Effects of Different Heat Treatments on Some Human Milk Constituents

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The 17 milk banks in France collect about 90,000 liters of milk per year (1). Since 1947, the heat treatment employed has been tyndalization (65°C for 20 min carried out three times) in accordance with the 1954 Department of Health Report (2). An up-to-date (1983) report reviewing the installation and working requirements in milk banks is at present being prepared.

This study was carried out to test equipment for pasteurizing human milk and also to measure the effect of heat treatment on some of the major milk constituents. Several papers have been published on this topic (3,4); the present study was carried out using commercial equipment currently available on the market.

MATERIALS AND METHODS

Milk samples were obtained from 20 to 40 mothers who donated their milk to the Paris Milk Bank (Lactarium). In most instances the milk was collected using an electric pump (Egnell) or a manual pump. The milk was frozen or kept at +4°C for no more than 48 hr before being collected from the mother's home. The samples were pooled in 4-liter batches. The milk used in each machine and cycle was obtained from different pools. The milk samples used for
biochemical studies were frozen at the milk bank before and after pasteurization and kept frozen until tested.

**Holder Pasteurization Equipment**

Holder pasteurization equipment comprised three machines that were lent to the Paris Lactarium. In all our experiments with the Holder pasteurizers, one bottle filled with milk was placed in the center of the bath, and the others were filled with water. The characteristics of each machine are as follows (Tables 1 and 2):

*The Oxford Human Milk Pasteurizer*

The bath temperature is 63°C, and milk is maintained at that temperature for 30 min. The milk containers are plastic and hold 100 ml. The total milk volume per cycle is 4 liters, and each cycle takes 100 min.

*The CM80 Pasteurizer*

The bath temperature is also maintained at 63°C for 30 min; 7.2 liters in 200-ml glass baby bottles can be pasteurized at one time, and the cycle takes 120 min. A feature of this machine is that the bottles are gently shaken during the cycle.

*The Lyon Pasteurizer*

Because technical assistance was available, it was possible to change the bath temperature easily. We used three different procedures: 65°C for 30 min and 58°C for 30 min done once or twice. The same milk bottles as for the CM80 were used (volume 200 ml). The total volume per cycle is 9.6 liters. The duration of a cycle is 120 min.

*Tyndalizer*

The Tyndalizer is a somewhat old-fashioned machine and is still used in milk banks in France. The milk is heated at ~65°C for 20 min three times at 24-hr intervals.
Thonon High-Temperature Short-Time Pasteurizer

The milk flows into a tube and is maintained at 70°C for 14 to 17 sec depending on the output, which is between 28 and 33 liters per hour. The temperature is reduced to 4°C in 30 to 45 sec.

BACTERIOLOGICAL TESTS

Bacteriological tests were carried out in the Paris Lactarium according to the procedures described in Table 2. At least 15 studies were performed for each type of equipment at each given temperature.

Protein Assay Techniques

Fractions enriched in secretory immunoglobulin A (sIgA), lactotransferrin, and lysozyme were prepared from thawed defatted milk. The latter was decaseinated and then fractionated using a concentration gradient of (NH₄)₂SO₄ and a pH gradient as described by Montreuil et al. (5). The sIgA was isolated from fraction P₄ under conditions described by Pierce-Cretel et al. (6). Lactotransferrin was purified from fraction P₇₋₈ by ion-exchange chromatog-

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Heating Temperature (°C)</th>
<th>Heating Duration (min)</th>
<th>Milk volume per container (ml)</th>
<th>Total milk volume per cycle (liters)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxford Human Milk Pasteurizer</td>
<td>63</td>
<td>30</td>
<td>100</td>
<td>4.0</td>
</tr>
<tr>
<td>CM80</td>
<td>63</td>
<td>30</td>
<td>200</td>
<td>7.2</td>
</tr>
<tr>
<td>Lyon Human Milk Pasteurizer</td>
<td>58</td>
<td>30</td>
<td>200</td>
<td>9.6</td>
</tr>
<tr>
<td>Human Milk Tyndalizer</td>
<td>65</td>
<td>20</td>
<td>200</td>
<td>20.0</td>
</tr>
<tr>
<td>Thonon HTST Pasteurizer*</td>
<td>70</td>
<td>14–17 secᵇ</td>
<td>Continuous flow</td>
<td>28–33 liters/hr</td>
</tr>
</tbody>
</table>

*a*High-temperature, short-time pasteurization.

