EFFECT OF VARIOUS METHODS OF COOLING ON SOME OF THE FACTORS AFFECTING QUALITY OF MILK

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INTRODUCTION

It is universally recognized and agreed that milk should be cooled soon after it is drawn from the cow. Those engaged in the production of milk for market purposes are to some degree cognizant of the increasing importance of producing high quality milk. Several factors that are commonly grouped together to determine milk quality are bacterial numbers and types, flavor and odor, sediment, and composition.

Sommer (27) states that under average conditions improper cooling of milk is the most important cause of a high bacteria count. Some dairymen are of the opinion that cooling milk over an aerator type of cooler improves the flavor, while others do not agree. Likewise the creaming ability is frequently discussed as being affected by the various methods of cooling. While the methods of cooling do not directly affect every factor concerned in milk quality, it is generally conceded that certain changes in milk are favored by the cooling methods used. Because of these changes that are alleged to take place, depending upon the cooling procedure, the subject of milk cooling has evolved into a matter highly controversial in nature.

Frequently in areas where the milk produced is primarily for bottling purposes, the methods of cooling and the time allowed for reaching a specified temperature after the milk is drawn from the cow, are set by ordinances. It is not uncommon to find a milk shed where the surface aerator type of cooler is recommended by authorities, while in an adjacent area the cold water tank type of cooling is advocated; and still another area may favor a combination of the two. For these reasons the average milk producer often is quite perplexed by the apparent or real confusion that exists in his own locality as to the best cooling method for the production of high quality milk.

This study embraces a consideration of the two more commonly used methods of cooling milk, namely, the surface aerator and the cold water tank cooler, with particular reference to comparison of the flavor, bacterial count, and creaming ability when cooled by the various methods.

The consumer today is more exacting in his demands for quality than ever before. Especially is this true in reference to food products in general and dairy products specifically. Flavor is rapidly taking its place as one of the more important requisites of milk quality. The consumer continues to be more discriminating in the desirable and undesirable qualities of flavors in milk and milk products that make up a large portion of his diet. Naturally the place to start flavor improvement is with the producer, but unfortunately in many instances he is the least informed of those who may better the situation. To add to his plight the confusion which exists on this one factor of cooling as related to flavors is but defeating to some degree the purpose of a universal effort to improve the flavor of milk.

The rate of bacterial reproduction depends upon temperature, presence of food nutrients, moisture, hydrogen ion concentration, oxygen supply, concentration of nutrients, and light. In controlling bacterial reproduction it is necessary to break their growth cycle by one or more of the factors on which their reproduction depends. In milk the one factor affecting their reproduction that is capable of being thus adjusted without materially changing the properties of milk is temperature. Bacteria, like all living matter have their minimum, optimum, and maximum growth temperatures. When temperatures above the maximum for an organism are encountered Gay (12) stated that death results due to protoplasm coagulation. Minimum temperatures do not bring about death but prolong the generative cycle. The destruction of bacterial life by heat is not adaptable to milk producing farms; therefore a temperature below their growth requirements which prolongs the rate of reproduction, is resorted to. Different species of bacteria vary in the temperature requirement to slow their growth processes. There is a close correlation between regenerative time and temperature within a given species of bacteria; however, this regenerative time varies materially in different milks because of the bacterial flora present. These factors have a tendency to be confusing in that opinions and evidence sometimes do not entirely agree on a definite temperature to accomplish the satisfactory retardation of bacterial reproduction.

Kelly and Clement (18) state:

> "If cans of warm milk are placed in a tank of cold water, much time is required to cool the large mass of milk. For this reason some form of a cooler which chills the milk instantly should be used."

Marquardt and Dahlberg (20) disapprove immediate cooling:

> "Warm fresh milk of good flavor should be placed immediately after milking into a well insulated water tank at 40° F. without previous cooling or agitation."

The latter statement is based on the contention that milk will inhibit bacterial growth for a few hours after it is drawn from the cow. The nature of this inhibition is founded in three schools of thought, namely, there actually is in milk a bacteriostatic substance; time is required for bacteria to adjust themselves to a new environment; and a combination of the two. Irrespective of the correct explanation, this characteristic does give an apparent excuse to delay the cooling of milk. It is with these factors in mind together with their relation to flavor, that delayed milk cooling is here considered with the hope of clarifying its relation to milk quality.

The creaming ability of milk is a characteristic indirectly related to milk quality; however, it is regarded by the consumer as very important in indicating richness of milk. The increasing popularity of homogenized milk and the introduction of non-transparent milk containers will tend to minimize the importance of creaming ability of milk. However, until these practices are more universally used, the creaming ability of milk will continue to be important.

Factors affecting the creaming ability of milk are numerous, but the two involved in cooling are agitation and temperature. The influence of varying temperatures will be disregarded as the study embraces a comparative analysis at one temperature only. The amount of agitation affects fat globule clustering, which is essential for creaming of milk; and, therefore, has been considered from the standpoint of the various cooling methods studied.

The purpose of a milk ordinance is to protect the consumer, insuring at all times a safe and healthful milk supply. It is not impossible to find milk producers who challenge the validity of such an ordinance with favorable results. While the bacteria count is our best indication of the sanitary practices that have been exercised in the production of milk, it may not be wise to dismiss the conjecture that has been made in more recent years regarding the significance of bacteria in producing desirable or inhibiting undesirable flavors in milk. If, however, it is found imperative in the production of quality milk to set definite time intervals and cooling temperatures the practice should receive universal and official sanction. Some authorities (27) (20) infer that regulations are sometimes imposed that discriminate against the milk producer, and if this is true such practices are injurious not only to the producer but to the manufacturer and consumer alike.

REVIEW OF LITERATURE

The cooling of milk under farm conditions has received rather generous experimental attention. The literature published on this subject shows conclusively that cooling practices have long been recognized as important in the production of high quality milk. Some of the publications pertaining to milk quality as affected by cooling are reviewed here.

Bacterial Count

Considerable controversy exists regarding bacterial contamination of freshly drawn milk. Kelly and Clement (18) expressed the opinion that milk must be cooled immediately. They stated that cans of warm milk placed in cold water required much time to cool the large mass of milk and for this reason some form of a cooler which chills milk instantly should be used. Macy (19) concluded after a study of delayed milk cooling that milk produced in accordance with approved methods should not have an excessive bacterial count for pasteurization purposes if received at the pasteurizing plant within five or six hours after milking. It should be understood that Macy was dealing with fresh milk to be pasteurized soon after milking and did not infer that cooling is unnecessary. However, the results of his work may be construed by some as a partial foundation to the argument that it is not necessary to immediately cool milk after it is drawn from the cow.

It is generally agreed among authorities that low bacterial counts are one of the more important requisites of quality milk. The significance of a high count, however, is sometimes questioned. Breed (3) in discussing the application of bacterial counts to sanitary milk control pointed out that not infrequently high count milk is milk undergoing normal lactic acid fermentation, a process that certainly is not to be regarded as filthy. Sommer (27) likewise stated that dirty milk does not necessarily give a high count, any more than low counts definitely prove that the milk is of high quality. Gamble (9) and Frayer (8) concluded that most high counts in milk are the result of bacterial reproduction and not the result of original inoculation as is sometimes implied. Regardless of the significance of the point in question, evidence (8) (7) (2) (23) substantiates the importance of bacterial counts and their relation to milk quality.

Bowen (2) showed conclusively the importance of cooling poor quality milk in controlling the bacterial count. In a survey including one hundred and two dairy farms known to produce average quality milk, the temperature to which the milk had been cooled was lowered from 62° F. to 54° F., which effected a reduction of 36 to 1 in the bacterial count. Frayer (7) and Downs and Lewis (6) concluded from their experiments on delayed cooling that milk must be cooled in 1 to 1½ hours if the keeping quality is to be insured. The longer cooling was delayed beyond this time limit, the poorer was the keeping quality. Frayer (7) pointed out that this injury to keeping quality is not evident at the time the milk is delivered and may not be manifested until the milk is two or three days old. It is the final recommendation of Frayer (8) that milk be cooled immediately to 40° F. or below if the best results with respect to quality, both present and future, are to be obtained. It is surmised that the conclusion is referring to bacterial count only and is not embracing the items of flavor and odor and their relation to quality.

A comparison of the effect of gradual and rapid cooling on the bacterial count was made by Marshall (22) and his results show very clearly that the bacteria count is greatly increased by the process of aeration. One series of Marshall's (22) experiments showed that the bacterial count increased from 8,400 per ml. to 31,950 per ml. in milk held in a closed can for 21 hours, while under the same conditions the bacterial count of milk which had been aerated increased from 8,400 per ml. to 14,096,400 per ml.

An exhaustive study has been made by Marshall (22) (21) regarding the interchange of gases and their affect on bacterial growth. He reported that the gas content of fresh milk directly from the udder was 8.0 per cent by volume, which consisted of 59.63 per cent carbon dioxide. He further believed that carbon dioxide was toxic to bacterial life. During the milking process part of the carbon dioxide was liberated, making the milk a more suitable medium for bacterial reproduction. Aeration of milk continued to liberate carbon dioxide until 73.63 per cent had escaped from the milk. The aeration process also makes possible the absorption of oxygen which is necessary for aerobic bacteria, and, therefore, makes the milk a more satisfactory medium than before aeration. Jordan (17), however, took issue with this theory when he stated:

> "Carbon dioxide is produced not only as a result of metabolic activities of bacteria but is apparently a necessary component of bacterial environment."

