THE GERMICIDAL PROPERTY OF MILK AS INFLUENCED BY THE TEMPERATURE OF STORAGE

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THE GERMICIDAL PROPERTY OF MILK AS INFLUENCED BY THE TEMPERATURE OF STORAGE

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INTRODUCTION

Many workers have regarded the germicidal property of milk as being too feeble to be of much importance.This was the view-point when the search was in progress for a substitution for pasteurization of milk.Although the germicidal property of milk cannot be relied upon for maintaining the safety of milk recent developments in the milk industry and further work in the field indicate that it may be of significance in maintaining milk quality.Before the milk reaches the milk plant in the present day milk industry it has to stand sometimes for hours at farms or in transport.During this time the germicidal property of milk may best serve its purpose.Hence conditions are ideal to make use of the germicidal property, of milk to keep down the growth of microrganisms during the period between milking and processing. The final quality of milk often depends greatly upon its initial handling.Milk with small populations of microrganisms from the start is generally superior in quality to the milk with large numbers of microrganisms at the start even though the number of microrganisms is much reduced

after processing.Also milk with a low number of bacteria when delivered to the plant may mean an additional income to the farmer by way of premiums.

Methods of preserving the germicidal property of milk in all its effectiveness over longer periods of time would fit in with this need of the farmer and the milk industry in general. Various studies on the germicidal property of milk have shown that the temperature factor is the most important one.The other factors are the pH of the milk,the type of microrganisms,time of holding and breed of cattle.The control of time and temperature is well within the means of the farmer and is of use in general.To this end studies reported herein were made.

REVIEW OF LITERATURE

The germicidal property of raw milk was first found by Fokker (1890) during his studies on goats milk.Hunziker (1901)observed that the number of bacteria might be reduced to one-tenth by its action.Jones(1930)showed a way of extracting the germicidal property which he called lactenin.

1-Temperature and time relationship.

Most of the investigators found that at lower temperatures the germicidal property of milk lasted for a longer period of time but was of a lesser intensity than at moderate temperatures and also that it could be heat-destroyed.Hunziker(1901) Heineman (1921) and Jomes (1928) confirmed the findings that the germicidal property was most effective at 37°C.At this temperature it may last from one to ten hours,but on an average it lasted for four to six hours.

Hunziker (1901) presented the following data:

Hours	Cooled naturally (No.of bact.)	70°F. (No.of bact.)	50°F. (No.of bact.)	70°F. for 3 hr then at 50°F. (No.of bact.)	70°F. 4½ hr. then at 50°F. (No.of bact.)
cow-warm after	5,055	5,055	5,055	5,055	5,055
3 hrs. after	8,545	4,280	4,080		
4½ hrs after	42,000	8,350	3,760	5,100	
6 hrs. after	182,000	15,250	3,982	4,570	6,580
9 hrs. after	inn.	180,000	4,960	7,480	19,500
30 hrs.	coag.	coag.	31,000	69,000	90,000

He noted that with natural cooling there was not only no decrease in the bacteria count but as early as three hours the number of microrganisms increased very markedly.When the milk was kept at 70°F. there was a slight decrease,while at 50°F. the bactericidal action was decidedly more distinct.

Hunziker (1901) also showed the effect of heat on the germicidal property of milk as noted by the data which follow:

Hours	Number of Bacteria					
	not heated		heated to 212°F. for 20 min.		heated to 176°F. for 40 min.	
	kept at 70°F.	kept at 50°F.	kept at 70°F.	at 50°F.	kept at 70°F.	k.at 50°F.
cow-warm inoculated	13,187		220	220	615	615
after 3 hrs	7,494	10,326	514	247	3,713	1,760
after 6 hrs	9,914	10,546	27,120	310	123,000	760
after 9 hrs	36,000	9,634	100,000	347	960,000	840
after 12 "	116,000	10,740	inn.	840	inn.	1,347
after 15 "	inn.	10,674				7,340

These data show that germicidal action in milk exposed to 212°F. and to 176°F. for 20 and 40 minutes respectively had ceased completely.The inoculated bacteria increased immediately after inoculation while the inocula introduced into parallel samples which were not subjected to heat and kept at exactly the same temperatures suffered a decided dimunition in their bacterial count.

