it is possible for milk producers to take short-cuts in dairy farm sanitation programs and still provide processing plants with milk having bacterial counts well under the legal maximum. These short-cuts may be effectively masked if efficient cooling equipment is employed to the extent that bacterial growth rates are temporarily retarded. Samples taken from such milk soon after production consequently are inadequate since they may indicate better than actual quality. Smillie et al. (31) found that cooling of milk immediately after production and holding it at low temperature results in a restriction of bacterial growth and multiplication, and these milks give lower counts when the standard plating method is employed within a few hours after production. They further suggested



that raw milks having initial bacteria counts of ten thousand or less per ml. and which did not have counts exceeding twenty-one times this initial count after being held at approximately 60° F. for twenty-one hours could be assumed to have been produced under conditions sanitary enough to meet present bacteriological standards in Scotland. Davis and Killmeier (7) conducted a study on raw milk using preliminary incubation before plating and also found this procedure to be valuable for detecting insanitary production practices. Before conversion to bulk handling systems, approximately 25% of producer samples coming into their Kentucky Laboratory had bacteria counts exceeding two hundred thousand per ml., and many producers were being degraded on this basis. After using bulk handling methods for four years not one single producer had been degraded due to bacteria counts exceeding two hundred thousand per ml. A bacteria count of ten thousand per ml. became routine among these producers, and this was realized to be inadequate in showing the actual milk quality since producer sanitation programs had not improved materially to effect such a change. A preliminary incubation at 55° F. for eighteen hours before bacteria counts were made was carried out on over six hundred other samples from the same producers, and it was found that 25% of them still had counts in excess of two hundred thousand per ml. A producer who was really efficient in his cleaning practices had an initial count of ten thousand per ml., but his count remained at this level or a little higher after a preliminary incubation. In many instances objectionable producers having initial counts of ten thousand per ml. had counts in excess of two hundred thousand or often in the millions per ml. after prelimin-



TABLE I

RESULTS OF BULK RAW MILK PRODUCED UNDER VARIOUS CONDITIONS  
AND STORED FORTY-EIGHT HOURS AT 36° F. TO 38° F. AND  
THEN INCUBATED FOR EIGHTEEN HOURS AT 55° F.

| Production conditions           | Before incubation<br>(per ml.) | After incubation<br>(per ml.) |
|---------------------------------|--------------------------------|-------------------------------|
| Good production methods         | 10,000                         | 10,000                        |
| (clean cows and equipment)      | 10,000                         | 30,000                        |
|                                 | 10,000                         | 20,000                        |
|                                 | 10,000                         | 50,000                        |
|                                 | 10,000                         | 10,000                        |
|                                 | 10,000                         | 40,000                        |
| Poor production methods         | 20,000                         | 420,000                       |
| (dirty cows and equipment)      | 60,000                         | 650,000                       |
|                                 | 70,000                         | 300,000                       |
|                                 | 10,000                         | 390,000                       |
|                                 | 20,000                         | 600,000                       |
|                                 | 30,000                         | 300,000                       |
| Very poor production methods    | 120,000                        | 1,200,000                     |
| (very dirty cows and equipment) | 80,000                         | 4,800,000                     |
|                                 | 10,000                         | 1,800,000                     |
|                                 | 10,000                         | 2,100,000                     |
|                                 | 40,000                         | 4,500,000                     |
|                                 | 10,000                         | 900,000                       |



ary incubation. Table I contains some results from this study which show how preliminary incubation detects those milks which have been produced while using poor methods but still have bacteria counts well under the legal maximum as determined by plate counts soon after production and rapid cooling.

Johns (15) found that the most serious fault with the methylene blue reduction test for assaying the bacteriological quality of high grade milk is that the degree of error increases as the reduction time is increased. He observed that variations encountered up to ten hours were not significant enough to require placement of the upper limit of accuracy below this point. With the upper limit of accuracy set at ten hours, the methylene blue reduction test would not be adequate in assaying very low count milk. It was decided that some modification of the test was needed to shorten the reduction period, and the idea of a preliminary incubation was selected as an alternative. Preliminary incubation of milk samples at  $12.8^{\circ}$  C. for eighteen hours before the reduction test showed a definite advantage of accuracy over the ordinary reduction test; especially among the higher grades of milks. Keeping quality was checked periodically, and a closer correlation was found between the results of the modified test and keeping quality than the correlation of results of the ordinary test and keeping quality. Johns (17) noted that preliminary incubation shortened the methylene blue reduction period more during winter months than during summer months. This was attributed to faster cooling because of low environmental temperatures during the winter which caused a reduction in the initial bacterial activity.