In this connection the toxic property of molecular oxygen in sufficient amounts must also be considered as detrimental to bacterial growth. Until the mechanisms of these effects are more completely understood it seems that compatible elements of truth exists in each theory.

Principles on Which Delayed Cooling is Based

Macy (19) has stated that those changes occurring in bacterial content during the first few hours after milking depend upon germicidal property, initial count, time, and temperature. This list may be more complete by the addition of those influences exerted by the lag phase and agglutination reactions. These influences exerted by the lag phase and germicidal property of milk constitute the points to be considered in this study of delayed milk cooling.

Stocking (28) inoculated fresh milk with various types of bacteria and found that growth of some species was not inhibited. He concluded there was no property or condition in milk that was germicidal in nature, but the phenomenon referred to as the germicidal period was nothing more than the natural result of ill-suited environment for various species. Sommer (27) likewise asserted that any germicidal property that may be present is so light in its influence that it would be very hard to distinguish it from the demonstrable lag phase.

Lactenin, a protein-like substance, was recovered from the serum of milk by Jones and Simms (16) that possessed bacteriostatic properties. These workers reported success in concentrating this substance from milk serum that had from 200 to 500 times the inhibitive properties of normal serum. As would be expected, its germicidal properties varied in inhibitory action on various species of bacteria. Orla-Jensen and Jacobson (24) and Jones and Little (15) also report the presence of a thermolabile bactericidal substance in milk. Jones (14) determined that the substance was capable of passing through the coarse and medium bacteriological filters but was retained by the fine filter.

Hammer (13) and Orla-Jensen and Jacobson (24) have also advanced the agglutination reaction as being a plausible explanation of the retarding of bacterial growth in fresh milk. The presence of group agglutinins would result in bacteria clumping or reduction in counts until all of the alexin (15) was fixed by the agglutination reaction.

The Relation of Milk Cooling Practices to Flavor and Odor

From the publications available it is evident that insufficient attention has been given to the important milk quality factors of flavor and odor. Marshall (22) in his study of gas interchange concluded that off-flavors and odors may be readily absorbed with the oxygen intake of milk during aeration. This theory demonstrates the advisability of aerating milk in a room that is free from undesirable odors. Gamble (10) asserted that milk should be cooled over an aerator immediately after milking for the most efficient results. If the greatest amount of off-flavors and odors are to be liberated by aeration the two processes of cooling and aeration cannot be carried on simultaneously according to Marshall (22).

Gamble and Kelly (11) fed varying amounts of different kinds of silage and found that the flavor of milk was improved by aeration in proportion to the amount of silage consumed. Their results indicated that cooling and aeration can be carried on as one operation.

Babcock (1) made a study of reducing off-flavors by aeration of milk from cows that were receiving varying quantities of alfalfa hay immediately before milking. Regarding each flavor score as an observation, his results showed that 46 per cent of the samples rated a normal flavor classification before aeration as compared with 56 per cent receiving normal classification after aeration.

Working with the cold water tank cooler, Marquardt and Dahlberg (20) found that good flavored milk could be cooled satisfactorily in the cans; however, their results favored aeration for the removal of some feed flavors. Associates of Rogers (26) pointed out that almost every variety of feed flavor has been reported as also being caused by bacteria. Naturally, until the source is removed such flavors will not be satisfactorily improved by aeration.

Creaming of Milk

Normally twenty-four hour creaming will produce a cream volume of 4.1 times the per cent fat (4). Many factors enter into variations that are common in the creaming ability of milk, the method of cooling being one of the less important. Dahlberg and Marquardt (5), making observations on the creaming of raw milk, reported that agitation above 60° F. slightly increased the cream volume, while agitation below 40° F. caused a slight yet perceptible decrease in cream volume. The same authors in another series of experiments (4) found that tubular cooled milk gave a greater cream volume when cooled to 60° F. than check samples which were not aerated. It was further determined that agitation resulting from hauling and stirring of full cans of milk was not sufficient to alter the creaming ability. Trout (29) and Whittaker, Archibald, Shere, and Clement (30) reported that the agitation caused by pumping is sufficient to reduce the cream volume slightly. Therefore, it seems logical to make the deduction that agitation caused by aeration would be insignificant in the creaming ability of milk.

Agitation of Milk While Cooling Gradually

Marquardt and Dahlberg (20) concluded that high quality milk may be cooled in the can without stirring. The flavor of milk was not impaired by this process and it also minimized the possibility of bacterial contamination. Price, Hurd and Copson (25) found that agitation of milk by stirring during gradual cooling was not necessary. Bowen (2) cooled milk at 95° F. in a tank with 37° F. water and reported that agitation during cooling is advisable in so far as rate of cooling is concerned.

PLAN OF STUDY

Products Used. The milk used in these experiments was produced by the College dairy herd. No effort was made to effect alterations in the normal procedure of milk production as practiced by the College Dairy Department. The condition of the barn, milk house, and milk utensils was representative of what one may find at any time the milk production facilities of the College are inspected.

Selection and Care of Cows. The cows producing milk for this series of experiments represent the Holstein, Jersey, and Guernsey breeds. They were not selected individuals but consisted of the same breeds in the two main divisions of the experiment. The cows were fed alfalfa hay approximately two hours before milking and while milking they were fed a concentrate mixture consisting of oats, corn, bran, and cottonseed meal.

Milking. The regular noon milking of the test cows was used in each instance. Two milkers were on duty at this time. For the first division of this study, the same two milkers did all of the milking. In the second division, one of the milkers in the first division was on duty and the other was replaced by a new milker. Neither the milkers nor herdsman were informed regarding the days experiments would be conducted. The experiments were generally started within fifteen minutes after a sufficient quantity of milk had been obtained.

Time of Year Experiments Run. The first division of the experiment was conducted during 12 weeks of the late fall and early winter of 1937. The second division was conducted during the spring and early summer of 1939. This makes a fair representation of the various seasons of the year and allows several conditions to be manifested that may not otherwise be encountered in any one season.

Equipment Used. The first series of these experiments were run in the College creamery. The surface cooler used was constructed of 16 three-quarter inch tinned tubes 16 inches long. Brine was used as a refrigerant, and the temperature was controlled by governing the flow of milk over the cooler and the amount of brine through the tubes. The milk cooled gradually from body temperature to 60° F. in a tank was controlled by adjusting the temperature of the cooling water, as was determined by preliminary trials, to produce the desired temperature in the desired time interval. The experiments of the second series were conducted in the milk house at the College dairy barn. The details were the same as in the first series with the exception that the refrigerant used in the surface cooler was cold water from the combination tank and surface cooler. The term aeration as used in these experiments implies the exposure of milk in a thin layer to atmospheric conditions during the cooling process.

Cooling Methods. Immediately following the straining of the milk by the barn employees, which was their last connection with the experiment, twenty gallons were thoroughly mixed by pouring from one can to another several times. The mixed herd milk was then poured into steam sterilized cans and maintained at a temperature between 95° F. and 98° F. by partly immersing the cans in warm water until each separate set was removed as will be explained presently.

From the cans of milk, a composite sample was taken for analytical purposes, cream volume observations, and flavor determinations.

Five portions of the milk were cooled by a surface cooler, and two portions were cooled in a can partly immersed in cold water. Table I shows the method of treatment of each portion. As each set was divided it was placed into a steam sterilized can in order that results might be as nearly comparable as possible.

TABLE I

Methods, Temperatures, and Time Intervals
Before Cooling

Set identification	Method of cooling	Temperature range before cooling	Temperature cooling process stopped	Time elapsed before finish of cooling process
A	surface cooler	95° F. to 98° F.	60° F.	0
B	surface cooler	95° F. to 98° F.	60° F.	30 minutes
C	surface cooler	95° F. to 98° F.	60° F.	60 minutes
D	surface cooler	95° F. to 98° F.	60° F.	90 minutes
E	surface cooler	95° F. to 98° F.	60° F.	120 minutes
F	cold water tank	95° F. to 98° F.	60° F.	60 minutes
G	cold water tank	95° F. to 98° F.	60° F.	120 minutes
control H	cold water tank	95° F. to 98° F.	50° F.	5 minutes

Flavor Samples. During the progress of the first series of these experiments a single flavor sample was taken from each set immediately after the final cooling temperature had been reached. These flavor samples were placed in sterile half-pint bottles and stored at 50° F. for 16 hours before scoring. In the second series of these experiments three flavor samples were taken from each set immediately after reaching the temperature for the method concerned. One group of these samples was held at 34° F. until the completion of the days experiment; another group of samples was held at the same temperature for 16 hours, while still another group was held at 50° F. for 16 hours. In each experiment control samples were taken.

