THE EFFECT ON BACTERIAL COUNTS OF THE GRADUAL WARMING OF MILK SAMPLES

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SUMMARY

Samples of retail pasteurized milk were allowed to stand at room temperature for 4 hr during which the temperature of the samples increased 33 F to an average maximum of 81 F. Coliform and standard plate counts were made during this period. Only 2 samples out of 16 showed extensive coliform growth. Increases in standard plate counts were surprisingly small.

In our area many in the dairy industry are convinced that a slight rise in temperature produces a marked increase in the coliform content of retail pasteurized milk. An increase of 1-2 to 4-5 F, in an indefinite but extremely short interval, is thought to induce such rapid growth of coliforms that the milk is by then beyond compliance with a coliform standard of not over 10 per ml. If this be the case, the sample collector or the laboratory may unintentionally allow samples to warm up enough to affect the counts. As a result, we decided to investigate bacterial growth in retail packaged milk in as simple and practical a manner as we could devise.

PROCEDURE

The samples consisted of Vitamin D homogenized, regular, and skim milk with the exception of one sample, No. 9, which was homogenized goat milk. They were processed by eight different plants, were pasteurized, and of one-quart size. Most were contained in paper cartons, but a few were in glass bottles. Samples were collected from trucks just prior to delivery and were kept well iced except during very cold weather. Upon reaching the laboratory, they were immediately placed in a refrigerator maintained at approximately 4 C until plated. Plating of the samples began at 12:00-12:30 p.m.

The coliform tests were made in duplicate as time permitted over a period of thirteen months. In addition, single standard plate counts were made on each sample. Violet red bile agar and plate count agar were used. Plating was carried out in close conformance to Standard Methods (1). Care was taken to see that clinging drops were not transferred with measured portions on the tips of pipettes. A mechanical convection incubator was used. Temperature readings averaged 35.0 C for the top shelf and 35.1 C for the bottom shelf.

Each sample was plated 10 times during a period of 4 hr. Platings were made initially and at the end of 5 min, 10 min, 15 min, 30 min, 45 min, 1 hr, 2 hr, 3 hr, and 4 hr. Samples were allowed to remain on the plating table at room temperature during the intervals between platings and care was taken to avoid undue warming from close proximity to gas burners. Only one sample was plated on a given day. The sample was shaken before each of the 10 dilutions was made, and 2 undiluted 1-ml portions were plated for coliforms and plates of 1:100 and 1:1,000 dilution were made for the standard plate count. The temperature of the sample was recorded as soon as possible after portions were removed for plating. The thermometer was allowed to stand in alcohol. Before use, the alcohol was removed by pouring two 99-ml quantities of sterile dilution water over the thermometer. After use, the milk was rinsed off the thermometer before it was placed in alcohol again. The room temperature was recorded at the beginning and the end of the 4-hr period.

The results are shown in Table 1 arranged according to platings intervals. In the interest of brevity, counts are reported for only the initial plating and as determined at the end of 15 min, 1 hr, and 4 hr. Sample and room temperatures and the month when each sample was examined are shown, also.

DISCUSSION

Only one sample showed a coliform count of over 10 on the initial plating, whereas four of the samples showed a standard plate count of over 30,000. On the final plating, after 4 hr of warming from an average temperature of 48 F to 81 F, four of the samples gave coliform counts of over 10 per ml and four gave standard plate counts of over 30,000 per ml. Sample No. 6 is assumed to be over 30,000 per ml on the final plating since a "spreader" resulted and the standard plate count at 3 hr was 44,000. Only one of the four samples having a high coliform count had a high standard plate count. One sample with an initial standard plate count of 38,000 gave a final count of 26,000. Thus, warming at room temperature for 4 hr

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caused three coliform counts to exceed 10 per ml but only one standard plate count to exceed 30,000 per ml.

