Thermoduric Organisms in Relation to High-Temperature Short-Time Pasteurization*

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INTRODUCTION

FROM the standpoint of temperature relationships, there are different kinds of bacteria: some that can live and grow at very low, some at very high, and some at temperatures in between. The bacteriologist classifies these three groups as follows:

Name	Temperature Range: Minimum	Optimum	Maximum
Cryophilic bacteria	32°F	59°F	86°F
Mesophilic bacteria	59°F	98.6°F	113°F
Thermophilic bacteria	113°F	131°F	158°F

The term "thermoduric" is not included in this classification. The reason is that it is a specialized term. Thermoduric in dairy bacteriology is used to designate a group of bacteria which will withstand the temperature of milk pasteurization, 140 to 145° F. for 30 minutes or 160–161° F. for 15 to 16 seconds, but will not grow at this temperature. *It is a matter of heat tolerance or resistance and not growth.* In contradistinction to this, the term *thermophilic* means *heat loving and growth only in the presence of heat.*

It, therefore, is evident that we should expect to find more thermophilic bacteria in milk pasteurized at 142 to 145° F. for 30 minutes than in milk pasteurized at 160 to 161° F. for 15 to 16 seconds since the pasteurizing temperature is within the growth range of thermophilic bacteria and there is sufficient time for them to grow, especially since milk may remain in the pasteurizing vat longer than the 30 minute interval. Conversely we should expect to find more thermoduric bacteria in the high temperature, short-time pasteurized milk than in the low-temperature, long-time pasteurized milk since they are heat resistant but are not able to grow at 142 to 145° F. but the longer time at this temperature is unfavorable to them. Thus, it becomes evident that the farm presents a greater possibility of thermoduric contamination in milk while for thermophilic bacteria, the holding type of pasteurizer is the chief source of contamination. In short, thermoduric bacteria are a problem of the producer, and thermophilic bacteria of the dealer.

EXAMPLES OF THERMODURIC BACTERIA

Keeping in mind that the term thermoduric refers to a group of bacteria and not any one species or even genus of bacteria, let us see the kind of bacteria that are able to withstand pasteurizing temperatures. Most investigators (7, 11, 21, and 26) agree that the micrococci predominate. Here are the names of some of the more common ones that survive pasteurization: *Micrococcus albus, Micrococcus aureus, Micrococcus candidus, Micrococcus conglomeratus, Micrococcus epidermidis, Micrococcus luteus,* and *Micrococcus varians.* The next most commonly found group are the streptococci such as *Streptococcus thermophilus, Streptococcus liquefaciens, Streptococcus bovis, Streptococcus*

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glycerinaceous, Streptococcus inulinaceus, Streptococcus fecalis, and *Streptococcus zymogenes.*

After the streptococci, sarcinae are most prevalent such as *Sarcina lutea* and *Sarcina rosea.* Next come the rod shaped bacteria mostly of the sporogenic type such as *Bacillus cereus* and *Bacillus subtilis.* Some idea of the relative numbers of each of these four groups of bacteria which survive pasteurization in milk is given by the data of Hileman *et al.* (7). They found in the laboratory pasteurization of 484 samples of milk from 49 producers that the surviving bacteria were composed of 79.3 percent micrococci, 7.4 percent streptococci, 8.1 percent sarcinae and 5.2 percent rods.

THE SOURCE OF THERMODURIC BACTERIA

There is evidence to indicate that one of the principal sources of thermoduric bacteria is the udder of the cow. Harding and Wilson (6) isolated 71 groups of organisms from 900 samples of milk drawn aseptically from the udders of cows. They found no spore formers and about 75 percent micrococci. Later Alice Breed (1) studied the micrococci present in the normal udder. Of the micrococci isolated from the udder by these investigators, more than 40 percent were thermoduric.

A second source of thermoduric bacteria in market milk are the farm utensils such as the milk pails, cans, and the milking machines. Robertson (24, 25, 30) has shown that they will survive the concentrations of chlorine and salt brines as used to sterilize milk cans and pails on farms. Utensils not properly drained and cleaned will contain sufficient nutrients for prolific growth of bacteria, many of which may be thermoduric types (24, 25, 31). When it comes to milking machines, there is ample evidence (3, 12, 14, 20, 23, 28) to show that they may be a prolific source of not only thermoduric but a great many other types of bacteria.

All evidence indicates that many of the thermoduric bacteria especially the micrococci found in milk originate in the udder and are carried by the milk to the pails, cans, and milking machines. If these farm utensils are not cared for properly, they may be a rich source of thermoduric bacteria. This of course does not exclude other sources of contamination such as soil, feed, etc.

That there are many bacteria with different degrees of sensitivity to heat is evidenced by the fact that there seems to be little relationship between bacterial counts of milk before and after pasteurization either by the holding or the high-temperature, short-time methods (20).