Bacterial Activity. It was desired to determine as nearly as possible the trends of bacterial activity for each of the various sets cooled. For this purpose samples for bacterial analysis were taken from each set at thirty minute intervals for two hours and again after 16 hours storage at 50° F. Table II shows more explicitly the schedule used in taking samples for bacterial analysis the first two hours. The samples for bacterial analysis taken as designated in table II, showed changes that may occur in morning's milk before it reaches the processing station. Those samples taken 16 hours later were comparable to the evening milk held over night on the producer's farm. Table III shows the schedule of samples taken for bacterial analysis after 16 hours.

Milk Inoculated with Bacterial Cultures. In the second series of these experiments it was desired to ascertain more definitely the influence of bacteria on flavor; thereby determining changes that might be expected to occur in high-count milk. This was accomplished by inoculating the milk before cooling with mixed cultures of organisms known to occur in milk. To check on the influence exerted, counts were made in accordance

TABLE II

Schedule of Samples Taken for Bacterial Analysis
for the First Two Hours

Set identification	Sample number	Method of cooling	Time elapsed before cooling started	Temperature	Explanation
A	1	--------------	0	95° to 98° F.	immediately before cooling
	2	surface cooler	0	60° F.	immediately after cooling
	3	surface cooler	0	60° F.	30 minutes after cooling
	4	surface cooler	0	60° F.	60 minutes after cooling
	5	surface cooler	0	60° F.	90 minutes after cooling
	6	surface cooler	0	60° F.	120 minutes after cooling
B	7	--------------	30 minutes	95° to 98° F.	immediately before cooling
	8	surface cooler	30 minutes	60° F.	immediately after cooling
	9	surface cooler	30 minutes	60° F.	30 minutes after cooling
	10	surface cooler	30 minutes	60° F.	60 minutes after cooling
	11	surface cooler	30 minutes	60° F.	90 minutes after cooling
C	12	--------------	60 minutes	95° to 98° F.	immediately before cooling
	13	surface cooler	60 minutes	60° F.	immediately after cooling
	14	surface cooler	60 minutes	60° F.	30 minutes after cooling
	15	surface cooler	60 minutes	60° F.	60 minutes after cooling
D	16	--------------	90 minutes	95° to 98° F.	immediately before cooling
	17	surface cooler	90 minutes	60° F.	immediately after cooling
	18	surface cooler	90 minutes	60° F.	30 minutes after cooling
E	19	--------------	120 minutes	95° to 98° F.	immediately before cooling
	20	surface cooler	120 minutes	60° F.	immediately after cooling
F	21	cold water tank	0		30 minutes after cooling
	22	cold water tank	0	60° F.	60 minutes after cooling
G	23	cold water tank	0		30 minutes after cooling
	24	cold water tank	0		60 minutes after cooling
	25	cold water tank	0		90 minutes after cooling
	26	cold water tank	0	60° F.	120 minutes after cooling
control H		cold water tank	0	50° F.	

TABLE III

Schedule of Samples Taken for Bacterial Analysis Immediately after Cooling to 60° F. and Held for 16 Hours at 50° F.

Set identification	Method of cooling	Time elapsed before cooling started	Time held after cooling	Holding temperature
A	surface cooler	0	16 hours	50° F.
B	surface cooler	30 minutes	16 hours	50° F.
C	surface cooler	60 minutes	16 hours	50° F.
D	surface cooler	90 minutes	16 hours	50° F.
E	surface cooler	120 minutes	16 hours	50° F.
F	cold water tank	cooled in 60 minutes	16 hours	50° F.
G	cold water tank	cooled in 120 minutes	16 hours	50° F.
H	cold water tank	0	16 hours	50° F.

with the procedure as outlined in tables II and III.

Cream Volume. The cream volume was determined after 18 hours of creaming at 45° F. by the use of 100 ml. graduated cylinders. In each instance the cylinder was filled immediately after the milk for that particular set had been cooled to 60° F.

Germicidal and Lag Phase. An attempt was made to demonstrate the effectiveness of any germicidal property that may be present in milk. This was done by inoculating freshly drawn milk immediately with known cultures, and 15 hours later inoculating a batch of the same milk under the same conditions and observing the trend of bacterial growth at thirty minute intervals for three hours.

Agitation While Cooling. In view of results published on the effect of agitation on the cooling of milk in 10 gallons cans, it was thought timely to conduct some experiments on this matter. Full 10 gallon cans of milk immersed in water to the shoulder of the can were used for these determinations.

Bacterial Count of Tank Cooling Water. Not infrequently has the writer heard inquiries regarding the bacterial contamination of water used for cooling in tank coolers. A series of counts were made on the water from the tank cooler at the College dairy barn under various operating conditions. The possibilities of controlling this contamination with chlorine were also studied.

Number of Bacteria. All bacterial counts were made on nutrient agar as outlined in Standard Methods of Milk Analysis.

Flavor and Odor. The flavor and odor in the first series of these experiments was evaluated by the writer. In the second series these determinations were made by judges who were members of the Dairy Department staff at Oklahoma Agricultural and Mechanical College. In no instance was the writer or other judges aware of the sample identification during the process of scoring.

EXPERIMENTAL RESULTS AND DISCUSSION

Analysis of Milk Used

The first series of experiments conducted in this study were on high quality milk. A composite sample was taken from each batch of milk for analytical purposes. Table IV reveals the average results of twelve analyses on milk used in the following experiments. Results show that the milk used in these experiments was of normal composition.

Effect of Cooling Practices on Bacterial Count of High Quality Milk

It was desired to study the influence of delayed surface cooling and gradual can cooling upon the bacterial count of high quality milk such as should be used for bottling purposes. In order that results might be more applicable to the customary procedure of milk producing farms, samples were taken soon after cooling, representing morning's milk, and again at the end of a 16 hour storage period, representing night's milk. Bacterial counts were also made at thirty minute intervals on the milk cooled by each method in order to determine bacterial activity during the first two hours after milking.

These experiments were made on twelve different lots of milk in as many weeks. All counts were made in duplicate. Ten lots of milk used had a bacterial count of less than 8,000 bacteria per ml., while the count of the other two lots did not exceed 24,000 per ml. The bacterial count is not to be considered as the important factor in these studies; instead, it is desired to show from an average of several experiments the trend of bacterial activity in milk cooled in accordance with methods outlined.

The per cent increase in bacterial count over the original count for each of the methods of cooling has been determined. The average of twelve

TABLE IV

Analysis of Milk Used

Per cent fat (Mojonnier)	3.99
Per cent fat (Babcock)	3.97
Per cent total solids (Mojonnier)	12.74
Per cent acid (calculated as lactic)	0.18
pH (Coleman electrometer)	6.53
Curd tension (curd test unit*)	51.33
Specific gravity (calculated)	1.032

*This unit manufactured by E. L. Cobb and Son.

trials for each set was calculated from these determinations. Figure 1 shows the trend of bacterial activity in fresh milk cooled to 60° F. immediately after this temperature had been reached by each of the various cooling methods. Bacterial activity as shown in set A, figure 1, is peculiar within itself. The 85.6 per cent increase over the original count occurred in approximately five minutes. No doubt a portion of this increase was contamination from the atmosphere and cooler, notwithstanding the fact that the cooler was cleaned well before using. An increase in bacterial count due to aeration was also reported by Marshall (22). The increase may have been influenced materially by gas exchange during aeration. The bacteria present having existed under unsuitable conditions due to the presence of excessive carbon dioxide, suddenly increased in number upon the liberation of carbon dioxide and the intake of oxygen which created a more suitable medium for aerobic types of organisms.

There was a decrease in bacterial numbers when surface cooling was delayed 30 minutes, set B, as compared with immediate cooling, set A. This phenomenon remains to be satisfactorily explained. As was previously stated such decreases are evidently effected by some germicidal property or agglutination reaction.

The longer cooling was delayed the greater was the bacterial count in surface cooled fresh milk. The results indicate that the cooling of milk by surface coolers should be done within 1½ hours after drawing if the bacterial count is to be held at a minimum. It is the opinion of the writer that high quality milk to be used for pasteurizing purposes, that reaches the processing plant within 3 hours after it is drawn, needs no previous cooling. It is to be understood of course that such practices apply only to milk from disease-free cows and produced under the most exacting conditions.