Jones (1928) working on the germicidal property of milk reported that 60°C for 30 minutes of boiling destroys this property.Milk heated at 63°C. for 20 minutes and at 70°C for 15 minutes still retained its inhibitory activities.

Hansen(1924) proposed that in view of this fact the germicidal property is different from alexines as they are much less heat resistant and are destroyed at 75°C for 15 minutes.He thought it was in close relation to oxidizing enzymes of milk.Rogers(1935) found that fresh milk with an average count of approximately 4,000 when held at 4.5°C showed approximately the same count after 24 hours.At 10°C the count was about 13,000 after 24 hours while at 15.5°C the average was about 1,500,000.Heineman(1921) found that freezing seemed to have no destructive influence upon the germicidal property of milk unless the time factor entered.Hillard and Davis(1917) showed that intermittent freezing of bacteria exerted a more effective germicidal action than continuous freezing due to the mechanical effect.The reduction of counts was much less in milk and cream than in pure water due to physical protection offered to bacteria by colloidal and solid matter in suspension. The degree of cold below freezing was not a very important factor in destruction of bacteria.There was no critical temperature below freezing where the germicidal effect was greatly accelerated. Crystallization probably resulted in mechanical

crushing.

Frayer(1932)proposed that cooling milk immediately to 50°F. was fairly satisfactory even when it was to be held overnight.Cooling morning milk to 60°F.was never quite satisfactory even though it was cooled immediately.Most consistently beneficial results were obtained by cooling milk immediately to and holding at 40°F. or below.

2-The nature of the germicidal property of milk.

Chambers(1920) was of the opinion that no common relationship between agglutination and bacterial inhibition was noted,except that both were destroyed by heating the milk and its specificity and reaction to heat suggest a serologic origin. Jones and Little(1927) in their experiment ruled out agglutination by using the streptococcus for which milk is a poor agglutin.They further said that if the substance was of blood origin the inhibitory substance should be greatly increased in the whey since with rennet coagulation there was a considerable concentration of blood proteins,as seen by the blood precipitin test of the whey.The view that the substance may have been "alexin"from the blood was not supported by their observations.The mixing of

fresh blood serum with a special medium containing casein and lactose did not inhibit the growth of the streptococcus.They also disproved Hansen's(1924) theory that bactericidal property of milk was closely related to the oxidizing enzymes of milk and that it depended upon the variation in the ration of the cow, by conducting their experiment with the same feed all through the year.

3-The properties of the bactericidal substance in milk. Jones(1928)noted the following characteristics:

a. Temperature. Germicidal property was destroyed at 60°C for 30 minutes or when boiled or at 80°C or 90°C for two minutes.Milk heated at 63°C for 20 minutes and at 70°C for 15 minutes still retained its inhibitory activities.

b. Influence of age and stage of lactation. The time and maximum concentration of the substance in the milk of young cows might be as great as in older cows.The inhibitory substance was present in colostrum of the first day was not quite as effective as it was in the milk obtained a few days later.

c. Quarters In their experiment the milk from the right half of the udder contained more principle than that from the left half but the difference was very little.

d. Reactivation Addition of active milk to inactivated milk showed only a slight degree of inhibitory effect.

e. Filterability Whey obtained by coagulation with rennet contained the the inhibitory substance.When this whey was filtered through a Berkefeld Candle almost all the inhibitory substance was removed.

f. Adsorbents A considerable portion of the inhibitory substance of raw milk was taken out by animal charcoal.

g. Dessication When dried in vacuo over sulfuric acid in a refrigerator,the milk or whey showed that the inhibitory substance would withstand drying.Some of the commercially dried milk powders showed inhibitory properties to a greater extent but the concentration of this substance was not the same as the original milk.Alcohol extraction destroyed the substance.

