Inoculation of Citric Acid-Fermenting Bacteria into Raw Milk in Farm Bulk Tanks

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ABSTRACT

Cultures of Streptococcus lactis subsp. diacetylactis and Leuconostoc cremoris were added together in the amount of 0.5% to raw milk in a farm bulk tank. This treatment did not significantly reduce the psychrotrophic or coliform population as hypothesized; however, the shelf-life was extended on products made from this raw milk by an average of 1 day. Also, the legal question of adding viable bacteria to the raw milk needs to be considered by state health departments and appropriate federal agencies. Since hydrogen peroxide is reported to be the toxic agent (to the psychrotrophs) released by the citrate fermenters, the obvious fact is noted that this agent can already be added to milk designed for cheese manufacture.

Work has been reported both by Sellars (2) and Juffs and Babel (1) regarding the killing effect exerted by lactic dairy cultures, specifically the citrate fermenters, on psychrotrophic bacteria. Work in the laboratory demonstrated this lethal effect on specific psychrotrophs. Juffs and Babel (1) postulated that the killing action was not due to the lactic acid produced by the cultures but more likely due to hydrogen peroxide. These workers supported this hypothesis quite effectively.

Since psychrotrophic bacteria are present in almost all raw milk supplies, they constitute the major problem concerning shelf-life reduction in milk and milk products. Nearly all pasteurized milk held at 4 C or below will eventually develop off-flavors due to psychrotrophic growth. Thus research which can reduce the number of psychrotrophs present in raw milk takes on added significance. Admittedly, the best way of reducing or retarding psychrotrophic growth is by preventing their entry into the milk at the farm. While prevention is the best method and one which is technically feasible, it is not easily achieved. Improper washing and drying procedures and improperly cleaned and sanitized farm bulk tanks head the list of factors which tend to permit the entry of psychrotrophs into the raw milk.

Therefore, the primary purpose of this study was to investigate the feasibility of adding dairy cultures capable of fermenting citric acid to raw milk on a commercial basis. The effect of these added cultures on the normal microflora would be observed. Moreover, the effect, if any, addition of these cultures would have on the shelf-life of dairy products made from this raw milk would be noted. This research was deemed important since conditions at the farm level are often hard to duplicate in the laboratory thus making long-term feasibility studies difficult.

MATERIALS AND METHODS

Cultures

Commercial lyophilized cultures of citrate-fermenting bacteria (Streptococcus lactis subsp. diacetylactis - #188 and Leuconostoc cremoris - CAF) were obtained from a commercial source. The cells were grown in reconstituted (10% MSNF) milk that had been steamed for 1 h. Initial inoculation was 1% with subsequent incubation at 21 C for 16 h. Following two transfers, bulk cultures were prepared and held at 4 C until used.

Initial laboratory testing

Before starting the study involving farm milk, attempts were made to simulate what would occur in the bulk tank. The situation of everyday pickup or every-other-day pickup would need to be considered. The effect of warming the milk as would occur during a normal milking would need to be considered. This is important since no matter how good a refrigeration system, there will be at the very minimum localized warming. This will obviously vary from farm to farm.

Growth responses (total count, pH, and titratable acidity) were obtained for the two organisms, S. lactis subsp. diacetylactis and L. cremoris, both in skimmilk and in raw whole milk. This was done in the laboratory before any fieldwork. Growth responses were measured at 4.4, 10, and 21 C. Finally, the responses were measured after 3 h at 4.4 C, 10 h at 10 C, again after 3 h at 4.4 C, 10 h at 10 C, etc. This was an attempt to predict the actual response of the cultures in the bulk tank. The temperature of the milk in the bulk tank at the University of Georgia Dairy Research Center rose to 10 C during milking but was back down to 4.4 C or less within 30 min postmilking; thus the conditions selected for the laboratory study were harsher than those the cultures would encounter during the actual experiment. The obvious reason for these safeguards was because S. lactis subsp. diacetylactis produces lactic acid.
Experimental procedure

When assurance was obtained from the laboratory studies that the cultures would not coagulate the milk in the farm bulk tank, the main thrust of the study commenced. An outline of the experimental procedure is depicted in Fig. 1.