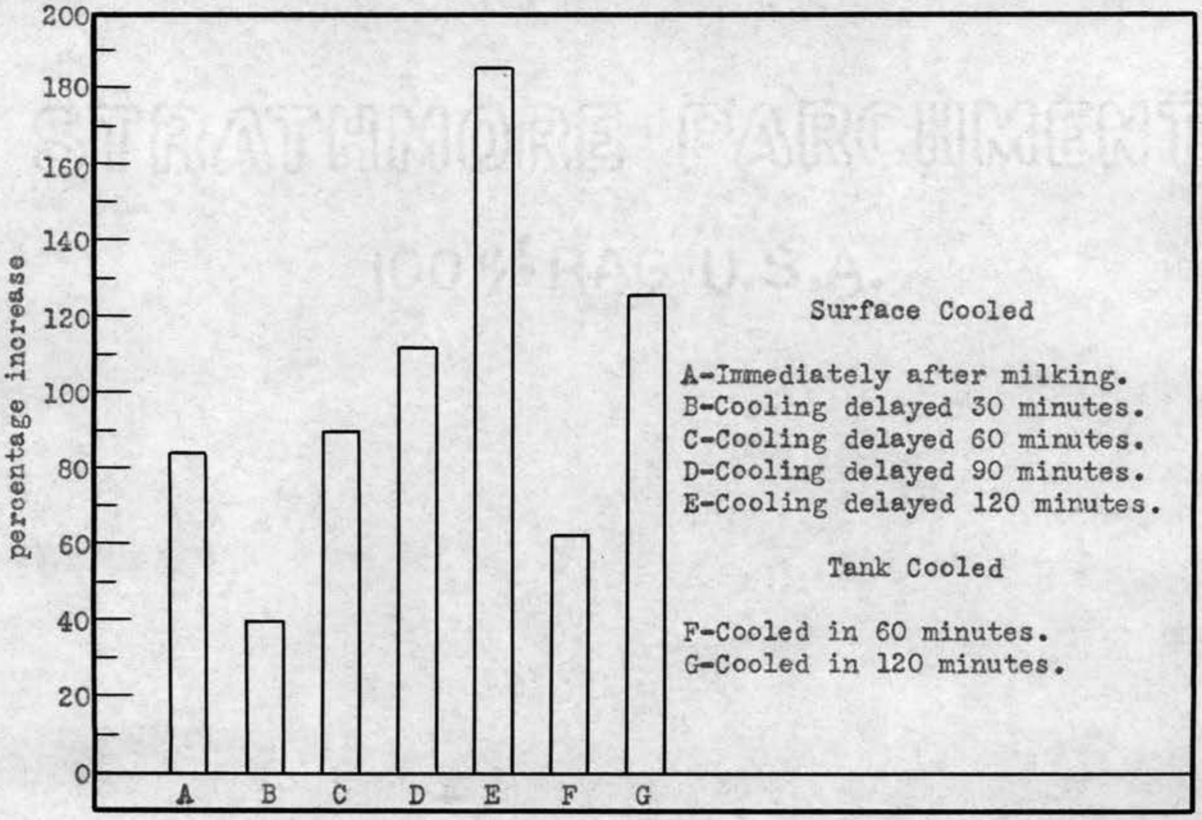

Figure 1

The Percentage Increase in the Bacterial Count of High Quality Milk Immediately after Cooling by the Methods Studied

So far as bacterial count is concerned in the immediate quality of milk, figure 1 shows that can cooled milk is superior. Factors responsible for this superior quality are minimum contact with utensils and atmosphere and the maintenance of a more nearly anaerobic condition.

To facilitate analysis of bacterial activity during the first two hours after milk was drawn, counts were made on each set of milk at 30 minute intervals before and after cooling. The results of such analysis are shown in figure 2. These studies further substantiate deductions previously discussed, namely: aeration of milk resulted in an immediate increase in bacterial count; when cooling was delayed longer than 1½ hours the bacterial count increased more rapidly the following hour; and milk cooled in the can contained a smaller number of bacteria than the same milk surface cooled.

The per cent increase in the bacterial count of milk caused by the process of surface cooling was determined by averaging the differences between the per cent increase immediately before and immediately after cooling. For the twelve experiments this increase was found to be 28.8 per cent. The increase in the bacterial count of milk in which surface cooling was delayed 1 hour, set C, was 27.1 per cent greater than set F, which was can cooled in 1 hour. The increase in the bacterial count of milk in which surface cooling was delayed 2 hours, set E, was 59.2 per cent greater than set G, which was can cooled in 2 hours.

The future trends of bacterial activity in the same milk are illustrated in figure 3. This figure shows that the increase in the bacterial count of high quality milk held over night at 50° F. is closely related to the length of time cooling is delayed. For lowest bacterial counts the surface cooling of milk to be held over night should not be delayed more than one hour as set C, figure 3 indicates. The increase

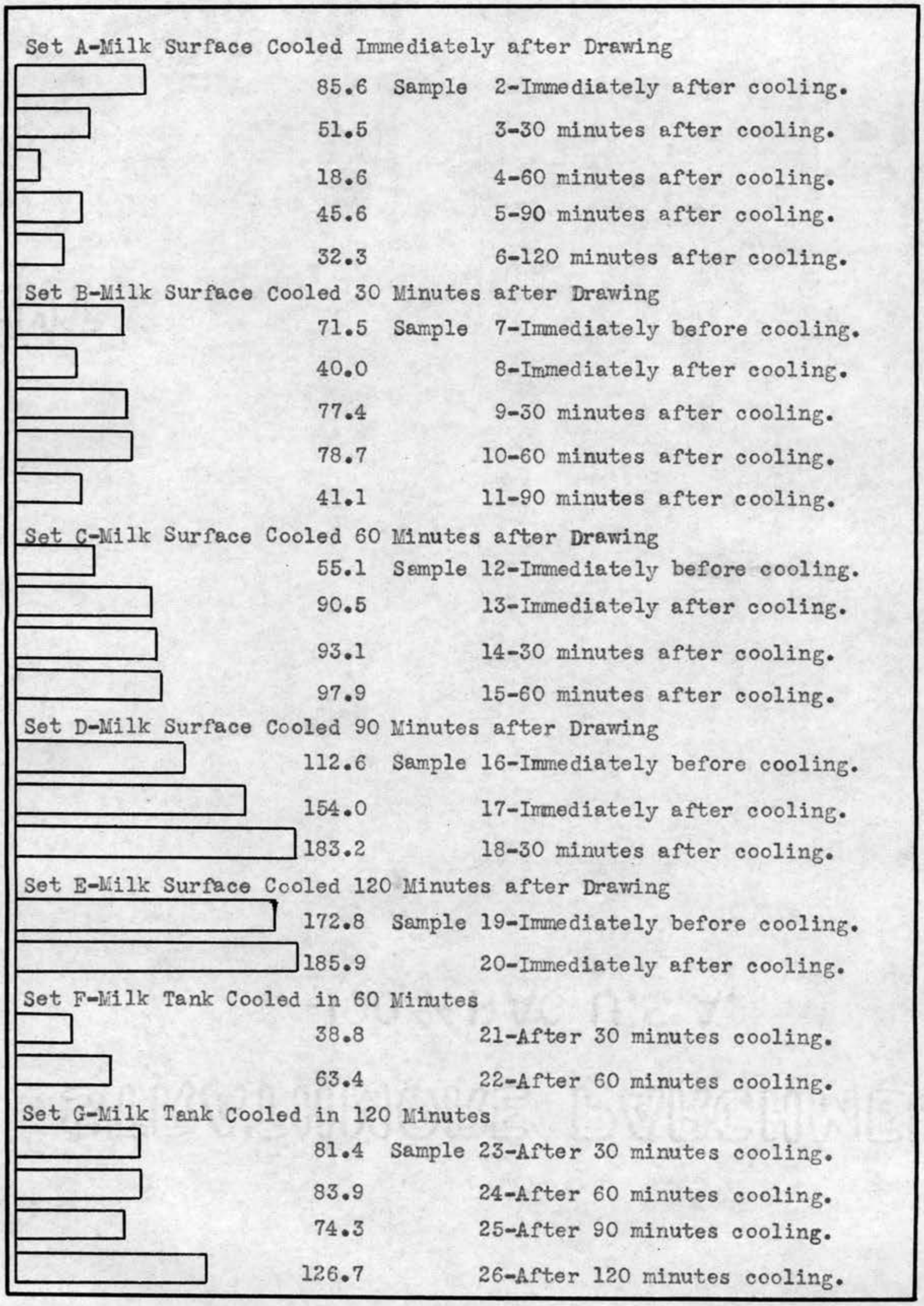

Figure 2

The Percentage Increase in Bacterial Count of Freshly-drawn Milk as Affected by Delayed and Gradual Cooling

