May 18, 1937

To the Committee on Graduate Study:

I submit herewith a thesis written by Mr. W. C. Patton and entitled "Quality Control of Market Milk", and recommend that it be accepted for nine quarter hours credit in partial fulfillment of the requirements for the degree of Master of Science in Agriculture, with a major in Dairying.

C. E. Wylie
Major Professor

At the request of the
Committee on Graduate Study,
we have read this thesis,
and recommended its acceptance.

Thos. B. Harrison
Paul W. Allen
C. E. Wylie

Accepted by the Committee

V. C. Smith
Dean

QUALITY CONTROL OF MARKET MILK

-o-

A THESIS

Submitted to the Graduate Committee
of
The University of Tennessee
in
Partial Fulfillment of the Requirements
for the degree of
Master of Science
in
Agriculture

by
W. C. PATTON

June 1937

PREFACE

The material in this thesis was secured from a study of some of the factors affecting the quality of market milk. The problem was planned so that the information could be of practical value to dairymen, milk dealers, health officials, and consumers.

A survey of the literature on this subject and related subjects is given in Part I. Many helpful suggestions on methods of procedure and presentation of results were found in this survey. Due credit has been given those earlier investigators whose work was included. The data in Part II were secured from an investigation made by the writer during two years of graduate study at the University of Tennessee and with the cooperation of the Knoxville Bureau of Health.

The writer wishes to express his sincere appreciation for the assistance given by the members of the University Dairy Staff and students. He is especially indebted to Professors C. E. Wylie and Thos. B. Harrison of the University of Tennessee Dairy Department for their able guidance during the investigation and writing of this thesis.

W. C. Patton

TO 37 M13 .90

146517

TABLE OF CONTENTS

	Pages
INTRODUCTION	vii
PART I. SURVEY OF LITERATURE:	
Flavors of Market Milk	1
Feed and Weed Flavors	2
Abnormal Conditions of the Cow	9
Handling of Milk	10
Unclean Flavors	10
Pasteurization	12
Oxidized Flavors	12
Creaming of Market Milk	14
Formation of Cream Layer	15
Cooling and Holding Temperatures	16
Pasteurization Temperatures	18
Holding Time	20
Agitation and Pumping	21
Homogenization	23
Bacteriology of Market Milk	25
Methods of Analysis	26
Microscopic Methods of Counting	27
Agar Plate Counting Method	27
Methylene Blue Reductase Test	29
Effect of Cooling	37
Effect of Pasteurization	39
Sterilization of Utensils and Equipment	40
Storage Temperatures	57
Acidity	58
Keeping Quality	59
Summary of Survey of Literature	60
PART II. ORIGINAL INVESTIGATION:	
Objects of Investigation	61
Plan of Investigation	62
Methods of Procedure	63

TABLE OF CONTENTS (Continued)

Flavors of Market Milk 65
Methods of Scoring 66
Influence of Aeration 66
Pasteurization Temperatures 74

Creaming of Market Milk 78
Methods of Measuring 78
In the Bottle 79
Graduated Cylinders 79
Pasteurization Temperatures 80
Influence of Storage 82

Bacteriology of Market Milk 84
Methods of Analysis 84
Agar Plate Counting 86
Methylene Blue Reductase 88
Influence of Cooling 96
Efficiency of Pasteurization 99
Sterilization of Cans 101

Conclusions 106

Bibliography 108

TABLES IN TEXT

Table		Page
I.	Summary of Initial Trials Comparing Sterilization of Cans by Steam and by Hot Air	50
II.	Germicidal Potency of Various Types of Chlorine Preparations Against B. Coli	54
III.	Plate Counts on B. Coli Inoculum After Treatments With Various Chlorine Germicides Containing Approximately 200 p.p.m. of Available Chlorine in Cold Solution	54
IV.	Plate Count on Milk Can Rinse Inoculum After Exposure to Germicidal Solutions Containing 200 p.p.m. of Available Chlorine in Cold Solution	55
V.	Growth of Bacteria in Milk When the Milk is Held at 50° F. and 68° F.	57
VI.	Scores for Good Flavored Milk Cooled by Different Methods Average of 3 Trials	70
VII.	Scores for Poor Flavored Milk Cooled by Different Methods Average of 6 Trials	71
VIII.	Scores for Medium Flavored Milk Cooled by Different Methods	73
IX.	Flavor Scores for Milk Samples Pasteurized at Different Temperatures	76
X.	Creaming of Milk Pasteurized at Different Temperatures	82
XI.	Creaming of Milk Pasteurized at Different Temperatures	83
XII.	Correlation Between Bacteria Counts in Thousands per cc. and Reduction Time in Hours	92
XIII.	Influence of Cooling on Bacterial Growth	98
XIV.	Efficienty of Pasteurization	99
XV.	Number of Bacteria per cc. of Rinse Water Before and After Sterilization of Cans by Steam	102
XVI.	Number of Bacteria per cc. of Rinse Water Before and After Sterilization of Cans by Dry Heat	103
XVII.	Number of Bacteria per cc. of 500 cc. Sterile Rinse Water Before and After Sterilizing with Chlorine Solutions Containing 50 Parts per Million of Available Chlorine	104

FIGURES

Page

1. Student Score Card 67

2. Surface Tubular Cooler 69

3. Milk Preheater 75

4. The Cherry Burrell Spray-type Pasteurizer 81

5. Material and Equipment Used For Making The Reductase Test . . . 89

6. Correlation Between Bacteria Counts in Thousands per cc. and Reduction Time in Hours 94

7. Correlation Between Bacteria Counts in Thousands per cc. and Reduction Time in Hours 95

INTRODUCTION

Dairy products constitute approximately one fifth of the diet of the average family in the United States. They appear daily on almost every family table. This requires over ten billion pounds of milk each year. Almost one half of this amount is consumed as fluid or market milk. Unusual care must be exercised throughout the production and handling of this milk in order for it to be of high quality when it reaches the consumer.

Quality in milk is a general term because it is governed by many factors, and yet, it may be applied as a specific term to mean a very highly standardized product. In this investigation, quality refers to the relative merits to be placed upon market milk as governed by the factors used in its production and handling. The factors included in this study are associated with the flavor, creaming, and bacteriology of milk. They determine to a great extent the standard of any market milk supply. Proper control methods are usually associated with high quality milk. Improper methods are usually associated with a corresponding sacrifice in quality.

Most of the available information on the quality of market milk has been secured through studies of the various factors affecting separate indications of quality. In problems which include only one point, a detailed series of trials can be made. In this problem, however, factors affecting three of the major indications pertaining to the quality of commercial market milk were studied. For the most part these studies were made for the purpose of obtaining some valuable information from sources

comparative to a commercial scale, rather than from a laboratory scale, which is the common procedure. In view of the fact that methods of control were very difficult for experiments which were conducted on a commercial scale, several trials were made for each series and the averages taken for reporting the results.

Several different viewpoints may be considered in making a study of the quality control of market milk. The producer and the distributor are interested in the economic control of the factors which affect the quality of their milk. There are those features which are primarily of interest to the consumer, and those features required by law or the legal health authorities. The consumer is primarily interested in the flavor of his milk, and the cream layer appearing on it. He depends on the health authorities for the nutritious and sanitary qualities. The health officials are governed largely by the city ordinances and the state dairy laws which define milk as to the minimum requirements of fat, serum solids, and the maximum bacteria counts. Since palatability does not permit legal definition, the consumer must employ his senses of taste and smell for determining this quality in milk.

PART I. SURVEY OF LITERATURE

Flavors of Market Milk.

Flavor is the most important quality in market milk. The consuming public expects and demands that this food be palatable. Milk which has an unpleasant flavor and aroma will be used only in limited quantities; whereas, it should be used in increasing amounts because of its high food value. When it is off-flavor, children oftentimes refuse to drink it. No other food can take the place of milk in the diet of growing children.

Many adults are fond of milk and drink it because it is refreshing and nutritious. The producer and the distributor should exercise the utmost care in the production and processing of market milk so that there can be no developments of off-flavors in the milk.

To most people good milk has a pleasant taste which is probably due to a combination of various ingredients found in milk; namely, fat, milk sugar, casein, albumin, and the minerals.

Normal milk is sweet because about 5% of it is milk sugar. When it is kept at warm temperatures, it turns sour because of the activity of certain bacteria. Milk readily absorbs odors that surround it and thereby may develop an off-flavor. The food the cow eats may greatly affect the flavor of the milk.

Cows vary somewhat in the quality of the milk they produce. A cow far along in her lactation period is apt to give milk with a somewhat salty flavor. Individual differences, however, are overcome to a certain extent when the milk from a number of cows is mixed.

Among the terms used to describe the off-flavors in milk, feed is probably the most common. Next to feed may come bitter, garlic or onion, and unclean. There are at present sixteen flavor criticisms on the score card recommended by the United States Department of Agriculture.

Feed and Weed Flavors.

Milk plants and consumers are objecting to milk with undesirable flavors and odors. This is a loss to the producer and the industry as a whole. The producer at one time was primarily interested in preventing the milk from souring before it reached the plant, giving very little attention to the objectionable flavors caused by improper feeding. The producer of today is faced with the problem of minimizing bacteria counts and preventing objectionable flavors caused by improper feeding. This may best be accomplished by a thorough study of the different feeds which cause undesirable flavors in milk, and the proper time for feeding to prevent these off-flavors.[53]

Gamble and Kelly,[22] after a systematic study of the effect of feeding silage upon the flavor and odor of milk, reported a wide variation between individual cows under normal condition which received the same feeds. When silage was fed one hour before milking, it was absorbed so quickly that it was noticeable in the milk from most of the animals in the experiments. In order to avoid undesirable flavors, it was recommended that silage be fed soon after milking, and that not more than 15 to 25 pounds of corn or legume silage be fed after each milking. These investigators also found that prompt aeration of warm milk removed slight silage flavors and reduced the more pronounced ones.

Among the other investigators who confirmed the conclusions of Gamble and Kelly, as a whole or in part, was C. J. Babcock,[3] who made an extensive study of feed flavors in milk during the period of 1923-1925 inclusive. These investigators reported on the use of turnips, green alfalfa, cabbage, potatoes, garlic, green rye, and green peas. The above feeds were found to produce objectionable flavors and odors if fed within one hour before milking. Where fed in moderate quantities, four to five hours before milking, only slight off-flavors and odors resulted from most of the above feeds.

Roadhouse and Henderson[50] found that garlic could be detected in samples of milk taken one minute after feeding and through the seventh hour following. As little as 10 pounds of green alfalfa or 5 pounds of alfalfa hay, fed two hours before milking, caused a distinct feed flavor and odor which was undesirable in market milk. Larger quantities caused a more pronounced off-flavor. When oat hay was substituted for about one half of the amount of alfalfa, the flavor was less pronounced. Moderate quantities of corn silage, 5 to 12 pounds, fed one to two hours before milking, produced an undesirable flavor; and when cows were fed 10 pounds, one hour before milking, a decided feed flavor and odor were present, which could be detected by the consumer of the milk. Cows that were fed hay in which a musty odor was observed gave milk with a musty flavor and odor, which were not present when this feed was discontinued. When the juice extracted from 25 pounds of green alfalfa was given to cows by force feeding just before milking, the following results were obtained: during the first twenty minutes, no feed flavor was present; a slight feed flavor at twenty to twenty-five minutes; and a noticeable feed flavor and odor at the end of

thirty minutes. The most distinct feed flavor was present from forty-five to sixty minutes after drenching.

Roadhouse, Regan, and Mead[51] found that there was a marked difference, as to the off-flavors present in individual cow's milk, when fed feeds of the same kind and amount. A pronounced flavor was found in the milk when alfalfa hay and green alfalfa were fed to a group of cows one to four hours before milking, and a less pronounced flavor was found when fed four hours or more before milking.

Most of the soluble feeds give the intensity of their flavor beginning within thirty minutes after feeding and reaching a maximum at the two or three-hour period. Practically all of this flavor has gone after the fourth hour. Under this division may be found pasture grasses and most of the hays and corn silage, all of which impart distinct feed flavors to milk. These flavors are usually not objectionable to consumers, but in some cases the milk is rejected. Hay and silage should, where conditions will warrant it, be fed after milking. These flavors may also be reduced by immediate cooling at the barn over surface coolers and by pasteurization of the milk.[53]

Succulent feed flavors are more commonly found in milk than those flavors caused by concentrates or hay. Silage made from corn, alfalfa, soy beans, cabbage, turnips, rape, and kale materially affect the flavor of milk; rye, cowpeas, potatoes, dried beet pulp, and carrots affect the flavor only slightly; and corn, oats, peas, pumpkins, and sugar beets have very little effect on the flavor.

Where proper feeding methods are employed, the intensity of the flavor

of most feeds will not be noticed by the average consumer. However, when very strong feeds are consumed in unusual large quantities, cabbage for example, the flavor of the milk will be affected in some cases for twelve hours after feeding.[53]

Feeding the cows at times when least objectionable flavors will result very often conflicts with other chores around the dairy. This gives rise to some of the improper feeding practices which are commonly followed.

Roadhouse and Henderson[50] found that very few of the concentrate feeds commonly fed to dairy cows produced objectionable flavors. They report a pleasing rather than an objectionable flavor in most instances. With wheat bran fed in quantities of from 4½ to 7 pounds one hour before milking, they reported a pleasing flavor. There was more flavor produced in the milk when the bran was fed than in the control samples where no bran was fed when other feed had been withheld for five hours previous to milking. The flavor, however, was an improvement over the control samples. Where 3½ pounds of dried beet pulp were fed one hour before milking, there was apparently no change in flavor, but when this amount was increased to 5½ pounds one hour before milking, a slight off-flavor was present, though not objectionable. Seven pounds of dried beet pulp produced milk with a slight off-flavor. It was not as desirable as the control when fed both one and two hours before milking, but this flavor would not ordinarily be noticeable to the consumer. Besides, 7 pounds is more than most feeders recommend.

Where a grain mixture consisting of 100 parts barley, 50 parts wheat

bran, 20 parts coconut meal, 2 parts salt, and 3 parts bone meal was fed in 5-pound quantities two hours before milking, a flavor was produced which was a little less pleasing than the control samples. With 7½ pounds of this mixture fed two hours before milking, the off-flavor was more pronounced. When 1 pound of cotton seed meal was added to the preceding ration, all samples gave a satisfactory flavor but left an unpleasant after-flavor.[50]

Besides the common feeds for cows, there are many weeds which produce undesirable flavors and odors in milk. When such weeds are present on pastures, the cows should be kept off, where conditions will permit, until the weeds are eradicated. When the cows are permitted to graze where these weeds are present, they should be removed several hours before milking. Garlic flavor may be somewhat reduced if cows are removed from seven to ten hours before milking, while bitter weeds may take a longer time.[3]

One of the most common objectionable flavors in milk, cream, and butter is that caused by garlic or wild onion. When cows are first turned on pasture in the spring, great care should be taken to prevent their eating onions. In many cases it causes great losses to the producer by his milk being returned. When the flavor has entered the milk, it is very difficult to neutralize or remove. Babcock[4] made a very extensive study of this to determine: (1) in what way the flavor entered the milk, (2) the length of time required after consumption for the flavor and odor to be noticeable, and (3) the length of time after consumption that the flavor would remain in the milk. He concluded that garlic flavor and odor were present in the milk one minute after the feed was consumed. The intensity

of this flavor increased with the time up to ten minutes. A very strong garlic flavor was present where cows were allowed to eat one pound of garlic four hours before milking. The intensity of the flavor was inversely proportional to the time interval between consumption of the feed and milking. Allowing the cows to inhale garlic odors also produced undesirable flavors. Where blood tests were taken from cows which had been fed garlic thirty minutes beforehand, positive results were reported. Stronger odors were found where forty-five minutes had elapsed.

MacDonald and Crawford[35] found that the onion flavor in milk was largely associated with the fat. This produces the greatest injury to cream - the most valuable part of the milk. Boiling, steaming, or blowing air through the milk will take out part of this flavor, but these methods injure the milk. The public health service forbids the addition of anything to milk - even clean water. This makes the use of chemicals out of the question.

Mineral oil with such a specific gravity to insure a good separation is suitable for removing onion or garlic flavor from milk. It is an excellent solvent for the material which is responsible for the onion flavor in milk. This oil separates from the milk and leaves no foreign material in the milk.

Their[35] recommended procedure is very simple. All that is necessary is to dilute the off-flavored milk with a good grade of mineral oil, four parts milk to one part oil, mix the two together well, allow the mineral oil to separate, and remove it from the milk. One treatment will usually

remove a slight onion flavor, while a second treatment is recommended for milk containing a strong onion flavor. This double treatment process necessitates the use of new oil for the second treatment if best results are desired. The mineral oils may be washed and used several times, thus reducing the cost.[35]

MacDonald and Crawford[36] found that the bitter flavor caused by the common dog fennel was associated with the milk serum and not the fat, as was the case with onions. This makes the problem even more complex. It can be washed from cream by using skim milk which is free from the bitter flavor, but this would not be recommended for removing it from milk, because this would require separation. Talbut[53b] says that a combination of mowing, fertilizing, and careful grazing will help to check the growth of bitter weeds.

Wylie[59] of Tennessee conducted an investigation to determine the practical value of the MacDonald process for the removal of onion flavor from milk and to determine the relation of the process to commercial possibilities. Briefly, the procedure was the addition of 5 pints of mineral oil to 7 gallons of milk and the mixture heated to 115° F. in a 40-gallon pasteurizer vat. The mixture was allowed to set undisturbed for three minutes for the oil to rise. The milk was then drawn through a "cotton pad strainer" and examined for onion flavor. "There was little if any evidence of onion flavor or odor in the milk." The milk after a second treatment showed no onion flavor. The oil was prepared for use again by washing with cold water, a 10% solution of washing soda, then heating with live steam until it was sterilized. There was less than one per cent of the milk lost and less than three per cent of the oil lost during the treatment.

Abnormal Conditions of the Cow.

Among the undesirable flavors which may be attributed to abnormal conditions of the cow are those resulting from udder disturbances and those which may be found in milk drawn from cows which are advanced in lactation, particularly those which have been milking more than a year. These flavors are not so easily recognized by the consumer, but may be noticeable to the extent that he would know that something was wrong with the milk.

Cows affected with udder disturbances resulting from inflammation of the udder tissues may produce milk with an abnormal composition which would influence the taste somewhat. When these chronic inflammations of the udder are present, the milk from the infected quarters may taste salty for the remainder of the lactation period.[50]

According to experiments by Roadhouse and Henderson[50] to compare the chloride percentages in the milk from the quarters which were known to be affected with mastitis with that from quarters which had not been affected with mastitis, it was found that the chloride content of the infected quarters was higher in every case, and that in most cases it was over 50% higher. The lactose per cent in each case was found to be lower, and consequently, a high chloride-lactose number in each case. The milk secured from the quarters of the udders which were infected in chronic rather than acute conditions appeared to be normal when the samples were tested for composition. When compared with milk from the other quarters it had a salty taste. The flavor was not as pleasing as that from the control group of cows.

A similar salty taste is the most common defect found in milk from cows advanced in lactation period. When this taste accompanies advanced lactation, the milk from all quarters is affected. The recommended procedure is to cease milking the cows that are giving these results. When they are again fresh the milk will, in most cases, be normal.[50]

Handling of Milk.

