A Research Note

Survival of Constitutive Microflora in Commercially Fermented Milk Containing Bifidobacteria During Refrigerated Storage

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ABSTRACT

The survival of constitutive microflora was studied in one batch (n = 50) of fermented milk containing bifidobacteria produced in Spain during storage at 7°C. Levels of Streptococcus salivarius subsp. thermophilus, Lactobacillus delbrueckii subsp. bulgaricus and Bifidobacterium spp. and the pH of the product were determined on the day of collection and after 10, 17, 24, 28, 31, 36, 42, 51, and 84 d of storage. Initial populations of streptococci, lactobacilli, and bifidobacteria were 2.6 x 10⁹, 5.1 x 10⁹, and 7.4 x 10⁹ CFU/g, respectively. The S. salivarius subsp. thermophilus population increased slightly after 10 d and then decreased during further refrigerated storage. Numbers of Bifidobacterium and L. delbrueckii subsp. bulgaricus decreased faster during storage. After 24 d (the reported shelf life of the product), levels of streptococci decreased only 10.7% as compared to decreases of 85.4 and 92.6% for lactobacilli and bifidobacteria, respectively. The pH values were between 4.57 and 3.81.

Fermented milks containing bifidobacteria are produced using either single strains of these organisms alone or in combination with other lactic acid bacteria, mainly Streptococcus and Lactobacillus spp. The latter is more common because slow acidification of milk by bifidobacteria is aided by other lactic acid bacteria (8).

Rasic’ and Kurmann (9) assert that the use of bifidobacteria in cultured milks produces the following nutritional and technological advantages: i) mild sour taste, ii) limited after acidification, iii) less bitterness, iv) beneficial as intestinal organisms, v) formation of physiological L(+)-lactic acid, and vi) nutritional-physiological properties typical of cultured milk.

The beneficial health aspects of bifidobacteria have been studied by Berrada et al. (3), who found differences in the survival of two Bifidobacterium strains contained in two different fermented milks, during gastric transit. For these authors, choosing an acidity-resistant Bifidobacterium strain was important for the manufacture of bifidus fermented milks.

The aim of this study is to determine the shelf life of fermented milk containing bifidobacteria during refrigerated storage.

MATERIAL AND METHODS

Preparation and sampling

Fifty samples from one batch of commercially produced fermented milk were transported to the laboratory in iceboxes maintained at 7°C, and analyzed for constitutive microflora: S. salivarius subsp. thermophilus, L. delbrueckii subsp. bulgaricus, and Bifidobacterium spp. Five samples were tested on the day of collection and the others were analyzed after 10, 17, 24, 28, 31, 36, 42, 51, and 84 d of storage at 7°C.

Samples were aseptically removed from containers and diluted by mixing 10 g with 90 ml of 0.1% Bacto peptone (Difco). Further dilutions were made as required.

Enumeration of S. salivarius subsp. thermophilus

M17 agar as described by Terzaghi and Sandine (13) was used to enumerate S. salivarius subsp. thermophilus. The pH of the medium was 7.0 ± 0.1. The inoculated plates were incubated at 37°C for 48 h. Under these conditions, S. salivarius subsp. thermophilus forms lenticular colonies with a diameter of 1-2 mm (J). The counts were expressed as CFU/g.

Enumeration of L. delbrueckii subsp. bulgaricus

MRS agar (Difco) acidified to pH 5.4 at 25°C was used to enumerate L. delbrueckii subsp. bulgaricus (4). The plates were incubated at 37°C for 3 d in an anaerobic culture jar (H₂/CO₂). Under these conditions, L. delbrueckii subsp. bulgaricus normally forms lenticular star-shaped colonies measuring 1- to 3-mm in diameter (J). The counts were expressed as CFU/g.

Enumeration of Bifidobacteria spp.

