Growth and Death of Selected Microorganisms in Ultrafiltered Milk

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(Received for publication October 4, 1985)

ABSTRACT

Studies were made to compare the growth and death of Staphylococcus aureus, Streptococcus faecalis and Escherichia coli in skim milk concentrated by ultrafiltration to that in unconcentrated skim milk. Behavior of the organism and total aerobes did not significantly differ in the four milk samples at either 7 or 13°C. For S. faecalis and E. coli, D-values for samples heated to 62.7°C did not significantly differ in the four milk samples (p>0.01). The D-value of S. aureus in water-diluted retentate was slightly but significantly lower than those in the other three milk samples (p<0.01), possibly due to the lowered lactose level in this sample.

Ultrafiltered milk has been used in Europe and Canada in the production of various cheeses (9,12,15,16), and has been proposed for use in the production of low-lactose dairy products (2,13).

The ultrafiltration process changes the composition of milk. Lactose, citrate, B-vitamins, non-protein nitrogen, and minerals pass through ultrafiltration membranes in various degrees, whereas fat and protein (and substrates that are bound to them) are concentrated by ultrafiltration (4,5,6,7,22,24). The buffering capacity of milk is increased by ultrafiltration due to the concentration of protein and phosphate components (10,14,21).

The purpose of this study was to investigate the growth/survival of selected pathogenic and/or spoilage microorganisms in ultrafiltered milk.

MATERIALS AND METHODS

Milk samples

Pasteurized skim milk was obtained from the Cornell University Dairy Plant which routinely monitors its milk for the absence of antibiotics using Delvotest P (GB Fermentation Industries, Des Plaines, IL). Skim milk was volume concentrated to 2× in laboratory-scale stirred UF cells. Behavior of the organism and total aerobes did not significantly differ in the four milk samples at either 7 or 13°C. For S. faecalis and E. coli, D-values for samples heated to 62.7°C did not significantly differ in the four milk samples (p>0.01). The D-value of S. aureus in water-diluted retentate was slightly but significantly lower than those in the other three milk samples (p<0.01), possibly due to the lowered lactose level in this sample.

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samples were inoculated with 0.1 ml (S. aureus and E. coli) or with 1.0 ml (S. faecalis) inoculum to achieve an initial count of $10^7-10^9$ cfu/ml. One-ml portions of the milk samples were then distributed into sterile 15×145 mm test tubes. Four test tubes (one from each milk sample) were simultaneously placed in a test tube rack which had been attached to an agitating bar in a temperature-controlled water bath set at 62.7°C. The rotor was immediately turned on. The rack was agitated approximately 3 cm in a back and forth motion at a speed of approximately 1 cycle/sec. The tubes remained in the water bath for a previously determined time interval and then were quickly removed and plunged into ice water. This procedure was repeated for several time intervals.

D-values for each organism in each milk sample were determined from appropriate portions (steep and linear) of the survival curves using a least squares regression analysis. For each organism the D-values in the four milk samples were compared for statistically significant differences using the general linear model method (19) and pairs of D-values were compared with the protected least significant differences test.

RESULTS AND DISCUSSION

Water activity and acidity of milk samples

Water activities of the four milk samples from numerous ultrafiltration trials were all within the range of 0.991-0.995 and pH values were all in the range of 6.6-6.7. The measured variations were within experimental error, indicating that ultrafiltration of milk to 2× did not change either $a_w$, or pH detectably.

Storage studies

Preinoculation counts on the experimental milks from numerous trials for S. aureus, S. faecalis, and E. coli on differential media were nearly always 0 cfu/ml and never exceeded 10 cfu/ml. The maximum count for total aerobes in milk samples before inoculation was 72 cfu/ml. Following inoculation of the various milks, typical growth curves for total aerobes, E. coli, S. faecalis, and S. aureus in milk samples stored at 13°C are shown in Fig. 1. In each instance, there were no appreciable differences among the growth curves of the specific inoculum in the four milk samples (2× retentate, 1× water-diluted retentate, 1× milk equivalent (retentate plus permeate), 1× skim milk). For each microorganism, the lag phases were of the same length in all four milks and the slopes of the exponential growth phases were parallel. The curves for the four milk samples for a given organism crossed repeatedly and rarely varied by more than half a logarithm. Growth curves for the organisms in the milks stored at 7°C also did not differ among milks and are not shown. Both S. faecalis and total aerobic counts increased at 7°C at a slower rate than at 13°C. The lag phase for S. faecalis was about 5 d at 7°C in all four milks compared to about 2 d at 13°C. At 7°C, both E. coli and S. aureus slowly died in all milks. The death curves for the four milks crossed repeatedly and, at their widest spread over periods up to 15 d, differed by less than one logarithm.