The flavor of milk after secretion by the cow usually cannot be improved. On the other hand, there is a natural tendency for it to deteriorate. It is the problem of the producer, the distributor, and the consumer to preserve the natural flavor of milk, and to prevent the development of undesirable flavors which are so closely associated with improper cooling, inefficient sterilization of equipment, exposure of milk to sunlight or hot metals, and unsanitary surroundings in general.[50]

Unclean Flavors. The dairy farmers of the United States suffer great losses each year because too large a proportion of the milk they produce is inferior in quality. The estimated loss is many millions of dollars. Some of the primary causes for this great loss are sour and off-flavored milk and cream. These inferior products are not readily marketed, and when the dairyman does find a market, he usually gets a comparatively low price.[30]

Milk of high quality not only brings better prices but it also helps to increase consumption. Milk of high quality may be defined as that milk coming from healthy cows, is of good flavor and free from dirt, and contains very few bacteria, none of which are harmful. This does not mean that milk is "clean" in the strict sense of the word because that would exclude milk

which contained any bacteria or foreign material whatever. All milk, except that produced under very exceptional conditions, has some bacteria present.[30]

Disease producing bacteria are not so common in milk. When they are present they may be attributed to diseased cows, unhealthy attendants handling the milk, or contaminated surroundings caused from water, flies, or filth. Relatively large numbers of bacteria get into the milk from neglect of proper cleaning of the cows, and lack of cleaning and sterilizing the milk utensils. The use of small top milking pails helps to reduce the number of bacteria and amount of dirt getting into the milk.

Bacteria find ideal conditions and food material in milk for rapid growth, unless the milk is held at low temperatures. The minute organisms are single-celled plants which cannot be seen with the naked eye. Some of them divide to form two bacteria at maturity, and, under favorable conditions, each of these repeat the process of division in thirty minutes, thereby resulting in two additional grown individuals. The optimum temperature range for the common types of organisms to develop in milk is from 80° to 100° F. Considerable multiplication takes place at 70° F., while at from 40° to 50° F. the growth is retarded.[30]

Many of the common types of organisms found in milk do not cause a change in the flavor. Other types might change the flavor with no apparent change in the appearance of the milk. Some of the most common types of bacteria change both the flavor and appearance of milk. As a result of these organisms producing acid from the milk sugar, many different flavors may occur. Where proper control measures are employed,

the flavors produced from bacterial growth should be reasonably clean. However, where this growth is associated with filth and dirty surroundings the flavors are usually very unclean.[30]

Pasteurization. While no definite figures are available to show what percentage of market milk sold in the United States is pasteurized, no one will dispute its rapid increase. This increase alone represents the endorsement of the general public. The larger cities are more inclined toward pasteurization than the smaller ones, probably due to the relatively long period between production and consumption, and the fact that epidemic diseases are more common in the larger cities.[29]

Pasteurization is desirable for bacteria reduction; however, the cream line and the change of the flavor have led to some objection on the part of the consumer. If pasteurization is to be generally adopted, it must be accomplished at temperatures low enough to avoid cooked flavors. This can be done only with the most accurate equipment and under the supervision of experienced operators. The solution to the problem of avoiding cooked flavors is the adoption of some method which does not depend on uncertainties for heat conduction.[23]

Oxidized Flavors. There are several terms in common use to describe the flavor sometimes found in market milk due to oxidation of the milk fat. Among them may be found oxidized, metallic, cappy, cardboard, oily, tallowy, and papery. This flavor may be caused in a number of different ways, or by a combination of factors, and yet the flavors produced are somewhat similar. This flavor varies in its intensity with the time of exposure to adverse conditions and its stage of development. It can

usually be traced to the contact of milk with certain metals. Some of these metals are acted upon by the milk in such a way that a small amount of the metal is dissolved. This in turn forms metallic salts which constitute the undesirable flavor.[50]

Since pasteurization has been so generally adopted, this flavor has become more noticeable. This may be partly explained by saying that the milk comes in contact with more metal than before pasteurization, and the metals are more soluble in hot milk. Refrigeration, which permits longer holding of the milk both by the distributor and the consumer, thus allowing more time for this oxidized flavor to develop, has been another factor to cause milk to develop these objectionable flavors.[50]

Guthrie[24] found that certain cows have produced milk which developed an oxidized flavor after one or two days in storage. This apparently was true when there was no contact with metals or no exposure to sunlight.

Roadhouse and Henderson[50] recommend the use of chrom-nickel-iron and chrom-nickel-alloy in milk vats and other equipment with which hot milk comes in contact. Copper, brass, bronze, monel metal, and nickel silver caused oxidized milk flavors in their experiments, and a loss in weight when brought in contact with hot milk.

Where milk is exposed to direct sunlight in clear glass bottles, it develops a definite oxidized, or tallowy, flavor. This flavor is noticeable to the consumer, though he may not detect the cause. When exposed for only a short time, ten minutes during summer months, this flavor may affect palatability. This is unlike oxidized flavors due to metals which develop in from one to three days.[50]

Creaming of Market Milk.

Market milk is very often judged by the consumer according to the amount of cream appearing on the bottle. The amount of cream forming on milk is of special commercial importance. The distributor stresses the cream layer as a sales advantage. It gives the consumer some idea of the richness of milk in regard to its butterfat content. However, this is sometimes a poor indication of the per cent of butterfat in milk. The consumer very often removes part of the upper layer for table cream. This gravity separation was once depended upon for obtaining cream for churning, but the centrifugal separator has about eliminated that practice.[10]

Another very important consideration for creaming properties of milk lies in the fact that the range of temperatures is very narrow between where pathogenic bacteria are destroyed and where the cream layer is shortened. Ordinary pasteurization temperatures (142° - 145°) do not materially affect the creaming ability of market milk when the holding time is not longer than thirty minutes. Temperatures above 145° F. for thirty minutes, or above 165° F. momentarily, produce a permanent precipitation of the calcium salts present in milk which do not dissolve when the temperature is lowered. The precipitation of these calcium ions partly destroy the creaming properties of milk.[10]

Dahlberg and Marquardt[10] say that the volume of cream rising on milk depends upon: (1) the per cent of fat in the milk before creaming begins, (2) the per cent of fat left in the skim after separation, and (3) the per cent of fat in the layer above the cream line.

Formation of Cream Layer.

The fat in milk exists as very small round globules, which rise to the top because they are less than nine-tenths as heavy as skimmilk. The fat globules, when separated as individuals, rise only a small fraction of an inch per hour. The time required for an individual globule to rise the entire depth of a milk bottle (approximately 7 inches) would be around 226 hours, or 9½ days. Since cream usually forms a good layer in two hours, it is self evident that the fat does not rise as individual globules.[10]

There is no conslusive evidence to prove the cause of the aggregation of fat into clusters, yet it is certain that these clusters are formed. They are very irregular in shape and size, and are loosely held together by some unknown force. Milk with excellent creaming properties has many comparatively large clusters which rise faster than the smaller ones. It is the rate of rising of the smaller clusters which determines the rate of creaming, because they must rise before a distinct line is formed, indicating complete creaming.[10]

Dahlberg[11] explains the formation of these clusters of fat globules as being the neutralization and combination of the minute particles of fat as a result of certain physical and chemical changes produced when the negative anions attract the positive cations. This causes the fat globules to come together in clusters.

The calcium salts in milk are present in such great amounts that they are not all dissolved, but some remain in suspension. In warm milk there is a maximum number of calcium ions and thus a maximum number of positive charges, because the solubility increases with temperature, thereby reducing

the formation of fat clusters. When the warm milk is quickly cooled to 40° F. or below, the calcium ion movement is reduced and clumping is speeded up. In that way the theory of best creaming at low temperature has been advanced.[10]

Cooling and Holding Temperatures.

Dahlberg and Marquardt[12] found that cooling temperatures were among the greatest influences on the creaming ability of market milk. On raw milk with good creaming properties, the maximum cream volume may be reached in two to four hours. This volume may be gradually decreased with age for the next twenty-four hours. The cooling and holding temperatures affect the rate at which the cream rises, the maximum length of the layer, and the shrinkage which occurs after the creaming process has been completed.

For ideal creaming, milk should be held at temperatures of from 35° to 40° F. Where milk was allowed to cream at an average temperature of 38° F. for 24 hours, a cream volume of 4.2 per cent for each 1 per cent of fat in the milk was found, while at 58° F., milk from the same samples gave cream volumes of 2.8 per cent for each 1 per cent of fat.[13]

Hammer[26] found that a greater cream volume appeared on milk when held at temperatures somewhat near that of ice water, than on milk at higher temperatures. When milk is creamed at room temperatures, there is a tendency for the fat to pack closer together. He concludes that the original temperatures, at which milk is held before creaming begins, have little affect on the cream layer. Separation and remixing causes a slight decrease. Milk creamed at low temperatures, then allowed to stand at room

temperatures, showed a noticeable decrease in cream volume, approximately 8% in raw milk. A marked increase of cream volume is noted on milk creamed at room temperature for 24 hours, then held at ice water temperatures for creaming. This increase, 50% in some cases, is possibly due to age.

Trout[57] found that a greater cream volume appeared on bottles creamed in air at 40° F. than when creamed in ice water at 32° F. at the 24-hour period. However, the creaming efficiency, as shown by the amount of fat remaining in the milk serum, was in favor of the ice water temperature. He explained this by saying that there was a greater concentration of fat in the cream layer creamed in ice water than when creamed at 40° F. Likewise, the fat test was lower in the milk serum creamed in ice water than in milk serum creamed at 40° F.

Creaming at 70° F. is a relatively slow process. A distinct line does not appear until after six hours. Cream rising at 70° F., when examined under a microscope, reveals a greater percentage of large fat globules than cream which rises at 32° to 40° F. The percentage of fat found in the cream layer where milk was allowed to set at 70° F. was higher than where creamed at 32° F.[57]

Raw milk creamed at 32° F. showed that 92% of the total cream had risen after two hours, 98% after four hours, and the maximum, or 100%, after six hours. At the end of 24 hours the volume had shrunk to approximately 90%. When creamed at 40° F., raw milk reached a maximum volume after 24 hours. In most cases where large volumes of cream appeared in a short time, the cream seemed to be bulky or coarse, and a decrease in volume resulted between the sixth hour and 24-hour periods. A shrinkage in cream volume of approximately 10% was found between the sixth hour and 24-hour period when raw milk was creamed at 32° F.; whereas, that creamed

at 40° F. showed an increase of over 3% in cream volume during the same periods.[57]

Pasteurization Temperatures.

The common practice for market milk plants of today is to pasteurize the milk at temperatures of from 140° to 145° F. and hold it at these temperatures for 30 minutes, after which it is cooled to 40° to 50° F. This procedure often affects the cream layer. Any procedure which will retard the natural rising of the cream should be of especial interest to the plant operator.[57]

Trout[57] found that milk pasteurized at 145° F. for 30 minutes showed an average depth of cream layer of approximately 14% when creamed at ice water temperature, as compared to 11% at 40° F., and 6% at 70° F. The per cent of fat remaining in the serum of pasteurized milk when creamed at 32° to 40° F. was more than twice as great as that found in the serum of raw milk creamed under similar conditions. Very little difference in the fat percentage remaining in milk serum was found in pasteurized and raw milk when creamed at 70° F.

Hotis and Babcock[29] say that heating milk to 140° F. for 30 minutes will kill the pathogenic bacteria present, provided all of the milk is heated to that temperature and held throughout the 30 minutes. There is a tendency for milk plant operators to pasteurize as near the minimum required by law as possible to avoid cooked flavors and so as to not injure the cream line.

The United States Department of Agriculture recommends that all of the milk be heated to a temperature not lower than 142° F. and held at

this temperature for not less than thirty minutes. When this is properly done, an ample margin of safety is not only insured, but a greater destruction of bacteria will result than when milk is pasteurized at 140° F. and held for the same period of thirty minutes. Only about 1% of the bacteria in milk remain alive when milk is pasteurized at 142° F. for thirty minutes.[29]

Parker[45] confirmed the statement of Professor Peter, of the Swiss Dairy School in Berne, that the quickness of creaming as well as the volume of cream increased up to a pasteurization temperature of 141.8° F. Beyond this point these properties decreased, but even at 145.4° F. they were greater than in raw milk.

Dahlberg and Marquardt[10] state that there is no known way of restoring the creaming properties of milk when pasteurization temperatures were over 145° F. for thirty minutes, or 165° F. momentarily.

Marcussen[37] points out that the temperature of pasteurization exerts an influence on the quantity of cream rising on pasteurized milk. He says that the volume of cream rising on milk which has been heated to 145° F. for thirty minutes is always less than the volume of cream rising on the same milk when pasteurized at 142° F. for thirty minutes. The average decrease in volume when pasteurized at 145° F. was 13% as compared to that pasteurized at 142° F.

Trout[57] states that pasteurizing milk at 145° F. for thirty minutes decreases the cream volume from 9 to 16%, the decrease depending on the temperature at which the milk was held for creaming.

Marquardt and Dahlberg[38] made a study of the creaming of milk pasteurized at high temperatures and found that the impaired creaming properties were more noticeable and the differences were greater at readings taken at the end of the second and fourth hours. However, a permanent reduction of the cream layer was noted in most cases where temperatures of 150° - 165° F. were used, their explanation being that the common methods of control were insufficient to hold all of the milk at uniform time exposures.

Holding Time.

Either method of pasteurization, whether it is the high temperature short holding or low temperature long holding process, is satisfactory for creaming of milk, provided the time of exposure to heat is in the proper proportion to the temperature. By this is meant that milk heated to 142° F. for thirty minutes retains very good creaming properties. Temperatures higher than 142° F. for thirty minutes seem to retard the natural rising of the cream. Holding periods for more than thirty minutes at 142° F. also seem to retard the natural rising of the cream. Furthermore, it is possible to heat milk as high as 160° F. for a period of twenty seconds without affecting the cream volume; whereas, the next twenty seconds of holding at 165° F. greatly reduces the cream volume.[38,29] Marquardt and Dahlberg[38] found that the holding time after the milk had reached the 145° F. required more attention when pasteurizing at high temperatures, than up to that temperature. Dahlberg and Marquardt[13], after a very extensive study of the influence on the time that milk was held at pas-

teurizing temperatures, concluded that the reduction in cream volume was a "time-temperature relationship". They found that a longer time was necessary for complete creaming where milk was held longer than thirty minutes at pasteurization temperatures. For example, milk heated to 145° F. and held at that temperature for 150 to 180 minutes (2½ to 3 hours) showed a greater cream volume at the 24-hour period than at the 2½-hour periods. Marquardt[39] says that frequently the cream line of milk is impaired by pasteurization temperatures which are too high, but numerous other things influence the creaming of milk. He also says that the factors which influence cream rising in milk are very often beyond the control of the distributor.

Agitation and Pumping.

Considerable disagreement exists among recent workers as to the affect of agitation of milk on its creaming properties. Most authorities agree on the critical temperatures at which pumping has the most harmful effect upon the cream volume. Whittaker and others[58] reported that their results would indicate that the tendency appeared to be slightly in favor of holding milk during pasteurization without agitation. However, their results were somewhat inconsistent. Their conclusions were that a reasonable amount of agitation during the thirty-minute holding period seemed to have no appreciable effect on the cream layer . . . . Holding and agitating at pasteurization temperatures had considerably less effect on the creaming properties than agitation at 60° F. to 100° F.

Kilbourne[32] reported that one of the most important factors influencing the creaming ability of milk was the amount of agitation to which the milk was subjected, especially while hot. Trout[57] and Dahlberg[10] found that agitation at pasteurization temperatures had very little effect on the creaming of milk.

Trout[57] found from an average of 92 trials that milk pasteurized at 145° F. for thirty minutes gave a cream volume equivalent to 85% of the original raw milk when samples were taken from the vat. When the milk was pumped at 145° F., a slight decrease of approximately 2% was evident. The samples taken from milk cooled back to 135° F. in the vat, then pumped at this temperature, gave a cream volume equivalent to 90% of the original raw sample. Other series of samples taken at 125°, 115°, and 105° F. gave similar cream volumes, but where the milk was pumped at 100° F. or below, the depth of the cream layer was reduced materially.

Erb[17] made a study of the effects of agitation on the cream layer of milk, extending over a one-year period. He found that any agitation between the temperatures of 40° and 105° F. seemed to impair the creaming properties of milk, the degree depending on the amount and kind of agitation and the distance from the mean. He also stated that the damage caused by agitation was greater in pasteurized milk than in raw milk.

There is a correlation between partial churning of the fat globules and the reduction of cream line as shown by examination under the microscope.[17] Agitation of milk at temperatures of from 105° to 144° F. had no effect on the cream line, neither did microscopic examination show any partial

churning of the fat globules. Erb[17] suggests that when the cream layer has been impaired by agitation, reheating to 140° F. momentarily will restore its rising. The identity of the fat globules is partly lost when the milk is re-heated to the melting point of fat.

Homogenization.

A comparatively new item in the bottle milk trade has been introduced in some sections of the United States; primarily in the North and East. Since this is a new product, information is limited to a few articles in the dairy journals. A complete history was not found, but apparently it is just getting into commercial trade. Some of the distributors are calling it "Homo" milk[53a]. The dairy at Ohio State University has been selling homogenized milk for more than two years with considerable growth in demand.

An interesting editorial recently appeared in one of the popular dairy journals[53a] with reference to the consumer's reaction toward "Homo" milk. The author says that "volumes have been written about why people should drink more milk. Little, however, has been said about making milk so good that everyone would drink more of it".

The process, as described by the writer[53a], involves heating the milk to 142° F., then passing it through a machine that puts a pressure of 2,000 to 3,000 pounds per square inch upon the milk. The fat globules are thereby broken up into many smaller particles. In this pulverized condition the fat globules do not clump together to form a cream layer. The milk is then pasteurized and bottled in the usual manner.

Dieticians have long contended that the more finely divided food was, the easier it would be digested. Theoretically, "Homo" milk should be more readily digested than regular milk.

This type of milk is ideal for infant feeding and is gaining in popularity for cooking as well as for general home consumption.[53a]

Bacteriology of Market Milk.

One of the most important factors to consider in the handling of market milk, intended for human consumption, is that of bacterial control. A great deal of attention has been focused on this problem recently, especially where a system of strict grading has become effective. Additional information on this subject should prove to be interesting and beneficial.

Bacteria are minute organisms so small that they cannot be seen without the use of a microscope. It was once believed that they belonged to the animal kingdom, but it is now agreed that they are small one-cell, colorless plants. They are normally found in milk, air, water, and practically everywhere. Normal milk, however, before any contamination, contains relatively few bacteria when drawn.

The problem of bacterial growth is of especial interest to the dairy industry and should be understood and properly controlled by skilled operators. While some bacteria grow at temperatures commonly used for pasteurization of milk, and others at the freezing point of water, a temperature range of from 70° to 100° F. with an optimum of around 95° F. is favorable for the development of most bacteria. The common elements necessary for bacterial growth are moisture, food, and oxygen.[31]

Evans[18] found that the bacterial flora of milk from apparently normal udders ranged from 0 to 300,000 per cubic centimeter. There were several different types of organisms present. Most of them were of the streptococcus

groups. About 15% of the samples contained so few bacteria that their significance was negligible.

After milk is drawn there are many sources of contamination. Some of the more common ones are the body of the cow, the air, the attendant, and the utensils and equipment. Specific disease producing organisms may be introduced from the attendant, flies, contaminated water supply, or manure.[31] In studying the action of bacteria on milk, several influences may be considered.

Methods of Analysis.

It would not only be impracticable, but physically impossible to actually count the number of bacteria in a quart of milk or even in a drop of milk. In analyzing milk for bacteria, recourse must be had to the making of estimates of numbers, not actual counts.[8]

The methods commonly employed for estimating the number of bacteria in milk are: (1) direct microscopic, (2) Agar plate counting, (3) Frost little plate, and (4) Methylene Blue Reductase test. All of the above mentioned methods have their advantages and disadvantages. However, this should not imply that none of them are satisfactory, because each of them has a place in the control of bacteria in milk.

