BACTERIOLOGICAL ASPECTS OF THE EVALUATION OF ADEQUACY OF PASTEURIZATION

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Increased interest in high-temperature, short-time pasteurization of various dairy products has emphasized the need for methods to determine the adequacy of pasteurization. One such method is described, but there is need for additional information. The thermal death time curves of various pathogens should be determined in different dairy products. A suitable heat resistant test organism such as Microbacterium MS-102 should be selected, approved by health officials and made available to others for controlled studies. Data should be accumulated so that curves could be prepared to show any combination of time and temperature which would result in adequate pasteurization.

Since the early 1900's it has been recognized that a heat treatment sufficiently severe to destroy all pathogenic microorganisms is needed to make dairy products safe for human consumption. This process has long been known as pasteurization. The long-hold method of pasteurization of dairy products is recognized as a safe and satisfactory method of heat treatment. The accepted standards for these methods are known to be more than adequate for making dairy products safe because not only is the holding time at 143°F in itself more than enough to insure destruction of pathogens in milk but also there is additional safety in the process provided by the long heating and cooling periods used.

When new combinations of time and temperature of pasteurization are considered for various dairy products, methods are needed to determine the adequacy or efficiency of the new process to make certain that the bacterial destruction is comparable to or better than that obtained by standard long-hold pasteurization treatments. It is the problem of this evaluation of high-temperature short-time (HTST) pasteurization and the determination of new standards for HTST pasteurization that are to be discussed in this paper.

PASTEURIZATION TEMPERATURES FOR MILK

The process of pasteurization was first applied to milk. Hence, the early investigators were interested in the determination of the time and temperature necessary to destroy the disease-producing organisms which might be present in raw milk. Perhaps it was the equipment and controls available in the babyhood days of pasteurization which necessitated the use of low-temperature long-time pasteurization methods. In any case the emphasis of bacteriological research was placed on the time required to kill pathogenic bacteria at temperatures in the range of 140°F to 150°F. The voluminous results reported show that the pasteurization treatment of milk at 143°F for 30 minutes gives satisfactory destruction of the pathogens which might possibly be found in the raw milk.

It was always believed that rapid heating to high temperatures (above 160°F) might be a more satisfactory pasteurizing method from an economic point of view as well as resulting in a better final product. Flash pasteurization, as the early HTST methods were called, has been practiced at intervals ever since the early 1900's. However, since the late 30's, improvements in the design of equipment and controls have been such that this HTST pasteurization method is now feasible and accepted as satisfactory by the dairy industry and health department officials for the pasteurization of milk.

In the meantime products other than milk were being subjected to pasteurization treatments. Fluid cream, skim milk, chocolate milk, ice cream mix, cream for butter making, milk for cheese making, all required pasteurization treatment. The long-hold pasteurization of these products produces satisfactory results but what HTST combinations of time and temperature are necessary for comparable bacterial destruction.

PROBLEM FOR OTHER MILK PRODUCTS

The processing of these products and application of HTST pasteurization to dairy products added to the problem of evaluation of the adequacy or efficiency of pasteurization treatments. Of course once a standard combination of time and temperature of heat treatment was decided upon, control of pasteurization was a fairly simple matter of checking thermometers, charts, and timing devices, aided by chemical tests for the destruction of phosphatase. It is the determination of new standards of time and temperature of the various heat treatments for these different dairy products which poses a problem for dairy bacteriologists, sanitarians, and health officials.

The problem of evaluation of adequacy of pasteurization is primarily bacteriological. At first glance it appears to be a relatively simple problem, but further investigation discloses many troublesome aspects. Many bacteriological aspects of the problem require investigation and most of the research must be proven in the laboratory before new pasteurization processes are used in the industry.

Such questions as the following must be answered:

1. Is Microbacterium tuberculosis the most heat resistant of the pathogens which must be destroyed by pasteurization treatments?
2. How much more time or heat is required to overcome the protective action of fat, sugar, or increased viscosity of the product?

3. How does the thermal death time curve of possible heat-resistant non-pathogenic test cultures compare with that of pathogenic bacteria?

4. What are the best methods for determining heat treatments which are comparable to longhold pasteurization?

5. Should not the thermal death time curves of pathogens inoculated into milk, cream, chocolate milk, and ice cream mix be determined?