During the first, or 5-min, interval none of the samples showed the alleged increase in coliforms; the temperature had increased an average of 2.6°F per sample. When warming was extended to 10 min, the temperature rose an average of 5.3°F and the samples failed to show marked increases in bacterial growth. The same was true at the end of 15 min when the temperature had increased an average of 8.4°F per sample. Even after 4 hours of warming during which the temperature rose an average of 33°F, from 48°F to 81°F, only six samples, Nos. 1, 2, 4, 5, 9 and 12, showed a distinct increase in coliform count. Only two of these samples, No. 2 and No. 5, both of which had a few coliforms initially, showed extensive coliform growth.

Significant increases in coliform counts for intervals not reported occurred in only three samples.

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**Table 1. The Effect of Gradual Warming of Milk Samples on Coliform and Standard Plate Counts**

<table>
<thead>
<tr>
<th>Platting interval</th>
<th>Temperature</th>
<th>Dup. coliform count</th>
<th>Single standard plate count</th>
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<th>Temperature</th>
<th>Dup. coliform count</th>
<th>Single standard plate count</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Sample No. 1, Feb.)</td>
<td>Initial: 80°C</td>
<td>Room: 76°C</td>
<td>Sample: 45°C</td>
<td>0</td>
<td>0</td>
<td>3,400</td>
<td>Initial: 77°C</td>
</tr>
<tr>
<td></td>
<td>15 minutes: 80°C</td>
<td>Room: 55°C</td>
<td>Sample: 51°C</td>
<td>0</td>
<td>0</td>
<td>5,500</td>
<td>15 minutes: 76°C</td>
</tr>
<tr>
<td></td>
<td>1 hour: 80°C</td>
<td>Room: 76°C</td>
<td>Sample: 62°C</td>
<td>0</td>
<td>0</td>
<td>4,300</td>
<td>1 hour: 76°C</td>
</tr>
<tr>
<td></td>
<td>4 hours: 82°C</td>
<td>Room: 79°C</td>
<td>Sample: 79°C</td>
<td>0</td>
<td>5</td>
<td>5,500</td>
<td>4 hours: 79°C</td>
</tr>
</tbody>
</table>

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During the first, or 5-min, interval none of the samples showed the alleged increase in coliforms; the temperature had increased an average of 2.6°F per sample. When warming was extended to 10 min, the temperature rose an average of 5.3°F and the samples failed to show marked increases in bacterial growth. The same was true at the end of 15 min when the temperature had increased an average of 8.4°F per sample. Even after 4 hours of warming during which the temperature rose an average of 33°F, from 48°F to 81°F, only six samples, Nos. 1, 2, 4, 5, 9 and 12, showed a distinct increase in coliform count. Only two of these samples, No. 2 and No. 5, both of which had a few coliforms initially, showed extensive coliform growth.

Significant increases in coliform counts for intervals not reported occurred in only three samples.
Sample No. 1 showed significant growth at the end of 3 hr, Sample No. 2 at 30 min and 3 hr, and Sample No. 5 at 2 hr and 3 hr.

The increase in standard plate counts was less than was anticipated; this was especially true after warming for 3 and 4 hr.

When first plated, the samples were undoubtedly within the period after pasteurization during which counts decline. It has been shown (2, 3) that the decline continues for 24 hr in a large percentage of samples and for at least as long as 72 hr in a few samples. With the temperature gradually increasing, apparently varying lengths of time up to 4 hr or more were required to reverse the downward trend or resolve the static condition of the bacterial population by the production of viable cells with rapid growth characteristics.

**Conclusions**

The counts showed no immediate surge of growth resulting from the first few degrees of increase in sample temperatures.

Only two samples showed rapidly increasing coliform counts. The count of the sample having a high initial coliform content increased very slowly. Increases in the standard plate count were either surprisingly small or the samples showed small decreases in count. In view of these results, it is concluded that the after-effect of pasteurization (2) was responsible for holding the bacterial growth of the samples to the magnitudes encountered.

It appears that the warming of samples, under the conditions studied, has a somewhat greater effect upon the coliform count than upon the standard plate count. However, more data are needed to establish this relationship.

The results reported certainly are not license to be lax with samples. Rather, these findings are assurance that when samples are handled according to USPHS recommended ordinance regulations (4) the processor and/or distributor may justly be held accountable for the condition of the products as collected by the sanitarian and examined by the laboratory.

**References**