Microscopic Methods of Counting Bacteria. One of the easiest ways of counting objects is by direct observation. This is almost impossible with the case of bacteria because they are too small to be seen with the naked eye. However, since the discovery of the microscope, more accurate estimates of bacteria may be made by direct observation. This method of estimation is comparatively new and has proved very valuable. There are

certain optical limitations when employing the microscope, possibly due to the (1) difficulty of inaccurate measurements in such minute quantities required for observation, (2) presence of dead cells, and (3) growth and division during preparatory steps for examination.[8] On the other hand, some idea of the types of organisms present may be had and results are quickly obtainable.[27]

Agar Plate Methods of Counting Bacteria. This method employs the use of inoculating some nutrient jelly, which is transparent, with measured quantities of the milk to be analyzed. This mixture of the nutrient is then incubated until the original bacteria have produced sufficient growth so that they may be counted with a low-power magnifying lens.

This method is an improvement over that introduced by Koch in 1881, which was conducted with the use of gelatin as a means of isolating pure cultures of bacteria. Nutrient agar has supplemented gelatin for analysis of milk largely through the work of the laboratory section of the American Public Health Association. It is now recognized by most authorities as a very satisfactory growing media.[8]

A modification of this method was introduced by Frost[27] about 1915, who employed the same media and preparations as are used in the large plate method. The greatest distinction being that in the "Little Plate" method, the colonies which develop on the media are counted under a compound microscope before they could be seen with the unaided eye. Its advantages and disadvantages are about the same as for the regular agar plate method.[8]

Some of the limitations for the culture media methods of estimating the bacteria in milk are:

a. All bacteria do not grow under the prevailing conditions.

b. Some colonies may grow so rapidly that other colonies are depressed, therefore an inaccurate estimation would result.

c. Growth from individuals cannot be distinguished from growth produced by clumps or clusters.

d. The possibility of contamination from technique is always present.

e. Measurements for dilutions cannot be absolutely accurate.

Of the above, faulty technique may have one of the greatest influences on inaccuracy. On the other hand, skill and care may reduce the difficulties to a minimum, but these handicaps have not yet been completely eliminated.[8]

Brew[8] concluded that actual counts in numbers of bacteria in milk were impossible as well as unnecessary. The plate method or the direct method may serve as an index to the quality of milk at hand, but neither of them will indicate the actual number of bacteria present. Laboratories are fortunate in getting the comparison of real value that they do find with the inaccuracies that are known to exist.

There is no necessity for knowing the actual number of bacteria in a given sample of milk. It matters not whether a sample of milk contains 5,000 bacteria per cubic centimeter or 6,000 bacteria per cubic centimeter. However, it is desirable in many instances to know the bacterial index of a milk supply. That is, if it would fall in a relatively high, medium, or

low class. Either the plate method or the direct count may be relied upon to give this information.[7]

Comparison between the microscopic and agar plate methods of counting. Breed[8] says that with the knowledge of conditions and technique under which both tests are conducted, the common assumption that the agar plate method gives more accurate results than the direct method has no basic value. He points out that for the plate count to be accurate it must not contain more than 300 colonies. This maximum must be adjusted by dilutions and measures which introduce error. Consequently, the plate method would be accurate only when low count milk was examined. In contrast to this, the direct microscopic method requires only a small but constant amount of milk regardless of the expected count. Therefore, it is less subjected to error in milk with high counts. Breed,[8] in summarizing the above, says that a failure of some investigators to appreciate these differences has caused unjustified criticism for bacterial analysis, but both methods are satisfactory.

The Methylene Blue Reductase Test. The reductase test is based on the fact that the color imparted to milk by the addition of a small amount of methylene blue solution disappears more or less quickly, depending on whether the bacterial content of the sample of milk is high or low. By the use of solutions of this dye made up to a definite strength and added in known dilutions, consistent results may be expected. However, it is very difficult to estimate the number of bacteria present in the sample of milk.

Those bacteria commonly found in milk but which do not develop very rapidly are of no commercial importance. Therefore, a method which determines the actual number that will grow in milk is of most importance to the fluid milk industry.

A brief consideration of all the methods suggested for determining the bacterial estimation would seem to indicate that the reductase method should come nearer to measuring the bacteria growing in the milk than any of the other methods, the principle being that the color disappears in proportion to the growth of the bacteria. This test, however, is made at temperatures at which milk should not be held. The growth and development of some types of organisms may result which, when held at comparatively low temperatures, would not function.[28]

One logical way to analyze the basic soundness of a given test would be to do as the chemist does; that is, to make up samples of solutions of known strength and conduct the test on these to determine if the results are consistent. The plate count makes use of this to some extent by making the different dilutions into sterile water blanks before plating.

It has been shown by Barthel, 1917, that sterile milk free from oxygen, upon heating or by passing hydrogen, nitrogen, or carbon dioxide through it, will reduce methylene blue solutions in about two hours when protected from atmospheric oxygen. This indicates that some constituent of the milk has a reducing power. If the milk alone did the reducing, about two hours would be a minimum time, while if the bacteria form a reductase (an enzyme), as is the case, the reduction time would be much shorter.[28]

The outstanding differences between the reductase test and the other methods for approximating the bacterial content are:

1. The agar plate and little plate methods give us counts, while the reductase test gives us an estimation or an index to quality.
2. The methylene blue test is less toxic in dilutions used, thus giving a point in its favor.

For the analysis of milk being delivered to distributing and manufacturing plants, the reductase test is gaining in favor. A supplement to this test has also been proved worthy of consideration - that of the Janus Green. It has been said to have given a more accurate test than the reductase method. The procedure is the same, except one part of the dye is used to 200,000 parts of milk.[28]

All who have made a careful study of the comparison of the plate and reductase test have found that the agreement was not very close. Either of them gives a fair indication of the quality of milk and the care used in its production. There is a direct relation between the reduction time and the leuccocytes present, which would indicate that the reductase method aids in picking out abnormal milk.[49]

A summary of this may be illustrated as follows:

Reduction time in hours - 1, 2, etc.
Leuccocyte in thousands - T
Millions - M

1	2	3	4	5	6	7	8	9	10	10+
12M	3M	1M	800T	300T	200T	150T	45T	40T	18T	13T

Ordinarily, the methylene blue reductase test for thermophylic bacteria in milk is the same as for raw milk, except that the tubes are incubated at pasteurizing temperatures (142-145) instead of the usual 98° F. A small

amount of glymol or light oil, e.g. mineral oil, should be added to form a layer on top of the milk to prevent the absorption of oxygen from the air which may interfere with the test. The results should be recorded in 30 minutes, 45 minutes, 2 hours, and 5 hours. These organisms appear to be associated with milkstone. Possibly, crevices in vats protect them. Pasteurizing milk, holding it for 24 hours, then remixing it with fresh milk, and repasteurizing causes this. At the above incubation temperatures all samples which retain the blue color over 5 hours contain very few themophylic bacteria.[52]

A combination of the methylene blue and fermentation test serves as an index to help to determine the kinds of bacteria present.

1. After reduction incubate 18 hours.
2. If the color is reduced in less than 5½ hours, there is an indication of improper cooling.
    a. A smooth, firm curd indicates growth of lactic acid bacteria.
    b. Channels showing gas are the result of:
        (1) Wet hand milking
        (2) Dirty cows
        (3) Improper cooling
3. If curd has appearance of being chewed up, this may be caused by proteolytic bacteria. These organisms are usually associated with utensils which have not been properly washed and sterilized. Unfortunately, all milk does not come in these classes; the classes overlap, thus requiring some interpretation of results.

The relations between plate count and reduction time as given by the U. S. Public Health Service in the code which accompanies the standard milk ordinance are:

Less than 50,000 per ml.	8 hours
50,000 - 200,000	6-8 hours
210,000-1,000,000	3½-6 hours
Over 1,000,000	less than 3½ hours

The above correlations are for raw milk.

Decolorization will eventually set up because of an enzyme termed aldelhyde reductase. Where a small amount of formalin is added, milk reduces in 10-24 hours.

Organisms found in the udder do not decolorize methylene blue at the same rate as those found on utensils. Neither do they grow as fast as the utensil flora. The organism S. lactis consumes oxygen rapidly, making methylene blue test practical.[44]

Experiments were conducted by Fay[19] to determine the reduction and plate count of milk to which formalin had been added. Dilutions were made where the final would have 1:100 and up to 1:1,000,000 parts of pure (HCHO) formalin. The dilutions were made in the milk and held at 21° C. (70° F.) for 24 hours. The standard plate count on the milk was 18,000. After 24 hours the milk containing concentrations of 1:2,500 or more showed no colonies in the 1/10 dilution plates. There was a very marked decrease in count with a 1:20,000 dilution, slight growth was evident in 1:25,000 dilution, and appreciable growth was evident in higher dilutions. It may be seen from these results that dilutions weaker than 1:25,000 failed to retard bacterial growth sufficiently to keep milk within legal standards.

Fay's[19] results indicated that the amount of formaldehyde necessary to inhibit growth varied with different samples of milk and those samples carrying high counts required relatively large amounts of (HCHO) formalin.

The reduction time for the original sample of milk was 660 minutes (11 hours). Addition of formalin, 1:100,000 parts concentration, shortened the reduction time to 259 minutes or 4 1/3 hours; similarly, the lower dilutions reduced quicker, the 1:1,000 requiring 21 minutes. However, with a dilution of 1:250 the reduction time was 440 minutes or 7 1/3 hours, and the 1:100 dilution inhibited reduction.

Using dilutions of 1:100 to 1:10,000 (HCHO) the reduction time decreased to a few minutes, apparently due to chemical changes rather than biological factors.[19]

The methylene blue reduction method helps to pick out suspicious samples of added formalin; however, it must be followed by more accurate methods to establish proof. Dilutions as high as 1:15,000 - 1:25,000 must be used if growth is retarded without being noticeable to the taste. The addition of formalin would require accuracy to .0125 cc., or 5 drops per gallon. This would necessitate more accuracy than most producers could depend on. Any samples with a low plate count that reduce in less than one hour may be said to be suspicious of HCHO added.[19]

Fay[19], in giving the normal limits of variation of the methylene blue reductase test, says that the results seem to indicate that duplicate samples check closer than duplicate plate counts. He points out that where 1774 tests were made on 19 samples of milk with specific control, the

reductase test gave an 82 per cent agreement to that of the direct count and somewhat the same results for sediment.

The advantages as suggested by Fay[19] are that it is simple, easy to interpret, and requires very little expense for materials. It is a good test for small plants and cities. Many larger cities use it for a general survey, then supplement it with more elaborate tests.

In an abstract given by Devereau,[14a] the results indicated that the comparison of the different methods may be summarized as follows: The reductase test was able to grade the milk into four classes equally as well as any of the others. The Brom thymol blue was also a good indication of quality.

A dipper is just as satisfactory as is a pipette provided it is thoroughly rinsed after each sample, preferably with cold water, then hot water, and then a chlorine solution. After a thorough washing, the tubes should be either steamed, subjected to boiling water, or filled with chlorine water at 200 P.P.M. before using again. The burette is quicker and just as accurate as a pipette for adding the methylene blue solution. The samples may be placed directly in the test tubes and should be started as soon as is practical; if they are to be held over 2 hours, they should be kept at from 50° to 32° F. The samples may be mixed by placing the thumb or a piece of rubber over the mouth of the tube. When milk reduces quickly, use the microscopic test to verify results.[16]

The reductase test apparently is a better indication of quality of milk due to delayed cooling than is the plate count.[19a]

Apparently, the geometric mean is more desirable than the arithmetic mean for recording counts of bacteria based on the time sweet milk will remain sweet. The curve based on arithmetic linear graphs seems worthless.

When used as an index the logarithmetic values of the plate count show less variation on milk with a low bacterial content, and the reduction method shows less variability with milk of high bacterial content. One method seems to be about as accurate as the other when the entire range of keeping qualities is considered. Both are more accurate than the actual bacterial plate count because they conform more to a curve relation.[19]

Hileman[28a] suggested using a single solution stain that was standardized by R. W. Newman. "We are not actively using the methylene blue reduction method for grading milk though we are satisfied it could be used as well as the direct method." Professor Troy of Cornell University spent the entire summer of 1924 in a large plant comparing the direct and reductase methods, and found very good agreement on the whole. Neither of the two methods are entirely satisfactory on low count milk. The plate count is probably more satisfactory than either of the above for milk containing small numbers of bacteria.

Effect of Cooling.

Bacterial growth and multiplication is much more important than the original count. High counts are not commonly found where growth is retarded soon after milking. Table V shows how rapidly bacteria multiply in a warm medium.

Improper cooling, or the failure to cool, is by far the greatest influence on high bacterial counts in milk which is received on the average market. Milk held at temperatures near those at which it is drawn permit bacteria to reproduce and double in number once every half hour. At this rate, one single organism would multiply to over 1,000 in 5 hours. Fresh milk containing 10,000 bacteria per cubic centimeter might contain at the end of 5 hours, 10,000,000. Fortunately, the growth does not take place at such a tremendous rate. Some of the organisms do not develop during the first few hours, while others die without producing any growth. However, there remains a rapid growth when milk is not properly cooled.[31]

Kelly and Clement[31] say that milk should be cooled as soon as it is drawn from the cow. Where cooling is delayed, the multiplication of bacteria takes place, resulting in a high count. Cooling milk in cans by placing it in cold water does not chill the milk as quickly as by allowing the milk to flow over a surface cooler. The quickest method for cooling is the most desirable from a quality viewpoint. In addition to the bacterial control by quick cooling, the surface cooler permits the milk to be shipped more quickly, especially the morning's milk, and saves time in cooling.

Gamble[21] says that water at 50° to 60° F. is available for cooling milk on most dairy farms. With water at this temperature, a very rapid, efficient, and economical cooler may be installed. By selecting a durable set of coils, one that may be easily cleaned, the cost of cooling over a surface cooler should be about the same as for can cooling. Each cow's milk should be cooled just as soon as milking is complete without waiting for the remainder of the cows to be finished. Great care should be given to the operation of the cooler in order to regulate it so that a continuous, thin stream of milk will flow throughout the entire milking period. When it is not necessary to cool the milk to be shipped immediately, an apparatus may be set up to allow the milk to be cooled over a surface cooler part of the way, then finished in a cooling tank. Where this method is practiced, cold water flowing through the tubes cools the milk to within a few degrees of the temperature of the water.

Downs and Lewis[14] found that cooling clean milk with a low count to 50° - 60° F. was a satisfactory procedure, and that cooling clean and medium clean milk to 50° - 60° F. was a fairly satisfactory procedure. When dirty or high count is not cooled below 60° F., the bacterial growth curve shows undesirable progress.

Effect of Pasteurization.

Kelly and Clement[31] say that about 99 per cent of the bacteria found in milk are usually killed by pasteurization. The percentage killed will vary somewhat with the original count and the types of organisms present. The percentage of decrease may not be a satisfactory indication of the quality of milk, because the efficiency of pasteurization may be as high as 99.5%, and the final count still be as high as 100,000 bacteria per cubic centimeter.

The types of organisms present may influence the efficiency of pasteurization due to the ability of some bacteria to survive ordinary pasteurization temperatures. Ayers and Johnson[55] found that four separate groups of bacteria survived pasteurization. Namely, acid formers, inert, alkali formers, and peptonizing organisms. They found further that the percentage of acid formers was increased by pasteurization, and the other groups were decreased. The average number of lactic acid forming bacteria that survive pasteurization is about 2 per cent of the number in the raw milk.

After a good grade of milk has been pasteurized, the types of organisms develop which may cause the milk to appear peptionized without the development of any acid. When the grade of milk is only fair, the acid group may develop and outgrow all of the other groups, and produce a normal curd. A very poor quality of raw milk may, after pasteurization, first become curdled as with a normal curd, then change to a broken-up curd.[1]

Pasteurization of milk at 142° to 145° F. for 30 minutes destroys any pathogenic bacteria that may be present. It reduces the number of lactic acid organisms and thereby adds to the keeping quality of the milk.[31]

Sterilization of Utensils and Equipment.

The sterilization of utensils and equipment is one of the essential factors in the production of milk with low bacteria counts. The minute organisms which very often cause off-flavors, souring, and sometimes human illness, multiply very rapidly on moist surfaces of improper sterilized milk utensils and equipment. Just the mere washing of milk containers is not satisfactory. The surfaces with which the milk comes in contact should be thoroughly washed, treated in some way to destroy the bacteria, and kept dry until ready for use again. They should then be sterilized to prevent contamination of the milk.

Some of the common methods of sterilizing dairy equipment are: (1) hot water, (2) steam, and (3) chemical. All of them have been used very extensively, plant practices being one of the major influences on the selection of the type.

Hot Water. Caulfield, Riddel, and Fay[9] of Kansas say that boiling water is effective as a sterilizing agent only when the utensil may be submerged for several minutes in water that is boiling vigorously. The common practice of scalding utensils by pouring water over the surface is very inadequate as a method of sterilization; however, this is the method most commonly used in Idaho as shown by a survey made by Theophilus and Atkeson[54]. A survey of 264 members of a dairy herd improvement association showed that 236 or 89.4% used hot water. Of the 236 using hot water for sterilization purposes, 216 heated the water on kitchen stoves, and the remainder used various other means. The survey showed that about 4 gallons of hot boiling water was required to properly sterilize the dairy utensils

used by the ordinary small producer which usually consisted of 3 cans, a strainer, a cooler, and the separator parts.

Prucha, Wheeter, and Chambers[47] of Illinois made a very extensive study of sterilization with hot water from the examination of 170 freshly washed cans (unsteamed) which showed the presence of large numbers of bacteria. Had these freshly washed cans been filled with sterile milk, the germ content of the milk would have varied from 197 to 2,557,000 bacteria per cc. with an average of 128,592 bacteria per cc. Their results from the observation on 170 cans suggest that milk cans, when washed in the ordinary manner, contain sufficient germ life to heavily inoculate the milk later placed in them. The results of successive rinsings with sterile water show that while the germ life removed by the first rinsings with sterile water amounts to a considerable portion of germ life in the can, it is by no means the entire germ life present. Accordingly, the germ content, as determined in this manner, is much lower than the actual number of bacteria present in the can under investigation.

In the preceding experiment it was shown that freshly washed cans invariably harbored large numbers of bacteria. Dairy utensils, however, are not commonly used for milk immediately after they are washed. This is especially true of cans in which milk is shipped from the farm to the plant. Such cans are usually washed and steamed at the plant, then covered with the lids and returned to the farm where they are frequently used for milk without any further treatment. Very often, one or two days will elapse between the time the cans are washed and when they are used.

Prucha, Wheeter, and Chambers[47] also studied the germ life in 160 8-gallon cans at the time they would ordinarily be used. These cans were examined after being washed at a particular dairy. 100 of them were steamed while 60 were left unsteamed.

The steaming consisted of holding each can over a jet of steam for 25 seconds at 15-pounds pressure. The pressure of the steam was measured by a guage placed between the valve and the jet opening. Other experiments on steaming cans in this manner showed that if cans so treated were filled with milk immediately afterward, they rarely ever added more than two bacteria per cc. to the milk. 50 of the steamed cans and 50 of those not steamed were inverted on a rack with the lids off. The other 50 steamed cans and 10 not steamed were closed immediately after washing. All the cans were then kept 30 hours in a room having a humidity of 40 and a temperature of 60° to 70° F. The number of bacteria found in each can was very interesting.[47]

The 50 cans that were washed, steamed, and held 30 hours uncovered and inverted on a rack were dry and free from objectionable odors with only a few bacteria present. Only 3 of the 50 cans had more than one million bacteria and 36 of them had less than 100,000. If the bacteria in the 50 cans had been added to 400 gallons of milk, the germ content of the milk would have been increased by only 8 bacteria per cc. Whether any bacterial growth took place in the cans during the 30 hours was not known, but the results show that the cans so treated have a negligible effect upon the germ content of milk.[47]

The 50 cans that were washed, steamed, and held 30 hours with the lids on were still damp and most of them had a pronounced odor. These cans had a much larger number of bacteria than those which were steamed, uncovered, and inverted on the rack. Only 3 of the 50 cans had less than 100,000 bacteria per cc. If the bacteria found in these 50 eight-gallon cans were added to 400 gallons of milk, its content would be increased by 1,816 bacteria per cc.[47]

The 50 cans which were washed but not steamed, and held 30 hours uncovered and inverted on a rack were dry, and none of them had a disagreeable odor. The numbers of bacteria in them were much larger than in the cans steamed and inverted. Only one of the 50 cans had less than one million bacteria, in 24 of them the numbers of bacteria were between one million and ten million, and 4 cans contained over a billion bacteria each. If the bacteria found in all of these cans were added to 400 gallons of milk, its germ content would be increased by 27,164 bacteria per cc. The effect of drying the cans upon the germ life present is evident from a comparison of the above results with those obtained from the freshly washed cans.[47]

Each of the 10 cans which were washed but not steamed, covered, and held 30 hours had a disagreeable odor, and they also contained a large number of bacteria. Nine of this number contained over a billion bacteria each. If the total number of bacteria found in these 10 eight-gallon cans were added to 80 gallons of milk, its germ content would have been increased by 128,730 bacteria per cc.[47]

It is evident from the above results that rapid bacterial growth took place in the cans which were covered and allowed to stand 30 hours.

