Effect of Emulsion Characteristics of a Low-Fat Dairy Spread on Bacterial Growth

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Abstract

The bacteriological keeping quality of a low-fat dairy spread was investigated and found to be closely related to the size of the serum droplets dispersed in the continuous fat phase. Standard plate and psychrophilic bacterial counts on 38.8% fat spreads stored at 7.2°C for four weeks increased by about one log cycle. The majority of the serum droplets were in the size range of 3 to 20 microns. In the 30.0% fat spreads, however, where many of the serum droplets exceeded 50 microns, the standard plate and psychrophilic bacterial counts increased by three to four log cycles over the four-week storage period.

During World War II and for some years thereafter, considerable research was conducted by several groups (3, 5, 6, 7) to develop a low-fat dairy spread which would be acceptable to the public. It may or may not have been coincidence that most of the spreads developed were fat-in-water-type emulsions, had little or no resemblance to butter, had a very limited shelf life, and were not generally accepted. In 1966 the development of a new low-fat dairy spread was reported (2). A typical composition was 35% fat, 17% calcium-reduced skim milk solids, and 48% water. The new spread was a water-in-fat-type emulsion and had a body somewhat like butter. Also, it had very good spreadability at refrigerator temperatures, was stable to repeated freezing and thawing, and had a flavor similar to butter. Contrary to expectations for a product which had a pH of about 6.5, which was not sterile, and which contained high percentages of protein and water, the preliminary report (2) indicated the spread had exceptionally good shelf life at refrigerator temperatures. Therefore, the purpose of the present investigation was to confirm the shelf-life finding and to discover the reason for it.

Experimental Procedures

Preparation of low-fat spreads. The fat source for all experiments was butter tempered to 21°C. The balance of the product consisted of a serum prepared by reconstituting calcium-reduced skim milk solids in water, heating to 77°C, holding 20 min, and cooling to 21°C. Two methods were used to prepare the spreads as water-in-fat emulsions. In the first method, referred to as the Hobart method, the butter and serum were blended together in 3.6-kg lots using a Model 120A Hobart food mixer. After creaming the butter in the mixer, the serum was added in 300-ml increments. Blending was carried out after each addition of serum, using medium speed for 30 sec. A final blending for 15 sec at high speed was used to promote a fine dispersion of serum in the fat phase. In the second method, sufficient serum was placed in 4-ml plastic bags, 33 by 60 cm, along with an appropriate amount of butter to make 3.2-kg lots. Tumbling the bags in a Gosselin churn caused the contents to form a water-in-fat emulsion. This is referred to as the churn method.

Photomicrographs. A Zeiss photomicroscope was used to study and photograph wet-mount preparations of the spreads.

Bacteriological tests. Samples of the spreads were placed in 140-ml ice cream cups and stored at 7.2°C within an hour of manufacture. Standard plate and psychrophilic bacterial counts were determined by official methods (1) on the freshly made product and again after two and four weeks of storage.

Experimental design. To study the keeping qualities of the prepared spreads as measured by bacterial growth during storage, five replications of three spreads containing 30.0, 35.0, and 38.8% fat, with 53.0, 48.0, and 44.2% water, respectively, were prepared. They were examined before and after the indicated storage periods to determine the changes in bacterial counts. Because all spreads contained 17% calcium-reduced skim milk solids, water-to-serum-solids ratios of 3.12, 2.82, and 2.60 were established in the spreads, respectively. In the statistical analysis of the results, treatments included the three compositions described, and three storage times at 7.2°C; namely, control (fresh sample), two weeks, and four weeks. The effects of treatments on standard plate and psychrophilic bacterial counts were evaluated.
Results and Discussion

The logarithms of standard plate counts for all treatments were determined and a highly significant $F$ value was obtained for treatment effects. Further analyses revealed highly significant $F$ values for main effects, namely, composition and storage time. The mean logarithmic standard plate counts (five replications and three storage times) for the 30.0, 35.0, and 38.8% fat spreads were 3.6300, 2.7211, and 2.2929, respectively, and were significantly different from each other ($\text{lsd}_{0.05} = 0.2270$) (4).

Similarly, the mean logarithmic standard plate counts (five replications and three compositions) for the control and the samples held for two- and four-week storage periods were 2.2571, 2.8391, and 3.5478, respectively, and were significantly different from each other. When the mean logarithmic counts for the different compositions were plotted against storage time in weeks, as illustrated in Figure 1, the reasons for a statistically significant interaction between composition and storage time became obvious. Bacterial growth rates during storage were considerably different in the three spreads, as indicated by diverging curves. The significance of mean difference between logarithmic counts was tested at each fat level by $\text{lsd}_{0.05} = 0.3926$. With the 30.0% fat spread, all the differences between means were significant. With the 35.0% fat spread the differences were significant for zero to four weeks' storage and for the two to four weeks' storage, but not for zero to two weeks' storage. With the spread containing 38.8% fat, the mean differences between logarithmic counts for the various periods were not significant, indicating very good bacteriological keeping quality over the four-week period.