Fewer attempts have apparently been made to compare the bacteria counts of pasteurized milk to keeping quality after subjecting it to a preliminary incubation. Berger and Anderson (3) suggest that at least twenty-four hours incubation time is required if bacterial analysis is to conform with keeping quality of the milk. They emphasize that keeping quality cannot be judged from analysis performed immediately after processing. Bertelsen et al. (4) stated that a coliform test on pasteurized milk and cream is most suitable for determining the keeping quality after preliminary incubation at 17° C. for twenty-four hours. They have set the upper limit at one hundred coliform bacteria per ml. after preliminary incubation for milk which is expected to retain good quality.

The effect of the initial number of bacteria present combined with the influence of temperature changes on bacterial populations would appear to be a basis for assuming that preliminary incubation might be valuable for predicting the keeping quality of pasteurized milk.

Hunter-Szybalska et al. (14) found that chilling decreases the rate of chemical reactions associated with nuclear division of bacterial cells, but its effect is apparently not uniform throughout the divisional cycle as certain stages are considerably more affected than others. Logarithmic multiplication in cultures of Escherichia coli were found to occur in a manner indicating simultaneous division of many cells. When actively growing cells of Bacillus megaterium which had been kept at 34° C. were subjected to a sub-optimal temperature of 10° to 15° C. for a short time, their nuclei became highly stainable masses made up of contracted chromosomes. Reincubation at 34° C. resulted in repeated cellu-



lar division, and when the temperature was allowed to reach 34° C. after the sub-optimal drop, separation of sister chromosomes occurred within five to fifteen minutes. Prow (26) found that 50.8% of the trials with coliform bacteria gave an average immediate increase of 52% in numbers when milk was plated at 5° C. and again at 10° C. only about five minutes later.

Dahlberg (9) collected pasteurized milk from six milk plants in the New York Metropolitan area and found three coliform positive samples out of fifty-four quarts. Holding this freshly pasteurized milk at room temperature to simulate failure to promptly refrigerate delivered milk showed immediate effects. The number of milks positive for coliforms was then one to three out of a total of eighteen milks on each of three consecutive weeks.

Green (12) has shown the effect of the temperature history on the growth response of psychrophiles when exposed to different incubation temperatures. He found that coliform bacteria lost their viability when transferred from 45° to 10° C. whereas cultures originally grown at 10° C. retained viability when subcultured at 45° C. The temperature at which a mother culture of psychrophiles has been held was found to have an effect on the growth and viability of subcultures incubated at various temperatures. This was observed by growing a culture of Pseudomonas fluorescens near its maximum, optimum, and minimum temperatures in nutrient broth and inoculating tubes of nutrient broth to be held at different temperature levels within this range. More growth was observed at 20° C. incubation temperature when subcultures were taken from cultures stored at 5° and 20°



C. than when the subcultures were taken from cultures stored at 37° C. Green (12) also found that pre-exposure to refrigeration temperatures, such as might be employed in a dairy plant, might accelerate bacterial growth at other refrigeration temperatures (e.g., in household refrigerators). The test was carried out at 2° and 8° C., and it was found that exposure of milk to the lower temperature followed by exposure to the higher temperature resulted in a greater increase in bacterial numbers than did exposure to the higher temperature before exposure to the lower temperature. It was suggested that this influence might be important from the legal aspect and the keeping time aspect.



## CHAPTER III

### EXPERIMENTAL METHODS

For the present study, fifty freshly pasteurized samples of packaged homogenized milk were obtained from ten processing plants in the Knoxville market milk area during two time intervals. Agar plate counts were employed to determine their bacterial content according to procedures outlined in Standard Methods for the Examination of Dairy Products (1); except colonies for psychrophilic and total counts were recorded as counted. Total count plates were incubated at 32° C. for forty-eight hours, (t three hours), coliform plates at 35° C. for twenty-four hours, and psychrophilic plates at 5° C. for seven days.

Twenty samples were assayed during January and February 1961 and the remaining thirty during September and October 1961.

All the samples were either quarts or one-half gallons and were obtained during the morning on the day of processing; either from filler conveyors or very soon after being pasteurized and placed in refrigerated holding rooms. The samples were immediately packed in flake ice for transport to the laboratory where they were placed in a refrigerator maintained at 40° F. until preparations for initial plating were made. The initial plating shall be referred to as that made on zero days and shall be used hereafter to indicate the day of processing.

The following testing procedure was used throughout the experiment. At zero days, total, psychrophilic, and coliform counts were made by



methods as discussed previously, and an evaluation of flavor was given by either two or three experienced dairy products judges using a five-x system to denote the intensity of any off-flavors present. More pronounced flavor defects were designated by recording more than one x after the defect; the number depending upon the intensity of the off-flavor.

For the preliminary incubation, two subsamples of one hundred ml. each were aseptically transferred from each sample to sterile dilution bottles and placed in water baths maintained at 15.5° C. and 18.0° C. (± .01° C.) respectively. These samples were incubated for twenty-four hours. After this preliminary incubation another total and coliform count was made on the subsamples.