Bacterial growth in general is influenced by: (1) temperature, (2) food, and (3) moisture. All of the cans in the above experiments were held at the same temperature and were washed in the same dairy by the same operator, so that the principal difference between the covered and uncovered cans was the presence of moisture in the covered cans. These results point to the conclusion that it is very difficult to wash cans so that no bacterial food is left in them, and if the cans are covered without drying and are allowed to stand for several hours, the bacterial growth increases rapidly.[47]

Bacterial studies were made by Prucha[47] and others using cans which were being washed and returned to the farm ready for use. In order to maintain the usual conditions in this dairy, no interference was made in any of the regular operations and the men doing the work were not aware of the experiment. No record could be obtained of the exact treatment of each can, but in general, each can was washed, rinsed, steamed over a jet, and covered with a lid. Steaming of the cans varied from 5 to 20 seconds.

The treatment of the cans at the farms was not uniform. Some of them were inverted on racks with the lids off, and others were not opened until they were used. The time intervening between the washing of the cans and their use varied from 6 to 40 hours.[47]

Just before the cans were filled with milk, they were rinsed with one liter of sterile water, and the bacterial content of this water was determined.

Of the 91 cans examined, 3 showed less than one million bacteria; 57 showed between one million and one hundred million; and 31 showed over one hundred million bacteria per can. If all the bacteria found in the 91 cans

were added to 728 gallons of milk (the total capacity of the cans), the bacterial content of this milk would be increased by 23,523 bacteria per cc.[47]

The above cans were inspected prior to the bacteriological examination and were found to be free from any dirt, and were dry in most cases. From extensive study of cans on a large number of farms and in dairies, the investigators were of the opinion that the 91 cans were cleaner and in a better condition than the average can used for milk.[47]

Steam. The most effective method of sterilizing dairy equipment, according to Caulfield, Hiddell, and Fay[40], is by exposing the utensil to live steam in a closed cabinet for 10 to 15 minutes. Steam jets commonly employed in dairy plants for sterilizing cans, pails, and other equipment are frequently not effective because of the short period of exposure. When exposed to a jet of steam for less than one minute, as is very frequently the case, little destruction of bacteria might be expected. The principal advantage of the use of a steam jet is that the utensils are heated so that subsequently they will become thoroughly dry.

Theophilus and Atkeson[54] made a study using electrically-heated steam sterilizers. They used three sterilizers made by different companies. The sterilizers studied were of the same size (4-can size), similar in construction, and all were of the cabinet type.

The sterilizers were studied from two viewpoints: first, as a means of sterilizing; and second, as a means of heating water for washing purposes around the dairy. In the first part of the study, 4 cans (two 10-gallon and two 5-gallon) used in the University of Idaho Creamery were washed but not sterilized. One of the 10-gallon cans was rinsed with 200 cc. of

sterile water and the bacterial content of this water was determined by the standard plate method. The sterilizer was then operated according to the directions of the manufacturer. After sterilization, the can previously checked for bacterial contamination was again checked by the same method. Sterilizing efficiency was expressed by the percentage of bacteria destroyed.[54]

In 7 of 13 trials with each electrically-heated steam sterilizer, the utensils were held in the sterilizer 20 minutes after the electric heat was automatically cut off by the thermostatic control. In three trials the utensils were held 15 minutes and in three other trials they were held only 5 minutes. The electric energy used in all trials was measured in kilowatt hours. The temperature of the cabinet was measured by an accurate long stem thermometer, the bulb of which was at the uppermost portion of the can.[54]

The average of 7 trials, when the utensils were held in the sterilizer 20 minutes after the maximum temperature was reached, showed each of the sterilizers to be 99.9 per cent efficient. Also an average of 99.9 per cent was obtained in 3 trials when the utensils were held in the sterilizer 15 minutes. The sterilizers showed a sterilizing efficiency of 99.5%, 99.1%, and 99.9% respectively in three trials when the utensils were held in the sterilizer 5 minutes. Not only do the averages of these different trials show a high degree of efficiency, but in no instance in any of these trials with any of the sterilizers did the sterilizing efficiency drop below 99.0%. The bacteria contamination of the original cans, measured as previously outlined, varied greatly, extending to as high as 14 million per cc.

After sterilization, however, the highest bacterial count obtained in 39 trials was 31 bacteria per cc.[54]

These results indicate that the cabinet-type electrically-heated steam sterilizer has a high degree of sterilizing efficiency, that of the sterilizers studied there was practically no difference in efficiency, and that leaving the utensils in the cabinet longer than 5 minutes after the maximum temperature is reached is not necessary. The third point is of particular importance when time is a factor in the sterilizing process, especially when more than one run of the sterilizer is necessary to handle all the utensils.

Eleven trials with each of the sterilizers averaged 22.7 minutes, 25 minutes, and 31.1 minutes respectively from the time the electricity was turned on until the thermostatic control cut off the heat. These sterilizers required 8, 16.5, and 18.0 pounds of water respectively for sterilization, and the thermostatic control operated at 190°, 193°, and 197° F. Thus, the time of operation is related to the amount of water heated for steam and the cut-off temperature. Therefore, when the utensils are held in the cabinet for 5 minutes, the complete sterilizing process varies from 28 to 36 minutes with the three sterilizers.[54]

Passon and Hotis[46] made some studies on the care of milk utensils on the farm. They say that if the steam is evenly distributed in the cabinet sterilizer, and a reliable thermometer in the top of the cabinet shows that a temperature of 200° F. or more has been maintained for 5 minutes or more, it is certain that the number of bacteria on the utensils will be greatly reduced; a period of time longer than 5 minutes gives a margin of safety.

Utensils such as cans, pails, and bottles should always be placed in the cabinet in an inverted position. If they are placed open end up it will take longer to heat the utensils, and the condensed steam cannot drain from them.

Results show that the bacteria contained in 10-gallon cans after they had been washed and rinsed were practically all killed by steaming the cans in a Galvanized-iron Box Steamer. Each of these cans contained on the average at least 80,000,000 bacteria before being treated, as compared to 2,100 after the treatment.[46]

Passon and Hotis[46] say that the effectiveness of the jet depends upon the steam pressure used, the size of the opening through which the steam is ejected, and the length of the time the utensils are steamed. They say that it usually requires about half a minute to steam a 10-gallon can thoroughly if the steam pressure is 20 to 25 pounds. Where a steam jet is used, the utensils should be steamed until they are too hot to handle with the bare hands. After treatment in this manner they will become dry from their own heat if placed right-side-up and left uncovered for a few minutes before they are inverted on the rack.

The influence of an unsteamed bottle filler upon germ content of milk, according to Prucha, Wheeter, and Chambers[47], when the bottle filler was carefully washed and steamed, exerted no appreciable effect upon the germ content of the milk passing through it. When it was similarly washed but not steamed, the germ content of the milk in the first bottles was increased on the average by 96,900 bacteria per cc. The continued use of the bottle filler gradually washed the larger part of the germ life from the machine, thereby reducing the contamination.

Dry Heat. A comparatively recent development is that of so-called "dry heat" sterilization of milk utensils. The method commonly employed is to transfer the utensils directly from the wash vat into a specially designed cabinet which is heated with electricity or some efficient type of burner. This method, when properly used, is very efficient. It has the additional advantage that the utensils are absolutely dry at the completion of the sterilizing process.

Nicholas, Sperry, and Tonney[43] made the following report on Humidified Hot Air Sterilizers: Two pails were used as a check on the bacterial efficiency of this sterilizer. Samples for bacteriological analysis were taken from each pail by rinsing with one liter of sterile saline solution before and after mechanical washing and hot air sterilization. These pails were hand washed in water, using an accepted dairy cleanser, rinsed in clear warm water, drained and placed in the sterilizer for treatment. The results obtained from a direct plate count, using one cubic centimeter of the rinse water as a unit of comparison, show the efficiency to be over 99%.

Dahlberg and Marquardt[14] conducted experiments with an Esco Sterilizer which had an electric heating unit and a mercoid control. Cans were placed in the unit to determine sterilization efficiency at temperatures ranging from 160° to 200° F. for various periods of holding. In this work the cans were washed and then rinsed with 500 cc. of contaminating water containing a miscellaneous milk-grown infection and a high contamination of colon-aerogenese bacteria as indicated by gaseous fermentation. One contaminated can, after draining, was washed with 500 cc. of sterile water. From this sterile water rinse, 0.1 cc. and 0.2 cc., and 1 cc. were plated in duplicate to obtain a knowledge of the bacterial condition of the cans.

In later work, 1 to 100 and 1 to 1,000 dilutions were also made. The results were all given as the bacterial contamination per can, this figure having been calculated from the count on 1 cc. from the 500 cc. (about 1 pint) of sterile rinse water used in each can. After the heat treatment the cans were rinsed with 500 cc. of sterile water, and 0.1 and 2 cc. portions were plated. Also a plate count of the contaminating material was made in duplicate in a dilution of 1 to 100,000.[14]

The contaminating material was prepared as follows: Into a solution containing 1% of raw milk and 2% of dehydrated broth, 1 gm. of dried pulverized cow manure was added per 100 cc. of solution. This solution was incubated for 24 hours at 98° F. This contaminating material was mixed flora representing many types of organisms encountered in the dairy industry.

The data presented in Table I gives the preliminary results in which the temperatures were secured by dry heat and by steam in the sterilizer heated by gas. They show that either steam or hot air can be successfully used to sterilize utensils on the farm. Exposure to a temperature of 212° F. for 15 minutes was sufficient for either steam or hot air. The results show further that a temperature of 230° F. for 25 minutes gave only slightly better results than 212° F. for 15 minutes.[14]

TABLE I

SUMMARY OF INITIAL TRIALS COMPARING STERILIZATION OF CANS BY STEAM AND BY HOT AIR

No. of Trials	Type of Heat	Temperature Degrees F.	Time in Minutes	Bacterial content of sterilized cans		
				Minimum No.	Average	Maximum
5	Steam	212	15	500	4,500	10,000
22	Hot air	212	15	2,500	6,500	13,500
5	Hot air	230	25	0	2,500	10,000

The cans were washed before heat treatment with 500 cc. of water containing in excess of 100,000,000 organisms per cc. After the heat treatment the cans were cooled and rinsed with 500 cc. of sterile water. The bacterial counts represent the total number present in the water after the rinsing.[14]

Chemical Sterilization. Many studies employing the use of chlorine sterilizers in the dairy industry have been made during the past few years. Their introduction was first opposed on the ground that chlorine may be intentionally placed in the milk as a preservative or gain access by accident, thus reducing its quality. Hale and Bleecher[25] state that active chlorine, in hypochlorites, acts as a germicide when put directly into milk and that bacteria counts are reduced in proportion to its concentration in the milk. Zoller[59] studied the rate of decomposition of hypochlorite and showed that sterile milk reduced the active chlorine content as rapidly as milk containing large numbers of bacteria, from which he concluded that chlorine in the form of hypochlorite when placed in milk exerted no selective action upon the chemical complexes of the bacterial cell. He further implies, differing from Hale and Bleecher[25], that the germicidal action of chlorine in milk was negligible in concentrations which did not affect its flavor and odor. The studies of Lochead and Johns[33] and of Purcha[47] tend to support the conclusions that the germicidal action of chlorine is relatively low in the presence of milk. The former subjected it to treatment with various amounts of hypochlorite for 18 hours without materially reducing the bacteria count or changing the curdling time of treated and untreated milks. The latter states that 5% of milk in a chlorine rinse solution practically destroys its germicidal actions.

Baker[5], studying sewage disposal, states that active chlorine showed greater germicidal powers than did other oxidation agents, such as permanganate which possess a higher oxidation potential. He concluded that its ability to kill bacteria was probably the result of reactions other than oxidation. Ayyar[2], Lochead and Johns[33], and Myers and Johnson[42] found that chlorine compounds decreased in germicidal effectiveness with increased alkalinity.

Mudge and Lawler[40] found that the extent of the germicidal action of alkaline solutions was determined largely by the measure of time, temperature, and pH values. Myers[41] found that increasing the pH of alkali washing compound solutions augmented; whereas, a similar increment in chlorine solutions reduced their germicidal effectiveness. He also compared the effectiveness of neutral sodium hypochlorite solutions containing 100 parts per million of active chlorine in water to a solution of 0.5 normal sodium hydroxide and found chlorine to be considerably more effective than the alkaline solution. A neutral sodium hypochlorite solution proved to be much more active than an alkaline solution, destroying 99% of suspended spores in less than a minute; whereas, it took 22 minutes to accomplish the same results when alkaline hypochlorite solutions were used.

Chloramine products have always shown less efficiency than hypochlorites. Prucha[47] reports that 200 parts per million of chlorine from chloramine-T compounds gave good results in practical tests, but equally good results were secured when only 50 parts per million of hypochlorite solutions were used. Lochead and Johns[33] tested representative chlorine compounds by observing their action in reducing the numbers of viable bacteria derived

from pure cultures of the types commonly present in milk and found chloramine products much slower in action than hypochlorites. Myers and Johnson[42] and Myers[41] secured similar results. They recommend higher concentration and longer contact periods where chloramine products are used instead of hypochlorites.

Apparently, the available chlorine content of a solution, when determined by chemical tests, does not give a true picture of its potency when used as a germicide. Myers and Johnson[42] studied the germicidal effectiveness of twelve commercial chlorine compounds relative to their chlorine concentration. Their observations seem to agree with those of Lochead and Johns[33], as well as previous studies made by Myers. That is, their results indicate that hypochlorites which approach neutrality are more effective than the alkali types, although the chlorine concentration of the hypochlorites may be only half as great. The organic compounds studied, although they were acid in reaction, required double the strength of chlorine to give results as effective as those afforded by hypochlorites of low alkalinity.

Tonney, Greer, and Leibig[55] found that extremely low concentrations of chlorine would destroy bacteria when foreign organic matter was absent. B. coli, the most resistant of 503 strains of the 48 species studied, succumb in from 15 to 30 seconds when 250 parts per million were used.

The following compounds were used in experiments by Loveless[34] and the results designated by letters assigned to them: sodium hypochlorite (A), calcium hypochlorite (B), chlormin-T (D), calcium hypochlorite with soda ash (E).

Table II shows the results secured from tests made with three types of hypochlorite compounds to determine their comparative effectiveness in killing the same test organism. The solutions contained approximately 100 parts per million of active chlorine. Triplicate tests were made and the average percentages of these surviving determined.

TABLE II

GERMICIDAL POTENCY OF VARIOUS TYPES OF CHLORINE PREPARATIONS AGAINST B. COLI

Seconds Exposed	[1] Initial Count	Percentages of bacteria surviving			
		Solutions 100 p.p.m.			106 p.p.m.
		A	B	E	D[2]
15	165,000	0	2.0454	3.7621	17.57
30	440,000	0	0.8181	1.0122	3.63
45	573,000	0	0.0000	0.0000	0.311
60	578,000	0	0.0000	0.0000	0.0346

[1] Initial counts of the diluted broth culture used.

[2] Also contained 0.1% H_2SO_4.

The results seem to indicate that the hypochlorites act very rapidly, and that so far as this organism is concerned they are very effective.[34]

TABLE III

PLATE COUNTS ON B. COLI INOCULUM AFTER TREATMENTS WITH VARIOUS CHLORINE GERMICIDES CONTAINING APPROXIMATELY 200 p.p.m. OF AVAILABLE CHLORINE IN COLD SOLUTION. INITIAL COUNT 15,400,000.

(Six trials for each compound)

Compound	Colony plate count after exposure to chlorine			
	15 sec.	30 sec.	45 sec.	60 sec.
E (Average count)	6,133	1,533	67	33
B " "	0	67	33	133
A " "	0	33	0	0
D " "	939,000	89,000	18,233	533

Comparison of the results obtained in these later trials with those in which a solution containing approximately 100 p.p.m. was used show a similar trend but not to the same degree. The increased concentration of available chlorine gave increased germicidal efficiency, even in cold solutions. Acidified and hot solutions were very efficient in the destruction of B. Coli. It would seem that from the results obtained by using sterile water at 180° F., that some of the increased efficiency in the hot solution is probably due to its temperature as well as to its active chlorine concentration or content.

Tests were also made on four germicidal products used in practical farm experiments. Cold water solutions were made up in 200 cc. of sterile water blanks to contain approximately 200 p.p.m. of available chlorine. The same inoculum was used.

TABLE IV

PLATE COUNT ON MILK CAN RINSE INOCULUM AFTER EXPOSURE TO GERMICIDAL SOLUTIONS CONTAINING 200 p.p.m. OF AVAILABLE CHLORINE IN COLD SOLUTION.
INITIAL COUNT OF INOCULUM 3,110,000 per cc.

(Six trials for each compound)

Compound	Colony Plate Count after exposure to chlorine			
	15 sec.	30 sec.	45 sec.	60 sec.
E (Average Count)	10,513	4,567	2,133	433
B " "	133	67	0	0
A " "	0		0	0
D " "	45,200	35,500	15,000	6,933

The results obtained in these trials further indicate a more rapid action of inorganic chloride compounds as compared to organic forms. Liquid sodium hypochlorite apparently has the greatest speed and efficiency of the

compounds tested. The small survival in the case of all hypochlorites tested had a tendency to make this type of germicide preferable to others for rapid rinsing with cold solutions when speed of action and efficiency were considered.

The above results secured by Loveless[34] indicate that (1) when solutions containing 200 p.p.m. of available chlorine were used, the higher concentrations increased the germicidal efficiency in cold solutions; (2) acidified and hot solutions were even more efficient; and (3) where sterile water solutions at 180° F. were used containing 200 p.p.m., the efficiency was apparently greater than in any of the other trials.

Storage Temperature.

The number of bacteria found in milk at delivery time depends very largely upon the temperature at which the milk has been kept during storage. The bacteria commonly found in milk have an optimum growth temperature of around 95° F. At 50° to 70° F. the rate of growth is much slower, and at 40° F. and below the rate of growth is very slow. However, a few types of organisms produce some growth at freezing temperatures.[30]

Kelly and Babcock[30] illustrate the rate of bacterial growth as shown in Table V.

TABLE V

GROWTH OF BACTERIA IN MILK WHEN THE MILK IS HELD AT 50° and 68° F.

Temperatures of milk	Number of bacteria per cubic centimeter				
	at beginning	at end of 6 hrs.	at end of 12 hrs.	at end of 24 hrs.	at end of 40 hrs.
50° F. . . .	10	12	15	41	62
68° F. . . .	10	17	242	61,280	3,574,990

At the rate of growth as shown above, milk with a count of 1,000 would increase to 4,000 in 24 hours when held at 50° F.; whereas, the same milk held at 68° F. would have a count of 6,000,000 bacteria per cubic centimeter.