Results of the psychrophilic bacterial counts followed the same pattern. The mean logarithmic psychrophilic bacterial counts (five replications and three compositions) for the 30.0, 35.0, and 38.8% spreads were 3.2592, 2.3481, and 1.6589, respectively, and were significantly different from each other ($\text{lsd}_{0.05} = 0.2284$). Similarly, the mean logarithmic psychrophilic bacterial counts (five replications and three compositions) for the control and the samples held for two- and four-week storage were 1.0800, 2.7347, and 3.4511, respectively, and were significantly different. Again, the interaction between composition and storage time was significant. (This is illustrated in Figure 2.) As before, the bacterial growth rates for the different compositions were significantly different from one another, but in this case significant psychrophilic bacterial increases occurred in all spreads. However, the magnitude of the mean differences indicated considerably more bacterial growth at two and four weeks of storage in the 30.0% fat spread than in the 35% fat spread, which in turn supported greater bacterial growth than did the 38.8% fat spread.

From these results it was apparent that bacterial growth decreased as the fat percentage in the spreads increased. Consideration was given to the possibility that higher osmotic pressures
in the sera of the high-fat spreads, resulting from the lower water-to-serum-solids ratios in the sera, might be responsible for the smaller bacterial increases. However, when serum having the same water-to-serum-solids ratio as that present in the 38.8% fat spread (i.e., 2.60) and, therefore, a similar osmotic pressure, was inoculated with bacteria isolated from a previously made 30% fat spread, a $10^3$-fold increase in standard plate count was obtained after five days' storage at 7.2°C. This did not support the suggestion that a higher osmotic pressure in the serum of the 38.8% fat spread caused an inhibition of bacterial growth.

Using a Zeiss photomicroscope, wet-mount preparations of spreads were studied. From the photomicrographs in Figure 3 it appeared that the serum in a 38.8% fat spread (A) was more finely dispersed than in a 30% fat spread (B). Therefore, considering the bacteriological results already obtained, it appeared that the fineness of serum dispersion was related to bacterial growth. If this were true, then it could be expected that bacterial growth in samples of spread removed at successive stages during the development of the emulsion would show a decreasing ability to support bacterial growth. To investigate this possibility the churn method was used to prepare two lots of spread containing 30.0 and 38.8% fat, and serum containing 28.0% calcium-reduced skim-milk solids. The churn method was chosen because it required much longer working times to develop the desired emulsion than did the Hobart method. Therefore, required working times between the removal of samples at successive stages in the development of the emulsion were not as critical as in the Hobart method, where the emulsion may be completed in as little as two minutes of working time. Also, the churn method allowed both lots of spread to be worked simultaneously under the same conditions of time and temperature, thereby eliminating these possible variables. Samples of the 30.0% fat spread were removed after 15, 45, 60, and 90 min of working. Because the 38.8% fat spread “worked” more quickly, samples were removed after 15, 30, 45, and 60 min.

From the results presented in Table 1, it was apparent that increased working time caused a decrease in the rate of bacterial growth. When the 30% fat spread was worked for 15 and 45 min, bacterial numbers exceeded five million at the end of four weeks of storage. However, when the working was extended to

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**Table 1. Effect of working time on standard plate counts of two low-fat spreads made by the churn method and stored at 7.2°C.**

<table>
<thead>
<tr>
<th>Weeks held at 7.2°C</th>
<th>30.0% fat</th>
<th>38.8% fat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Working time (min)</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>330</td>
<td>340</td>
</tr>
<tr>
<td>45</td>
<td>1,700,000</td>
<td>1,800,000</td>
</tr>
<tr>
<td>60</td>
<td>3,700,000</td>
<td>5,200,000</td>
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bacterial growth. The explanation of the suppressed bacterial growth may be that most of the bacterial population is isolated in serum droplets not much larger than the bacterial cells or clumps of cells. Under these conditions certain nutrients required for growth may be quickly exhausted, or growth may be inhibited by an accumulation of metabolic products in the tiny droplets.

These keeping-quality results are not considered to be novel, for it has been known for many years that fine moisture distribution in butter, resulting from good workmanship, has a bacteriostatic effect on the bacterial population. However, one might not expect a similar situation in this low-fat dairy spread which, instead of having one part of serum dispersed in four parts of fat (as in butter), has about seven parts of serum dispersed in four parts of fat.

This investigation has shown that when the dairy spread contained as little as 30% fat, the desired serum distribution was not achieved and this allowed excessive bacterial growth during storage at 7.2°C. The 35% fat spreads supported considerably less growth, while the 38.8% fat spread, in which the serum was finely dispersed, was most acceptable, as indicated by very little bacterial growth during storage.

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References