Sterile two-liter Erlenmeyer flasks were used for storing the remainder of the original samples at 40° F. for later testing. These flasks were equipped with dust proof caps to prevent possible recontamination.



## CHAPTER IV

### RESULTS AND DISCUSSION

Fifty freshly pasteurized and packaged samples of homogenized milk from ten processing plants in the Knoxville, Tennessee area were assayed for bacterial content on the day of processing and again after a preliminary incubation. This incubation was continued for twenty-four hours, and the influence on bacterial growth from incubation at two temperatures, 15.5° C. and 18.0° C. ( $\pm 0.01^\circ$  C.) during this time was observed.

An attempt was made to compare increases in numbers of coliform and total bacteria counts after the preliminary incubation with the keeping quality of the samples. The data in Table II indicate very little consistency in relationships between coliform counts and keeping quality, however eleven of forty-eight samples had coliform bacteria counts exceeding one thousand per ml. after preliminary incubation at 15.5° C. All of these samples except three, (numbers forty, forty-four, and forty-eight), developed fruity, unclean, rancid, or stale flavors after seven days of storage at 40° F. These off-flavors had intensities ranging from two-x to five-x in the scoring system as previously explained. Two of these three samples, (numbers forty-four and forty-eight), developed slightly stale flavors, (designated by one x), and the third, (number forty), was so severely cooked during pasteurization that other possible off-flavors might have been masked. However, a low concentration of coliform bacteria after preliminary incubation for twenty-four hours at



TABLE II

COLIFORM BACTERIA COUNTS FROM PASTEURIZED MILK ON THE DAY OF PROCESSING AND AFTER PRELIMINARY INCUBATION AT 15.5° C. AND 18.0° C., ( $\pm$  0.01° C.) FOR TWENTY-FOUR HOURS WITH FLAVOR EVALUATION OF THESE MILKS AFTER STORAGE FOR SEVEN DAYS AT 40° F., ( $\pm$  1° F.)

| Sample number | 0 days <sup>a</sup><br>(per ml.) | After P.I. <sup>b</sup><br>at 15.5° C.<br>(per ml.) | After P.I.<br>at 18.0° C.<br>(per ml.) | Flavor criticism<br>and intensity <sup>c</sup> |
|---------------|----------------------------------|-----------------------------------------------------|----------------------------------------|------------------------------------------------|
| 1             | 0                                | 0                                                   | 1,660                                  | acid xx                                        |
| 2             | 0                                | 0                                                   | 0                                      | acid x; unclear xx                             |
| 3             | 1                                | 113                                                 | 3,200                                  | unclear xxx; none                              |
| 4             | 0                                | 12                                                  | 170                                    | none; unclear xx                               |
| 5             | 6                                | LA <sup>d</sup>                                     | LA                                     | acid x; unclear xxx                            |
| 6             | 0                                | 0                                                   | 0                                      | stale xxx; musty xx                            |
| 7             | 0                                | 14                                                  | LA                                     | stale xxx; musty xx                            |
| 8             | 0                                | 0                                                   | 610                                    | rancid x; unclear xx                           |
| 9             | 0                                | 0                                                   | 0                                      | stale xx; unclear x                            |
| 10            | 0                                | 0                                                   | 0                                      | cooked xxx; cooked xxx                         |
| 11            | 2                                | 213                                                 | 2,710                                  | unclear xx; stale xx                           |
| 12            | 6                                | 2,230                                               | 5,000                                  | unclear xx; stale x                            |
| 13            | 0                                | 390                                                 | 2,600                                  | cooked x; unclear xx                           |
| 14            | 0                                | 1                                                   | 7                                      | fruity xxx; unclear xxx                        |
| 15            | 0                                | 2                                                   | 9                                      | fruity xxx; cooked xxx                         |

<sup>a</sup>Day of processing

<sup>b</sup>Preliminary incubation

<sup>c</sup>x--slight; xx--moderate; xxx--distinct; xxx--intense.

<sup>d</sup>Laboratory accident



TABLE II (continued)