Cultures were added to approximately 0.5% by weight following the evening milking. The average weight of milk for the two milkings was 2165 kg (5500 lb.). The milk was picked up daily from the University Farm following the morning milking. Thus, the culture was added at 0.5% on the estimated quantity of milk that would be present after two milkings.

Stage one represents the raw milk before treatment. The Standard Plate Count (SPC), coliform count (COLI), 10-day psychrotrophic count (PSY), pH, and titratable acidity (TA) were determined.

Stage two represents the raw milk immediately (5 min) following addition of culture. The same measurements as for stage one were taken. Stage three represents the same milk taken from the tank truck.

Stage four represents the same milk taken from the raw milk holding tank at the University processing plant. The pH and TA were not taken on the samples from any subsequent stage.

Stage five represents a 10-ml sample taken from the raw milk both before and after addition of starter. The sample was pasteurized in the laboratory at 63 C - 30 min — the SPC being determined both at 32 and 21 C (48-h incubation). Coliform and psychrotrophic counts were determined.

Stage six represents the finished product (homogenized milk and chocolate milk) made from the same supply of raw milk. The samples were evaluated for flavor and bacterial estimates.

Stage seven represents a 10-ml sample taken again from the raw milk both before and after addition of culture. These samples were incubated raw at 7 C for 5 days after which bacterial estimates were made.

Stages eight and nine represent samples of finished product examined after refrigerated (4.4 C) storage for 7 and 10 days, respectively. The samples were tasted following plating.

Stage ten represents the resulting shelf-life of the finished samples, i.e., days required before off-flavor development.

The procedure was repeated weekly for 9 weeks. Untreated samples were taken from six different loads of milk coming into the UGA processing plant (stage three). Untreated samples were taken to give a baseline and a picture of the natural microflora of the raw milk.

RESULTS AND DISCUSSION

The two strains of citrate fermenters grew adequately in reconstituted (10%) NFDM. Initially, there was some difficulty in obtaining sufficiently large numbers of L. cremoris (CAF). The amount of inoculum was increased to 6% with a resultant increase in numbers following incubation. The mean count of the CAF culture was 98 x 10⁶ cfu/ml while the mean count for 188 was 1.96 x 10³ cfu/ml. The two organisms grew quite well in both types of media, skim milk, and raw milk.

With regard to pH, culture CAF did not lower the pH below 6.6 even after 36 h of alternating incubation temperatures between 4.4 C (3 h) and 10 C (10 h). With culture 188, however, the situation was a little more critical since the pH was reduced to 4.8 after the same 36 h. Thus, when the study was initiated, the amount of culture used to inoculate milk in the farm bulk tank was carefully monitored with 75% of the total starter being CAF and 25% made up of 188 (Table 1). With this combination, there was no appreciable rise in acidity when using everyday pickup from the farm. The mean pH after addition of the milk from the second milking was 6.75 with a mean TA of 0.18. Actually a higher percentage of CAF alone may eventually prove to be best, depending upon the degree of psychrotrophic reduction obtained. If culture 188 were solely used with less than...
adequate refrigeration in the tank, the acidity could most assuredly increase to prohibitive levels.

In contrasting stages 1 and 2 (Fig. 1) which involve the raw milk before and after addition of culture, there was a highly significant \( P < 0.01 \) difference between stages only in SPC values. This significance was expected since actively growing cells were added which readily showed up on plates incubated at 32 C for 48 h. Data in Table 2 show the actual difference.

In following the milk through the outline (Fig. 1), the milk is pumped into the tank (stage 3) where it is transported to the processing plant (stage 4). There were no significant differences between these two stages either in counts or in acidity (pH or TA). There were some differences (treated vs. nontreated), however. These differences are depicted in Table 3. The high psychrotrophic count in the treated samples indicates that a portion of the cultures are at least capable of some growth at 7 C. Gram staining of cells from plates made of starter cultures are at least capable of some growth at refrigeration temperatures. In the first comparison, stage 2 (after addition of culture) is contrasted with stage 3 (non-treated only) for SPC, PSY, and COLI. These data are in Table 4. No significant differences were noted.