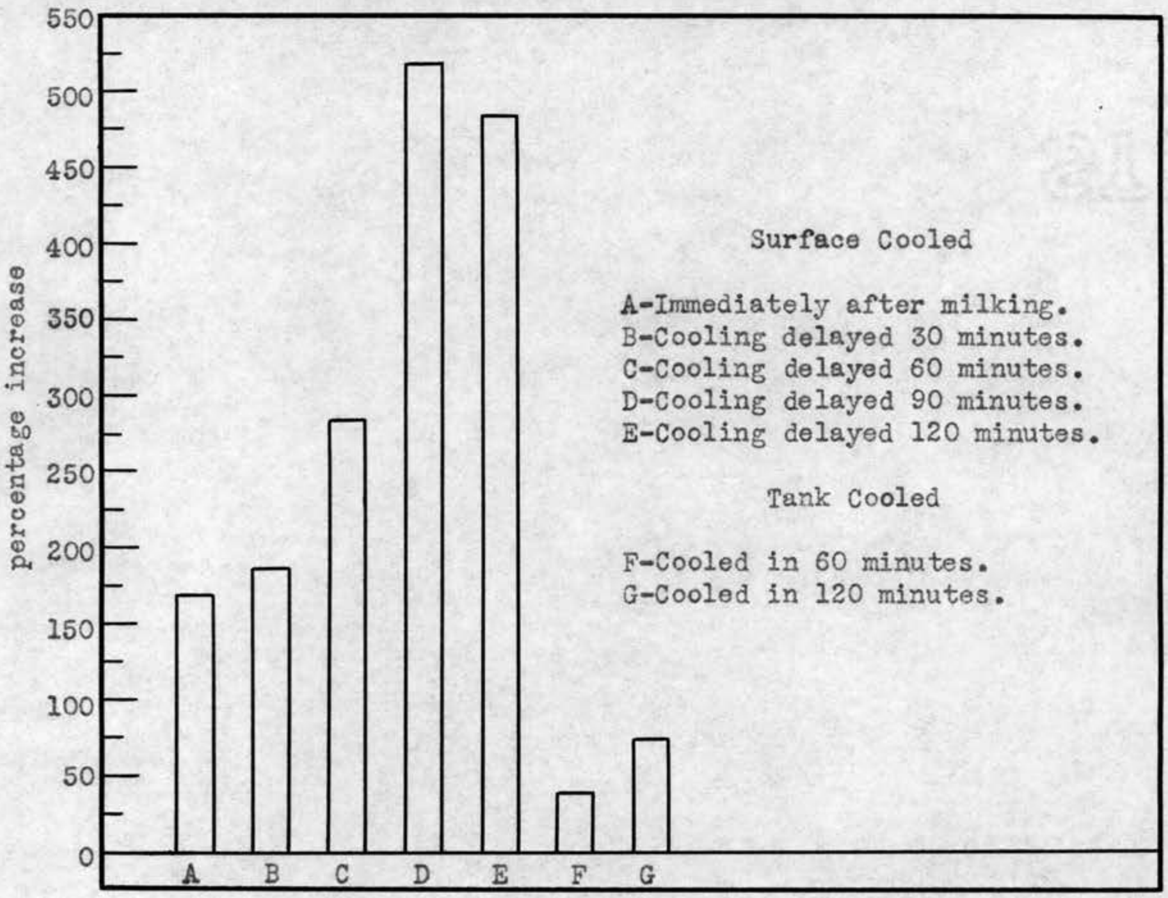

Figure 3

The Percentage Increase in the Bacterial Count of High Quality Milk Held 16 Hours at 50° F. after Having Been Cooled by the Methods Studied of 287.2 per cent in milk where cooling was delayed one hour is not sufficient to cause a change in grade where the original count is less than 10,000 per ml.

Cooling delayed one hour did not in any instance lower the flavor classification. It will be pointed out in the discussion on flavor of high quality milk that an increase of 534.9 per cent was concurrent in some instances with a decrease in flavor classification.

The trends of bacterial activity in can cooled milk that are shown in figure 1 are demonstrated more strikingly in figure 3. The results of these experiments indicate that reproduction in can cooled milk stops at a temperature of 50° F. or the inhibitory properties offset the actual reproduction.

Effect of Cooling Practices on Flavor of High Quality Milk

Ninety-six observations were made on twelve different lots of low count milk to determine the influence of various cooling methods on the flavor of such milk. As stated previously, flavor samples for these series were taken immediately after each set had been cooled, placed in a closed sterile bottle, stored for 16 hours at 50° F., and scored by the writer. In scoring the flavor and odor in this series of experiments the following classification was used: perfect flavor score 25; excellent, 23 and up; good, 21 and 22; fair, 18, 19, and 20; poor, 17 and below. A classification of the flavor and odor of the 96 observations are given in table V.

An analysis of table V reveals that in five trials out of twelve the method of cooling did not sufficiently influence the flavor and odor to change the classification of milk. Set D in which surface cooling was delayed $1\frac{1}{2}$ hours, scored below the most common classification for a single

TABLE V

Classification of the Flavor and Odor
of Low Count Milk

G-good, F-fair, P-poor, s-slight, v-very

Trial	Method of cooling								
number	A	B	C	D	E	F	G	H	Remarks*
1	F	F	F	P	F	F	P	F	cowy, oxidized
2	G	G	G	F	F	G	G	G	cowy, feedy
3	F	F	F	F	F	F	F	F	oxidized
4	G	G	G	G	G	G	G	G	s. feedy, s. cowy
5	F	F	F	F	P	F	F	F	cowy, s. feedy
6	F	F	F	F	F	F	F	F	feedy
7	F	F	F	F	F	F	P	P	cowy, s. feedy
8	F	F	F	F	F	F	F	F	cowy, oxidized
9	F	F	F	F	F	F	F	G	s. feedy, v. s. cowy
10	F	F	G	F	F	G	F	G	feedy
11	G	G	G	G	F	G	G	F	feedy
12	F	F	F	F	F	F	F	G	feedy, s. cowy

*Scored by the writer only.

experiment in two trials out of twelve. Set E in which cooling was delayed two hours, scored below the common classification in three trials out of twelve. Set G which was gradually cooled in two hours, scored below the common classification in two out of twelve trials. The conclusion is made from these experiments that one hour delayed surface cooling of high quality milk does not impair flavor and odor, nor do such cooling practices raise the flavor and odor classification above that of milk cooled gradually in one hour. Can cooling does not impair flavor if a temperature of 60° F. is reached in one hour after the milk is drawn.

One observation, that has instigated a study that shall be later considered, is the decrease in flavor classification of set G while the bacterial count remained low.

The method of flavor and odor classification in these studies did not indicate that one method was superior to another in improving the flavors and odors encountered. Feed flavors in these studies were not sufficiently pronounced to facilitate actual classification as to source.

Effect of Cooling Practices on Bacterial Count of Low Quality Milk

The experiments discussed have been concerned with milk that was generally recognized as a high quality product. In the following studies, milk that would ordinarily be regarded as a poor quality product from the standpoint of bacterial count was used. As in previous experiments, it was desired to study bacterial activity as influenced by various cooling procedures and the relation of bacteria to flavor and odor. In order that results may be more closely checked and the possible trends be of more significance, fresh milk was inoculated with prepared bacterial cultures. This procedure allowed for the scoring of control samples, therefore, more definitely determining the influence of bacteria.

From pure cultures on agar slants, transfers were made to nutrient broth medication tubes for preparation of the mixed culture to be used in these experiments. The medication tubes were incubated at 98° F. for 12 hours. From each one of the pure cultures 0.1 ml. of broth was transferred to a common 200 ml. flask containing 100 ml. of sterile skim milk. The skim milk was then incubated for 12 hours at 98° F. and a portion of this culture used to inoculate 10 gallon cans of milk. The following organisms were used in preparing this culture: Aerobacter aerogenes, Pseudomonas fluorescens, Pseudomonas fragi, Escherichia coli, Alcaligenes lipolyticus, Acetobacter putrefaciens, and a butter culture. The bacterial count of all inoculated samples of milk immediately after inoculation averaged 735,000 per ml. The average count before inoculation was 13,500 per ml. Samples for counts were taken immediately after each set had been cooled and again at the end of a 16 hour storage period at 50° F. Figure 4 gives the results of these experiments.

The per cent increase in the bacterial counts of these inoculated samples was small as compared to increases in higher quality milk. While an increase of 40.0 per cent in the count of low quality milk amounts to more bacteria than in the previously discussed results, the fact remains that an increase here of 50.0 per cent is no more significant from the standpoint of increase than a 50.0 per cent increase in high quality milk. Supposedly the influence of bacterial content becomes more pronounced in proportion to the numbers of bacteria present; however, some of the results obtained by the author do not substantiate this supposition. The per cent increase in the bacterial count of inoculated milk immediately after cooling resembled very much the trends in fresh milk. The decrease in count after storage is a phenomenon not understood. These results indicate that cooling procedure has little influence upon the bacterial count of low