Hickey et al. (10) found that growth rates of S. cremoris BK5 in milk retentate and in milk equivalent (retentate plus permeate) did not differ at either 15 or 45°C (near the minimal and maximal temperatures for growth), although at temperatures near the optimum for growth (30-32°C), growth rate was greater in milk retentate than in milk equivalent. They also found that growth rate at 30°C was greater in milk equivalent than in milk not ultrafiltered. They suggested that the increased growth in milk equivalent could have been due to alteration of milk components or release of stimulating factors in milk as a result of ultrafiltration. The present findings indicate that at refrigeration temperatures and somewhat above, there are no appreciable differences in growth or death of any of the organisms studied as a result of changes in milk from ultrafiltration.

Heat inactivation studies

Typical death curves for E. coli, S. faecalis, and S. aureus in the four milks heated to 62.7°C are shown in Fig. 2. D-values from the steep and linear portions of the curves for these microorganisms are given in Table 1. No significant difference was found between the D-values in the four milks for either S. faecalis or E. coli (p>0.01). For S. aureus, the D-value in sample B (water-diluted retentate) was significantly lower than those in the other samples (p<0.01), indicating a lowered
BEHAVIOR OF MICROBES IN ULTRAFILTERED MILK

3. a-lactose in milk samples heated to 62.7°C. Symbols as in Figure 1.

TABLE 1. D-values (min) for S. aureus, S. faecalis, and E. coli in milk samples heated to 62.7°C.

<table>
<thead>
<tr>
<th>Milk sample</th>
<th>E. coli</th>
<th>S. faecalis</th>
<th>S. aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.94c</td>
<td>1.26c</td>
<td>1.66c</td>
</tr>
<tr>
<td>B</td>
<td>0.80c</td>
<td>1.09c</td>
<td>1.32d</td>
</tr>
<tr>
<td>C</td>
<td>0.80c</td>
<td>1.17c</td>
<td>1.67c</td>
</tr>
<tr>
<td>D</td>
<td>0.79c</td>
<td>1.25c</td>
<td>1.81c</td>
</tr>
</tbody>
</table>

aUF retentate (2 ×), B-Water-diluted retentate (1 ×), C-Milk equivalent (retentate + permeate (1 ×), D-Skim milk (1 ×).

Different letters in a column indicate statistically significant differences (p<0.01).

thermal resistance in this sample. This lowered thermal resistance of S. aureus in water-diluted retentate may be due to the lowered concentration of lactose in this sample compared to the other samples since sugars have a protective influence on the thermal destruction of microorganisms in general, and S. aureus in particular (1,20). The D-values for the other three samples (A, C, and D) were not significantly different.

Grieme and Barbano (8) found that the D-values for S. aureus MF31 in whole milk retentates concentrated 1.35 × by reverse osmosis were significantly greater than those in unconcentrated whole milk but not significantly greater when the retentates were concentrated by ultrafiltration. Reverse osmosis membranes are ‘tighter’ than ultrafiltration membranes and retain the lactose. D-values for S. aureus and E. coli at comparable temperatures re-

References


Table 2 shows that there was a significant decrease (P< 0.001) in the number of airborne spores in the dust during the sampling period. Even though the numbers of airborne spores did not remain constant for the entire sampling period, this method for comparison of air samplers remains valid since all samplers were exposed to this same circumstance of decline in airborne spores over time.

The Andersen 6-stage sampler was efficient for determining size of particles with which A. flavus spores were associated. Six different trials were made in the model system during which spores tended to associate with particles of different sizes, making results erratic. When no consistent counts are found on each stage after sampling, no real conclusions can be drawn in this situation.

In conclusion, the Millipore and the absorbent cotton samplers had almost identical sampling efficiencies, while that of the all glass impinger was slightly lower. Differences in spore-to-dust particle size association made comparisons of the Andersen multi-stage sampler with the other impossible. All of these samplers would be useful in sampling regimens for the airborne spores of A. flavus from agricultural grain commodities such as corn.

REFERENCES


Haggerty and Potter, con’t. from p. 235