SANITARY SIGNIFICANCE OF THERMODURIC BACTERIA

There is no evidence to indicate that the thermoduric bacteria cause disease. From what has been said so far, it should be clear that the presence of excessive bacterial counts in milk due to thermoduric organsms would indicate improper care of milking utensils such as milk pails, cans, and milking machines. This type of carelessness is not good sanitation and should not be condoned.

METHODS OF PASTEURIZING MILK

There are in use in this country today two commonly used methods of pasteurizing milk: (1) The holding method which consists in heating the milk to 142 to 145° F., and holding it for 30 minutes. (2) The high-temperature, short-time method which consists in heating the milk to 160–161° F. for 15 to 16 seconds. A third method, the so-called "High-Pasteurization" method should be mentioned since it is widely used in continental Europe. In this method the milk is heated to 176 to 185° F. and held momentarily. However, we shall confine our remarks to the first two methods since they are in general use in America today. Each of the first two methods has its adherents and each has advantages and disadvantages. These may be found elsewhere in the literature (8, 22).

One of the first questions that should interest you is, Do both methods kill pathogenic bacteria? The answer is yes. There is no question about the holding method since it has been used successfully for years. Whenever there is a question of this nature raised, it is in connection with the short-time, high-temperature method of pasteurization. Why should there be any doubt about this method? The real reason, I believe, is to be found in the history of pasteurization in this country.

One of the first methods of pasteurization used for market milk was the so-called "flash pasteurization" one, starting about 1890. It was used extensively in the United States from about 1900 to 1914. As practiced at that time, flash pasteurization consisted in rapidly heating the milk to 160° F. and immediately cooling. There were no automatic temperature controls such as we have today. Naturally with such an uncontrolled method there were slips and inefficient pasteurization. As a result, there were disease epidemics traced to flash pasteurized milk. Consequently this method came into disrepute. It is the memory of these early experiences which still lingers in the minds of many of the older milk control officials and this has built up the prejudice which still exists in their minds against high-temperature short-time pasteurization.

WHAT ARE THE FACTS?

About 1925 an electrical method of high-temperature short-time pasteurization of milk was introduced, and in 1927, it was tested and accepted by the Pennsylvania State Health Department (5) and put into large scale commercial use in Pittsburgh. In 1928, the New York State Health Department (29) tested this type of pasteurization. It was accepted by them, as well as the U. S. Public Health Service, Massachusetts State Health Department, and many cities, especially those operating under the U. S. Public Health Service Milk Ordinance. By 1935 according to Irwin (13), there were 76 plants in the United States using either the electric or hot water type of high-temperature, short-time pasteurizing equipment. I have seen no figures available on the number of installations of this type since Irwin's, but every one familiar with the facts knows that a very great many units have been installed not only in the United States but also in Canada and the British Isles.

Why has high-temperature short-time pasteurization again come into favor after being in the "dog-house" for a decade or so? The first reason is because of the mechanical changes which have been made in the equipment so as to make it reliable and foolproof. When the milk is in contact with the pasteurizing temperature only a few seconds, usually 15 to 16, there must be no slips and no guess work. The machines must deliver the goods and they have been designed to do just that.

The second reason which is really based on the first is that numerous investigators (5, 10, 16, 17, 19, 29) have shown that the temperatures and times used did and would actually kill pathogenic bacteria under commercial conditions. And finally a third reason, predicated on the second, is the large number of units of high-temperature short-time pasteurizers which have been operated successfully since 1927 without a single epidemic of disease having been traced to them. Of course there are other reasons, economic and engineering, which should be considered in a full discussion of the subject but we are interested only in the bacteriology of the subject.

ADVANTAGES AND DISADVANTAGES BACTERIOLOGICALLY

Dahlberg (2), taking the data of North and Park (19) and plotting them on semilog graph paper, has shown that, accepting 142° F. for 30 minutes as the proper pasteurization standard, a series of standards could be calculated which were comparable from the standpoint of margins of safety in protecting the public against disease germs in milk. He found that "while the total time variation became less with increased temperatures of pasteurization, the time required to destroy the tubercle bacillus was exceeded at each temperature variation by the same percentage of the total variation. Pasteurization at 142° F. permitted a maximum time variation of 20 minutes while 160° F. gave a safety period of five seconds." From the standpoint of temperature variation, "if the holding time was exactly 30 minutes, the maximum temperature variation was from 136° to 144° F. but at exactly 16 seconds holding, the temperature variation was from 159° to 161.25° F." The 5-second safety period at 160° F. means, when translated into practical terms, that if milk is held 11 seconds instead of 16 seconds or if the temperature is allowed to drop below 159° F. when the holding period is actually 16 seconds, then infected milk may result.