EXPERIMENTAL PROCEDURE

The reduction in bacterial population in milk was used as a criterion of the existance of the germicidal property.The bacterial population was determined by the standard plate count method. This consisted in plating milk or its dilutions in sterile water,into standard plates.The standard tryptone-glucose-extract-milk agar with the following composition was used:

Composition

Agar,best quality,not oven dried.....1.5 percent
Beef extract.....0.3 "
Tryptone.....0.5 "
Glucose.....0.1 "
Distilled water

Reaction pH 6.6 to 7.0

Preferred reaction pH 7.0

One percent sterile skim milk was added just before plating.This is a modification over the standard method wherein skim milk was added just before final sterilization.No sterile skim milk was added for dilution 1:10 or lower.

The plates after the solidification were inverted and incubated at 37°C for 48 hours. Colony counts were made with a Quebec colony counter. Plates having colony counts between 30 and 300 were considered for compilation of the data.

The samples were collected from cows in the Michigan State College Dairy herd.The milk samples were collected in sterile Erlenmeyer flasks from the milking machines.The flasks were carried in a vacuum jug to prevent change of temperature. The lapse of time between milking and first plating (called the zero hour reading)varied from 15 to 20 minutes.

Two organisms were used for simulating conditions of heavy contamination by coliforms and pathogens.The organisms used were Escherichia coli and Staphylococcus aureus.Twenty-four hour culture in F.D.A.broth(Food and Drug Administration)incubation temperature being 37°C,was used for inoculation.The inoculum used was at the rate of 0.1 ml.per 100 ml. of milk.

Detailed procedure was different in parts of the study as shown in the following:

Table No.1: In this experiment the effect of the temperature upon the growth curve of the inoculated milk was determined.The sample was collected as above and then one portion was inoculated and plated,while the other portion was plated as such.The inoculated,as well as the uninoculated milk was divided into three parts and kept at three different temperatures,that is, 40°F.,60°F.,and 80°F.Dilutions of the milk held at each temperature were plated again after one hour,two hours,four hours,eight hours,eighteen hours,twenty-four hours and every twenty-four hours until one hundred and twenty hours were completed or until the milk curdled.

Table No.2: In this experiment,instead of keeping milk immediately at storage temperature of 40°F.,60°F. and 80°F. cooling was delayed for two hours at room temperature.The rest of the procedure was the same as the above.

Table No.3: The milk sample was collected as before and one portion was inoculated and plated while the other was plated as such.After this each of these portions were divided into three parts and kept at the temperatures of 40°F.,60°F. and 80°F.for a

period of ten hours.After ten hours plates were made for the six samples and then all samples were held at 70°F. After this they were plated at intervals of 2,4,8,12,23 hours and after this every 24 hours for 120 hours or until the milk coagulated.

Table No.4: In this experiment the same procedure as in Experiment three was followed except that the cooling was delayed at room temperature for two hours before it was kept at storage temperatures of 40°F.,60° F. and 80°F. for eight hours.

RESULTS

The influence of cooling upon the germicidal property of milk.

Four to five trials were run on the milk of two cows to ascertain the effect of cooling the milk to various temperatures on the inhibition of growth of the bacteria present. The data are included in Tables 1 and 2, Part 1. Here it will be noted that the germicidal property of milk is effective for less than four hours, when the milk is cooled to 80°F. When the milk is cooled immediately to and held at 60°F. the germicidal effect is extended to 18 hours or longer. On the other hand when the milk was cooled immediately to 40°F., it appeared that the germicidal property maintained its action for at least 48 hours. Some variations in the data occurred; however, the data would seem to indicate clearly that an inhibitive effect on bacterial growth exists and its influence extended over a longer period when the milk is cooled immediately to 40°F.

The influence of the germicidal effect on bacterial growth when the milk is contaminated.

