Inactivation of Milkborne Foot-and-Mouth Disease Virus
at Ultra-High Temperatures

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ABSTRACT

Many reports indicate that milk obtained from foot-and-mouth disease (FMD) virus-infected cows (4, 10-13, 15) and the derived milk products such as cheese (1) and casein (8) may be insidious sources of infectious FMD virus. For example, FMD virus can survive conventional high temperature-short time (HTST = 71.7 C/15 sec) pasteurization of whole milk, skim milk, cream and cellular debris from the milk of experimentally infected dairy cows (2). We have shown persistence of infectious FMD virus up to 42 days in heat-dried casein produced by acid precipitation of HTST-pasteurized skim milk from infected cows (7, 8).

Therefore, milkborne FMD virus may pose a serious threat to FMD-free countries, and reliable procedures are needed to inactivate FMD virus in milk. Rapid technological advances associated with the application of ultra-high temperature (UHT > 130 C/2 sec) procedures, enabled production of sterile milk with a long shelf life at ambient temperatures (16). Therefore, milk from cows infected with FMD virus was treated at different times and temperatures, and inactivation of FMD virus is reported.

MATERIALS AND METHODS

Experimental design

Results of experiments with bacterial spores reported by Westhoff and Doores (16) suggested to us that a temperature range of 102 to 149 C would include the heat inactivation conditions for FMD virus. Therefore, milkborne FMD virus was exposed to 102, 123, 130, 138 or 148 C for 2 to 5 sec — 1 to 5 replicates for each temperature.

Immediately after UHT treatments, milk was cooled in a tap water bath (7 C), transferred directly into sterile plastic bottles and stored at 4 C.

Virus inoculum

The virus used was FMD virus, type A, subtype 3, strain Mecklenburg (FMD, A-3M). It was prepared as a 5% suspension of bovine vesicular material in fetal bovine serum. Virus inoculum (10^6.3 PFU/ml) for dairy cows was 1 ml intravenously and 1 ml each in the right fore and left hind quarters with a teat cannula and syringe.

Cattle

Groups of three nonregistered Holstein dairy cows were milked twice daily with a portable automatic milking machine. Milk from each group was pooled during collection, and morning and evening pools were combined when necessary. Housing of cattle was described in detail previously (11).

Steers used for infectivity testing were nonregistered Herefords about 18 months old. Housing and maintenance of the steers have been described elsewhere (5).

Ultra-high temperature apparatus

An indirect heat exchanger raised the milk temperature to the desired treatment levels. The heat exchanger (Fig. 1) consisted of a steel pressure tank containing 20.56 m of coiled stainless-steel tubing with an inside diameter of 7.5 mm. The heat exchanger coil contained 906 ml of fluid. Milk was pumped through the exchanger by a variable-speed, stainless-steel, positive displacement centrifugal pump. Before addition of infectious milk, the apparatus was adjusted to the desired temperature by recycling distilled water and adjusting steam pressures. Variations of steam pressure and pump speed provided the various temperatures and transit times. Milk volume varied from 8-15 liters/experiment.

The apparatus was decontaminated between experiments by thorough rinsing with large volumes of tap water followed by a 5-min recycling of hot (150 C) 2% NaOH. The NaOH was then flushed from the system with running tap water, which was replaced by 10 liters of distilled water. The distilled water was recycled and heated to 150 C for 5 min and allowed to remain in the system until the next experiment.

Assay methods

A sample of pooled milk from each milking was assayed for FMD virus titer; standard plaque procedures were used. Plate titers (PFU/ml) were determined for milk pools before and after

1 Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

2 Conde 600 Milking Machine Unit with pneumatic pulsator, and S. S. RHT pail; Conde Milking Machine Co., Inc., Sherrill, NY.
Experimental treatments. The inoculum (0.1 ml) was allowed to adsorb to drained primary bovine kidney cell cultures for 1 h at 37 °C before it was overlaided with 0.6% gum tragacanth in culture fluid.

Infectivity of UHT-treated milk samples was assayed in two steers each by inoculation with 2 ml of sample distributed over the dorsal surface of the tongue in 20 intradermal lingual (IDL) needle tracks (7) and with 48 ml intramuscularly; thus, 100 ml of each sample was inoculated into steers. The IDL route was used because of its optimal sensitivity (8) for detecting very small amounts of FMD virus. Sera from steers that did not develop FMD 14 days after test inoculations were assayed for virus neutralizing antibodies as previously described (9).

Infectivity of foot-and-mouth disease virus infected cows' milk before and after ultra-high temperature treatments.