6. Should not a satisfactory heat-resistant non-pathogenic test organism be selected, studied, approved by health officials, and made available for bacteriological evaluation of adequacy or efficiency of pasteurization processes?

These questions can be answered but it will require cooperation of universities, experiment stations, commercial research laboratories, dairy companies, dairy equipment companies, and health departments and officials to obtain data that can be accepted by all concerned. An organization such as the International Association of Milk and Food Sanitarians might well set up and sponsor a program for the unification of a plan of action. There is little doubt in the minds of many that there is need for such a program.

**NEEDED STUDIES IN HTST PASTEURIZATION**

It is felt that there are certain questions which must be answered sooner than others. For example the increased interest in the HTST pasteurization for dairy products other than fluid milk necessitates the determination of standards of HTST pasteurization for these products. At present such standards for ice cream mix are of paramount importance if the advantages of this method of pasteurization are to be realized. Later the time and temperature combinations used with cream and chocolate milk should be evaluated.

Standards are in effect for the pasteurization of milk, and everyone accepts the HTST treatment of 161°C for 16 seconds as being comparable to 143°F for 30 minutes. The HTST pasteurization of ice cream mix is assuming more and more importance to the dairy industry. Presently accepted standards require a pasteurization treatment of 155°F for 30 minutes but no standards have been approved for HTST pasteurization, although tentative approval has been granted for a treatment of 175°F for 25 seconds.

Let us consider for a moment a few of the bacteriological problems which are involved in the determination of a HTST pasteurization standard for ice cream mix. The first problem facing the research investigator is, of course, the determination of HTST temperature and time combinations which result in satisfactory bacterial destruction.

A search of the literature shows that much of the early work on the heat resistance of pathogens was concerned with the destruction of *M. tuberculosis* in milk. There are almost as many thermal death times reported for this organism in milk as there are investigators reporting. However, North and Park reported that 138.2°C for 30 minutes destroyed the tubercle bacillus and stated that probably the variance in thermal death times reported by others was due to methods of study and not to resistance of strains. A few studies have been reported using cream as the heating medium and even fewer using ice cream mix.

Oldenbusch, Frobisher, and Shrader reported on studies using cream and ice cream mix. They showed the tubercle bacillus to be the most resistant of the pathogens studied and that it was killed within 6 minutes at 145°F and within 3 minutes at 150°F. Using this data, Ball has determined the thermal death time curve for *M. tuberculosis* in cream and shown the slope of this curve to be 12°C°F.

These studies show that satisfactory destruction of pathogens is obtained by longhold pasteurization of 155°F for 30 minutes but what about temperatures of 160°, 170°, 180°, or even higher, for seconds of holding time. Studies should be undertaken to determine if possible the destruction of these organisms at high temperatures or at least to determine the thermal death time curves in various dairy products.

In seeking approval of HTST pasteurization of ice cream mix, it is necessary to show that the time and temperature of treatment results in bacterial destruction comparable to that obtained by the presently accepted pasteurization treatment of 155°F for 30 minutes.

Three approaches can be taken to this problem. (1) The determination of the destruction of the normal bacterial flora at these temperatures as compared to the destruction obtained at 155°F for 30 minutes. (2) Actual studies at these temperatures using the tubercle bacillus as a test organism; or (3) studies at these temperatures using a heat-resistant non-pathogenic test organism.

The destruction of the normal bacterial flora is probably the most simple approach to the problem. It requires that standard plate counts be made on samples of the raw mix, of the mix pasteurized in the laboratory at 155°F for 30 minutes and of the mix pasteurized by whatever HTST combinations are being studied. The percent destruction of the normal flora by each treatment is then calculated. As long as a laboratory pasteurized control is used, the comparable destructiveness of various combinations of time and temperature can be determined. However, the normal flora is variable and, therefore, there is nothing known about the organisms present in mix or their thermal death rates. It is this fact which has prompted health officials to request studies using test cultures of known heat resistance with a known thermal death time curve.