Hammer[27] found that the storage temperatures not only influenced the rate of growth, but also had an effect on the types of organisms that would develop. At temperatures around 70° F. the development of the S. Lactis organism is very rapid. These organisms tend to utilize the bacterial food at 70° F., thereby checking the growth of the other types present.

Thurston and Olson[56] say that pasteurization of low count milk sometimes produces a renin-like enzyme which causes a slow development of acid, and coagulation of the milk at low acidities, possibly due to the change in bacterial flora. They[56] found no tendency for peptonizing bacteria to outgrow other types at storage temperature of around 40° F. However, there was a decided tendency for the pasteurized-bottled milk to develop a "cappy" flavor after two days storage at temperatures of around 40° F.

All milk should be held at temperatures not higher than 40° F. until delivery. There is only a slight increase in bacteria in pasteurized milk during the first 24 hours when held at this temperature.[29]

Acidity.

High acidity in market milk is an indication that the milk is being soured by the action of bacteria on the milk sugars. This is, of course, a very undesirable characteristic. This acid flavor and odor, when present in milk, is noticeable to the average consumer at an acidity of around .3 per cent, while experienced judges begin to notice the undesirable flavor at around .2 per cent, because it does not have the desired keeping qualities.[31]

Acidity in milk may be attributed to two causes, (1) the presence of acid phosphates, and (2) the formation of lactic acid as decomposition products of milk sugar which has been acted upon by the bacteria commonly found in milk. High acidity may be best prevented by quick cooling to a low temperature, and holding in this state until delivery.[31]

Keeping Quality.

One of the primary requisites of market milk is that of good keeping qualities. The consumer desires milk which will keep from one meal to another without souring. Evidently, the foregoing pages have covered many of the factors which influence the keeping quality of milk. A combination of cooling, pasteurization, and bacterial control largely determine the length of time that milk will keep.

Conn[9a] found that a specimen of milk which had been held in a cold room for four days had a final bacterial count of ten million bacteria per cubic centimeter; whereas, a sample of the same milk after standing in a warm room for seven hours increased a hundredfold.

In order for milk to keep well, the producer, distributor, and consumer must cooperate to prevent exposure to warm temperatures, sunlight, and unsanitary surroundings.

Summary of Survey of Literature.

1. There are many feeds which have a tendency to impart a slight off-flavor to milk.
2. Feeding silage soon after milking produces a better flavored milk than feeding it before milking.
3. Garlic and wild onion usually produce undesirable flavors when cows are allowed to eat them anytime within several hours before milking.
4. Relatively few of the concentrate feeds commonly fed to dairy cows produce objectionable flavors in milk.
5. The flavor of milk after secretion cannot usually be improved; there is a tendency for it to deteriorate.
6. Ordinary pasteurization temperatures have a tendency to injure the flavor and creaming of milk.
7. Raw milk forms a maximum cream layer more quickly than pasteurized milk.
8. The direct microscopic, agar plate counting, and methylene blue reduction test are satisfactory methods for making bacterial estimates in milk.
9. Duplicate samples of the reduction method check closer than duplicates for the other methods in determining the bacteria in milk.
10. In general, the quickest method for cooling milk is the most desirable from a quality viewpoint.
11. Approximately 99% of the bacteria commonly found in milk is killed by pasteurization.
12. Effective sterilization of milk utensils and equipment may be accomplished by the use of steam, hot water, dry heat, or chemicals.
13. Chemical sterilization is apparently the most effective method for use on milk utensils and equipment.
14. Relatively low storage temperatures are desirable for holding market milk.

PART II. ORIGINAL INVESTIGATION

Objects of Investigation.

1. To study the influence of the following factors on the flavor of market milk:
    - A. Aeration
    - B. Pasteurization
2. To study the creaming of market milk as affected by:
    - A. Pasteurization temperature
    - B. Storage time
3. To study the bacterial content of market milk and its relation to:
    - A. Cooling
    - B. Pasteurization
    - C. Sterilization of cans

Plan of Investigation.

This investigation was essentially divided into three sections, so that a detailed study of several of the factors which influence the quality of market milk could be made. All of the factors which determine quality do not enter each milk distributor's problem, even though the fundamental principles are closely related.

A raw milk dealer is not confronted with the partial destruction of the creaming ability of milk due to heating; whereas, this is a common problem in the pasteurization of milk. On the other hand, milk which has been properly pasteurized and given careful attention after pasteurization is said to be free from any danger of carrying disease-producing bacteria.

Pasteurized milk very often has a slight cooked flavor. This flavor is not objectionable to most people, especially after they have become accustomed to pasteurized milk. However, the pasteurized flavor has led to considerable objection on the part of some consumers.

The plan of this investigation included a study of some of the different flavors commonly found in market milk, the causes for the presence of these flavors, and an effort to determine the factors necessary for preventing or reducing the intensity of some of the undesirable flavors by methods practiced by commercial milk distributors.

It was further planned to study the effect of pasteurization temperatures and storage time on the creaming ability of milk by determining variations which would normally be noticeable to the consumer.

An attempt was made to determine the effects of cooling milk on the bacterial growth, the efficiency of bacterial destruction by pasteurization,

and the effect of sterilization of cans as a source of reducing contamination commonly encountered in the handling of market milk. Sterilization, as applied in this problem, shall refer to the partial destruction of the bacteria present.

The plan of the investigation was outlined so that most of the trials could be made on a commercial scale, and not on a laboratory scale which would not be practicable for modern dairies and milk pasteurizing plants.

Methods of Procedure.

The major part of this investigation was limited to problems directly related to the market milk industry. Most of the problems are very common in creameries. The data presented were secured from experiments comparative to methods commonly employed in the commercial routine of handling milk. The equipment used for the experimental work was designed for modern dairies and milk plants, and for technical work in quality control laboratories.

The milk used in this study was obtained from the University of Tennessee dairy herd and the Knoxville Milk Producers' Association. The University herd at the time of the experiment consisted of Holsteins and Jerseys, while the herds supplying the Association were of mixed breeding with the Jersey breed predominating.

Duplicate samples of each lot of milk were taken for each trial for the flavor tests. The samples were held in quart milk bottles except as otherwise indicated. These samples were held in the storage room at 40° F. When the milk was ready to be scored for flavor, it was warmed to a

temperature of from 80° to 90° F. and scored at that temperature.

Eight students, with considerable experience in the judging of milk, were available for scoring the samples for flavor. Three students were used for each series of samples; however, the same three were not always present throughout the investigation. These three students were selected from two different judging teams. Each of the two teams made excellent records at the Students National Dairy Products Judging Contest, 1935 and 1936.

A detailed procedure of the scoring methods may be found in the section devoted to flavors of milk.

The creaming tests were made by two different methods. When the experiment was first begun, the cream volume was measured as it appeared on the bottle. The chief object was that of representing the comparative cream volume of the milk as it could be seen by the consumer. This method, however, was later replaced by the use of 100 cc. graduate cylinders. The preparation and handling of the samples will be discussed under the section devoted to the methods of measuring the creaming of milk.

The bacteria analyses in this investigation were based on the technique as given in the standard methods of milk analysis and recommended by the American Public Health Association. The reductase tests were conducted in the dairy laboratory at the University of Tennessee. The agar plate counting was conducted, in part, in the dairy laboratory of the University of Tennessee, and, in part, by the milk sanitation division of the Knoxville Bureau of Health. The methods used for securing the data on bacteriology studies will be given in the section devoted to the bacteriology of market milk.

Flavors of Market Milk.

Milk is either pleasing, flat, or disagreeable in flavor to the ordinary consumer. Fortunately, the fluid milk industry has established a reputation for good-flavored milk. As a result of the confidence which the consumer has for the palatability of milk, he is usually not suspicious of off-flavors being present. This psychological feature is perhaps more favorable toward milk consumption than for the consumption of many of the other so-called essential foods.

Market milk has the endorsement of most of the leading physicians of the nation. It has consistently gained favor with nutrition specialists and various other health authorities. It is generally assumed that all normal milk is palatable to the consumer. As suggested above, fortunately, milk is usually palatable; however, there are many factors connected with the production and care of milk which have a tendency to cause abnormal and undesirable flavors. These off-flavors have been attributed to abnormal conditions of the cow, improper feeding methods, and careless handling of the milk.

The problem of preventing and controlling off-flavors which are sometimes imparted to milk is somewhat complex, and yet undesirable flavors are seldom reported by the consumer. This is probably due to the scientific knowledge and effort employed by the producer and distributor to reduce the intensity of the flavor to such a point that it is not noticeable. Sometimes the consumer fails to notice the undesirable flavors which are actually present in the milk. Nevertheless, an investigation to include a study of the factors influencing the flavors of milk should prove very beneficial for both the milk industry and the milk consuming public.

Methods of Scoring.

The methods employed for scoring the samples of milk for flavor in this investigation were similar to those recommended by the Rules Committee for the "Students' National Dairy Products Judging Contest". An illustration of the student score card used in 1936 is given in Figure 1. The milk in all of the trials on flavor was scored on a comparative basis using the student score card for a guide. The method of sampling will be indicated later in this section. In order to make this a comparative study of some of the different factors which affect the flavor of milk, a number of preliminary trials were conducted so that good, medium, and poor flavored samples could be selected for the experimental work. These preliminary trials with samples from the different milk patrons shipping to the University Creamery were helpful in selecting: (1) the best flavored milk being received at the time of the experiment, (2) a typical silage flavor, and (3) an unclean flavored milk. These series of samples were selected for the purpose of studying the effect of aeration on the flavor of milk.

Influence of Aeration.

Many investigators have found that aeration during the cooling process helped to reduce the off-flavors imparted to milk by certain feeds.

Aeration may consist of allowing warm milk to flow over a surface tubular cooler containing circulating water, or water and brine as the cooling agents. This practice rests on the theory that when warm milk is exposed to the air in thin films, the air has a tendency to remove some of the feed flavors which are often present.

MILK SCORE CARD

For Students' National Contest in Judging Dairy Products

Contestant No. _____ Sample No. _____

	Student	Official	Grades		Criticisms
			Score	Criticism	
Flavor (25)					**Flavor** Bitter, Cardboard, Cooked, Cowy, Disinfectant, Feed, Flat, Garlic or onion, High acid, Malty, Metallic, Musty, Rancid, Salty, Unclean, Weedy
Sediment (10)					**Sediment** Score cottons as per photograph in U. S. Department of Agriculture Circular 384.
Bottle and cap (5)					**Bottle and Cap** Absorbent cap protector, Chipped mouth, Dirty bottle, Leaky cap, Lip not protected, Lip partially protected, Loose cap cover, Not full
Placing					
Grade on criticism					
Total Grade					

8—7813 U. S. Government Printing Office

Figure 1. Student Score Card.

In this investigation, samples of milk were obtained from two different patron's who were shipping to the University Creamery. Special effort was made to select samples from the best flavored milk being received at the time the experiment was started and from a relatively poor flavored milk. These trials were conducted during July 1936, at which time most of the milk being received at the creamery had a slight feed flavor. The same patron's milk was used for samples throughout each series. The data shown in Tables VI and VII were obtained from samples and duplicates which had been taken from the warm cans of morning's milk, divided, and cooled by two different methods. The temperature of the milk when received was between 75° and 80° F. Part of the milk was put in regular quart milk bottles which were immediately submerged in ice water and cooled to 40° F. Another portion of milk from the same can was poured over the tubular cooler (Figure 2) until the temperature at the bottom of the cooler had reached 40° F., at which time samples were taken. All of the samples were placed in the milk storage room and held at 40° F. until they were ready to be scored.

The time required for cooling the samples in glass bottles surrounded by cold water from the original temperature to 40° F. with the temperature of the water from 34° to 36° F. ranged from 1 to 1½ hours. This is representative of the time required to cool milk in cans in electrically operated units where the water is agitated around the can. The time required to cool the milk over the tubular cooler with a circulation of brine at 20° F. through the coils was less than one minute.

The samples were scored for flavor during the training period for the dairy products judging team. The students were not aware of the treatment

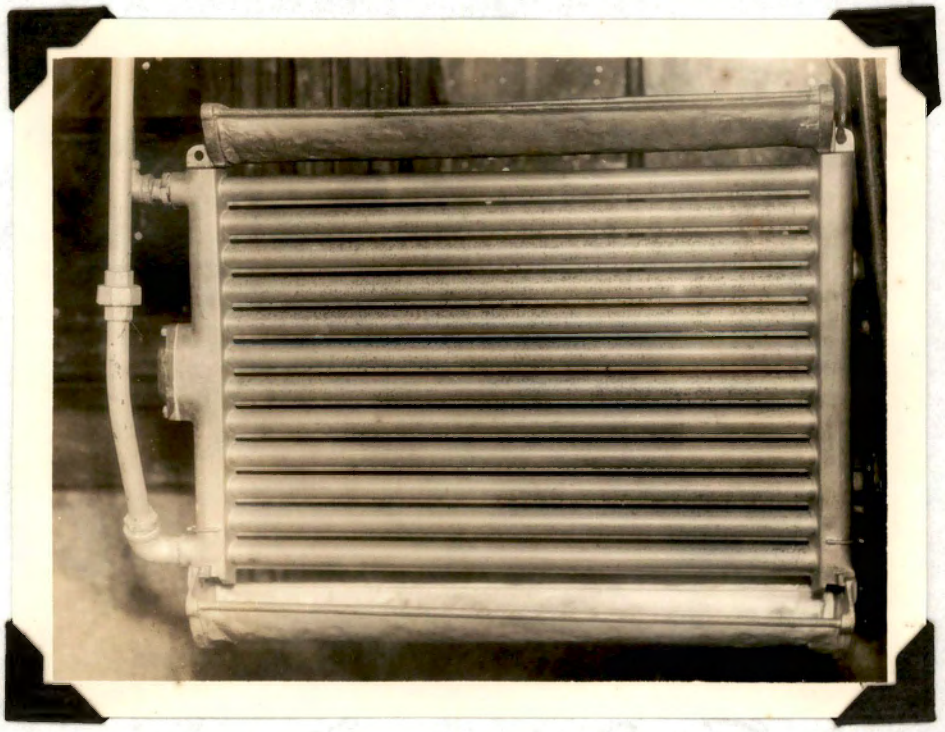


Figure 2. Surface Tubular Cooler

which had been given the samples. They were, however, requested to pick out duplicates, or determine differences where possible. When disagreement as to scores to be given to a sample existed, averages were recorded provided the differences did not amount to more than one point. When the differences amounted to more than one point, a second score was secured by passing the sample around after several others had been scored.

TABLE VI

SCORES FOR GOOD FLAVORED MILK COOLED BY DIFFERENT METHODS

Trial		Cooling Method			
		In bottle		Aerated	
		Scored		Scored	
		6 hr.	24 hr.	6 hr.	24 hr.
1	Score	22	21	22	22
2	Score	22	21.5	22	18
3	Score	22.5	22	22	22
Average		22.16	21.16	22	20.66

The average score at the six-hour period was 22.16 for the samples cooled in the bottle as compared to 22 for the samples cooled over the aerator. At the 24-hour period the average score for the samples cooled in the bottle was 21.16 as compared to 20.66 for the samples cooled over the aerator.

The results as given in Table VI indicate that there was very little difference between the flavor scores of milk cooled in bottles without agitation and that cooled over an aerator. The original milk was considered good in flavor with only a slight feed flavor present. These results

indicate further that cooling milk of good flavor within one to two hours was just as satisfactory as to flavor score as cooling more quickly. Preliminary trials showed that samples cooled soon after drawn scored higher in flavor at the 6 and 24-hour periods than at the 48-hour period.

The data in table VII show the effects of aeration of milk on a strong feed flavor and slightly unclean in flavor when handled in a similar manner to the samples used in the above experiments.

TABLE VII

SCORES FOR POOR FLAVORED MILK COOLED BY DIFFERENT METHODS

Trial		Cooling Method			
		In bottle		Aerated	
		Scored		Scored	
		6 hr.	24 hr.	6 hr.	24 hr.
1	Score	17	18	17.5	18
2	Score	18	17.5	18	18
3	Score	17	12*	17	16.5*
Average		17.33	15.83	17.5	17.5

*Criticized high acid.

The average score for the 6-hour period for the samples that were cooled in the bottles was 17.33 as compared to 17.5 for those cooled over the aerator. The samples cooled in the bottles had an average score of 15.83 as compared to 17.5 for those cooled over the aerator at the 24-hour examination.

The results as given in Table VII indicate that aeration in these trials failed to remove much of the strong feed and unclean flavor from

milk which was decidedly poor in flavor. They show further that very little change in flavor is brought about between the 6th and 24th-hour examination, except in the third trial where an off-flavor was present. This was possibly due to acid development which apparently had taken place at the 24th-hour examination.

A series of trials was conducted to determine the effect of aeration on milk with a noticeable silage flavor. This milk was taken from the University herd during October 1935. At that time each of the cows was being fed from 30 to 35 pounds of corn silage per day at milking time. They were also getting a regular grain mixture consisting of corn and cob meal, wheat bran, cottonseed meal, and all of the good hay they would eat. This milk was taken from the morning milking which consisted of a mixture from the Holsteins and Jerseys just as they were normally being milked.

The milk was drawn by machine, after which it was poured into 40-quart cans. When the cans were about three-fourths full, the milk was well mixed and the temperature taken and found to range from 82° to 88° F. Half of this milk was set in the electrically operated Daniel's milk cooler in the milk room at the barn and cooled to 40° F. The time required for cooling was from 1 to 1½ hours. The other half of the milk, at the temperatures of 82° to 88° F., was carried to the creamery and cooled over the tubular cooler to 40° F. This method required less than one minute from the time cooling began. Not more than twenty minutes elapsed from the time the milk was first mixed until it was cooled over the aerator. Samples were taken in duplicate and scored as explained in the preceding trials.

TABLE VIII

SCORES FOR MEDIUM FLAVORED MILK COOLED BY DIFFERENT METHODS

Trial		Cooling Method			
		In can		Aerated	
		Scored 6 hr.	Scored 24 hr.	Scored 6 hr.	Scored 24 hr.
1	Score	21.5	21.5	21.5	21.5
2	Score	21.5	21.5	22	21.5
3	Score	21.5	21	21.5	21
Average		21.5	21.33	21.66	21.33

The average score for the samples that were cooled in the can at the six-hour period was 21.5 as compared to 21.66 for the samples that were aerated and scored at the six-hour period. The samples that were cooled in the can and those cooled over the aerator had the same average score of 21.33 when scored at the 24-hour period.

The results as given in Table VIII indicate that aeration had very little effect on the flavor of milk which originally had a decided silage flavor. The scores and criticisms were practically the same in the milk just after it was drawn, after it was cooled in the can, and after it was cooled over the tubular cooler.

Pasteurization Temperatures.

Pasteurization is universally recognized as one of the leading safeguards against milk-borne epidemic diseases. Apparently, the general public is in favor of pasteurized milk as evidenced by the rapid trend toward its use. The milk distributor is interested in the advancement of pasteurization because it improves the keeping quality of his milk and increases milk sales. One of the claims which has led to objection to pasteurized milk on the part of some consumers is that it does not possess a natural milk flavor. This may be attributed to the removal of some of the flavors which are actually present in the raw milk. For example, the heating may possibly reduce the intensity of certain feed and weed flavors present in the raw milk, thus leaving a flat taste, or the heating may possibly destroy some of the characteristic flavor properties of normal milk. Pasteurization, even though it may in some cases improve the flavor of milk, should be accomplished at relatively low temperatures if the pleasing milk flavor is to be retained.