ᵇCooling to 4°C, 30–45 sec.
TABLE 2. Bacteriological tests

<table>
<thead>
<tr>
<th>Before pasteurization</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Blood agar medium (total mesophilic flora); 0.1 ml of milk diluted 1/1,000 and 1/10,000</td>
</tr>
<tr>
<td>2. Chapman medium (Staphylococcus); 0.1 ml of milk diluted 1/10</td>
</tr>
<tr>
<td>3. Drigalsky medium (enterobacteria); 0.1 ml of milk diluted 1/100</td>
</tr>
<tr>
<td>After pasteurization</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Blood agar medium (total mesophilic flora); 0.1 ml of undiluted milk</td>
</tr>
</tbody>
</table>

raphy according to the procedure described by Spik et al. (7). Lysozyme was obtained from fraction P_{7-8} by ion-exchange and gel-filtration chromatography. Monospecific antisera to sIgA, lactotransferrin, and lysozyme were produced in rabbits according to the procedure of Vaitukatis et al. (8).

Levels of sIgA and lactotransferrin were determined by the radial immunodiffusion technique of Mancini et al. (9).

The iron-binding capacity of lactotransferrin was determined after saturating defatted milk with sufficient FeCl$_3$ in citrate/bicarbonate pH 8.6 reagent (10). Free iron was removed by ion-exchange chromatography, and the protein precipitated in acid conditions. The iron released from the lactotransferrin was measured using sulfobathophenanthroline reagent (11).

Lysozyme activity was assayed using the enzymatic lysoplate, which measures the lysozyme-mediated degradation of heat-killed Micrococcus lysodeikticus cells (12).

**Carbohydrate Assay Techniques**

After thawing at room temperature, the milk samples were defatted by centrifuging at +4°C. Ten milliliters of each sample was dialyzed overnight at +4°C against 100 ml of distilled water. All dialyzable fractions were lyophilized and analyzed by electrophoresis and chromatography (13,14). For electrophoresis, 10 mg of each fraction was placed on the anode side of No. 3 Whatman filter paper. Electrophoresis was carried out over 15 hr at 7 V/cm using a water–acetic pyridine–acid buffer at pH 5.4, and spots were stained using aniline oxalate reagent at 105°C.
For chromatography, 4 mg of each fraction was put on No. 3 Whatman paper and 4 mg on No. 3 MM Whatman paper. Chromatography was performed for 15 and 36 hr, respectively, using a solution of pyridine:ethyl acetate:distilled water (1:2:2). Spots were stained by the same procedure used after electrophoresis.

RESULTS

Bacteriology

The total mesophilic colony count before pasteurization varied considerably (10,000 to 10,000,000 CFU/ml). Our criterion for adequate pasteurization was that the total mesophilic flora should be <10 CFU/ml. This criterion was met using all the procedures with the exception of the 58°C/30 min method with the Lyon Human Milk Pasteurizer; however, after a second treatment at the same temperature, the total mesophilic count was <10 CFU/ml with this machine.

Changes in Milk Constituents

Table 3 shows the modifications of sIgA, lactotransferrin, iron-binding capacity, and lysozyme activity after pasteurization. Several experiments were done with each type of equipment and procedure. Table 3 shows the mean results. The values after pasteurization are expressed as a percentage of the initial values. In view of the small number of studies, care should be exerted in making comparisons, and we consider our results as preliminary.