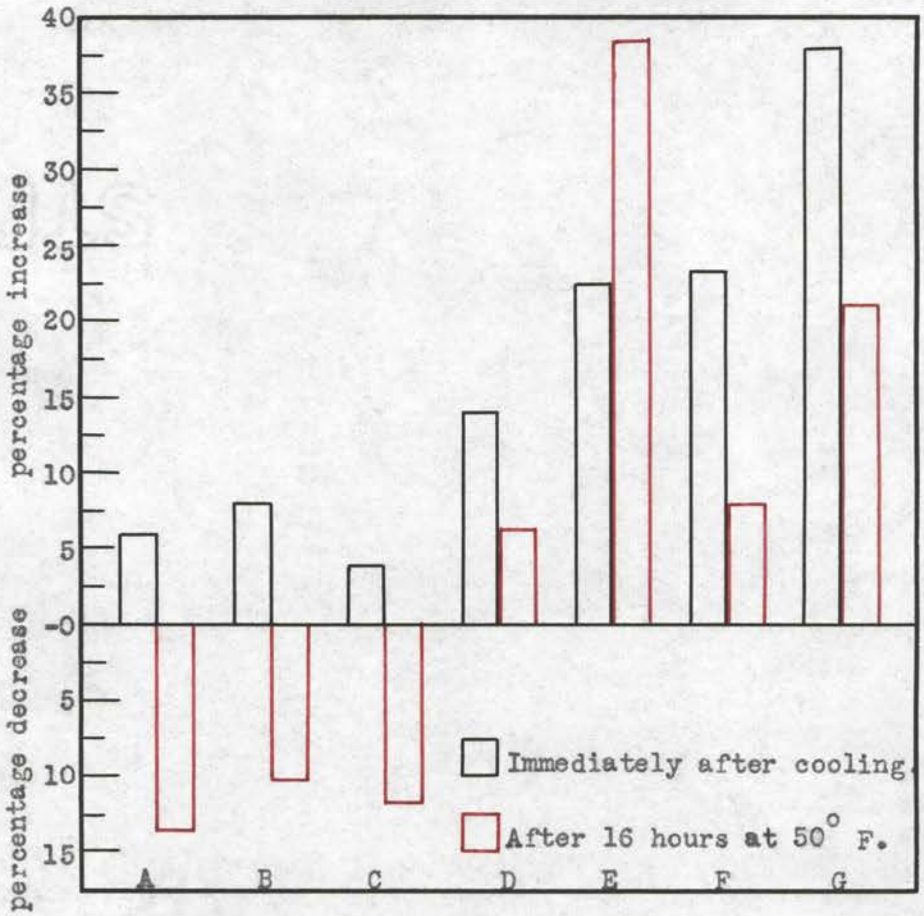

Figure 4

Trends of Bacterial Activity
in Inoculated Milk

Surface Cooled

A-Immediately after milking.
B-Cooling delayed 30 minutes.
C-Cooling delayed 60 minutes.
D-Cooling delayed 90 minutes.
E-Cooling delayed 120 minutes.

Can Cooled

F-Cooled in 60 minutes.
G-Cooled in 120 minutes.

quality milk so long as cooling is effected in a reasonable short time after milking. There is a possibility that results obtained in these studies may not compare in detail with results that would be obtained when working with natural contamination.

Effect of Cooling Practices on Flavor of Low Quality Milk

From the results of one phase of study in the experiments dealing with high quality milk it was noted that increased flavor and odor scores were not consistent with decreased bacterial counts. These peculiar results have led to a study of the relation existing between flavor and bacterial count.

In these experiments the inoculated milk used for bacterial analysis was used also for flavor and odor determinations. The samples were scored by members of the Dairy Department staff on basis of 25 being a perfect flavor and odor score. The scores of all judges were averaged for each set and each trial, and are expressed in numerical value.

Three controls and three samples from each set were taken for these studies. It was desired to determine how rapidly the influence of high bacterial counts may become noticeable; therefore, one sample from each set was placed immediately in ice and water and scored at the end of that experiment. To determine the influence of high counts when held at a low temperature one sample from each set was held in ice and water for 16 hours before scoring. The final sample was held at 50° F. for 16 hours. A compilation of the results of 120 observations are given in figure 5.

Results of scoring milk following each experiment that had been held at 34° F. indicate that the bacterial count does not materially influence flavor and odor immediately. That is to say, the presence of such organisms as those in the culture added did not cause noticeable changes.

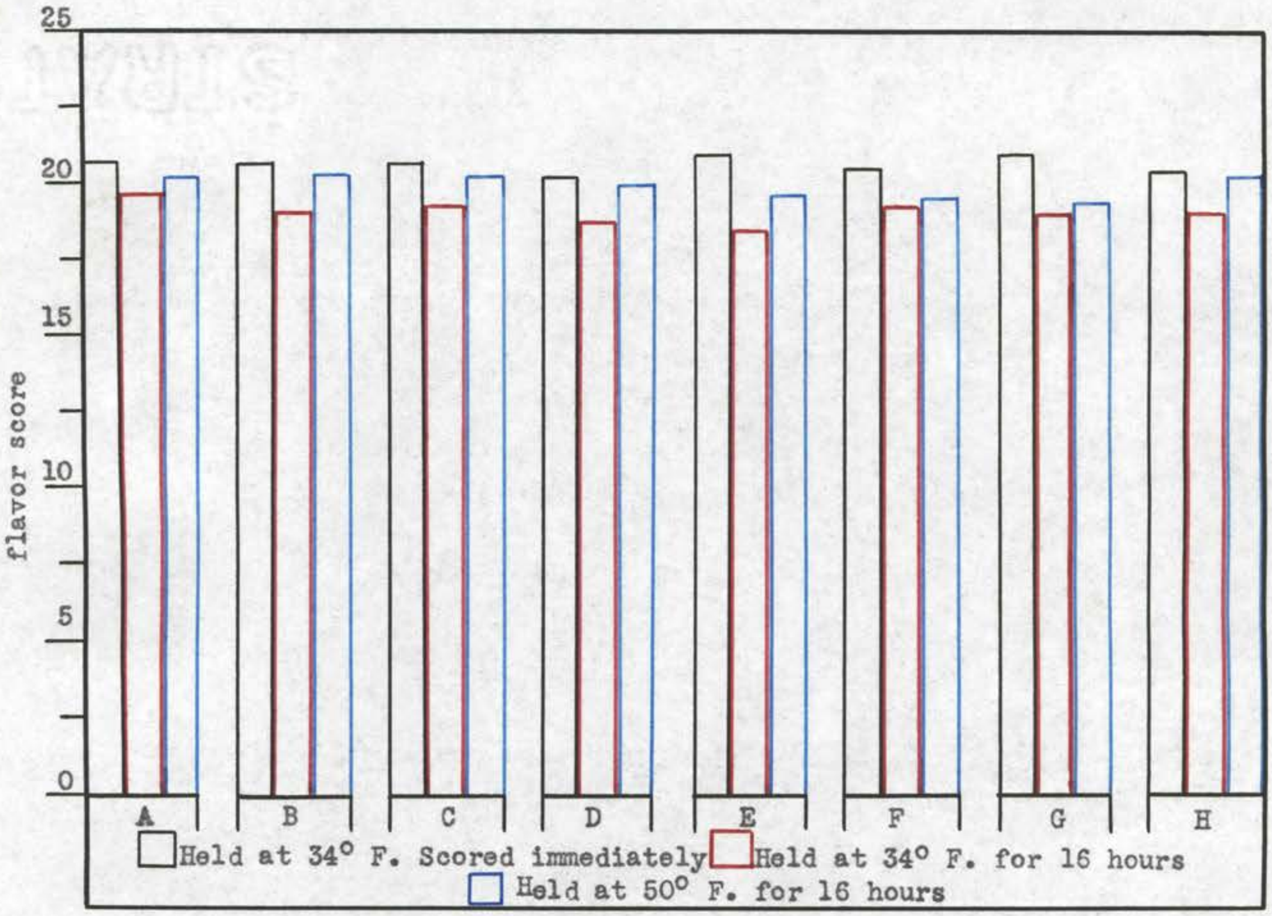

Figure 5

Relation Between High Bacterial Counts and Flavor and Odor

Surface Cooled

A-Immediately after milking.
B-Cooling delayed 30 minutes.
C-Cooling delayed 60 minutes.
D-Cooling delayed 90 minutes.
E-Cooling delayed 120 minutes.

Can Cooled

F-Cooled in 60 minutes.
G-Cooled in 90 minutes.
H-Control.

The deduction is made, therefore, that any changes which bacteria incite are the result of their metabolic processes. Although the flavor and odor score variations are probably insignificant it is interesting to note the trend in figure 5. Surface cooling delayed $1\frac{1}{2}$ hours, set D, and control samples H were the lowest scoring samples in this study, while set E and set G, in which the bacteria had been active the longest time before cooling were the highest scoring samples.

The flavor and odor score of milk cooled to 34° F. and held for 16 hours introduces factors which are out of the scope of this study. In comparing these results with bacterial activity some correlation is noted; however, the importance of these relations are questionable as the samples from which bacterial counts were made had been stored at 50° F. The results do show that there is a minimum temperature near 34° F. at which milk cannot be stored and still maintain its most desirable flavor and odor.

The results of flavor and odor score on milk stored at 50° F. for 16 hours indicate again that bacteria are not an important factor in influencing flavor and odor changes during the first $1\frac{1}{2}$ hours after gaining entrance into milk. This $1\frac{1}{2}$ hour period corresponds closely with the inhibiting factors previously discussed. Comparing the bacterial activity from figure 4 to the flavor and odor score of figure 5 one notes a decrease in score corresponding to an increase in bacterial count. These results are in direct opposition with a similar comparison made on milk stored at 34° F.

Flavor Comparison in Milk of High and Low Bacterial Counts

The more or less inconsistent results obtained from studies on flavor and odor variations in high count milk has instigated further study on the subject. The point at issue being: Are the variations in flavor and odor score of high count milk caused by bacteria or would the changes occur regardlessly?

Freshly drawn mixed milk was divided into equal portions and held at a temperature of 98° F. One lot of the milk was inoculated with the bacterial cultures previously described, while the other was used for control or check samples. The regular cooling procedure was followed. Two samples were taken for scoring immediately after the final cooling temperature had been reached. One set of samples was held at 50° F. for 16 hours, the other placed in ice and water and scored soon after each experiment was completed. The results of 96 observations are given in figure 6.