A second disadvantage of the high-temperature, short-time method of pasteurization is the higher bacterial counts obtained with many milk supplies when this method is used (3, 8, 20, 23). Comparisons made with the same milk pasteurized by the holding and the high-temperature, short-time method showed this to be the case. This has increased the cost of laboratory and field work to hold the counts down to within legal limits. From what has been said previously it is evident that a part of the increased count is directly attributable to thermoduric organisms which are better able to withstand the higher temperatures and shorter pasteurization times than they are at the lower temperature and longer pasteurization times.

Another factor which is also responsible not only for the higher total counts in raw and pasteurized milk but for relatively larger percentages of thermoduric bacteria in raw milk is the change to a more nutritious official medium and an optional lower incubation temperature (9).

The phosphatase test is of value as a control and an additional means of checking adequate pasteurization since Mattick and Hiscox (17) found that tubercle bacilli in the numbers in which they occur in naturally infected milk were killed at 158° F. in 11.5 seconds, after being in the regenerating and heating sections, at a temperature culminating at 158.5° F. for about 16 seconds. The phosphatase test remains positive at holding temperatures below 160° F. for 11.5 seconds. They concluded that 162° F. for 15 seconds afforded a sufficient margin of safety, and any significant departure from this time and temperature would be revealed by the phosphatase test which is still positive at temperatures and times at which the tubercle bacilli are unable to survive.

METHODS OF DETECTING THERMODURIC BACTERIA

There are several methods for determining the individual farms that are contributing excessive numbers of thermoduric bacteria to a milk supply. One commonly used method, first suggested by Taylor (28), is to pasteurize samples of milk from individual producers in the laboratory and then plate them. This is expensive and time consuming. To cut down the expense, Myers and Pence (18) inoculated a standard loopful, 0.001 or 0.01 ml. of laboratory pasteurized milk into an oval test tube containing melted standard milk agar, mixed well, and incubated at 37° C. for 48 hours. Their data show very good agreement between the standard plate count and this less expensive method.

Another method (8) is to pasteurize the samples in the laboratory at 161° F. for 16 seconds, then incubate them for seven hours at 37° C., after which they are examined microscopically. Mallmann *et al.* (15) incubated the raw milk for two hours at 58° to 60° C. after which a microscopic examination was made. They claim that the viable thermoduric bacteria present in raw milk may be determined in this manner since all the non-thermoduric bacteria have been destroyed and will dissolve during the two hour incubation period. They propose a standard of not more than 40,000 thermoduric bacteria in raw milk as determined by this method. Most investigators (20, 23, 31) find that laboratory pasteurization tends to give lower bacterial counts than commercial pasteurization, irrespective of the time and temperature used.

It is also interesting to note that Parfit (20) found that methylene blue tests were not an accurate index of thermoduric bacteria. Thus, shippers' milk might pass a satisfactory methylene blue test and yet be the source of thermoduric bacteria in the milk supply.

SUMMARY

Thermoduric bacteria are a group of bacteria which are able to withstand pasteurizing temperatures but are not able to multiply at these temperatures. Four groups of thermoduric bacteria are commonly found in milk, viz., micrococci, streptococci, sarcinae and bacilli. Of these four groups, the micrococci are by far the most common. One of the principal sources of thermoduric bacteria is the cow's udder since milk drawn aseptically from the udder predominates in micrococci. Many species of these have been demonstrated to be the same as the thermoduric bacteria found in milk. Other sources of thermoduric bacteria are poorly cleaned and improperly sterilized milk pails, cans, and milking machines contaminated with milk from the udder and from other sources.

There are three methods of pasteurizing milk, viz., the low-temperature, long-time method, commonly known as the holding method; the high-temperature short-time method; and the high-temperature method. The first two methods are in common use in the United States while the latter method is used mostly in continental Europe.

From a bacteriological standpoint, the high-temperature, short-time method of pasteurization is effective in killing all pathogens commonly found in milk as the numerous experiments carried out on a commercial scale have proven. Additional and more convincing evidence is the absence of disease epidemics caused by milk pasteurized in this type of pasteurizer. Such milk has been sold to and consumed by thousands of people every day for the past decade or more. However, it is quite generally agreed that the high-temperature, short-time pasteurization gives higher bacterial counts with many milk supplies than the holding method. A part of the increase in count is due to thermoduric bacteria. There seems to be little relationship between counts on raw milk and the counts on milk pasteurized by either method.

There are three different methods of determining the presence of thermoduric bacteria in milk, viz., laboratory pasteurization, then plating; laboratory pasteurization and incubating 7 hours at 37° C., then examining microscopically; and incubating raw milk 2 hours at 58° to 60° C., then examining mcroscopically. Laboratory pasteurized milk tends to give lower counts than commercial pasteurization, irrespective of the time and temperature used.

The control of thermoduric bacteria is a producers' problem while the control of thermophilic bacteria is a dealers' problem.

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