Good results were obtained with the Oxford Human Milk Pasteurizer at 63°C for 30 min, but only three experiments were done. Considerable variations were observed using the same procedure (63°C for 30 min) or similar (65°C for 30 min) with different types of equipment. The first results obtained with the Lyon Human Milk Pasteurizer (58°C for 30 min repeated twice) were particularly encouraging. The results of the Thonon High-Temperature Short-Time continuous-flow pasteurizer were also satisfactory.
<table>
<thead>
<tr>
<th>Equipment</th>
<th>No. of studies</th>
<th>Heating Temp. (°C)</th>
<th>Heating Duration (min)</th>
<th>IgA (% survival)</th>
<th>Lactotransferrin (% survival)</th>
<th>Iron-binding capacity (% initial value)</th>
<th>Lysozyme (% initial activity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxford Human Milk Pasteurizer</td>
<td>3</td>
<td>63</td>
<td>30</td>
<td>87.1</td>
<td>61.6</td>
<td>78.3</td>
<td>ND</td>
</tr>
<tr>
<td>CM 80/6</td>
<td>2</td>
<td>58</td>
<td>30</td>
<td>71.1</td>
<td>17.3</td>
<td>4.8</td>
<td>95.8</td>
</tr>
<tr>
<td>Lyon Human Milk Pasteurizer</td>
<td>2^b</td>
<td>58</td>
<td>30</td>
<td>92.7</td>
<td>89.3</td>
<td>ND^a</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>2^c</td>
<td>58</td>
<td>30</td>
<td>99.2</td>
<td>100.0</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Human Milk Tyndalizer</td>
<td>3</td>
<td>65</td>
<td>30</td>
<td>56.8</td>
<td>15.8</td>
<td>ND</td>
<td>92.4</td>
</tr>
<tr>
<td>Thonon HTST Pasteurizer</td>
<td>3</td>
<td>70</td>
<td>15 sec</td>
<td>81.3</td>
<td>39.0</td>
<td>55.3</td>
<td>ND</td>
</tr>
</tbody>
</table>

^aND, no data.
^bOne cycle.
^cTwo cycles.
No modification of acid oligosaccharides, monosaccharides (galactose, glucose, fucose, N-acetylglucosamine, N-acetyleneuraminic acid) and polysaccharides (tri-, tetra-, penta-, and hexasaccharides) was observed with any of the methods used.

DISCUSSION

The aim of heat treatment is to kill bacteria with the minimum loss of milk constituents. All the equipment and procedures proposed by the manufacturers produced efficient bacterial killing. We therefore tried to lower the heating temperature to 58°C for 30 min but obtained poor bacteriological results. Effective pasteurization was, however, obtained by repeating the cycle twice.

The results obtained with the Oxford Human Milk Pasteurizer at 63°C for 30 min confirmed the results of others using the same equipment and the results of experiments carried out previously at the same temperature (3,4,16). Immunoglobulin A seems to be preserved better than lactotransferrin and iron-binding capacity. This is confirmed by the results obtained with the CM80 and the Lyon Human Milk Pasteurizer. Our data confirm the stability of lysozyme previously observed by Ford (3) and Eyers (15).

Discrepancies in results obtained with the 63°C/30 min method suggest that factors other than time and temperature should be taken into account. In particular, it should be noted that only the Lyon Human Milk Pasteurizer records milk temperature during Holder pasteurization. In other types of equipment, only the bath temperature is measured. We carried out further studies to investigate this point. Results obtained with the Lyon Human Milk Pasteurizer using the same milk show that the type of milk container (glass or plastic) does not seem to be important. Similarly, exposure to heat for 20 min instead of 30 min is not associated with better biochemical preservation. Among other factors, the volume of each milk container and shaking might modify the thermokinetics. Another factor that might modify the protein survival is the pH. At 58°C for 30 min with the Lyon Human Milk Pasteurizer, the destruction of IgA and lactotransferrin tends to decrease as the pH increases.
HEAT EFFECTS ON HUMAN MILK

Depending on the daily quantity of milk dealt with by a milk bank, we propose the following recommendations:

1. A milk bank that collects more than 6,000 liters a year could use the High-Temperature Short-Time Pasteurizer by which 30 to 40 liters can be pasteurized per hour with a good protein preservation. Precautions should be taken to avoid bacterial contamination when filling bottles after pasteurization.

2. A milk bank that collects <6,000 liters/year could pasteurize batches of 7 to 10 liters at a time at a given temperature in 200-ml bottles. As a bacteriological test is done to check the heat treatment after each cycle, it would be best to try to pasteurize at a temperature as low as possible (17). If the result is unsatisfactory, we suggest pasteurizing twice, because at 56 or 58°C, the loss of IgA and lactotransferrin is less after two exposures at these temperatures than after one exposure at temperatures >60°C. With a 200-ml container, 60°C seems to be a critical temperature above which proteins are damaged.

3. Similar considerations apply to milk banks that receive only small amounts of milk daily; small containers (100 ml) should be used so that the milk is heated and cooled quickly.

ACKNOWLEDGMENT

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REFERENCES

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