A modified Rogosa’s agar (11) containing 0.04 g of oxytetracycline (Fluka Chemie AG, Switzerland), 30 g of sodium propionate (Merck), and 6 g of lithium chloride (Fluka AG) was used to enumerate Bifidobacterium spp. The pH was 7.1 ± 0.1. Bifidobacterium formed colonies measuring 0.5 to 3.0 mm in diameter after anaerobic incubation at 37°C for 5 d (11). The results were expressed in CFU/g.

pH determination

The pH was measured using a digital pH-meter (Crisson, Model 501) with a combination electrode.
RESULTS AND DISCUSSION

Streptococci, lactobacilli, and bifidobacteria were initially present at levels of \(2.6 \times 10^6, 5.1 \times 10^7\), and \(7.4 \times 10^6\) CFU/g, respectively. After 24 d of storage (maximum shelf life for yogurt in Spain [2]), numbers of \(S.\ \text{salivarius}\ \text{subsp.}\ \text{thermophilus}\) decreased only 10.7\%, while populations of \(\text{Bifidobacterium}\ \text{spp.}\) and \(L.\ \text{delbrueckii}\ \text{subsp.}\ \text{bulgaricus}\) decreased 43.3 and 85.4\%, respectively. At the end of the storage, streptococci were present at a level of \(2.2 \times 10^6\) CFU/g (0.8\% of the initial population). Counts on the remaining constitutive microflora were below our detectable limit of 10 CFU/g. The initial pH of 4.57 decreased to 4.25 and 3.81 after 24 and 84 d, respectively (Table 1).

\(S.\ \text{salivarius}\ \text{subsp.}\ \text{thermophilus}\) decreased moderately during storage with the exception of a slight increase at day 10. After 51 and 84 d of storage, viable populations of streptococci decreased 30.5 and 99.2\%, respectively, as compared to initial levels. Populations of \(L.\ \text{delbrueckii}\ \text{subsp.}\ \text{bulgaricus}\) generally decreased during refrigerated storage, particularly between day 10 and 24, when populations decreased 64.5 and 85.4\%, respectively. Only \(7.4 \times 10^6\) viable organisms remained in the product after 24 d of storage, which was 14.6\% of the initial number. From day 42 onward, the counts were <1 \times 10^6 CFU/g. \(\text{Bifidobacterium}\) also had a tendency to decrease, although less sharply than \(L.\ \text{delbrueckii}\ \text{subsp.}\ \text{bulgaricus}\). Although numbers of viable bacteria remained relatively stable between day 17 and 42, the population in 84-d-old product was only 13.5\% of the original level (Table 1).

Hamann and Marth (5) found that the population of viable yogurt organisms increased initially after manufacture reached a maximum and then decreased in the product during refrigerated storage. Numbers of \(L.\ \text{delbrueckii}\ \text{subsp.}\ \text{bulgaricus}\) decreased faster than did those of \(S.\ \text{salivarius}\ \text{subsp.}\ \text{thermophilus}\) in one of three different commercial yogurts tested, while numbers of each yogurt organism decreased similarly in the other two commercial yogurts.

The yogurt organism survival curves were quite different for the three yogurts tested. Manufacturing practices and the various strains of starter cultures employed by the different manufacturers had a major effect on the survival of \(S.\ \text{salivarius}\ \text{subsp.}\ \text{thermophilus}\) and \(L.\ \text{delbrueckii}\ \text{subsp.}\ \text{bulgaricus}\).

Regarding the evolution of constitutive microflora in fermented milks containing bifidobacteria, Smaczny and Reinartz (12) verified that after 30 d of storage at 4°C the streptococci population in Biogarde (a milk product fermented with \(S.\ \text{salivarius}\ \text{subsp.}\ \text{thermophilus}\), \(L.\ \text{acidophilus}\) \text{acidophilus}\) and \(\text{Bifidobacterium}\ \text{bifidum}\) remained at over \(1 \times 10^8\) CFU/g in 80\% of the samples, while 40\% of these samples contained \(1 \times 10^6\) to \(1 \times 10^7\) bifidobacteria CFU/g. In our analysis, numbers of viable \(S.\ \text{salivarius}\ \text{subsp.}\ \text{thermophilus}\) and \(\text{Bifidobacterium}\) were within the range of the values reported by Smaczny and Reinartz (12) until day 51 of storage at 7°C. Furthermore, Hekmat and McMahon (6) detected reductions in \(L.\ \text{acidophilus}\) and \(B.\ \text{bifidum}\) populations of 97.3 and 96\%, respectively, in fermented ice cream after 17 weeks of storage at -29°C.