**HTST AS COMPARED WITH LONG-HOLD PASTEURIZATION**

The ideal method of evaluating HTST pasteurization methods would be the inoculation of the most heat resistant pathogen into the product, pasteurization of the product using various combinations of time and temperature and finally the determination that the organism was killed by these treatments. It is understood that there are such studies being conducted with HTST pasteurization of ice cream mix and it is hoped that the results will be made known to the dairy industry.

However, there is understandable reluctance toward the use of pathogenic bacteria in a dairy plant. Therefore, the third approach of using a heat-resistant non-pathogenic test organism may provide the necessary information for the evaluation of the adequacy of a pasteurization process. Several heat-resistant cultures have been used for this purpose. Some of the early milk work...
made use of Escherichia coli strain 3U. Speck\(^8\) has reported on the use of Micrococcus freundii. Tracey et al.\(^1\) have studied M. freundii, Streptococcus faecalis, and an unidentified spore-forming bacillus. Barber and Hodes\(^3,4\) reported using an unidentified Micrococcus MS-102.

The selection of a satisfactory heat-resistant test culture is important. To be of value in the evaluation of HTST treatments the culture should survive heating at high temperatures (above 175\(^\circ\) or 180\(^\circ\) F); it should be easily recognized by distinctive colony formation on agar plates; it should grow profusely so that large numbers of cells can be harvested for inoculum; it should have uniform heat resistance; it should be more resistant to heat than the most resistant pathogen and yet have a thermal death time curve slope as close as possible to that of the pathogen. The Micrococcus MS-102 meets all of these requirements. It survives a heat treatment of 180\(^\circ\) F for 15 seconds, produces a typical golden yellow colony on yeast extract "N-Z-Amine" agar, grows profusely on this medium at 35\(^\circ\) C, has uniform heat resistance when transferred properly, is more resistant to heat than \textit{M. tuberculosis}, and has a thermal death time curve slope in milk of 11.4\(^\circ\) F as compared with the \textit{M. tuberculosis} thermal death time curve slope of 12.6\(^\circ\) F.

Two of the foregoing methods for the evaluation of adequacy of pasteurization were used in recent studies on the HTST pasteurization of ice cream mix at National Dairy Research Laboratories. Preliminary studies were made in which the destruction of the normal bacterial flora of the mix was determined for temperatures of 165\(^\circ\), 170\(^\circ\), 175\(^\circ\), and 180\(^\circ\) F with holding times of 37.5\(^\circ\), 32.7\(^\circ\), 29.7\(^\circ\), and 25 seconds at each temperature. Laboratory long-hold pasteurization for these studies was 160\(^\circ\) F for 30 minutes. The results indicated that destruction of the normal flora obtained at 175\(^\circ\) to 180\(^\circ\) F for 25 seconds was comparable to that obtained at 160\(^\circ\) F for 30 minutes.

Further studies with a different HTST unit were made using temperatures of 160\(^\circ\), 165\(^\circ\), 170\(^\circ\), 175, and 180\(^\circ\) F for 25 seconds and 180\(^\circ\), 190\(^\circ\), 200\(^\circ\), 220\(^\circ\), 240\(^\circ\), and 260\(^\circ\) F with no holding tube in the equipment. Normal flora destruction at 175\(^\circ\) F for 25 seconds and temperatures above 190\(^\circ\) F with no holding tube was comparable to laboratory long-hold pasteurization destruction at 155\(^\circ\) F for 30 minutes.

Health officials requested further studies using a heat-resistant test culture. The experimental HTST unit was installed at the National Dairy Research Laboratories and studies were made using the heat-resistant culture Micrococcus MS-102. The mix was inoculated so that there were between 50,000 and 1,000,000 cells of the test organism per ml of mix. Laboratory pasteurization of the inoculated mix was at 155\(^\circ\) F for 30 minutes. The mix was pasteurized in the experimental unit at 165\(^\circ\), 175\(^\circ\), and 185\(^\circ\) F for 25 seconds and at 190\(^\circ\), 210\(^\circ\), 240\(^\circ\), and 260\(^\circ\) F for 1.4 seconds. The bacteriological results indicated that destruction of the heat-resistant test culture at 175\(^\circ\) F and above for 25 seconds and at 190\(^\circ\) F and above for 1.4 seconds was comparable to that obtained at 155\(^\circ\) F for 30 minutes.