| Sample number | 0 days <sup>a</sup><br>(per ml.) | After P.I. <sup>b</sup><br>at 15.5° C.<br>(per ml.) | After P.I.<br>at 18.0° C.<br>(per ml.) | Flavor criticism<br>and intensity |
|---------------|----------------------------------|-----------------------------------------------------|----------------------------------------|-----------------------------------|
| 16            | 7                                | 430                                                 | 7,100                                  | fruity x; stale xx; cooked xx     |
| 17            | 21                               | 940                                                 | 30,300                                 | stale x; cooked x; feed xx        |
| 18            | 50                               | 2,580                                               | 44,800                                 | rancid x; rancid xx, feed xx      |
| 19            | 10                               | 590                                                 | 12,700                                 | stale x; stale xx                 |
| 20            | 6                                | 130                                                 | 970                                    | flat x; cooked xx                 |
| 21            | 3                                | 2,890                                               | 17,400                                 | stale x; musty x; fruity xx       |
| 22            | 0                                | 1,980                                               | 99,000                                 | stale x; musty x; fruity xx       |
| 23            | 0                                | 10                                                  | 33                                     | stale x; musty x; fruity xxx      |
| 24            | 0                                | 620                                                 | 153                                    | stale x; musty xx; fruity xxx     |
| 25            | 2                                | 660                                                 | 12,160                                 | cooked xxx; musty x               |
| 26            | 0                                | 0                                                   | 0                                      | feed xx; stale x                  |
| 27            | 0                                | 85                                                  | 660                                    | feed xx; cooked x; none           |
| 28            | 2                                | 1,280                                               | 48,000                                 | fruity xx; rancid x; stale x      |
| 29            | 0                                | 5                                                   | 27                                     | stale x; musty x                  |
| 30            | 0                                | 470                                                 | 12,300                                 | cooked xxx; cooked xi             |
| 31            | 0                                | 0                                                   | 0                                      | stale xx; stale x                 |
| 32            | 0                                | 6                                                   | 510                                    | chlorine xxx                      |
| 33            | 0                                | 0                                                   | 0                                      | stale xx; stale x                 |
| 34            | 36                               | 1,880                                               | 44,000                                 | stale xx; cooked xx               |
| 35            | 0                                | 800                                                 | 11,000                                 | cooked xxx                        |
| 36            | 0                                | 0                                                   | 0                                      | stale x; cooked xx                |
| 37            | 0                                | 0                                                   | 0                                      | stale x; cooked x                 |
| 38            | 4                                | 11,500                                              | 767,000                                | stale xx; cooked x                |
| 39            | 0                                | 45                                                  | 1,700                                  | stale x; feed xx                  |
| 40            | 6                                | 12,000                                              | 147,000                                | cooked xxx                        |



TABLE II (continued)

| Sample number | 0 days <sup>a</sup><br>(per ml.) | After P.I. <sup>b</sup><br>at 15.5° C.<br>(per ml.) | After P.I.<br>at 18.0° C.<br>(per ml.) | Flavor criticism<br>and intensity |
|---------------|----------------------------------|-----------------------------------------------------|----------------------------------------|-----------------------------------|
| 41            | 69                               | LA                                                  | 825,000                                | stale xx; stale x                 |
| 42            | 7                                | 1,200                                               | LA                                     | stale xx; stale x                 |
| 43            | 0                                | 8                                                   | 22                                     | stale x; stale x                  |
| 44            | 24                               | 1,400                                               | 10,300                                 | cooked x; stale x                 |
| 45            | 0                                | 5                                                   | 63                                     | cooked xx; stale x                |
| 46            | 0                                | 12                                                  | 780                                    | feed x                            |
| 47            | 0                                | 1                                                   | 200                                    | stale x                           |
| 48            | 0                                | 1,450                                               | 27,100                                 | stale x                           |
| 49            | 0                                | 236                                                 | 1,460                                  | cooked xx                         |
| 50            | 0                                | 730                                                 | LA                                     | cooked xxx                        |



15.5° C. does not assure that similar off-flavors will not develop. It was noted that nineteen samples had coliform counts of ten or less per ml. and eleven of these developed off-flavors with intensities equal to those occurring in samples having more than one thousand coliform bacteria per ml. after preliminary incubation. Similar observations can be made from counts made after a preliminary incubation at 18.0° C., and these do not appear to be any more valuable in predicting keeping quality than counts made at 15.5° C.

Further observation of the data in Table II disclose that 64% of the samples tested were coliform negative on the day of processing. However, after a preliminary incubation for twenty-four hours at 15.5° C. the percentage of negative samples was reduced to 24%, and only 18% were negative when the incubation temperature was increased to 18.0° C. Also on the day of processing, 10% of the samples had coliform bacteria counts of ten or more per ml. After preliminary incubation at 15.5° C. for twenty-four hours, 58.3% had counts exceeding ten per ml. while incubation at 18.0° C. resulted in 76% of the samples having ten or more coliform bacteria per ml.