The original score of the fresh milk, both normal and inoculated, was 20.5. A small difference in score of milk was perceptible on each cooling method, however, the trend of the changes were in the same direction for all surface cooled milk. The trend of score for surface cooled milk held 16 hours was in general the same as for fresh milk. These results indicate that the various species of bacteria used did not bring about noticeable changes in flavor and odor score. The score of surface cooled milk fluctuated with cooling methods regardless of bacterial count.

The theory that bacteria satisfy some requirement for development of desirable flavor is exemplified strikingly by cooling method G which was can cooled in two hours. In all instances the inoculated milk scored immediately after cooling by method G received a higher flavor and odor score than the normal milk. Frequently the judges, while not knowing the identification, commented that the inoculated samples possessed a "fuller" and "more balanced" flavor than the control sample of normal milk.

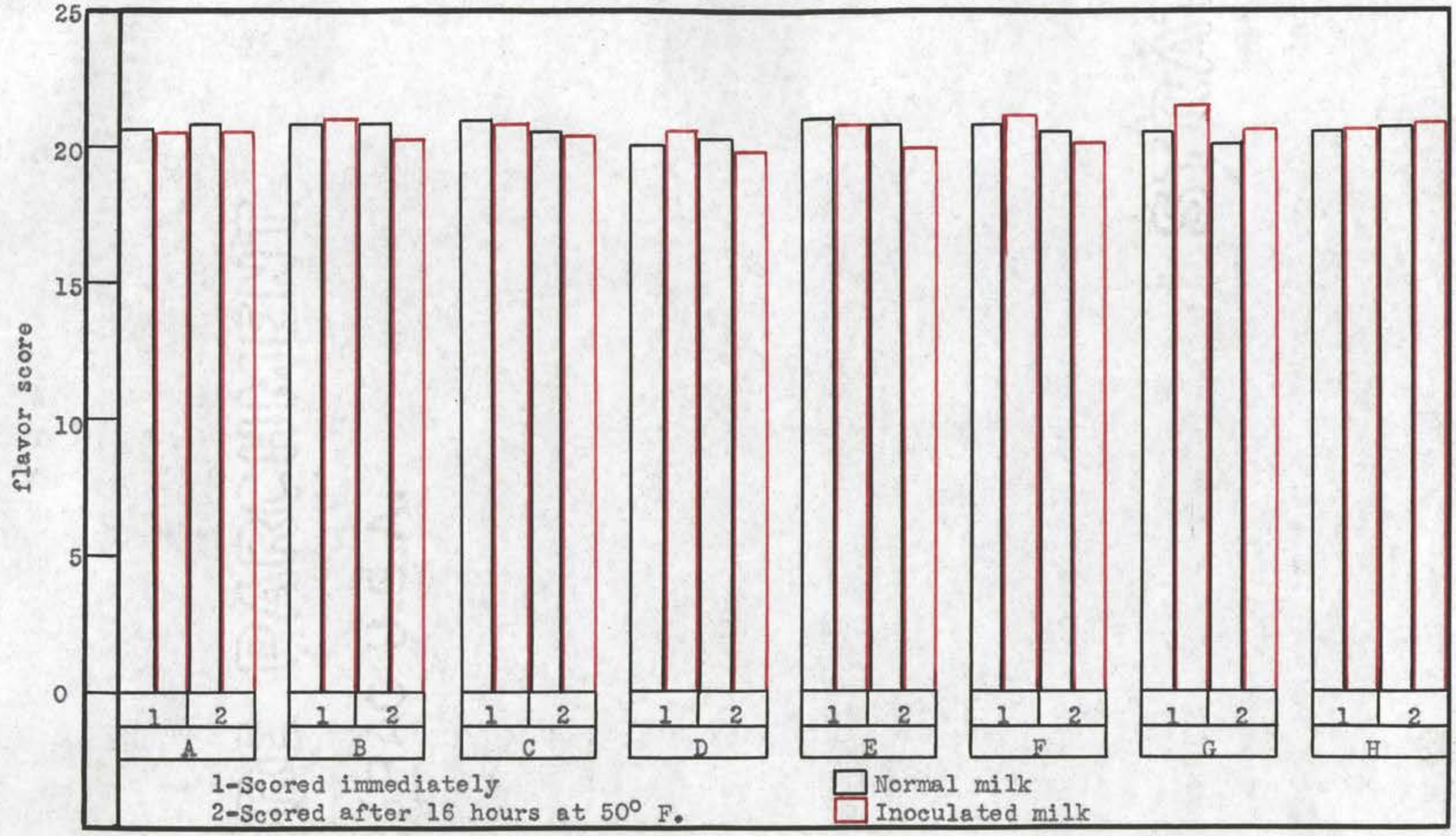

Figure 6

Comparison of Flavor and Odor of Normal and Inoculated Milk

A-Immediately after milking.
B-Cooling delayed 30 minutes.
C-Cooling delayed 60 minutes.
D-Cooling delayed 90 minutes.
E-Cooling delayed 120 minutes.
F-Can cooled in 60 minutes.
G-Can cooled in 120 minutes.
H-Control.

Effect of Cooling Practices on the Cream
Volume of Milk

In a series of 11 trials, 88 observations were made on the creaming ability of milk cooled according to methods previously described. To measure the cream volume, 100 ml. graduated cylinders were used. The cylinders were filled with milk from each of the various sets immediately after the cooling temperature had been reached. The cylinders were stored for creaming in a water bath at 45° F. for 18 hours.

The frequency of the maximum cream volume forming on each of the various sets was determined. In several instances the maximum cream volume was the same on more than one set within a given experiment, therefore, more than one set may be classified as showing the maximum volume. Table VI shows the results of such an arrangement. It is evident that surface cooling delayed 30, 60, and 90 minutes produced the greatest cream volume. The average of the differences between the minimum and maximum cream volume formed on 11 trials of aerated milk was 0.7 per cent. This difference is so small that for practical purposes the cream volume for all surface cooled samples were averaged together for comparison with the cream volume of can cooled milk. The results of such a comparison are shown in table VII. The 11 trials presented show that surface aeration produces a very slightly greater cream volume. This difference is regarded as insignificant.

Bacterial Contamination of Water in Tank Coolers

The ability of certain species of bacteria to live and reproduce at low temperatures affords the possibility of bacterial life existing in the water of tank coolers. From observations it seems to be difficult to prevent some milk spillage into the water compartment of tank coolers. Milk thus spilled serves as a source of contamination to the cooling water

TABLE VI

Frequency of Maximum Cream
Volume Formation

Set identification	Maximum cream volume	Set identification	Maximum cream volume
A	4	E	5
B	7	F	1
C	6	G	0
D	8		

TABLE VII

A Comparison of the Creaming Ability of
Surface and Can Cooled Milk

	Trial number										
	1	2	3	4	5	6	7	8	9	10	11
Percentage cream in total volume when surface cooled	15.6	16.1	15.0	17.3	19.6	15.0	15.7	16.0	15.6	14.2	16.6
Percentage cream in total volume when can cooled	15.7	16.0	14.2	16.5	18.5	14.0	14.7	15.0	15.5	14.0	15.5
Per cent difference in cream volume	0.1	0.1	0.8	0.8	1.1	1.0	1.0	1.0	0.1	0.2	1.1

and as a source of nutrients for bacterial life. The seriousness of such contamination depends largely upon the care exercised in keeping the tank clean.

Bacterial counts of the water were made on nutrient agar. Samples were taken under various operating conditions of the cooling system. The average count on those samples taken several hours after the water in the cooling compartment had been changed was 4,717 bacteria per ml. The average count under the more common operating conditions when the water was slightly cloudy with milk was 34,612 per ml. Several samples were taken when the water was contaminated to such an extent that it would constitute very unsatisfactory operating conditions. The average number of bacteria per ml. in these samples was 103,500. From these results the importance of keeping the cooler tank sanitary may be fully appreciated. Especially should care be taken to prevent water in the cooling compartment from gaining entrance into milk cans as certain species of bacteria responsible for ropy milk often are found living under such conditions.

It is possible to control to a large extent the tank water contamination by treating with chlorine compounds. The efficiency of such treatment depends largely upon the amount of organic matter in the water and the amount of chlorine used.

Effect of Agitating Milk in the Can on Rate of Cooling

Experimental data in the literature reveal that various workers do not agree upon a comprehensive recommendation regarding the agitation of milk in a can during the cooling process. A study was conducted to determine the advisability of such a practice.

Two full 10 gallon cans of milk were mixed and brought to a standard temperature. The cans were immersed to the shoulder in an agitated chilled water bath. One can of milk was agitated at 10 minute intervals, the other was not agitated until the completion of the trial. Figure 7 gives the average results of six trials.

The temperature of the cooling water was 36° F. The temperature of the milk which had been agitated at 10 minute intervals for 70 minutes was 50° F. The temperature of the milk which had not been agitated at the end of the same period was 60° F. After agitation the temperature of this can was 56.5° F. All temperature readings were made in the center of the can about 8 inches from the surface which is the warmest point in the can. Under farm conditions the agitation of several cans of milk with a single stirring rod at 10 minute intervals would be a source of bacterial contamination, the seriousness of which depends on the human element. The reproduction of bacteria is suppressed by inhibitory properties of milk and by a temperature of 60° F. It is therefore concluded that the agitation of milk during the cooling process is unnecessary if a temperature of 60° F. is reached within $1\frac{1}{2}$ hours.