From the results just described it would appear that the HTST standard for ice cream mix might be 175\(^\circ\) F for 25 seconds. Other investigators have reported numerous other time and temperature combinations (table 1).

### Table 1

<table>
<thead>
<tr>
<th>Temperature ((^\circ) F)</th>
<th>Time (Sec)</th>
<th>Investigator</th>
</tr>
</thead>
<tbody>
<tr>
<td>175</td>
<td>25</td>
<td>Minthorn</td>
</tr>
<tr>
<td>175</td>
<td>25</td>
<td>Barber-Hodes(^8, 4)</td>
</tr>
<tr>
<td>175-180</td>
<td>25</td>
<td>Speck(^9)</td>
</tr>
<tr>
<td>180</td>
<td>16</td>
<td>Armstrong(^1)</td>
</tr>
<tr>
<td>180</td>
<td>19</td>
<td>Dowd-Anderson(^9)</td>
</tr>
<tr>
<td>185</td>
<td>6.1</td>
<td>Tracy et al.(^11)</td>
</tr>
<tr>
<td>190</td>
<td>1.4</td>
<td>Barber-Hodes(^8, 4)</td>
</tr>
<tr>
<td>194</td>
<td>0.75</td>
<td>Tracy et al.(^11)</td>
</tr>
</tbody>
</table>

A chart (figure 1) has been prepared which shows graphically the information obtained in these studies on HTST pasteurization of ice cream mix. Curve 1 is the thermal death time curve of MS-102 in ice cream mix (Data for this curve is shown in table 2). Curve 2 is a curve drawn through the points 155\(^\circ\) F and 30 minutes (1800 seconds) parallel to curve 1. Curve 3 is the reported thermal death time curve for \textit{M. tuberculosis}. Also shown on the chart are time and temperature combinations reported for HTST pasteurization of ice cream mix. It can be seen that these points all fall well above curve 2 which represents time and temperature combinations comparable to 155\(^\circ\) F for 30 minutes.

It is felt that this method of obtaining and presenting data can be applied to dairy products such as milk, skim milk, cream, and chocolate milk. The foregoing data are presented as a suggestion for the evaluation of adequacy of pasteurization and the determination of HTST pasteurization standards.

### Table 2

<table>
<thead>
<tr>
<th>Temperature ((^\circ) F)</th>
<th>Time (Sec)</th>
<th>Number of Trials</th>
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<tbody>
<tr>
<td>155</td>
<td>3780</td>
<td>10</td>
</tr>
<tr>
<td>160</td>
<td>1650</td>
<td>7</td>
</tr>
<tr>
<td>165</td>
<td>642</td>
<td>8</td>
</tr>
<tr>
<td>170</td>
<td>210</td>
<td>7</td>
</tr>
<tr>
<td>175</td>
<td>48</td>
<td>8</td>
</tr>
<tr>
<td>180</td>
<td>9.6 to 15</td>
<td>5</td>
</tr>
</tbody>
</table>

**Problems Needing Study**

To return to the questions which were raised earlier in this paper, what should be done to improve the evaluation of new pasteurization processes? It would seem that there are definite points which require investigation, the result of which may do much in providing a firm, well established foundation for the determination of HTST pasteurization standards for various dairy products.

First, the thermal death time curves of various pathogenic microorganisms...
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organisms should be determined in milk, cream, and ice cream mix.

Second, a heat-resistant, non-pathogenic test organism should be found whose heat-resistance is greater than that of the most heat-resistant pathogen. The slope of the thermal death curve of this test organism in various dairy products should be nearly equal to that of the most resistant pathogen.

Third, this test organism could be approved by health officials and made available to others for the determination of adequacy of pasteurization treatments.

Fourth, sufficient studies could then be made using the test culture in various dairy products so that curves could be prepared to show any combinations of time and temperature which would be comparable to presently accepted standards of pasteurization.

There will be ever-increasing requests by the dairy industry for the approval of new time and temperature combinations of HTST pasteurization for milk, ice cream mix, cream, chocolate milk, sherberts, and possibly other dairy products. A unification of methods of study and presentation of results will do much to bring about a more rapid approval of these pasteurization treatments.

REFERENCES

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