<table>
<thead>
<tr>
<th>UHT pool</th>
<th>Temp. (°C)</th>
<th>Time*</th>
<th>Cultured cell infectivity**</th>
<th>Cattle Infectivity***</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before heat</td>
<td>After heat</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>102</td>
<td>2-3</td>
<td>6.1</td>
<td>&lt;0.0</td>
</tr>
<tr>
<td>2</td>
<td>123</td>
<td>2-3</td>
<td>5.1</td>
<td>&lt;0.0</td>
</tr>
<tr>
<td>3</td>
<td>123</td>
<td>2-3</td>
<td>6.3</td>
<td>0.92</td>
</tr>
<tr>
<td>4</td>
<td>123</td>
<td>2-3</td>
<td>6.2</td>
<td>&lt;0.0</td>
</tr>
<tr>
<td>5</td>
<td>130</td>
<td>2-3</td>
<td>4.9</td>
<td>&lt;0.0</td>
</tr>
<tr>
<td>6</td>
<td>130</td>
<td>2-3</td>
<td>6.3</td>
<td>&lt;0.0</td>
</tr>
<tr>
<td>7</td>
<td>130</td>
<td>2-3</td>
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<td>8</td>
<td>138</td>
<td>2-3</td>
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<td>138</td>
<td>2-3</td>
<td>5.2</td>
<td>&lt;0.0</td>
</tr>
<tr>
<td>10</td>
<td>138</td>
<td>2-3</td>
<td>5.4</td>
<td>0.94</td>
</tr>
<tr>
<td>11</td>
<td>138</td>
<td>2-3</td>
<td>5.2</td>
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</tr>
<tr>
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<td>2-3</td>
<td>3.7</td>
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</tr>
<tr>
<td>13</td>
<td>148</td>
<td>2-3</td>
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<td>&lt;0.0</td>
</tr>
<tr>
<td>14</td>
<td>148</td>
<td>2-3</td>
<td>5.2</td>
<td>&lt;0.0</td>
</tr>
</tbody>
</table>

*Time in seconds that milk was held at indicated temperature.
**Values are log10 plaque-forming units/ml; <0.0 = < 1 PFU/ml.
***One or more steers developed FMD after inoculation of UHT milk; steers failed to develop signs of FMD during 14 days after test inoculation, and 14-day sera did not contain virus neutralizing antibody to FMDV A-3M.

DISCUSSION

Large amounts of FMD virus can be found in cows' milk before FMD is even suspected (4). Data in Table 1 show that UHT treatment at 148 °C for at least 3 sec can inactivate FMD virus in milk from infected dairy cows. Further studies have been designed to refine UHT inactivation conditions for FMD virus in cows' milk. Thus, UHT treatment of fresh, whole milk in the dairy industries can eliminate regional or international distribution of infectious FMD virus in dairy products.

Although organoleptic qualities of UHT-treated milk were not studied here, reports in the literature (10) indicate that UHT-treated milk can be a palatable food. Current research in several countries involves removal of objectionable odors and flavors caused by UHT treatments of milk (4). Use of UHT-treated milk in the cheese industry may present technical problems involving loss of coagulability. We expect that such problems can be resolved by applied research. We have also produced dried casein from UHT-treated (148 °C) milk according to methods described previously (8) and have observed no manufacturing difficulties.

The versatility of UHT technology is apparent when one considers that mobile UHT units can be rapidly moved into an outbreak area and attached to existing bulk milk processing equipment. Mobile units would provide a practical alternative for safe disposal of infectious milk in an FMD outbreak and would salvage a valuable source of high-quality nutrients for man.
Acknowledgments

We are indebted to Mr. R. Trower for valuable technical assistance in this study. We are also indebted to Drs. J. H. Graves, P. D. McKercher and J. W. McViear of Plum Island Animal Disease Center who were helpful in the initiation of the study; and to Dr. V. Jones, North Carolina State University and Dr. Locke Edmonson, Eastern Regional Research Center for their encouragement and technical advice.

References


Ice Cream’s Contents List Won’t Indicate Its Quality

Next July ice cream will become one of the first “standard of identity” foods requiring a list of ingredients on the package. The U.S. Food and Drug Administration (FDA) will continue to regulate the ingredients in ice cream, however.

The standard of identity guarantees consumers that all ice cream contains minimum amounts of certain ingredients, according to William Winder, food scientist at the University of Wisconsin-Madison (UW-Madison).

At first glance, the FDA’s description of the minimum composition of ice cream appears simple:

At least 10 percent of ice cream must be milk fat. At least 20 percent must be milk solids which includes milk fat, protein, lactose (milk sugar) and minerals.

A gallon of ice cream must weigh at least 4.5 pounds. This controls the amount of air the producer may whip into the ice cream to increase its volume.

A gallon of ice cream must contain at least 1.6 pounds of food solids or digestible ingredients. The remainder can be water.

All ice cream contains emulsifiers to prevent the fat and water from separating, and stabilizers to help maintain ice cream’s smooth texture. Emulsifiers and stabilizers together can’t exceed five-tenths of 1 percent of the final product.

One reason why the price and quality of ice creams vary is that producers have a long list of acceptable ingredients to choose from, says Winder.

For example, producers can choose from several sources of milk solids, including nonfat dry milk solids or whey to comply with the standard of identity. Whey solids, which can constitute only one-fourth of the milk solids not fat, cost about one-fourth as much as non-fat dry milk solids, but contain less protein.

Producers can also choose from a list of sweeteners, emulsifiers, stabilizers and milk fat sources.

Some ice creams contain more than the minimum amounts of milk fat and solids. And some producers whip in less air than the standards allow.

“Ice cream grading is unique among dairy products,” notes Winder.

“Beverage, for example, is graded for quality. The grades AA, A and B listed on butter containers clearly identify the quality of butter. These grades also help explain price differences to consumers,” he adds.

Ice cream is not graded for quality. Instead, the standard of identity establishes the minimum quantity of the ingredients used in ice cream. The producer can move up from the minimum level, but there’s no grading scale to indicate he has done so.

Winder doesn’t foresee ice cream grading in the near future. Although he thinks grading, if done properly, would make it easier for consumers to compare ice creams, he points out that it would also increase the cost.

If you equate quality with price in ice cream, you could be missing out on some very good competitively priced ice cream.

“Talking about price and ingredients only scratches the surface of what constitutes quality ice cream. Processing, handling and flavoring are also important to quality. The fact is that there is quality ice cream on the market today that is competitively priced,” Winder concludes.