Effect of the Germicidal Property and Lag Phase in Inhibiting Bacterial Reproduction

In this series of experiments it was desired to demonstrate the relative influence exerted by the germicidal properties of milk and the lag phase in bacterial reproduction.

Lots of freshly-drawn milk were divided into two equal portions, one portion being cooled immediately to 50° F. and stored at that temperature for 15 hours. The other lot was inoculated with a culture and held at a temperature of 98° F. for $2\frac{1}{2}$ hours. At the end of the 15 hour period the first portion of milk was warmed to 98° F. and inoculated with the same amount of culture. The average bacterial count of all samples immediately

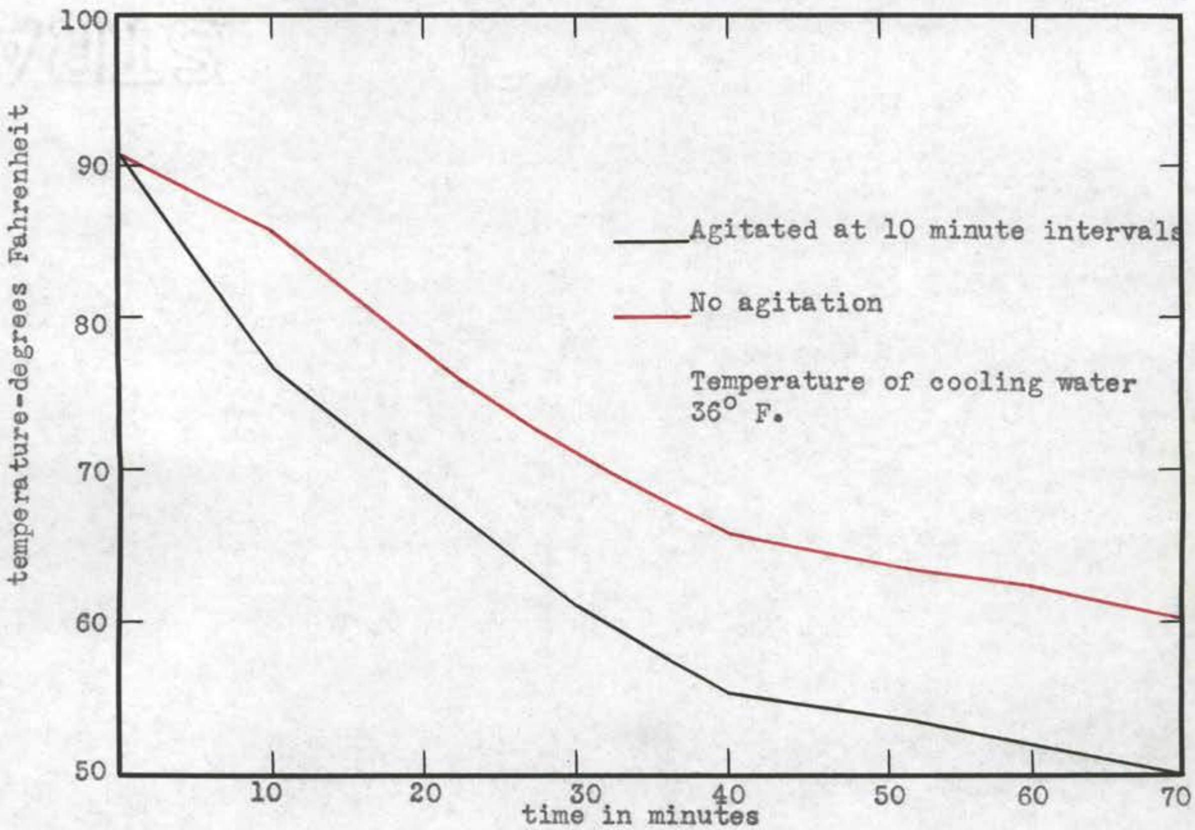

Figure 7

Effect of Agitating Milk in the Can
on Rate of Cooling

after inoculation was 69,000 per ml. Thirty minutes after the inoculation of each lot and each 30 minutes thereafter for $2\frac{1}{2}$ hours samples were taken for bacterial counts. The average per cent increases at the end of each thirty minutes are compared in figure 8.

The results of these studies show a marked similarity in the rate of growth of bacteria in fresh and aged milk for the first 90 minutes. This similarity demonstrates the influence of the lag phase of bacterial reproduction when introduced into a new environment. It is apparent as shown in figure 8 that this culture has adequately adapted itself to its new environment in 90 minutes. The per cent increase in both fresh and aged milk during the 90 minute to 120 minute period is greater than any previous period of growth, indicating that at least one factor in inhibiting normal reproduction has been eliminated. The failure of the per cent increase in fresh milk to follow the curve of increase in aged milk must be attributed to some unstable bacteriostatic property of milk. The per cent increase at the end of the lag phase in both fresh and aged milk indicates that the influence exerted by this bacteriostatic substance is insufficient to inhibit bacterial growth. Bacterial reproduction when influenced by both of these properties was effectively retarded for 90 minutes.

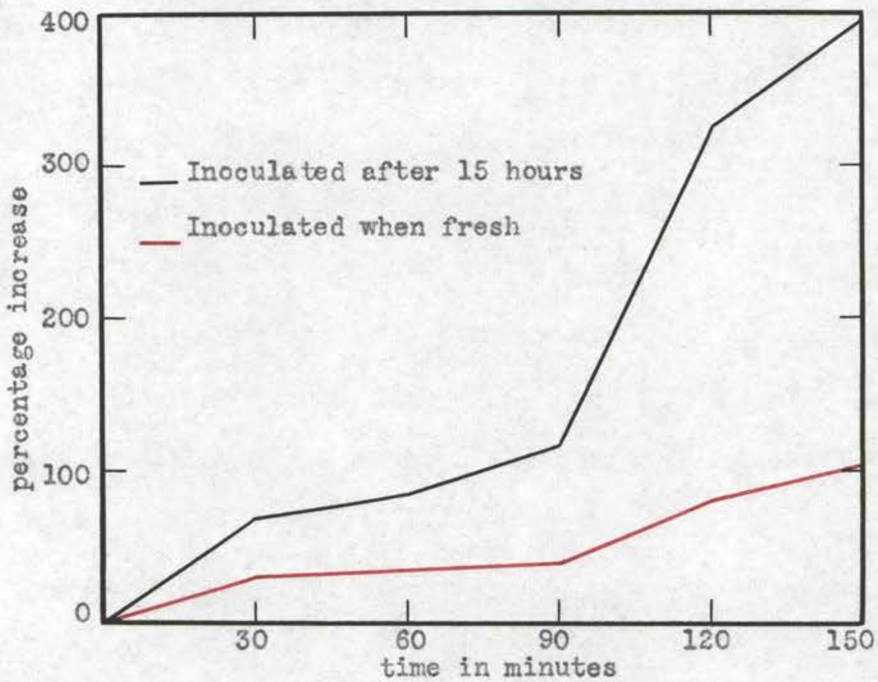

Figure 8

The Effect of the Age of Milk When
Inoculated on the Rate of Growth
of Added Organisms

CONCLUSIONS

1. The bacterial count of high quality milk increased immediately when cooled over an aerator.

2. If the bacterial count of aerator cooled milk is to be held at a minimum the cooling process should not be delayed longer than one hour.

3. High quality milk cooled to 60° F. contained fewer bacteria when can cooled within two hours than the same milk surface cooled 30 minutes after milking. The difference in the bacterial count was sufficient to distinctly favor can cooling where it is desired to produce milk with the lowest possible bacteria count.

4. The percentage increase in the bacterial count of low quality milk cooled to 60° F., either by surface cooling or in the can was for all practical purposes the same.

5. For the highest flavor classification in milk of low bacterial count a temperature of 60° F. should be effected within one hour after the milk is drawn regardless of cooling method employed.

6. Bacteria did not cause detectable changes in flavor and odor of milk during the first three hours after it had been inoculated with bacterial cultures regardless of cooling procedure. Milk with high bacterial counts was stored at 50° F. for 18 hours without detrimental effect to flavor and odor.

7. Varying intervals of delayed cooling resulted in slight variations in flavor scores, however, no connection was established between these variations and bacterial activity.

8. Irrespective of cooling procedure when milk was stored at a temperature near the freezing point for 15 hours undesirable flavors and odors developed.

9. The creaming ability of surface cooled milk is greater than the

creaming ability of can cooled milk, however, the difference is so slight that it is regarded as insignificant.

10. The water compartment of the tank cooler became seriously contaminated with bacteria when adequate sanitary precautions were not exercised.

11. If a tank cooler is equipped with a device to circulate the cooling water it is unnecessary to agitate the milk during the cooling process.

12. Milk contained some germicidal property which in combination with the lag phase of bacterial reproduction was effective in inhibiting bacterial growth for $1\frac{1}{2}$ hours at 98° F.

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