The samples of milk,data of which were reported on Part 1,Table 1 and 2,were inoculated with E. coli and Staph. aureus to ascertain the effect of the natural germicidal property of milk upon the growth of contaminating bacteria.The data are presented in Tables I and II,part 2,and 3. Again the data seem to indicate that the germicidal property of milk was prolonged when the milk was cooled immediately and held to a low temperature. In fact,the germicidal effect was more marked in the inoculated milk than in that milk containing the organisms present when the milk was first drawn from the udder.The effect seemed to be greater of Staph. aureus than on E.coli at low temperatures. This may be due to the fact that the optimum temperature for the favorable growth of Staph.aureus is 37°C (98.88°F.)while E.coli grows well between 10°C (50°F.)and 45°C (113°F.)

Destruction of organisms in uninoculated and inoculated milk.

Temperature (°F.)	Bacteria count at 0 hours*	Bacteria count at 2 hours*	Bacterial destruction Number	Bacterial destruction Percent
UNINOCULATED				
80	180,000	37,000	143,000	80
60	180,000	36,000	144,000	80
40	620,000	170,000	450,000	62
INOCULATED WITH *E.coli*				
80	1,400,000	420,000	420,000	70
60	1,400,000	770,000	630,000	45
40	1,400,000	580,000	820,000	58
INOCULATED WITH STAPH. aureus				
80	1,100,000	350,000	750,000	68
60	1,100,000	370,000	730,000	66
40	1,100,000	230,000	870,000	79

* Figures are taken from Table I only.

From comparisons of the non-inoculated and inoculated milk it appeared that although the number of organisms destroyed was greater in the case of inoculated milk the percentage of destruction was more in the case of uninoculated milk.

When samples inoculated with *Staph. aureus* were held at 40°F. the percentage of destruction as well as the total number of organisms destroyed was greater than in the case of the uninoculated milk.This may be due to the fact that the milk was being held at a temperature which was almost 60°F. below the optimum temperature for the organism.

The influence of the temperature of cooling upon its germicidal property when the cooled milk was warmed to a favorable growth temperature after 10 hours storage.

Several trials were run on milk cooled to 80°,60°,and 40°F. and after 10 hours storage at those temperatures was held at 70°F.to determine if the germicidal property persisted and was sufficiently strong to inhibit subsequent bacterial growth.The data are presented in Table 3.Here it will be noted that when the milk was cooled to 40°F. immediately after being drawn from the udder that the germicidal property was maintained and was effective in inhibiting bacterial growth for at least 4 hours after having been warmed to favorable growth temperature.Although the germicidal property of milk was still present when the milk was cooled and held at 60°F.,its inhibitive action was not so prolonged when the milk was warmed after 10 hours storage at favorable growth temperature.Also,when milk was cooled and held to 80°F.then tempered to 70°F.after 10 hours storage,no germicidal activity whatsoever was evident.Likewise when the milk was contaminated with E.coli and Staph. aureus 10 hours after cooling and the milk allowed to warm to

favorable growth temperature,some germicidal property existed in that milk cooled and stored at 40°and 60°F. From the data obtained it appears therefore that adequate and prompt cooling of milk stored 10 hours might warm up in transit without an appreciable increase in bacteria population,if the milk is delivered with two to four hours after warming up.

The effect of a delay in cooling milk upon its germicidal property.

Two trials were run on fresh milk to ascertain the effect of a two-hour delay in cooling the milk upon its germicidal property. The data are included in Table 4.Here it will be noted again that the germicidal property of milk is present when the milk is cooled finally to 40° and 60°F. respectively after a two-hour delay following production.However,the germicidal property did not seem to be present when the milk was cooled only to 80°F.The effect of the germicidal property was noticed particularly when the milk was warmed to a favorable growth temperature after 10-hour storage.The germicidal property still

persisted at 40°F.,was less at 60°F. and did not exist at 80°F.Even when the milk was contaminated with E.coli and Staph. aureus,the germicidal effect was apparent at 40°F.

Table I. The influence of the temperature of cooling upon the germicidal action of milk. (Guernsey cow No.37.)