There was very little consistency in relationships between total bacteria counts after preliminary incubation and keeping quality, but the data in Table III show 12% of the samples to have total counts of one hundred thousand or more per ml. after incubation at 15.5° C. for twenty-four hours. All of these, after seven days of storage at 40° F., had stale, musty, fruity, rancid, or unclean flavors with at least two-x



TABLE III

TOTAL BACTERIA COUNTS FROM PASTEURIZED MILK ON THE DAY OF PROCESSING AND AFTER PRELIMINARY INCUBATION AT 15.5° C. AND 18.0° C., (± 0.01° C.) FOR TWENTY-FOUR HOURS WITH FLAVOR EVALUATION OF THESE MILKS AFTER STORAGE FOR SEVEN DAYS AT 40° F., (± 1° F.)

| Sample number | 0 days <sup>a</sup><br>(per ml.) | After P.I. <sup>b</sup><br>at 15.5° C.<br>(per ml.) | After P.I.<br>at 18.0° C.<br>(per ml.) | Flavor criticism<br>and intensity <sup>c</sup> |
|---------------|----------------------------------|-----------------------------------------------------|----------------------------------------|------------------------------------------------|
| 1             | 1,200                            | 3,000                                               | 91,000                                 | acid xx                                        |
| 2             | 1,240                            | 5,900                                               | 147,000                                | acid x; unclear xx                             |
| 3             | 860                              | 1,300                                               | 9,500                                  | unclear xxx; none                              |
| 4             | 430                              | 94,000                                              | 960,000                                | none; unclear xx                               |
| 5             | 440                              | 24,500                                              | 610,000                                | acid x; unclear xxx                            |
| 6             | 320                              | 2,400                                               | 13,600                                 | stale xxx; musty xx                            |
| 7             | 6,000                            | 8,900                                               | 18,000                                 | stale xxx; musty xx                            |
| 8             | 870                              | 75,000                                              | 860,000                                | rancid x; unclear xx                           |
| 9             | 2,120                            | 4,800                                               | 4,600                                  | stale xx; unclear x                            |
| 10            | 100                              | 1,900                                               | 8,100                                  | cooked xxx; cooked xxx                         |
| 11            | 1,330                            | 3,800                                               | 17,300                                 | unclear xx; stale xx                           |
| 12            | 2,060                            | 124,000                                             | 3,120,000                              | unclear xx; stale x                            |
| 13            | 1,230                            | 15,000                                              | 22,400                                 | cooked x; unclear xx; rancid xx                |
| 14            | 3,300                            | 5,600                                               | 25,000                                 | fruity xxx; unclear xxx                        |
| 15            | 5,100                            | 10,300                                              | 11,600                                 | fruity xxx; cooked xxx                         |

<sup>a</sup>Day of processing

<sup>b</sup>Preliminary incubation

<sup>c</sup>x--slight; xx--moderate; xxx--distinct; xxxxx--intense



TABLE III (continued)

| Sample number | 0 days<br>(per ml.) | After P.I.<br>at 15.5° C.<br>(per ml.) | After P.I.<br>at 18.0° C.<br>(per ml.) | Flavor criticism<br>and intensity |
|---------------|---------------------|----------------------------------------|----------------------------------------|-----------------------------------|
| 16            | 890                 | 6,700                                  | 87,000                                 | fruity x; stale xx; cooked xx     |
| 17            | 18,400              | 88,000                                 | 1,420,000                              | stale x; cooked x; feed xx        |
| 18            | 11,500              | 1,205,000                              | 4,230,000                              | rancid x; rancod xx; feed xx      |
| 19            | 10,300              | 15,200                                 | 179,000                                | stale x; stale xx                 |
| 20            | 4,100               | 4,100                                  | 21,200                                 | flat x; cooked xx                 |
| 21            | 15,700              | 152,000                                | 1,760,000                              | stale x; musty x; fruity xx       |
| 22            | 7,800               | 13,200                                 | 650,000                                | stale x; musty x; fruity xx       |
| 23            | 7,800               | 5,800                                  | 44,000                                 | stale x; musty x; fruity xx       |
| 24            | 4,900               | 16,700                                 | 400,000                                | stale x; musty x; fruity xxx      |
| 25            | 5,500               | 7,100                                  | 89,000                                 | cooked xxx; musty x               |
| 26            | 87                  | 5,400                                  | 54,000                                 | feed xx; stale x                  |
| 27            | 6,600               | 7,800                                  | 59,000                                 | feed xx; cooked x; none           |
| 28            | 10,700              | 159,000                                | 5,800,000                              | fruity xx; rancid x; stale x      |
| 29            | 1,471               | 4,400                                  | 66,000                                 | stale x; musty x; stale x         |
| 30            | 550                 | 9,100                                  | 272,000                                | cooked xxx; cooked xxx            |
| 31            | 8,700               | 7,800                                  | 74,000                                 | stale xx; stale x                 |
| 32            | 6,200               | 6,900                                  | 128,000                                | chlorine xxx                      |
| 33            | 5,100               | 7,600                                  | 48,000                                 | stale xx; stale x                 |
| 34            | 3,500               | 8,100                                  | 420,000                                | stale xx; cooked xx               |
| 35            | 5,000               | 7,800                                  | 117,000                                | cooked xxx                        |
| 36            | 7,800               | 8,600                                  | 11,000                                 | stale x; cooked xx                |
| 37            | 8,400               | 13,600                                 | 23,600                                 | stale x; cooked x                 |
| 38            | 4,100               | 192,000                                | 3,800,000                              | stale xx; cooked x                |
| 39            | 3,200               | 5,900                                  | 19,900                                 | stale x; feed xx                  |
| 40            | 490                 | 98,000                                 | 4,700,000                              | cooked xxx                        |