A series of experiments, consisting of six trials with each of the three common pasteurization temperatures, (142^{0} - $143\frac{1}{2}^{0}$ - 145^{0}), were used in this investigation to determine the effect of pasteurization temperatures on the flavor of market milk. These experiments were conducted in the University Creamery, using the Cherry Burrell spray-type pasteurizing vat. The milk samples used for these experiments were secured from the regular runs of the pasteurized milk as carried through the daily routine at the creamery. The only variations from the regular practice were the pasteurization temperatures. A "drip" sample was caught at the preheater (Fig. 3)

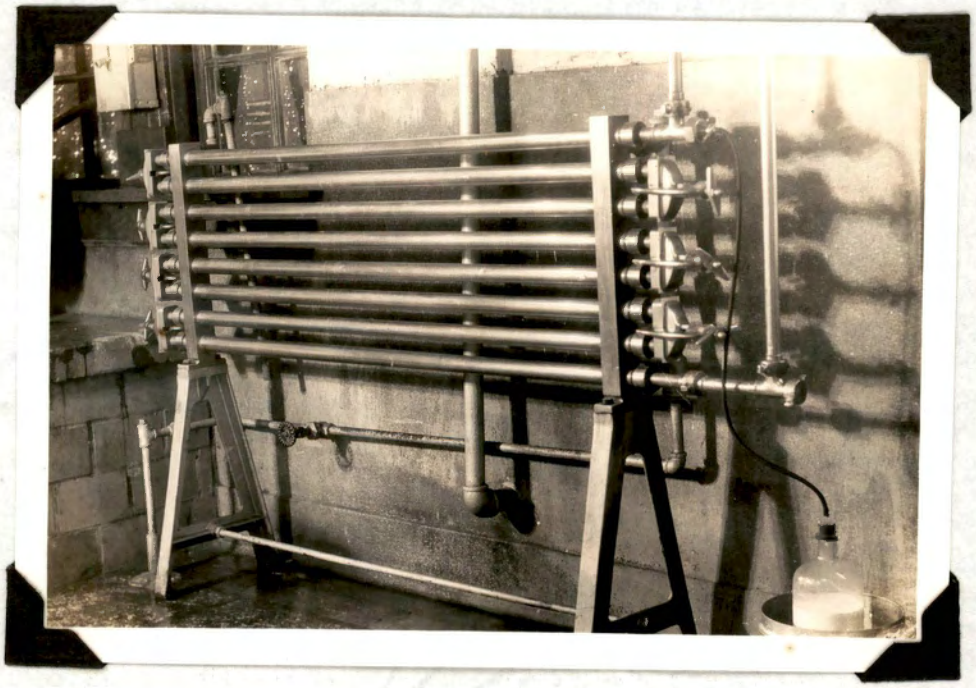


Figure 3. Milk Preheater

which served as a control for comparing the flavor of the raw and pasteurized milk. This sample represented a composite of the milk flowing into the vat after it had been heated to a temperature of 95° F. and was secured by allowing the milk to drip through a clean rubber tubing which extended from the end of the top coil of the preheater to a small-top glass bottle which was kept in ice water so that the milk was cooled as it dropped into the bottle.

Samples of the pasteurized milk were taken from the bottle-filling machine after they had been held in the vat for thirty minutes at their respective pasteurization temperatures, then partially cooled in the vat to 130° to 135° F., and the cooling finished to 38° to 40° F. over a surface tubular cooler. These samples with duplicates were held in quart milk bottles in the milk storage room with the control samples which had been transferred to quart bottles until they were scored by procedures similar to those outlined under the heading - Methods of Scoring.

TABLE IX

FLAVOR SCORES FOR MILK SAMPLES PASTEURIZED AT DIFFERENT TEMPERATURES

Pasteurizing Temperature		Raw	Pasteurized	
		6 hr.	6 hr.	24 hr.
142°	Score	21.9	22.25	22.1 -
143½°	Score	21.5	22	21.75
145°	Score	21.5	22	22*

*Average of 3 trials.

Table IX represents an average of six trials using different pasteurizing temperatures as indicated. The pasteurized milk from each of the three standard temperatures scored a little higher in flavor than the corresponding raw milk. The judges for these samples were accustomed to drinking pasteurized milk; however, they were also accustomed to scoring raw milk samples. With the difference between the raw and pasteurized milk being so small, the consumer would not likely notice any change. Some consumers may notice a slight cooked flavor present in the pasteurized

samples; whereas, it had replaced a slight feed flavor in the raw samples.

Preliminary experiments in November 1935, conducted at Southern Dairies and Avondale Farms Creamery, commercial plants of Knoxville, showed different results from the above. Using water at 180° F. for the heating medium in the coil-type Cherry Burrell vats, the results seemed to indicate: (1) no apparent change in flavor of milk pasteurized at 142° F. for thirty minutes; (2) a cooked flavor when pasteurized at 143½° F. for thirty minutes, possibly lowering the score an average of .5 points; and (3) a decided cooked flavor at 145° F. with at least a one-point cut in score for cooked flavor.

These results, although the number of trials were not sufficient to draw specific conclusion, indicate that milk may be pasteurized at any of the above temperatures in the spray-type vat without materially affecting its fine flavor. They further indicate that relatively high pasteurization temperatures have a tendency to produce an undesirable cooked flavor when the coil-type vat is used with water at 180° F. for the heating medium.

Creaming of Market Milk.

The length of the layer of cream appearing on a bottle of milk when it is delivered to the consumer may be influenced by several different factors. Many of these factors can be controlled by the milk distributor in processing plants, but some of them are beyond his control. For example, he cannot control the breed of animals on the producing farm, yet the breed sometimes influences the creaming of milk. This cream layer is of considerable commercial importance. It is commonly accepted by the consumer as an index to the richness of the milk.

The factors influencing the cream layer of milk, which were studied in this investigation, have to do with some of the major problems encountered in commercial pasteurizing plants. The temperatures to which milk is heated during pasteurization and the time bottled milk is held in storage are of vital importance from the standpoint of the resulting cream layer on milk at the time of delivery.

Methods of Measuring.

The methods commonly used for measuring the creaming of market milk are: (1) those which reveal the length of the layer as it appears on the bottle, and (2) those which make use of graduate cylinders. Both of the above methods were used in this investigation. The samples for the creaming tests were taken from the milk received at the University Creamery which constituted the regular pasteurized supply. This milk, as stated in the foregoing section, consisted of a mixture from Holsteins, Jerseys, and grades. All of the milk was standardized to 4% butterfat as shown by the Babcock test.

The raw samples were secured by the "drip" method as described in the section devoted to flavors. Standardization was accomplished by separating the required amount of skimmilk and pouring it back into the receiving tank so that it could be pumped back through the lines, thus giving the "drip" test a butterfat test of 4%. The pasteurized samples were secured from the milk after it had been cooled to 130° - 135° F. in the vat, then pumped over a surface tubular cooler and cooled to 38° - 40° F.

Measuring In Bottle. When the creaming tests were first begun, the cream layer was measured as it appeared in the bottle. The samples were secured as described under methods of measuring. When the samples were ready to be measured for cream volume, the bottles were carried from the storage room to the laboratory and placed on a level table, care being given to avoid destroying the line of demarcation. By extending a pair of calipers over the entire length of the layer and transferring this measurement to a rule graduated in sixteenths of an inch, the length of the layer was determined.

One feature which makes this method attractive is the volume of cream indicated that may be seen by the consumer.

Measuring In Graduated Cylinders. This method of measuring the creaming of milk required the use of 100 cc. clear glass cylinders graduated to 1 cc. divisions. The cylinders used in this investigation were approximately seven inches in height and of an even diameter of about one inch. By using cylinders of this height, the fat clusters rose about the same distance as they normally would in a regular quart milk bottle. Other features which make this method attractive are that the results are easily interpreted, and they may be expressed in percentages.

Samples and duplicates for the creaming tests in the cylinders were secured as described under the methods of measuring. The raw samples which were used for controls were taken from the bottle which represented the composite "drip" test. The pasteurized samples were taken from the bottles as they came from the filling machine. The milk was poured from the bottle to the 100 cc. mark on the cylinders. All of the cylinders were placed on a level shelf in the milk storage room and held at 40° F. until the creaming tests were made.

Pasteurization Temperatures.

Many investigators have shown that pasteurization at the temperatures commonly used in commercial creameries causes a decrease in cream volume when compared to the cream volume of the original raw milk. Most authorities agree that this decrease may be only a slight one when proper control methods are practiced. This investigation included a study of the three most common temperatures of pasteurization and their effects on the creaming ability of milk. The trials on pasteurization were conducted in the 200-gallon Cherry Burrell spray-type pasteurizer (Figure 4). The basis for comparison was the cream volume of the raw milk.

Averages were secured from three trials using each of the following processing series: (1) raw milk, (2) pasteurized at 142° F., (3) pasteurized at 143½° F., and (4) pasteurized at 145° F. All of the pasteurized samples were held for thirty minutes at their respective temperatures. Of the three trials, not more than one-sixteenth of an inch variation was found between the original and the duplicate samples. Not more than two-sixteenths of an inch variation was found between the individual trials in any one of the above series.

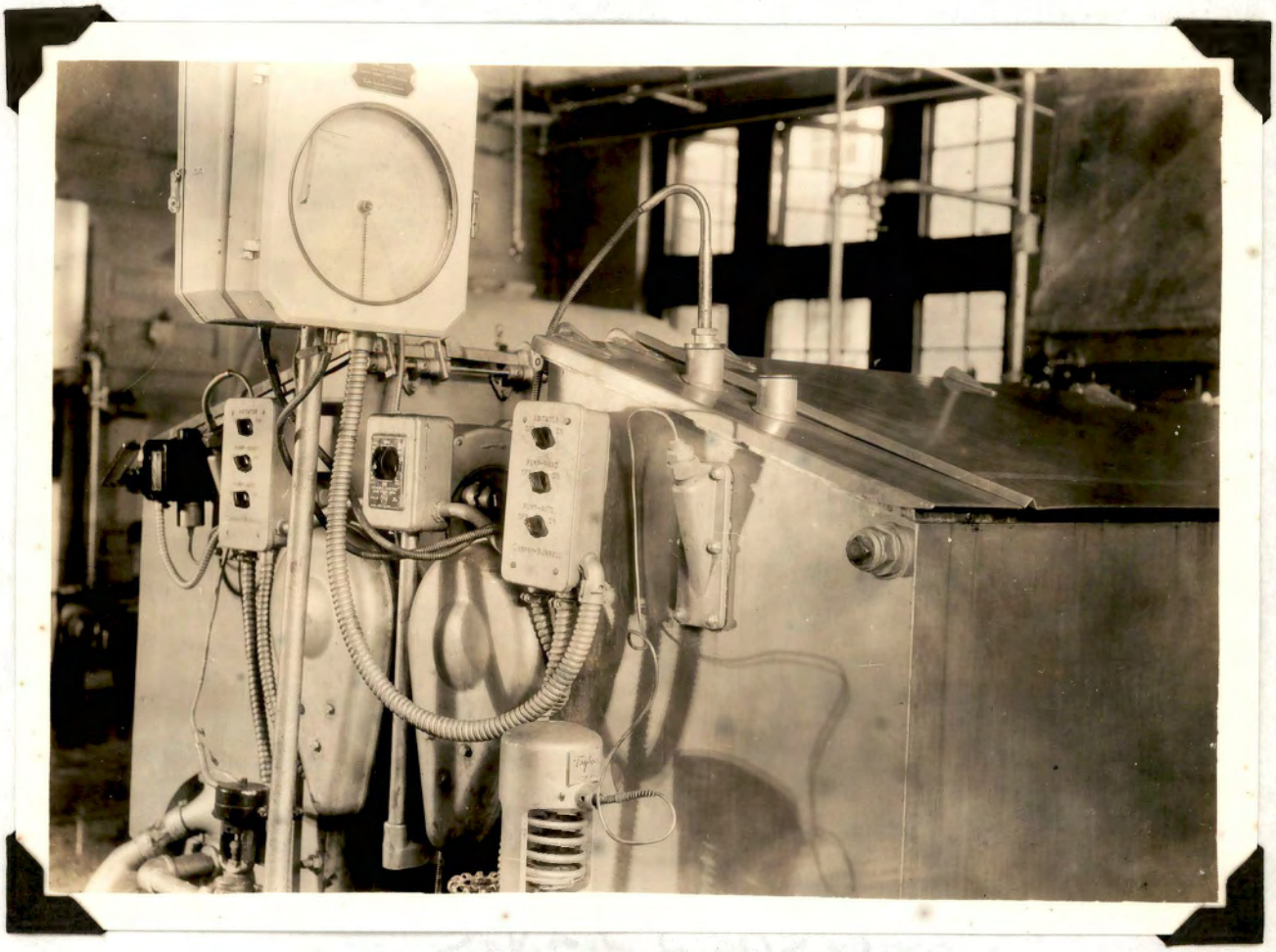


Figure 4. The Cherry Burrell Spray-type Pasteurizer.

TABLE X

CREAMING OF MILK PASTEURIZED AT DIFFERENT TEMPERATURES

AVERAGE OF 3 TRIALS

	Creaming Time	
	6 hours	24 hours
	cream volume	cream volume
Raw (Controls)	3 3/16 inches	3 9/16 inches
Pasteurized at 142° F.	3 8/16 inches	3 12/16 inches
Pasteurized at 143½° F.	3 6/16 inches	3 10/16 inches
Pasteurized at 145° F.	3 5/16 inches	3 7/16 inches

Measurement in the bottle

The results as shown in Table X indicate that milk pasteurized in the Cherry Burrell spray-type vat has a smaller cream volume than raw milk at the six-hour examination. In general, the higher the pasteurization temperature, the smaller the cream volume. They further indicate that the samples pasteurized at 142° and 143½° F. have a larger cream volume than the corresponding raw samples at the 24-hour examination. However, samples pasteurized at 145° F. showed a smaller cream volume than the corresponding raw samples at the 24-hour examination.

Influence of Storage.

The time elapsing between the bottling and delivering of market milk is of considerable importance. The time relationship for raw milk is different from that of pasteurized milk. Raw milk shows its maximum cream layer more quickly after bottling than pasteurized milk. The cream layer on raw milk after reaching a maximum has a tendency to shrink with age.

This shrinkage has been attributed to a higher concentration of the fat in the cream. The cream layer on pasteurized milk, even though it is usually a little shorter than that of raw milk according to Tables X and XI, did not shrink as rapidly as that of raw milk.

The data presented in Tables X and XI show the relationship of the different temperatures of pasteurization to the resulting cream layer, and the relationship between storage and the change in the volume of cream.

TABLE XI

CREAMING OF MILK PASTEURIZED AT DIFFERENT TEMPERATURES

AVERAGE OF 6 TRIALS

	Creaming Time	
	6 hours	24 hours
	cream volume	cream volume
Raw (controls)	16 cc.	14 cc.
Pasteurized at 142° F.	14 cc.	16 cc.
Pasteurized at 143½° F.	14 cc.	15 cc.
Pasteurized at 145° F.	13 cc.	14 cc.

Measurement in 100 cc. graduated cylinders

The data in Table XI indicate that raw milk has a larger cream volume at 6 hours than at 24 hours. Milk pasteurized at 142°, 143½°, and 145° F. had a larger cream volume at 24 hours than at 6 hours. Milk pasteurized at 142° - 143½° F. had a larger cream volume than raw milk at 24 hours. The raw milk at 6 hours had a greater cream volume than any of the pasteurized milk. The milk pasteurized at 145° F. had the smallest cream volume of the group; it was the same as raw milk at 24 hours.

Bacteriology of Market Milk.

The bacteria in market milk largely determine its keeping qualities. The original bacteria found at the time the milk is drawn are not considered as significant as those resulting from multiplication. This may be attributed to the relatively low counts and to the types of organisms present in normal fresh milk. However, milk known to contain only a few tubercular or typhoid organisms would be considered very dangerous for use as a human food. There are a number of sources from which bacteria may enter milk and each of them should be safeguarded against disease-producing types of organisms. This investigation covers a study of some of the fundamental requirements which are necessary to produce, process, and distribute milk with a relatively low bacterial count, and to destroy disease-producing types of bacteria.

Methods of Analysis.

There are four popular methods used for estimating the number of bacteria in milk. Each method has been very valuable to the market milk industry. The measurement of their practical value is influenced largely by the quality of the milk being handled and the desired information.

Some milk contains several different types and species of bacteria. Each of the different types in milk has peculiarities which require various preparation and treatment for determining their presence. Thus, negative results for a given type are not at all uncommon when employing some methods; whereas, a change in methods or a combination of methods may give entirely different results.

Both the agar plate counting method and the methylene blue reductase test have been used very extensively for milk analyses. These two methods were compared in this investigation. The data were secured from a series of trials with the use of each method on milk from the same source in order to get the correlation between the two methods. The procedures are given later in this section.

The agar plate counting method was used for determining the influence of cooling on the bacterial content of raw milk in this investigation. This method was also used for studying the efficiency of pasteurization. Preliminary reductase tests on pasteurized milk gave inconsistent results. Some investigators believe that the organism responsible for the production of the enzyme, aldehyde reductase, is killed during pasteurization. Accordingly, the color change upon which the reductase method is based would not be brought about, even though there were bacteria present in large numbers. The inconsist results found by using the reductase method for tests on pasteurized milk may have been partly due to the relatively long time required for decolorization. Only a few of the samples decolorized in eight hours. Some of the samples in the preliminary trials were held until they had coagulated and the blue color still had not completely disappeared.

The bacterial analyses for the series of experiments conducted on sterilization of cans were limited to the agar plate method. The chief object of these experiments was to determine the efficiency of some of the different methods of sterilization. The plate method was used for analyzing the bacterial content of the rinse water which was used in the cans.

These studies were intended for estimating the total number of bacteria present and not a particular group. However, it is generally agreed that most of the bacteria present in milk are members of the S. lactis group.

Agar Plate Counting. This is probably the most common method used for estimating the number of bacteria in milk. It has proved to be, for the most part, a very satisfactory index to the quality of milk. The principle of this method is based on the growth of individual cells or organisms into comparatively large colonies. The term "counting" is perhaps a little misleading because the individual bacteria are not actually counted. The count or estimation is made on the colonies which are presumably produced from a single cell. Some of the unfavorable criticism for the plate method has resulted from the theory that many types of bacteria appear in pairs, and when colonies are produced from their growth, each colony may easily represent the growth of two bacteria. This is not at all impossible. A colony may result from several individual cells and be counted as though it were a single bacterium. However, if consistent results are obtained from the plate method, it still has some very distinct advantages over other methods. It is the index, or approximation of numbers, which helps to establish standards for grading milk according to bacterial content.

The material and equipment used in the agar plate method include:

1. Dilution bottles in which 99 cc. water can be sterilized.
2. A supply of 1 cc. pipettes for making the dilutions; with one .1 cc. graduation.
3. A liquifiable solid media for growing the colonies. (Beef extract nutrient agar)

4. A steam pressure autoclave for sterilizing 99 cc. water blanks, and melting agar.

5. A dry heat oven for sterilizing the plates and pipettes.

6. An automatic temperature controlled incubator in which to grow the colonies.

7. A solution of lysol for disinfecting the working table.

In addition to the above equipment a wax pencil for marking the plates and a counting glass are desirable for part of the work.

The procedures followed in this investigation were similar to those recommended by the United States Public Health Service in the Standard Methods of Milk Analysis. Briefly, they were as follows:

General: The agar was made according to standard methods. When the plates were ready to be poured it was melted and cooled back to about 100° F. The dilution blanks were sterilized at 15-pounds pressure for one hour. All of the pipettes and plates used were sterilized in the dry heat oven at 300° F. for two hours.

1. Dilutions of 1 to 100 were made by pipetting 1 cc. of the sample of milk into 99 cc. of sterile water.

2. From this dilution, .1 cc. and 1 cc. respectively were transferred to plates, giving duplicate plates for each sample in different dilutions.