Time after milking (hours)	Bacteria per ml. by standard plate count when milk was cooled immediately to and held at temperatures indicated.					
	No. of trials	80°F.	No. of trials	60°F.	No. of trials	40°F.
Part 1. NONINOCULATED MILK						
0	4	180,000	4	180,000	4	620,000
2	4	37,000	4	36,000	4	170,000
4	4	64,000	4	71,000	4	240,000
8	4	350,000	4	55,000	4	500,000
18	2	85,000,000	3	270,000	4	470,000
24	2	1,400,000,000	3	140,000	4	670,000
48		coagulated	2	160,000	4	520,000
72			2	520,000	4	760,000
96			2	25,000,000	4	1,200,000
120			1	230,000,000	4	1,400,000
Part 2. INOCULATED WITH E.coli AT 0 HOUR						
0	4	1,400,000	4	1,400,000	4	1,400,000
2	4	420,000	4	770,000	4	580,000
4	4	400,000	4	1,100,000	4	510,000
8	4	5,500,000	4	670,000	4	530,000
18	1	1,200,000,000	3	10,000,000	4	3,800,000
24	1	6,200,000,000	3	900,000	4	1,200,000
48		coagulated	2	35,000,000	4	3,000,000
72			2	980,000,000	4	3,900,000
96			1	340,000,000	4	17000,000
120			1	730,000,000	4	9,300,000
Part 3. INOCULATED WITH Staph.aureus AT 0 HOUR						
0	4	1,100,000	4	1,100,000	4	1,100,000
2	4	350,000	4	370,000	4	230,000
4	4	620,000	4	770,000	4	690,000
8	4	12,000,000	4	440,000	4	310,000
18	1	270,000,000	3	1,100,000	4	250,000
24	1	7,400,000,000	3	1,300,000	4	410,000
48		coagulated	2	1,100,000	4	1,200,000
72			2	35,000,000	4	560,000
96			2	100,000,000	4	740,000
120			1	730,000,000	3	2,900,000

Table II The effect of delay in cooling upon the germicidal action of milk. (Jersey cow No.143)

Time after milking (hours)	Bacteria per ml. by standard plate count when there was a 2-hour delay in cooling before storage at: 80°F. No. of trials	80°F.	60°F. No. of trials	60°F.	40°F. No. of trials	40°F.
Part 1. UNINOCULATED MILK						
0	5	2,000	5	2,000	5	2,000
2	5	1,700	5	1,900	5	1,800
4	5	13,600	5	3,700	5	1,300
8	5	150,000	5	47,000	5	1,800
18	5	59,000,000	5	58,000	5	29,000
24	4	260,000,000	5	280,000	5	220,000
48	3	1,800,000,000	5	31,000,000	5	22,000
72	2	18,000,000,000	5	180,000,000	5	23,000
96		coagulated	5	270,000,000	5	48,000
120			3	1,100,000,000	4	65,000
Part 2. INOCULATED WITH E.coli AT 0 HOUR						
0	4	950,000	4	950,000	4	950,000
2	4	770,000	4	720,000	4	490,000
4	4	2,100,000	4	2,200,000	4	660,000
8	4	12,000,000	4	1,000,000	4	320,000
18	4	310,000,000	4	4,700,000	4	1400,000
24	4	29,000,000,000	4	220,000,000	4	870,000
48	2	2,800,000,000	4	110,000,000	4	1500,000
72	2	3,400,000,000	4	670,000,000	4	2800,000
96		coagulated	4	320,000,000	4	2300,000
120			2	1,700,000,000	3	1600,000
Part 3. INOCULATED WITH Staph.aureus AT 0 HOUR						
0	3	370,000	3	370,000	3	370,000
2	3	270,000	3	270,000	3	260,000
4	3	340,000	3	320,000	3	300,000
8	3	1,400,000	3	500,000	3	410,000
18	3	110,000,000	3	470,000	3	290,000
24		coagulated	3	1,700,000	3	310,000
48			3	61,000,000	3	370,000
72			3	470,000,000	3	350,000
96			3	1,100,000,000	3	370,000
120			2	2,600,000,000	2	260,000

Table III The effect of the temperature of cooling milk upon bacterial growth when the cooled milk was warmed after ten hours storage to a favorable growth temperature (70°F.) (Guernsey cow No.37)