TABLE III (continued)

| Sample number | 0 days<br>(per ml.) | After P.I.<br>at 15.5° C.<br>(per ml.) | After P.I.<br>at 18.0° C.<br>(per ml.) | Flavor criticism<br>and intensity |
|---------------|---------------------|----------------------------------------|----------------------------------------|-----------------------------------|
| 41            | 1,770               | 102,000                                | 16,800,000                             | stale xx; stale x                 |
| 42            | 5,900               | 19,500                                 | 141,000                                | stale xx; stale x                 |
| 43            | 4,200               | 4,000                                  | 13,800                                 | stale x                           |
| 44            | 4,000               | 74,000                                 | 1,230,000                              | cooked x; stale x                 |
| 45            | 15,400              | 20,500                                 | 153,000                                | cooked xx; stale x                |
| 46            | 1,270               | 6,100                                  | 97,000                                 | feed x                            |
| 47            | 700                 | 5,700                                  | 210,000                                | stale x                           |
| 48            | 3,700               | 5,600                                  | 72,000                                 | stale x                           |
| 49            | 7,700               | 5,500                                  | 90,000                                 | cooked xxx                        |
| 50            | 360                 | 43,000                                 | 540,000                                | cooked xxx                        |



intensity. Low total counts after preliminary incubation at  $15.5^{\circ}$  C., however, do not assure that similar off-flavors will not develop. This is emphasized in that 8% of the samples having total bacteria counts per ml. of three thousand or less after incubation of which 75% had off-flavors after seven days of storage at  $40^{\circ}$  F.

Further observation of the data in Table III show that none of the samples had total bacteria counts on zero days which exceeded the legal maximum of thirty thousand per ml. for Grade A Pasteurized Milk. After preliminary incubation at  $15.5^{\circ}$  C. for twenty-four hours, 28% of the samples failed to comply with this standard and 72% failed to comply after incubation at  $18.0^{\circ}$  C.

There appeared to be even less consistency in relationships between psychrophilic bacteria counts made on the day of processing and keeping quality than the relationships between total and coliform bacteria counts made at this time. The data in Table IV show that samples, (numbers one and two) which had the lowest psychrophilic bacteria count at zero days, developed off-flavors with two-x intensity during seven days of storage. Then the sample which had next to the highest count at zero days, (number seventeen), developed an off-flavor with only one-x intensity.

In general, off-flavors from bacterial activity did not develop in the samples until after four days of storage at  $40^{\circ}$  F. Likewise the greatest increases in total and psychrophilic bacteria numbers occurred after this time, as illustrated in Table IV and Table V, page thirty-two.

As a final observation in the study, the samples from each of the



TABLE IV

PSYCHROPHILIC BACTERIA COUNTS ON PASTEURIZED MILK MADE ON THE DAY OF PROCESSING AND AFTER FOUR AND SEVEN DAYS OF STORAGE AT 40° F. (2 1° F.) WITH REFERENCE TO KEEPING QUALITY AT SEVEN DAYS

| Sample number | 0 days <sup>a</sup><br>(per ml.) | 4 days<br>(per ml.) | 7 days<br>(per ml.) | Flavor criticism<br>and intensity <sup>b</sup> |
|---------------|----------------------------------|---------------------|---------------------|------------------------------------------------|
| 1             | 20                               | 60                  | 1,400               | acid xx                                        |
| 2             | 20                               | 150                 | 800                 | acid x; unclear xx                             |
| 3             | 50                               | 200                 | 1,000               | unclear xxx; none                              |
| 4             | 30                               | 170                 | 19,200              | none; unclear xx                               |
| 5             | 100                              | 250                 | 8,100               | acid x; unclear xxx                            |
| 6             | 140                              | 170                 | 49,000              | stale xxx; musty xx                            |
| 7             | 90                               | 170                 | 10,200              | stale xxx; musty xx                            |
| 8             | 270                              | 880                 | 370,000             | rancid x; unclear xx                           |
| 9             | 90                               | 110                 | 10,900              | stale xx; unclear x                            |
| 10            | 100                              | 190                 | 12,000              | cooked xxx; cooked xxx                         |
| 11            | 840                              | 3,200               | 4,600               | unclear xx; stale xx                           |
| 12            | 1,010                            | 4,100               | 367,000             | unclear xx; stale x                            |
| 13            | 460                              | 2,400               | 545,500             | cooked x; unclear xx; rancid xx                |
| 14            | 240                              | 2,300               | 4,000               | fruity xxx; unclear xxx                        |
| 15            | 230                              | 2,300               | LAC                 | fruity xxx; cooked xxx                         |