Survival rates and numbers of viable bifidobacteria in fermented milks during storage vary depending on their use (9). Thus, a large number of viable bacteria is important in a cultured milk for dietetic-therapeutic use, while variations in numbers of such organisms are less important when these products are consumed as a foodstuff.

According to Rasic' and Kurmann (9), the numbers of bifidobacteria will likely decrease 2 logs in cultured milks (pH 4.3-4.7) during 1-2 weeks of storage. After 17 d of storage, the \(\text{Bifidobacterium}\) in our milk decreased from \(7.4 \times 10^6\) CFU/g (pH 4.57) to \(4.5 \times 10^6\) CFU/g (pH 4.33), which represents a reduction of 39.2\%. Although the initial number of bifidobacteria had decreased by 86.5\%, after 51 d of storage, the organism was still \(1 \times 10^8\) CFU/g (pH 4.24). At the end of the storage period, the surviving bifidobacteria population was less than \(1 \times 10^4\) CFU/g (pH 3.81). Kurmann (7) considers pH values of 4.5 and 4.2 to be critical marks for the survival of \(\text{Bifidobacterium longum}\). The possible variations in viable organisms and the pH value of cultured milks would be related to the storage temperature, the initial number of bacteria, the storage time, and the kind of bifidobacteria strain used, among other factors (9).

### Table 1. Number and percentage of surviving constitutive microflora in commercial fermented milk containing bifidobacteria (n = 50) during refrigerated storage (7°C).

<table>
<thead>
<tr>
<th>Storage time (d)</th>
<th>pH</th>
<th>(S.\ \text{salivarius}\ \text{subsp.}\ \text{thermophilus})</th>
<th>(L.\ \text{delbrueckii}\ \text{subsp.}\ \text{bulgaricus})</th>
<th>(\text{Bifidobacterium}) spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.57</td>
<td>262.6(^a) 100(^b)</td>
<td>50.7(^a) 100(^b)</td>
<td>7.4(^a) 100(^b)</td>
</tr>
<tr>
<td>10</td>
<td>4.34</td>
<td>265 100.9</td>
<td>46.2 91.1</td>
<td>5.8 78.3</td>
</tr>
<tr>
<td>17</td>
<td>4.33</td>
<td>241.6 92</td>
<td>18 35.5</td>
<td>4.5 60.8</td>
</tr>
<tr>
<td>24(^c)</td>
<td>4.25</td>
<td>234.6 89.3</td>
<td>7.4 14.6</td>
<td>4.2 56.7</td>
</tr>
<tr>
<td>28</td>
<td>4.28</td>
<td>220 83.7</td>
<td>5.4 10.6</td>
<td>4.5 60.8</td>
</tr>
<tr>
<td>31</td>
<td>4.19</td>
<td>205.7 78.3</td>
<td>3 5.9</td>
<td>4.4 59.4</td>
</tr>
<tr>
<td>36</td>
<td>4.15</td>
<td>196.2 74.7</td>
<td>3.2 6.3</td>
<td>4.6 62.1</td>
</tr>
<tr>
<td>42</td>
<td>4.23</td>
<td>194.6 74.1</td>
<td>0.4 0.7</td>
<td>4 54</td>
</tr>
<tr>
<td>51</td>
<td>4.24</td>
<td>182.6 69.5</td>
<td>0.002 0.0004</td>
<td>1 13.5</td>
</tr>
<tr>
<td>84</td>
<td>3.81</td>
<td>2.2 0.8</td>
<td>&lt;0.00001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

\(^a\) Mean values \(\times 10^6\) expressed in CFU/g from five samples.

\(^b\) Percentage of surviving bacteria.

\(^c\) Maximum shelf life.
Finally, Schuler-Malyoth et al. (10) consider that a good cultured milk should contain $1 \times 10^6$ to $1 \times 10^8$ bifidobacteria per ml. After 51 d of refrigerated storage, our fermented milk still contained a bifidobacteria population of $1 \times 10^6$ CFU/g. Hence, this product is still likely to be of some dietetic-therapeutic benefit when consumed.

REFERENCES