3. The warm agar was poured into the plate and the two mixed gently and placed on a level table until the agar had solidified, after which they were inverted and placed in the electrically heated oven and incubated at 37° C. (98.6° F.) for 48 hours.

4. The counts were made and averages taken from the count recorded for the 1 - 100 and 1 - 1000 dilution.

The above procedure gives a general summary of the methods used for all of the plate counting in this investigation except in the trials conducted on sterilization of cans. There were no dilutions made for plating the rinse solutions taken from the cans. The procedures were the same as those outlined for milk samples except 1 cc. of the rinse solution was plated in duplicate and the average taken from the two.

Some of the advantages claimed for the plate method are: (1) it indicates the growth of the living organisms; dead or inactive organisms are not considered important, (2) dilutions may be made to secure estimates of different grades of milk, and (3) the method has been more uniformally standardized for milk analysis.

Among the disadvantages for the plate method may be: (1) the expense of the material and equipment, (2) less generally useful for high count milk, (3) the incubation temperatures are different from those at which milk is generally held, (4) results are not obtained very quickly, (5) not all of the bacteria grow under the conditions of the test, and (6) not much knowledge of the type of organisms present is given.

Methylene Blue Reductase. The methylene blue reductase test was used in this investigation to determine its relation to the plate method as to the bacterial action in milk. The principle of the test is based on the action of an enzyme formed from bacteria on methylene blue dye solutions. The action of this enzyme aldehyde reductase produces a color change, or complete decolorization. This change in color and the time required is said to be directly related to the activities of the bacteria.

The reductase method requires the use of a comparatively large quantity of milk, which apparently is favorable toward its accuracy. Many investigators have found a very close agreement between the samples and duplicates. Some arguments for this method are that it is simple and inexpensive, the results are usually obtained in a comparatively short time, and the method permits the use of a follow test. (The fermentation test). The material and equipment used for making the reductase tests are shown in Figure 5.



Figure 5. Material and Equipment Used For Making The Reductase Test.

A list of the material and equipment used for making the reductase test as shown in Figure 5 are:

1. A supply of sterile test tubes
2. A 10 cc. pipette for measuring the milk
3. A 1 cc. pipette for measuring the methylene blue solution
4. An incubator equipped with a water bath
5. A standard methylene blue solution made by dissolving one tablet of dye in 50 cc. of sterile water and diluting this to 200 cc.

Note: The tablets may be obtained from the National Aniline and Chemical Company, Inc., New York.

The procedures followed were similar to those used by Ellenberger[16] and others. Methods for securing milk samples used for the reductase test for making the comparison were similar to those practiced by the dairy inspector. The samples were secured directly from the can. The trials were planned so that the samples for the reductase test could be taken at the same time the milk inspector from the Knoxville Bureau of Health took samples. The milk inspector was taking samples at least once each month during the entire year of 1936 in which the experiment was conducted.

Samples were taken from the milk of each of the patrons shipping to the University Creamery. A 10 cc. pipette was used for all of the sampling just after the city inspector had taken his sample. In case a patron had more than one can, the sample was secured from the different cans in proportion to the milk in each. The pipette was first rinsed with a sample of the milk which was to be secured, then a pipette full was drawn and transferred to a sterile test tube. When all of the samples were taken

they were carried to the laboratory and 1 cc. of the standard methylene blue solution was added. After thorough mixing they were incubated at 98° F. until the blue color had disappeared.

Table XII shows the relation of the number of bacteria per cc. in thousands to the reduction time in hours. The tubes for the reductase test were incubated for a maximum time of 8 hours and examined and recorded at 15-minute intervals. Since the test has been recommended by authorities primarily for milk with high counts or short reduction time, the tubes retaining the blue color for 8 hours and over were recorded as decolorizing at 8 hours. The results are recorded in thousands of bacteria in round numbers and hours, half and quarter hours for the reduction time. These trials were conducted for the entire year of 1936 using a sample from each patron once each month. It was found that some of the patrons did not ship regularly, so only the samples from ten of the regular shippers were recorded in the results.

The averages as shown in Table XII indicate that there is a fairly close correlation between the reductase method and the agar plate counting method. In general it may be said that the shorter the reduction time the higher the bacteria count.

The averages taken from each patron's column, as given in Table XII, and shown on a graph (Figure 6), indicate that the coefficient of variation is higher than the normal allowance for experimental error. For example, a diagonal line from 350 in the bacteria column to 7.8 in the reduction column should fall very close to the mean. The variation from this mean may be due, in part, to experimental error and, in part, to unknown factors which distinguish color reduction from the number of bacteria present.

TABLE XII

CORRELATION BETWEEN BACTERIA COUNTS IN THOUSANDS PER CC. AND REDUCTION TIME IN HOURS
(Thousands omitted)

Patron	No. 1		2		3		4		5		6		7		8		9		10	
	Bacteria per cc.	Reduction Time hrs.	Bacteria per cc.	Reduction Time hrs.	Bacteria per cc.	Reduction Time hrs.	Bacteria per cc.	Reduction Time hrs.	Bacteria per cc.	Reduction Time hrs.	Bacteria per cc.	Reduction Time hrs.	Bacteria per cc.	Reduction Time hrs.	Bacteria per cc.	Reduction Time hrs.	Bacteria per cc.	Reduction Time hrs.	Bacteria per cc.	Reduction Time hrs.
Jan.	2	8	32	8	10	8	1	8	3	8	4	8	1	8	1	8	1	8	8	8
Feb.	7	8	10	8	27	8	4	7	17	8	2	8	2	8	17	8	2	8	3	8
March	276	6	4	8	39	8	2	8	6	8	5	8	189	6	83	7	87	6	44	8
April	27	8	3	8	4	8	1	8	360	4¾	5	8	17	8	--	--	2	8	62	7
May	58	7	62	8	--	--	95	7	158	5¾	36	8	750	6	750	4½	48	8	40	8
June	90	8	750	5	10	8	660	4	195	5½	23	8	750	5½	6	8	660	4	53	8
July	510	4	750	4	285	5	750	4	39	8	750	6	750	4	750	4	480	5	263	5
Aug.	22	8	750	4	23	8	150	5	320	5	70	8	450	6	750	6	480	4½	40	8
Sept.	750	4	124	5½	237	5	660	4½	513	4	540	6	750	5½	12	8	540	4¼	372	6
Oct.	--	--	50	7	7	8	7	8	4	8	2	8	12	8	5	8	2	8	--	--
Nov.	20	8	4	8	5	8	29	8	300	8	1	8	1	8	8	8	1	8	5	8
Dec.	500	4½	29	8	14	8	2	8	--	--	--	--	2	8	--	--	--	--	4	8
Average	206	6.6	214	6.8	60	7.5	197	6.6	174	6.6	130	7.6	306	6.7	217	6.3	209	6.4	81	7.5

Reading from left to right on the graph (Figure 6), the indexes for patrons 6 and 8 are extremely contrary to the theoretical correlation for the two methods. The other patrons' indexes are probably within the normal range of experimental error.

The correlation for the individual months (Figure 7) is much closer than the averages taken from the year as shown in (Figure 6). The range of bacteria counts as taken from averages in Table XII is from 6.5 thousand to 530 thousand. The average range for the reduction time is from 4.9 hours to 8.0 hours. According to the data secured from the averages, (Figure 7), the relation between the methylene blue reduction method and the bacterial plate count is very close in cases where the plate count is over 5 thousand.

For example, the months of June, July, August, and September, (Figure 7), have a very high average bacterial index. The proportional reduction time for the months listed above is relatively consistent with the bacteria counts. For the months not mentioned above, the bacterial indexes are comparatively low. The average reduction time was not as consistent nor were the correlative indexes as close as they were for the four months with high bacterial indexes.

The ten patrons' milk, as shown in the Table, represents the different samples used in this investigation. Each patron's milk was kept separate and recorded by the Knoxville Milk Producers' contract number until all of the data were summarized. The results in the Table indicate that for some months during the year, or some seasons of the year, the variations between the methylene blue reductase test and the agar plate count were greater than the others.

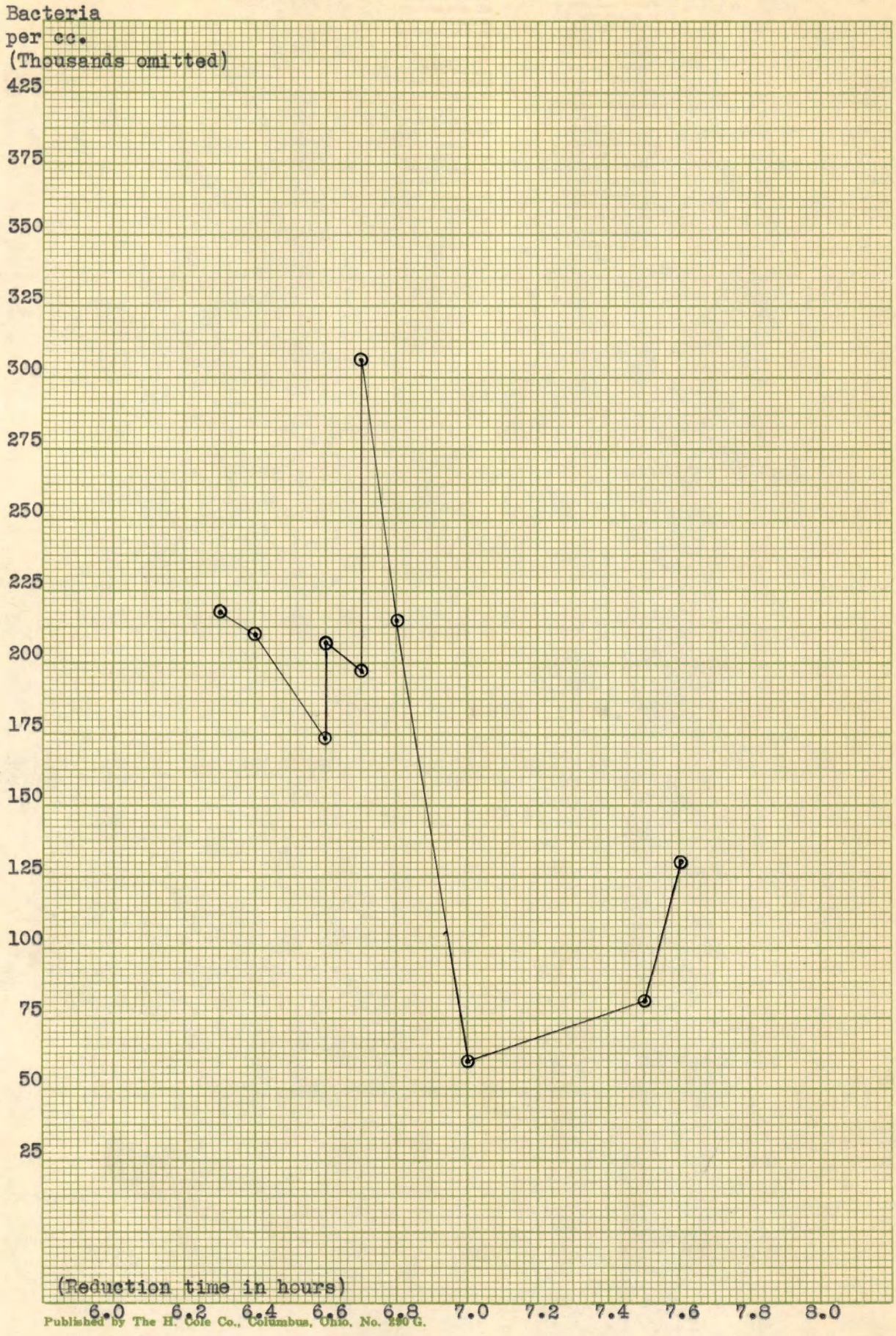


Figure 6. Correlation Between Bacteria Counts in thousands per cc., and reduction time in hours.

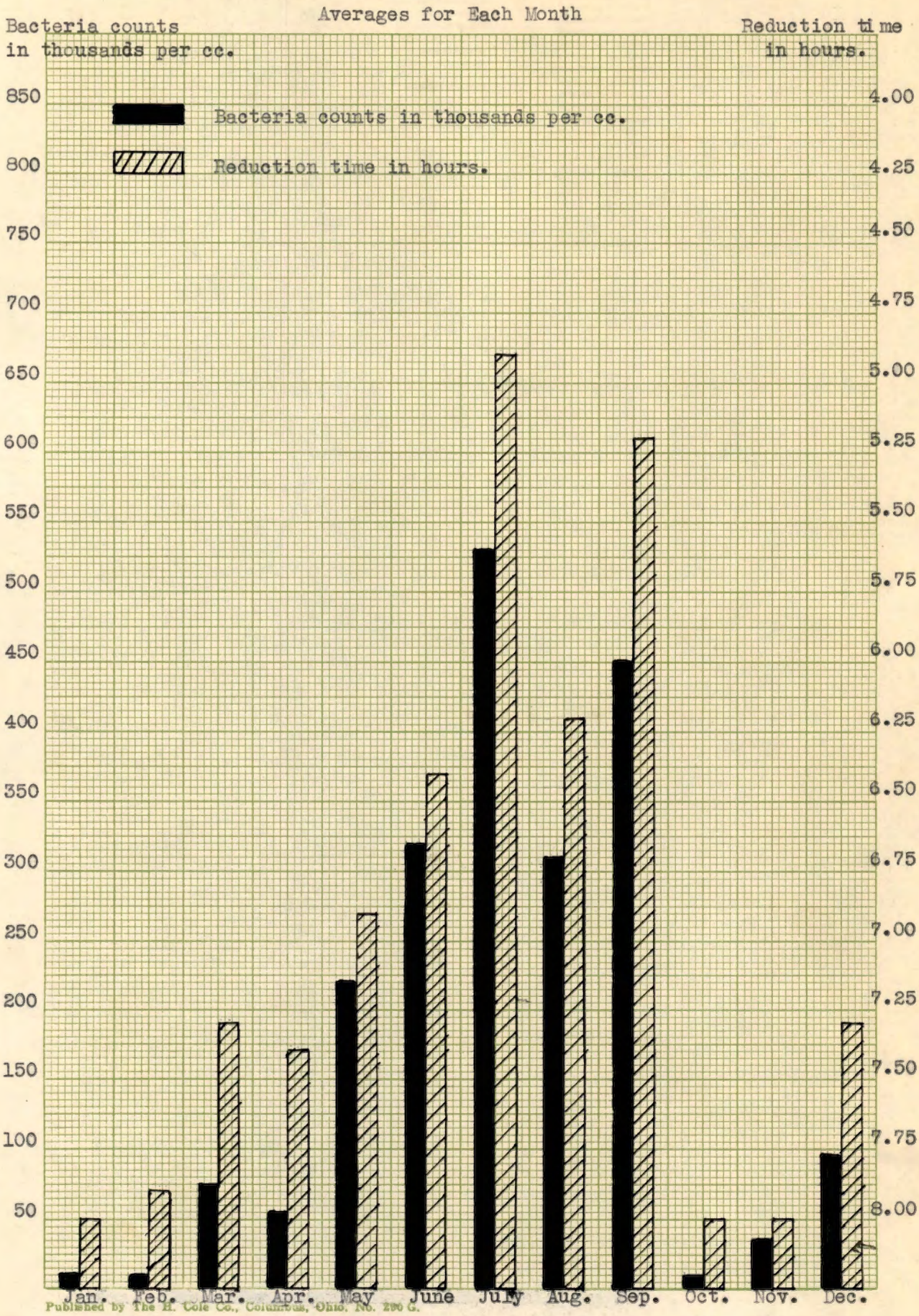


Figure 7. Correlation between bacteria counts in thousands per cc. and reduction time in hours.

Influence of Cooling.

Bacteria that are usually responsible for the souring of milk apparently have an optimum growth temperature of 98° to 100° F. Some of the other factors which influence bacterial growth are moisture and oxygen. Therefore, the conditions in milk at the time it is drawn from the cow are said to be almost ideal for bacterial growth because the temperature, oxygen, and moisture are near the optimum requirements. When the milk is held at temperatures around the optimum range for two to four hours, the bacteria counts indicate that a very rapid growth has taken place; whereas, when milk is cooled to relatively low temperatures immediately after it is drawn, the bacterial growth is retarded.

This investigation included a series of six trials made by using milk soon after it was drawn at the University dairy barn. The milk was carried from the barn in 10-gallon cans and cooled to different temperatures over the surface tubular cooler shown in Figure 2.

Control samples for making the bacteria plate counts were taken from the cans just before cooling the milk. The other samples in each series were cooled as indicated in Table XIII. The maximum elapse between the time the milk was drawn and when it was cooled was not more than thirty minutes.

This part of the investigation was planned so as to represent common practices on various dairy farms. For example, the better milk producers usually cool their milk to 40° F. immediately after it is drawn. This practice usually requires a mechanical refrigeration unit or the purchasing of ice. It is quite obvious that cooling by the above methods should help to retard bacterial growth. There are also some producers who use spring

water or well water to cool their milk. The average temperature range to which milk can normally be cooled throughout the year in most sections of the United States, using water as the cooling agent, would probably be from 60° F. to 70° F. The series cooled to 70° F. was selected to represent cooling practices by the producer who is trying to produce good milk with a minimum investment for cooling equipment. In addition to the above mentioned classes of producers, there are some who deliver milk to processing plants without cooling it at all. They try to make deliveries soon after milking to avoid cooling. Some producers deliver twice each day. These conditions may be represented by the trials in which no cooling was done.

The same supply of milk was used for the three different temperature series. A sample was taken for bacteria plating, then the can of milk was divided into three separate portions, one of which was cooled to 40° F., another to 70° F., and the other was not cooled. All samples were transferred to quart milk bottles which had been washed and steamed for handling commercial market milk. Duplicate samples were held from each trial for each temperature, one for the sixth and one for the 24-hour plating. The caps were carefully placed on the bottles by hand in such a way as to avoid contamination.

The samples which were cooled to 40° F. were held in the milk storage room at that temperature; those cooled to 70° F. were held in a water bath at that temperature; and those which were not cooled were held in a room with a temperature of 68° to 72° F., representing the temperature of the average milk house. These samples were removed and plated at the periods indicated in Table XIII using the regular agar plate method with two dilutions.

TABLE XIII

INFLUENCE OF COOLING ON BACTERIAL GROWTH - AVERAGE OF SIX TRIALS

	Plating Time After Cooling		
	Original Count	6 hours	24 hours
	Average number of bacteria per cc.		
Cooled immediately to 40° F.	9,500	21,000	30,000
Cooled immediately to 70° F.	9,500	89,000	418,000
Not cooled	9,500	458,000	over 1 million

The results secured from the series of six trials, using three different cooling temperatures, indicate that prompt cooling to relatively low temperatures is essential for producing milk with a minimum number of bacteria.

Table XIII shows that from an average of six trials, milk with an original count of 9,500 when cooled immediately to 40° F. and held 6 hours contained an average of 21,000 bacteria per cc. This series had a variation of from 10,000 to 60,000 bacteria per cc.

The average count from the samples cooled to 70° F. at the 6-hour plating was 89,000 bacteria per cc., and at the 24-hour plating the average count was 418,000 bacteria per cc.

The average count from the samples which were not cooled but were held in a room at 68° to 72° F. at the 6-hour plating was 458,000 bacteria per cc., and at the 24-hour plating, over one million bacteria per cc. The estimates from each of the six trials on the 24-hour plating of the samples not cooled were over one million bacteria per cc.

Efficiency of Pasteurization.

The percentage of bacterial decrease due to pasteurization, or efficiency of pasteurization, is of vast importance to the pasteurized milk industry. Health authorities require a relatively low bacterial count for pasteurized milk. In order for the milk plant operator to market pasteurized milk with low counts, his equipment and methods must be efficient.