<sup>a</sup>Day of processing

<sup>b</sup>x--slight; xx--moderate; xxx--distinct; xxxx--intense

<sup>c</sup>Laboratory accident



TABLE IV (continued)

| Sample number | 0 days<br>(per ml.) | 4 days<br>(per ml.) | 7 days<br>(per ml.) | Flavor criticism<br>and intensity |
|---------------|---------------------|---------------------|---------------------|-----------------------------------|
| 16            | 420                 | 620                 | 28,200              | fruity x; stale xx; cooked xx     |
| 17            | 570                 | 820                 | 141,000             | stale x; cooked x; feed xx        |
| 18            | 600                 | 194,000             | 21,800,000          | rancid x; rancid xx; feed x.      |
| 19            | 570                 | 680                 | 8,900               | stale x; stale xx                 |
| 20            | 400                 | 540                 | 8,800               | flat x; cooked xx                 |
| 21            | 190                 | 180                 | 440                 | stale x; musty x; fruity xx       |
| 22            | 200                 | 200                 | 580,000             | stale x; musty x; fruity xx       |
| 23            | 290                 | 190                 | 20,000              | stale x; musty x; fruity xx       |
| 24            | 300                 | 200                 | 280                 | stale x; musty x; fruity xxx      |
| 25            | 320                 | 260                 | 260                 | cooked xxx; musty x               |
| 26            | d                   | 120                 | 330,000             | feed xx; stale x                  |
| 27            | —                   | 220                 | 230                 | feed xx; cooked x; none           |
| 28            | —                   | 1,340               | 26,650,000          | fruity xx; rancid x; stale x      |
| 29            | —                   | 100                 | 160                 | stale x; musty x; stale x         |
| 30            | —                   | 430                 | 23,530,000          | cooked xxx; cooked xx             |
| 31            | 100                 | 1,670               | 1,840,000           | stale xx; stale x                 |
| 32            | 220                 | 80                  | 1,650,000           | chlorine xxx                      |
| 33            | 50                  | 50                  | 5,100               | stale xx; stale x                 |
| 34            | 60                  | 20                  | 1,490               | stale xx; cooked xx               |
| 35            | 230                 | 150                 | 7,000               | cooked xxx                        |

d<sup>1</sup>Missing data



TABLE IV (continued)

| Sample number | 0 days<br>(per ml.) | 4 days<br>(per ml.) | 7 days<br>(per ml.) | Flavor criticism<br>and intensity |
|---------------|---------------------|---------------------|---------------------|-----------------------------------|
| 36            | —                   | 770                 | 1,069,000           | stale x; cooked x                 |
| 37            | —                   | 280                 | 26,800              | stale x; cooked x                 |
| 38            | —                   | 411,000             | 7,210,000           | stale xx; cooked x                |
| 39            | —                   | 280                 | 430                 | stale x; feed xx                  |
| 40            | —                   | 330                 | 1,340               | cooked xxx                        |
| 41            | 190                 | 310                 | 480,000             | stale xx; stale x                 |
| 42            | 260                 | 350                 | 54,000              | stale xx; stale x                 |
| 43            | 300                 | 390                 | 230                 | stale x                           |
| 44            | 260                 | 260                 | 7,600               | cooked x; stale x                 |
| 45            | 310                 | 400                 | 1,160               | cooked xx; stale x                |
| 46            | 320                 | 320                 | 660                 | feed x                            |
| 47            | 370                 | 370                 | 13,000              | stale x                           |
| 48            | 260                 | 260                 | 6,300,000           | stale x                           |
| 49            | 7,700               | 200                 | 8,000               | cooked xx                         |
| 50            | 360                 | 260                 | 2,010,000           | cooked xxx                        |



TABLE V

TOTAL BACTERIA COUNTS ON PASTEURIZED MILK MADE ON THE DAY OF PROCESSING  
AND AFTER FOUR AND SEVEN DAYS OF STORAGE AT 40° F. (± 1° F.)