In looking at only the treated milk before and after addition of culture, a somewhat clearer picture is obtained. These data (for stage 7) are in Table 5. The SPC shows a significantly higher number of bacteria present in the treated sample — an expected happening. There was a slight decrease in the number of coliforms in the treated samples but a slight increase in the number of psychrophilic bacteria. An unexpected and undesirable event. This comparison is of particular importance since raw milk is being held for extended periods and the nature of the microflora of the raw milk needs to be examined.

The most important criterion comes, however, with stages 6, 8, and 9, i.e., the finished product (homogenized milk and chocolate milk) and the shelf-life of these products (stage 10). Initially, comparing stages 6 (day of packaging), 8 (after 7 days of storage at 7 C), and 9 (after 10 days of storage at 7 C) for homogenized milk, highly significant differences were obtained for SPC.

### Table 1. Composition of dairy culture used to inoculate raw milk in farm bulk tank.

<table>
<thead>
<tr>
<th>Culture used</th>
<th>Total starter</th>
<th>Approximate No. cells (log)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leuconostoc cremoris (CAF)</td>
<td>75</td>
<td>( 9 \times 10^3 )</td>
</tr>
<tr>
<td>Streptococcus lactis subsp. diactylacti (188)</td>
<td>25</td>
<td>( 16 \times 10^3 )</td>
</tr>
</tbody>
</table>

1. Represents total number of cells added of each bacterial strain.

### Table 2. Mean differences between bacterial estimates \(^1\) of raw milk before and after addition of citrate fermenting bacteria.

<table>
<thead>
<tr>
<th>Stage</th>
<th>SPC</th>
<th>PSY</th>
<th>COLI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Before)</td>
<td>3.4983 (^2)</td>
<td>2.0089</td>
<td>1.9864</td>
</tr>
<tr>
<td>2 (After)</td>
<td>5.5572 (^2)</td>
<td>2.6734</td>
<td>1.9112</td>
</tr>
</tbody>
</table>

\(^1\) All values are log means.

\(^2\) \( P < 0.01 \).

### Table 3. Differences among bacterial counts \(^1\) and acidity levels for raw milk both treated with culture and non-treated.

<table>
<thead>
<tr>
<th>Test</th>
<th>Mean (^2)</th>
<th>Level of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPC - Treated</td>
<td>5.2299</td>
<td>( P &lt; 0.01 )</td>
</tr>
<tr>
<td>SPC - Non-treated</td>
<td>3.4691</td>
<td>( P &lt; 0.01 )</td>
</tr>
<tr>
<td>PSY - Treated</td>
<td>4.5765</td>
<td>( P &lt; 0.01 )</td>
</tr>
<tr>
<td>PSY - Non-treated</td>
<td>2.1899</td>
<td>( P &lt; 0.01 )</td>
</tr>
<tr>
<td>COLI - Treated</td>
<td>1.6538</td>
<td>N.S. (^3)</td>
</tr>
<tr>
<td>COLI - Non-treated</td>
<td>1.9184</td>
<td>N.S.</td>
</tr>
<tr>
<td>pH - Treated</td>
<td>6.73</td>
<td>( P &lt; 0.01 )</td>
</tr>
<tr>
<td>pH - Non-treated</td>
<td>6.74</td>
<td>N.S.</td>
</tr>
<tr>
<td>TA - Treated</td>
<td>1.8</td>
<td>N.S.</td>
</tr>
<tr>
<td>TA - Non-treated</td>
<td>1.8</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

\(^1\) Log values.

\(^2\) Means of each treatment over stages 3 and 4.

\(^3\) Not significant.

### Table 4. Comparison of log means of bacterial counts involving milk to which culture was added and a non-treated sample after holding raw milk at 7 C for 5 days.