Time after milking (hours)	Bacteria per ml. by standard plate count when the milk was warmed to 70°F. after ten hours storage at:					
	No. of trials	80°F.	No. of trials	60°F.	No. of trials	40°F.
Part 1. UNINOCULATED MILK						
0	4	31,000	4	31,000	4	31,000
10	4	180,000,000	4	150,000	4	30,000
Time after warming (hours)						
2	4	3,600,000,000	4	160,000	4	36,000
4	3	560,000,000	4	2,500,000	4	77,000
8		coagulated	3	45,000,000	4	600,000
12			3	510,000,000	3	120,000,000
24				coagulated		coag.
Part 2. INOCULATED WITH E.coli						
Time after milking (hours)	Bacteria per ml. by standard plate count when the milk was warmed to 70°F. after ten hours storage at:					
	No. of trials	80°F.	No. of trials	60°F.	No. of trials	40°F.
0	4	740,000	4	740,000	4	970,000
10	4	55,000,000	4	2,100,000	4	780,000
Time after warming (hours)						
2	4	160,000,000	4	660,000	4	760,000
4	4	440,000,000	4	5,000,000	4	11000,000
8		coagulated	4	39,000,000	4	56000,000
12			4	89,000,000	3	46000,000
24			1	150,000,000		coag.
48				coagulated		

Table IV The effect of a 2-hour delay in cooling of milk (at room temperature) upon bacterial growth when cooled milk was warmed after 10 hours storage to a favorable growth temperature (70°F.) (Guernsey cow No.37)

Time after milking (hours)	Bacteria per ml. by standard plate count with delayed cooling and storage at: No. of trials	80°F.	No. of trials	60°F.	No. of trials	40°F.
Part 1 UNINOCULATED MILK.						
0	2	44,000	2	44,000	2	44,000
10	2	1,800,000	2	25,000	2	30,000
Time after warming (hours)						
2	2	8,100,000	2	250,000	2	24,000
4	2	17,000,000	2	220,000	2	42,000
8	2	71,000,000	2	7,900,000	2	4300,000
12	1	178,000,000	2	28,000,000	2	71000,000
24		coagulated		coagulated		coag.
Part 2 INOCULATED WITH E.coli						
Time after milking (hours)	Bacteria per ml. by standard plate count with delayed cooling and storage at: No. of trials	80°F.	No. of trials	60°F.	No. of trials	40°F.
0	2	1,300,000	2	1,300,000	2	1300,000
10	2	72,000,000	2	1,400,000	2	860,000
Time after warming (hours)						
2	2	100,000,000	2	2,600,000	2	990,000
4	2	250,000,000	2	25,000,000	2	560,000
8	2	690,000,000	2	40,000,000	2	660,000
12	1	1,500,000,000	1	70,000,000	2	30000,000
24		coagulated		coagulated		coag.

DISCUSSION

The influence of temperature upon the germicidal property of milk was the same as that usually seen in the common germicides.The germicidal property of milk became more effective at higher temperatures.Also at higher temperatures,this property was exhausted in a short period of time, while it was retained over a longer period when the temperature was low.This finding is in full agreement of reports of the previous workers(7,9,10).

Most of the workers (7,9,10)have reported that the germicidal effect is more pronounced in inoculated milk than in uninoculated milk.From the findings herein reported it seemed that the above observation was true only when the number of organisms destroyed was taken into consideration.If,however,the percentage of destruction was taken into consideration the effectiveness of the germicidal property seemed to be greater in uninoculated milk than in inoculated milk.

SUMMARY

The criteria of the reduction of the bacteria count in the milk was used to demonstrate the germicidal property.The influence of the germicidal property can be used to reduce the bacterial count if the milk is stored at 40°F. and delivered with 72 hours,if kept at 60°F.it should be delivered within eight hours,and if kept at 80°F.it should be delivered within four hours.

This may be one of the reasons why morning milk which is delivered within eight hours without being cooled,does not have a very high bacteria count.

As the percentage of destruction of organisms was greater in uninoculated milk it may be suggested that in order to make the most effective use of this property the contamination of milk should be kept down to a minimum.

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