| Sample<br>number | 0 days <sup>a</sup><br>(per ml.) | 4 days<br>(per ml.) | 7 days<br>(per ml.) |
|------------------|----------------------------------|---------------------|---------------------|
| 1                | 1,200                            | 1,200               | 800                 |
| 2                | 1,240                            | 800                 | 1,000               |
| 3                | 860                              | 1,800               | 800                 |
| 4                | 430                              | 900                 | 9,600               |
| 5                | 440                              | 1,000               | 4,300               |
| 6                | 320                              | 420                 | 760                 |
| 7                | 6,000                            | 6,600               | 7,800               |
| 8                | 870                              | 1,270               | 158,000             |
| 9                | 2,120                            | 1,920               | 5,400               |
| 10               | 100                              | 120                 | 240                 |
| 11               | 1,330                            | 1,920               | 1,420               |
| 12               | 2,060                            | 1,960               | 23,800              |
| 13               | 1,230                            | 1,750               | 2,290               |
| 14               | 3,300                            | 8,800               | 5,220               |
| 15               | 5,100                            | 9,200               | 7,700               |
| 16               | 890                              | 1,500               | 11,400              |
| 17               | 18,400                           | 12,950              | 132,000             |
| 18               | 11,500                           | 61,000              | 1,070,000           |
| 19               | 10,300                           | 9,300               | 184,000             |
| 20               | 4,100                            | 220                 | 670                 |
| 21               | 15,700                           | 9,000               | 11,600              |
| 22               | 7,800                            | 6,400               | 490,000             |
| 23               | 7,800                            | 6,800               | 12,900              |
| 24               | 4,900                            | 1,830               | 6,400               |
| 25               | 5,500                            | 4,900               | 6,200               |
| 26               | 87                               | 900                 | 1,235,000           |
| 27               | 6,600                            | 4,800               | 41,000              |
| 28               | 10,700                           | 6,100               | 14,620,000          |
| 29               | 1,471                            | 4,000               | 1,590               |
| 30               | 550                              | 630                 | 1,520,000           |

<sup>a</sup>Day of processing



TABLE V (continued)

| Sample<br>number | 0 days<br>(per ml.) | 4 days<br>(per ml.) | 7 days<br>(per ml.) |
|------------------|---------------------|---------------------|---------------------|
| 31               | 8,700               | 10,800              | 9,910,000           |
| 32               | 6,200               | 9,000               | 740,000             |
| 33               | 5,100               | 5,500               | 5,800               |
| 34               | 3,500               | 4,200               | 17,200              |
| 35               | 5,000               | 4,300               | 8,700               |
| 36               | 7,800               | 8,800               | 3,440,000           |
| 37               | 8,400               | 9,000               | 26,700              |
| 38               | 4,100               | 126,000             | 9,100,000           |
| 39               | 3,200               | 49,000              | 4,400               |
| 40               | 490                 | 920                 | 202,000             |
| 41               | 1,770               | 8,800               | 710,000             |
| 42               | 5,900               | 80,000              | 66,000              |
| 43               | 4,200               | 4,800               | 7,000               |
| 44               | 4,000               | 1,040               | 10,200              |
| 45               | 15,400              | 18,800              | 23,800              |
| 46               | 1,270               | 6,500               | 950                 |
| 47               | 700                 | 410                 | 750                 |
| 48               | 3,700               | 5,200               | 1,120,000           |
| 49               | 7,700               | 5,700               | 1,760               |
| 50               | 360                 | 4,900               | 243,000             |



ten plants were grouped in an attempt to compare the relationship of increases in bacterial numbers brought about by preliminary incubation with official plant inspection records for the years 1960 and 1961. It was believed that lower increases in bacterial counts might parallel inspection records showing less criticism and higher increases might be accompanied by records containing more criticism. If this were true then preliminary incubation might be beneficial in the evaluation of dairy plant sanitation programs. In order to make this analysis, average logarithmic counts for each plant were computed for total and coliform bacteria, and the plants were ranked according to the rate of bacterial increases occurring in their respective samples after preliminary incubation. Finally an average position was derived for each plant from its position in each of the original rankings.

The only relationship that could be found from this approach was that the plant which had the highest rate of bacterial increase at both of the preliminary incubation temperatures for both total and coliform counts had had its cream temporarily suspended from the market during the year 1960. Only one other plant had had a product temporarily suspended in 1960 or 1961, and it ranked fifth in rates of total and coliform bacteria increases.



## CHAPTER V

## SUMMARY AND CONCLUSION

Fifty freshly pasteurized and packaged samples of homogenized milk from ten processing plants in the Knoxville, Tennessee area were assayed for bacterial content on the day of processing and again after a preliminary incubation. This incubation was continued for twenty-four hours, and the influence on bacterial growth from incubation at two temperatures,  $15.5^{\circ}\text{C.}$  and  $18.0^{\circ}\text{C.}$  ( $\pm 0.01^{\circ}\text{C.}$ ) was determined.

An attempt was made to compare increases in numbers of coliform and total bacteria counts after the preliminary incubation with the keeping quality of the milk samples and milk plant inspection records to determine whether or not this type of test would be suitable as a basis for predicting the keeping quality of pasteurized milk or for evaluating milk plant sanitation programs.

The data indicated very little consistency in relationships between the increases in bacteria counts brought about by a preliminary incubation of this nature and the keeping quality. There was nothing to indicate, within the limits of this experiment, that preliminary incubation is a reliable method for determining the keeping quality of pasteurized milk or for evaluating milk plant sanitation programs.



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