<table>
<thead>
<tr>
<th>Test</th>
<th>Stage</th>
<th>Mean</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPC</td>
<td>2(^2)</td>
<td>7.1279</td>
<td>N.S.</td>
</tr>
<tr>
<td>PSY</td>
<td>2(^3)</td>
<td>6.7207</td>
<td>N.S.</td>
</tr>
<tr>
<td>COLI</td>
<td>2(^3)</td>
<td>6.4814</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

\(^2\) Stage 2 = after addition of culture

\(^3\) Stage 3 = non-treated milk.

### Table 5. Comparison of log means of bacterial counts involving treated milk both prior to addition of culture and after with both samples subsequently being held raw for 5 days at 7 C.

<table>
<thead>
<tr>
<th>Test</th>
<th>Stage</th>
<th>Mean</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPC</td>
<td>Before</td>
<td>6.0811</td>
<td>( P &lt; 0.05 )</td>
</tr>
<tr>
<td>PSY</td>
<td>After</td>
<td>7.1279</td>
<td>N.S.</td>
</tr>
<tr>
<td>COLI</td>
<td>Before</td>
<td>6.0780</td>
<td>N.S.</td>
</tr>
<tr>
<td>COLI</td>
<td>After</td>
<td>3.0454</td>
<td>N.S.</td>
</tr>
<tr>
<td>COLI</td>
<td>After</td>
<td>2.6628</td>
<td>N.S.</td>
</tr>
</tbody>
</table>
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PSY, and COLI counts (Table 6). The differences were among stages and not for treatment, i.e., treated vs. non-treated. The increase in mean psychrotrophic count closely paralleled that of the total aerobic, mesophilic count (SPC).

### TABLE 6. Differences of bacterial counts after five and seven days of refrigerated storage of pasteurized, homogenized milk made from milk treated with citrate fermenting bacteria.

<table>
<thead>
<tr>
<th>Test</th>
<th>Stage</th>
<th>Mean(^4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPC</td>
<td>6(^a)</td>
<td>1.3947 (a)</td>
</tr>
<tr>
<td></td>
<td>8(^b)</td>
<td>3.3872 (b)</td>
</tr>
<tr>
<td></td>
<td>9(^c)</td>
<td>5.1183 (c)</td>
</tr>
<tr>
<td>PSY</td>
<td>6</td>
<td>0.4985 (a)</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>3.6332 (b)</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>4.4865 (c)</td>
</tr>
<tr>
<td>COLI</td>
<td>6</td>
<td>0.0000 (a)</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.9084 (b)</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>1.0227 (b)</td>
</tr>
</tbody>
</table>

1 Stage 6 = Day of bottling.
2 Stage 8 = After 5 days of storage at 7 C.
3 Stage 9 = After 7 days of storage at 7 C.
4 Log values

Any means not followed by the same lower case letter differ significantly (\(P < 0.05\)).

When F tests were made on the chocolate milk, almost exact counts were obtained as had been obtained for the homogenized whole milk. Thus significant increases occurred among counts over refrigerated storage but no significant differences were noted for treated vs. non-treated samples.

Shelf-life values for both the homogenized milk and chocolate milk for treated (culture added) and non-treated samples are shown in Table 7. The shelf-life values indicate that the milk tended to last 1 day longer with rather than without the citrate fermenting bacteria added to the raw milk. While this is not a tremendous extension, it is a promising start and future research is needed to further extend this work.

### TABLE 7. Shelf-life values for homogenized whole milk and chocolate milk which either did or did not have added citrate fermenting bacteria added to the raw milk before processing.

<table>
<thead>
<tr>
<th>Product</th>
<th>Treatment</th>
<th>Shelf-life (days - mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenized whole milk</td>
<td>Treated(^1)</td>
<td>16.3</td>
</tr>
<tr>
<td></td>
<td>Non-treated(^2)</td>
<td>15.3</td>
</tr>
<tr>
<td>Chocolate milk</td>
<td>Treated</td>
<td>13.5</td>
</tr>
<tr>
<td></td>
<td>Non-treated</td>
<td>12.4</td>
</tr>
</tbody>
</table>

1 Treated = Citrate fermenters added to raw milk.
2 Non-treated = No bacteria added.

**REFERENCES**