This investigation consisted of a series of six trials for each of the following pasteurization temperatures, 142° , 143½° , and 145° F., as indicated in Table XIV. The experiments were conducted, using the regular pasteurized milk at the University Creamery, with the raw drip samples for controls. Bacterial analyses were made by the agar plate method at the 6 and 24-hour periods. All of the samples were plated in duplicate using 1 - 100 and 1 - 1000 dilutions. The counts were recorded in round thousands.

TABLE XIV

EFFICIENCY OF PASTEURIZATION

Average of 6 Trials
(Thousands omitted)

	Plating Time After Pasteurization			
	Raw (Controls)	6 hours	24 hours	Average % Efficiency
	Bacteria per cc.			
Pasteurized at 142° F.	183	3	7	97
Pasteurized at 143½° F.	126	2	3	98
Pasteurized at 145° F.	200	4	4	98

Of the series of six samples pasteurized at 142° F., the original count when raw varied from 60 to 500 thousand, and after pasteurized, the range

was from 1 to 8 thousand at the 6-hour period, and 1 to 25 thousand at the 24-hour period. Those pasteurized at 143½° F. varied from 85 to 225 thousand when raw; from 1 to 5 thousand when plated at the 6-hour period; and 1 to 5 thousand at the 24-hour period after pasteurization. Those pasteurized at 145° F. varied when raw from 60 to 600 thousand; 1 to 10 thousand at the 6-hour period; and 1 to 10 thousand at the 24-hour period after pasteurization.

The results in Table XIV indicate that from an average of six trials, each of the different pasteurization temperatures had a very high percentage of efficiency. These figures were obtained by getting an average from the six trials of the raw samples and getting the per cent of decrease using an average of the six trials as taken from the 6 and 24-hour plating periods. For illustration, the average for the six trials in the raw milk, in the 143½° F. series, was 126 thousand. The counts on the pasteurized samples at the 6 and 24-hour plating periods were 2 and 3 respectively, or an average of 2.5. The per cent of efficiency may be calculated as follows:

$$126 - 2.5 \text{ or } \frac{123.5}{126} \times 100 = 98 - \% \text{ efficiency.}$$

The other series were calculated in a similar manner.

Sterilization of Cans.

Procedure: The cans used in this investigation were regular 10-gallon milk cans in use at the University Creamery. Some of the cans had not been washed since their use; some of them had been washed and steamed and allowed to set in the wash room for several hours; and some of them had been washed the day before they were used for the experiments. The lids were removed from the cans because the steaming apparatus did not provide for steaming the lids.

Bacteria counts were secured by plating 1 cc. portions of 500 cc. of sterile water which had been used to rinse each can. The water used for rinse solutions was sterilized in quart milk bottles for a period of one hour at 15-pounds steam pressure in an autoclave. The differences in the bacterial counts, as shown in Tables XV and XVI, were determined by first rinsing the cans which were to be sterilized with 500 cc. of sterile water and 1 cc. of this solution plated. The differences between the bacteria counts for the separate platings constitute the efficiency of sterilization.

Steam Sterilization. The cans were first rinsed with 500 cc. sterile water which was poured back into the bottle to be plated later. The cans were next steamed over a jet with approximately 60-pounds boiler steam pressure for one minute, accurately timed by a stop watch. After steaming, they were dried over a hot air (135° F.) jet for 1½ minute. After the cans had cooled, another 500 cc. of sterile water was used to rinse the cans in the same manner as before sterilizing. The standard plate method was used for determining the number of bacteria per cc. in the rinse solutions. The results were given in bacteria per cc. from the 500 cc. of rinse

solution, and not the total number of bacteria in each can.

Data from the six trials with steam sterilization are given in Table XV. All of the plates were incubated for 48 hours before the counts were made. This was also the incubation period for all of the experiments on sterilization that follow.

TABLE XV

NUMBER OF BACTERIA PER CC. OF RINSE WATER BEFORE AND AFTER STERILIZATION OF CANS BY STEAM.

Trial	Colony Plate Count Before and After Steaming	
	Before	After
1	6,200	36
2	3,300	74
3	9,000	23
4	1,000	70
5	23,000	11
6	2,000	41
Average	7,417	42

The data in Table XV show that steam killed the majority of the bacteria present in the cans. However, quite a number of bacteria survived after treatment. The efficiency of sterilization by steam according to these trials was over 98%. Some advantages for steam are that it is generally accessible and is therefore inexpensive, and it is also a very quick method of sterilization.

Dry Heat Sterilization. The electrically heated Esco Sterilizer at the dairy barn was used for these experiments. Each of a series of 10-gallon cans was rinsed with 500 cc. of sterile water before and after

sterilization the same as with steam. The cans were inverted and placed in the electric cabinet, and the switch turned on. They were left in this cabinet from one milking until another which was a period of about 12 hours. During sterilization, the temperature went to approximately 212° F. at which point the switch was cut off by a thermostat. The temperature remained high for at least one hour and then went down gradually. However, the cans were still warm at the end of 12 hours.

The data for these trials are given in Table XVI. The high counts obtained before sterilizing were only approximate.

TABLE XVI

NUMBER OF BACTERIA PER CC. OF RINSE WATER BEFORE AND AFTER STERILIZATION OF CANS BY DRY HEAT

Trial	Colony Plate Count Before and After Sterilization	
	Before	After
1	320,000	169
2	21,000	2
3	256,000	20
4	76,800	5
5	134,400	280
6	102,000	45
Average	151,500	87

The data in Table XVI indicate that the efficiency of sterilization in these trials was over 99.9%. The above results show that dry heat is also a very effective method of sterilization. The initial counts in these cans were relatively high. That, however, should not have affected the results enough to cause any marked inaccuracy.

Chemical Sterilization. Three of the most common chemical sterilizers for dairies were used for sterilizing the cans in this series. When referring to chemical sterilization or sterilizers, they are generally called chlorine sterilizers because chlorine produces the germicidal action. The trade names for the sterilizers used were as follows: Diversol, H-T-H, and B-K.

The cans were treated as outlined in the above procedure. Several trials were run using solutions of each product containing 50 parts per million, 100 parts per million, and 200 parts per million of available chlorine. The cans were rinsed with sterile water and plated for controls. They were next rinsed with one gallon of the sterilizing solution made from the different chlorine compounds of known strength. After thorough exposure to the chlorine solutions, the cans were well drained before they were rinsed with sterile water. They were then rinsed and 1 cc. of the rinse water was plated. The data shown in Table XVII were secured by using solutions containing 50 P.P.M. of available chlorine.

TABLE XVII

NUMBER OF BACTERIA PER CC. OF 500 CC. STERILE RINSE WATER BEFORE AND AFTER STERILIZING WITH CHLORINE SOLUTIONS CONTAINING 50 PARTS PER MILLION OF AVAILABLE CHLORINE

Sterilizers	Average Colony Count Before and After Treatment		
	Trials	Before	After
B-K	2	1,612	1
H-T-H	2	4,400	6
Diversol	4	4,750	4
Average of the three		3,578	4

The average efficiency of sterilization, according to the trials shown in Table XVII, was a little less than 99.9%. Each of the chemical sterilizers used in these trials was apparently very satisfactory. The difference in results for the three sterilizers used was not great enough to be considered important.

One trial for each sterilizer containing 100 P.P.M., and one trial for each sterilizer containing 200 P.P.M. of available chlorine was made. The data indicate that the efficiency for the higher concentrations was not much greater than the lower ones where a thorough exposure was given.

The above results indicate that chemical sterilization is a very effective method of sterilizing milk cans. In all the trials with chemical sterilization the percentages of efficiency were greater than those obtained from steam and equal to those obtained by dry heat methods. These results further indicate that in sterilizing milk cans, using 50 parts per million of available chlorine was just as efficient as 100 or 200 parts per million. The differences in results probably were not great enough to warrant the use of more than 50 parts per million in sterilizing cans. However, there are some disadvantages connected with chlorine sterilization which steam and dry heat do not have. Chemical products are usually more expensive. They do not retain their strength unless properly stored, and they sometimes produce a chlorine flavor in the milk. This flavor is considered very objectionable. However, this flavor can be prevented if the chemicals and the utensils are handled properly.

Conclusions.

The results obtained from the research in this investigation indicate that the following conclusions may be justifiable:

1. Aeration, to the extent used in these trials, did not remove the feed and weed flavors from milk. There was no evidence that the flavor was improved by aeration over the flavor of that milk cooled by other methods.

2. Pasteurization at temperatures of 142° to 145° F. improved the flavor of milk slightly. The feed flavor was not as noticeable in the milk after it had been pasteurized. However, there was usually a slight cooked flavor present in the pasteurized milk.

3. Pasteurization at temperatures of 142° to 145° F. for 30 minutes caused a slight decrease in cream volume compared to raw milk at the 6-hour creaming period. Samples pasteurized at 142° to 143½° F. had a larger cream volume than raw control samples at the 24-hour creaming period. Samples pasteurized at 145° F. had a smaller cream volume than raw samples at the 24-hour creaming period.

4. Pasteurization apparently retards the rising of cream. In general, the higher the temperatures of pasteurization the slower the rising of the cream.

5. Raw milk had a larger cream volume at the 6-hour creaming period than at the 24-hour creaming period. Pasteurized milk had a larger volume of cream at the 24-hour period than at the 6-hour period.

6. A very close correlation was found between the methylene blue reductase test and the agar plate method for estimating the bacterial content in raw milk. The plate method was apparently more satisfactory for determining the number of bacteria in pasteurized milk.

7. Cooling milk to 40° F. immediately after it was drawn from the cow retarded the bacterial growth throughout the following 24 hours. Cooling to 70° F. helped to retard the bacterial growth for the 6 hours after the milk was drawn then rapid multiplication took place between the 6 and 24-hour periods.

8. Failure to cool milk resulted in a very rapid bacterial growth before the end of the 6-hour period. Millions of bacteria were present at the end of the 24-hour period.

9. Pasteurization at temperatures of 142° to 145° F. for 30 minutes killed a very high percentage of the bacteria in milk. There apparently was very little difference between the efficiency of pasteurization at 142°, 143½°, and 145° F.

10. Steam, dry heat, and chemical sterilization were all very effective methods for killing the bacteria in milk cans. Steam was apparently the most economical and chemicals were probably the quickest of the methods used in this investigation. Dry heat was very effective and may be recommended where conditions will warrant its use.

Bibliography

1. Ayers, S. Henry, and Johnson, W. T. Jr., A Study of the Bacteria Which Survive Pasteurization, U. S. D. A., B. A. I. Bulletin No. 161; 1913.

2. Ayyar, C. S. Ram, A Comparative Study of the Germicidal Efficiency of Electrolytic Chlorogen and Formalin on Bacterial Spores, Journal of India, 25; 1930.

3. Babcock, C. J., (No title) U. S. D. A. Leaflet No. 25.

4. Babcock, C. J., Effect of Garlic on the Flavor and Odor of Milk, U. S. D. A. Bulletin No. 1326.

5. Baker, J. C., Chlorine in Sewage and Waste Disposal, Indus and Eng. Chem., 17; pp. 1059-1060.

6. Brew, James D., Dairy Short Course Lecture, University of Tennessee, February 1937.

7. Brew, Dottener, and Hall, How Bacteria are Counted in Milk, New York Ag. Exp. Sta. Bulletin No. 439.

8. Breed, R. S., and Stocking, W. A. Jr., The Accuracy of Bacterial Count from Milk Samples, New York Ag. Exp. Sta. Bulletin No. 75.

9. Caulfield, E. J., Riddel, W. H., and Fay, A. C., Cleaning and Sterilizing Dairy Farm Utensils, Kansas State College Circular 176; 1935.

9a. Conn, H. W., Milk Fermentations, U. S. D. A. Farmers' Bulletin No. 9.

10. Dahlberg, A. C., and Marquardt, J. C., How the Cream Layer Forms on Milk, New York Ag. Exp. Sta. Bulletin No. 591 (Geneva) 1931

11. Dahlberg, A. C., Dairy Short Course Lecture, University of Tennessee February 1937.

12. Dahlberg, A. C., and Marquardt, J. C., The Creaming of Raw Milk, New York Ag. Exp. Sta. Bulletin No. 593 (Geneva) 1931.

13. Dahlberg, A. C., and Marquardt, J. C., The Creaming of Raw and Pasteurized Milk, New York Ag. Exp. Sta. Bulletin No. 157 (Geneva) 1929.

14. Dahlberg, A. C., and Marquardt, J. C., Sterilization of Dairy Farm Utensils, New York Ag. Exp. Sta. Bulletin No. 612; 1932.

14a. Devereau, E. D., Michigan Ag. Exp. Sta., Journal Dairy Science, September 1929.

15. Downs, P. A., and Lewis, E. B., Cooling Milk on Nebraska Farms, University of Nebraska Ag. Exp. Sta. Bulletin No. 266; 1932.

16. Ellenberger, H. B., and Moody, Ruth I., Preparing Samples for the Methylene Blue Reductase Test, Vermont Ag. Exp. Sta. Bulletin No. 312; 1930.

17. Erb, J. H., Effect of Agitation on Cream Layer of Milk, Milk Dealer 18, pp. 53-54, February 1929.

18. Evans, The Bacteria of Milk Freshly Drawn from Normal Udders, Journal of Infectious Diseases, Vol. 18, No. 5.

19. Fay, A. C., Methylene Blue Reductase Test, Journal of Dairy Science, May 1935.

19a. Frayer, J. M., Vermont Agr. Exp. Sta. Bulletin No. 313; 1930.

20. Frost, W. D., Rapid Method of Counting Bacteria in Milk, Sci., 42, pp. 255-256; 1915.

21. Gamble, J. A., Cooling Milk and Cream on the Farm, U. S. D. A. Farmers' Bulletin, 976.

22. Gamble, J. A., and Kelley, Ernest, The Effect of Silage on the Flavor and Odor of Milk, U. S. D. A. Bulletin No. 1097; 1-24.

23. Getchell, B. E., Electric Pasteurization of Milk, Diag. Ag. Eng. 16; pp. 408-10; 1935.

24. Guthrie, E. S., The Cow as a Source of "Oxidized" Flavors in Milk, New York (Cornell) Exp. Sta. Bulletin No. 606.

25. Hale, H., and Bleecher, W. L., Active Chlorine as a Germicide for Milk and Milk Products, Journal Agr. Rsch., 26, 8; pp. 375-381; 1923.

26. Hammer, B. W., Studies on the Creaming Ability of Milk, Iowa Agr. Exp. Sta. Rsch. Bulletin No. 31; 1916.

27. Hammer, B. W., Dairy Bacteriology, John Wiley & Sons, Inc., New York.

28. Hastings, E. G., Journal of Dairy Science, 2; pp. 293-311; 1919.

28a. Hileman, J. H., "Abstract", Dairy League Cooperation, Newark, N. J.

29. Hotis, R. P., and Babcock, C. J., The Present Status of the Pasteurization of Milk, U. S. D. A. Bulletin No. 342; 1932.

30. Kelly, Earnest, and Babcock, C. J., Production of Clean Milk, U. S. D. A. Farmers' Bulletin No. 602; 1932.

31. Kelly, Earnest, and Clement, Clarence E., Market Milk, New York.

32. Kilbourne, H., The Pasteurization of Milk from a Practical Viewpoint, New York, 1916.

33. Lochead, A. G., and Johns, C. K., The Preservative Effect of Chlorine Compounds in Milk, Com. Canada Exp. Farms, Rpts., 1929.

34. Loveless, W. G., The Use of Chlorine Products as Germicides on Dairy Farms, Vermont Exp. Sta. Bulletin No. 369; 1934.

35. MacDonald, Margaret B., And Crawford Ester M., Removal of Onion or Garlic Flavor and Odor From Milk, University of Tennessee Agr. Exp. Sta. (Knoxville), Circular No. 14 (Revised) 1927.

36. MacDonald, Margaret B., and Crawford, Ester M., Removal of Bitter Flavor from Bitterweed Cream, University of Tennessee Agr. Exp. Sta. Circular No. 26.

37. Marcussen, W. H., Comparative Study of Cream Volume of Milk Pasteurized at 142° and 145° F., I. A. M. D. Gen. Sessions Proceedings Com. 21; 48-75 Illus.; 1928.

38. Marquardt, J. C., and Dahlberg, A. C., The Creaming of Milk at High Temperatures, N. Y. Ag. Exp. Sta. Tech. Bul. No. 180 (Geneva)

39. Marquardt, J. C., Creaming Properties of Milk, Milk Dealer, 19; 60, February 1930.

40. Mudge, C. H., and Lawler, B. M., Effect of Alkali Solutions on Bacteria Found in Unwashed Milk Bottles, Indus. and Eng. Chem. 20, 4; p. 378; 1931.

41. Myers, R. P., The Germicidal Properties of Alkali Washing Powders, Journal Agr. Rsch. 38, 10; pp. 521-563; 1929.

42. Myers, R. P., and Johnson, A. H., A Critical Study of the Methods for Determining Bacterial Properties of Chemical Sterilizers, Proc. Interntl Assn. Milk Dealers, 25; pp. 21-55; 1932.

43. Nicholas, J. E., Sperry, J. H., and Tonney, L. F., Electrically Heated Dairy Utensil Sterilizers, Pa. Ag. Exp. Sta. Bul. 296; 1933.

44. Parbitt, E. H., Milk Plant Monthly, p. 66; January 1936.

45. Parket, H. N., City Milk Supply, New York, 1917.

46. Passon, R. J., and Hotis, R. P., Care of Milk Utensils on the Farm, U. S. D. A. Farmers' Bulletin No. 1675.

47. Prucha, M. J., Wheeter, H. M., and Chambers, W. H., Germ Control of Milk, University of Illinois Ag. Exp. Sta. Bulletin No. 204, 1918.

48. Prucha, M. J., Chemical Sterilization in the Dairy Industry, Proceedings Interntl Assn. Dairy and Milk Inspectors, 16; pp. 319-328; 1927.

49. Prucha, M. J., Comparison of the Methylene Blue and Plate Counts in Grading Milk, Milk Dealer, September 1929.

50. Roadhouse and Henderson, California Ag. Exp. Sta. Bulletin No. 595; 1935.

51. Roadhouse, Regan, and Mead, 1926 California Ag. Exp. Sta. Rept. 26; p. 62; 1925.

52. Ruhe, H. A., Milk Plant Monthly, January 1936, p. 62.

53. (No author's name) The Flavor of Milk, N. Y. Ag. Exp. Sta. 1936, Circular No. 167, Geneva, N. Y.

53a. Southern Dairy Products Journal, March 1937, p. 62.

53b. Talbut, M. W., Bureau Plant Industry, U. S. D. A.

54. Theophilus, D. R., and Atkenson, F. W., Sterilizing Dairy Utensils on the Farm, University of Idaho, Bulletin No. 183; 1931.

55. Tonney, F. O., Greer, F. N., and Leibig, G. F., The Minimal Chlorine Death of Bacteria, Am. Jour. Public Health, 20; pp. 503-508; 1930.

56. Thurston, L. M., and Olson, H. C., The Bacterial Flora of High-Grade Milk Before and After Pasteurization, West Va. Ag. Exp. Sta. Bulletin No. 255; 1933.

57. Trout, G. Malcolm, Cream Line Studies of Market Milk, West Va. Ag. Exp. Sta. Bulletin No. 229; 1930.

58. Whittaker, H. A., Archibald, R. W., Shere, L., and Clement, Effect of Various Factors on the Creaming Ability of Market Milk, U. S. D. A. Dept. Bulletin No. 1344.

59. Wylie, C. Elmer, The Adaptation of the MacDonald Process for the Removal of Onion Flavor and Odor in Milk to Creamery Practice, Journal of Dairy Science, Vol. XI, No. 1, January 1928; pp. 106-108.

60. Zoller, Harper F., Sodium Hypo-Chlorite, The Rate of Decomposition of Hypochlorite in Cow's Milk, Jour. Dairy Science 6, 4; pp. 310-319; 1923.