Effectiveness of Soy Milk as Food Carrier for Lactobacillus Acidophilus

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ABSTRACT

Three mild-fermented milk beverages prepared from soy milk and cow’s milk were compared for their ability to preserve the cell viability of Lactobacillus acidophilus during refrigerated storage. In associative growth with Lactobacillus casei and Streptococcus thermophilus. The highest survival rate was obtained by using soy milk as substrate. The presence of L. casei in the starter culture had no influence on the viability of L. acidophilus, while the streptococcal cells showed a harmful effect. The culture activity measured as proteolysis and acid production remained fairly constant during the shelf life, despite the variations in colony counts observed for the different fermented milks analyzed.

In recent years, increased emphasis has been laid on the therapeutic effect of Lactobacillus acidophilus, which plays an important role in the microbial ecology of the intestinal environment (9,12). In relation to this, several kinds of products containing L. acidophilus have been proposed as dietary adjuncts. For many years a cultured acidophilus milk was available, which had an unpleasant taste and was not well-accepted by the consumers. In addition, the cultures rapidly died during refrigerated storage (10). Dugan et al. (6) described the preparation of an “unfermented acidophilus milk” with a high number of viable cells suspended in it, but stressed the need for consuming 950 ml of milk daily to reach the “suggested daily intake” of L. acidophilus. A third type of dietary adjuncts are dried products belonging to the “health food” and the “pharmaceutical groups”. L. acidophilus does not survive drying very well, and there are some doubts about the real number of viable cells that reach the consumers (2). However, little attention was paid to the possibility of using other substrates, e.g., soy milk, as food carrier for this microorganism.

Taking the above considerations into account, we undertook the present study for the purpose of developing a mild fermented acidophilus product from soy milk that might prove useful as a dietary adjunct in lactase-deficient individuals.

MATERIALS AND METHODS

Organisms

Lactobacillus acidophilus sp., L. casei CRL 200, and Streptococcus thermophilus CRL 417 were obtained from the stock culture collection of the Centro de Referencia para Lactobacilos.

Growth conditions

MRS (Oxoid) and LAPTg (11) broth were used for the cultivation of lactobacilli and streptococci, respectively. Cultures were incubated at 37°C for 14 h. The cells were harvested and washed once by centrifugation at 3,000 g for 15 min and resuspended in sterile distilled water to the same initial volume. This cell suspension was used as the inoculum.

Preparation of the milk substrates

Soy milk was prepared as follows: the overnight soaked soybeans were blended with distilled water in a 9:1 ratio of water to beans. The slurry obtained was heat processed for 1 min at 100°C, immediately cooled, and pressed to obtain the soy milk. The standard control was 10% nonfat skim milk. The milk substrates were sterilized at 121°C for 15 min and cooled to 37°C before being used. Each one was inoculated with the 12-h cultures at the rate of 2% (vol/vol) inoculum, comprising 107 cells per ml, as follows: i. L. acidophilus + L. casei + S. thermophilus, ratio 1:1:1 (ACT-Milk); ii. L. acidophilus + L. casei, ratio 1:1 (AC-Milk); and iii. L. acidophilus and L. casei were inoculated separately to both soy milk and cow’s milk and mixed together in a ratio 1:1 after fermentation. This third type of product was called AC2-Milk.

All the inoculated samples were incubated at 37°C for 4 h, chilled in an ice bath, and stored at 5°C during 21 d. Bottles were removed one at a time for assays on days 0, 7, 14, and 21. The assays performed included proteolysis, acid production, and enumeration of viable lactic acid bacteria.

Viability assays

Appropriate dilutions were duplicate pour plated in MRS and LAPTg agar for the enumeration of the lactobacilli and streptococci, respectively. MRS agar without glucose plus 2% melezitose (MRSme) was employed for the selective counting of L. casei. To determine the number of L. acidophilus in the mixed culture, the differences in CFUs on MRS and MRSme were used. Plates were incubated at 37°C for 48 h, after which the resulting colonies were counted.

Titratable acidity

Samples (10 g) withdrawn from the culture flask were titrated to the endpoint (pH 8.0) with 0.1 N NaOH. Results were expressed as percentage of lactic acid produced. The pH of the fermented milks was determined by potentiometric methods.

Proteolytic activity

Proteolytic activity was measured according to the method of Hull (8) and modified by Citti et al. (3). Results obtained were expressed as milligram of tyrosine released per 100 ml trichloroacetic acid filtrate.

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RESULTS AND DISCUSSION

The storage stability of different acidophilus products made from soy milk and cow’s milk (ACT-, AC-, and AC2-Milk) was evaluated. L. casei and S. thermophilus were added to the starter culture in order to improve the flavor of the final product. The survival rate of the lactic acid bacteria during storage at 4°C is shown in Fig. 1-3. During the first day of storage, an increase in the number of the microorganisms was observed. This effect could be due to the fact that the cells continued to grow for a time after being cooled.

A definite decline in the survival rate of L. acidophilus and S. thermophilus was found for ACT-Milk in both substrates (cow’s and soy milks), while L. casei remained fairly constant during the storage period considered (21 d). This latter microorganism showed a similar behavior in the other fermented milks considered in this study.

It is well-known that streptococcus strains have a high sensitivity to pH’s below 6.0. The decreased viability of L. acidophilus, however, cannot be ascribed to a pH effect because of the acidic nature of the microorganism, but rather, to the presence of some substances produced during the associative growth with other bacteria. Indeed, analyses of ACT-Milk with Perid Test (Boehringer & Mannheim) gave positive results for the presence of hydrogen peroxide. This substance may be the main agent responsible for the loss in viability. Our results are in agreement with those reported by Guilliland and Speck (7) who found a marked decrease in the survival rate of L. acidophilus when growing in association with a yogurt culture.

Experiments were conducted to determine the cause of the viability lost in ACT-Milk. When S. thermophilus was removed from the starter culture, the survival rate of L. acidophilus was markedly improved (P < 0.05), as shown in Fig. 2. The viability remained fairly constant in AC-soy milk, while the colony counts dropped steadily in cow’s milk. However, the decrease in survival was not as sharp as that found for ACT-Milk (Fig. 1). These results put in evidence that the streptococcal strain would be responsible for the inhibition observed in ACT-Milk. As regards to L. casei, no changes in viability were observed in either soy milk or cow’s milk, independent of the elaboration procedure employed.

To elucidate if the latter lactobacillus had some effect on the shelf life of L. acidophilus, the milk substrate was separately inoculated with each lactobacillus, incubated as before, and mixed together before storage (AC2-Milk). The behavior of L. acidophilus in AC2-Milk was similar as in AC-Milk (Fig. 2), although the survival rate was lower (1 log unit) in the former product. The difference observed in colony counts, however, was not significant (P > 0.05).

The increase in the survival rate of L. acidophilus when grown in soy milk in relation to the lactobacilli cultured in cow’s milk might be attributed to the presence of certain protective factors present in the former substrate (Fig. 3). If mild or nonfermented milks containing the lactobacillus are to be used as dietary adjuncts, it is important that the microorganism maintains both viability and activity during the shelf life of the product. In this study, proteolysis and acid production were determined as a measure of culture activity. Results obtained with the different fermented milks are presented in Tables 1-3.

Despite the poor viability observed in ACT-Milk (Fig. 1), the microorganisms retained good activity during the

Reproducibility

All results presented are the mean of three replicate assays.

Figure 1. Changes in the microbial flora of ACT-Milk during storage at 4°C. A: soy milk; B: cow’s milk; (●) total lactic flora; (▲) L. acidophilus; (■) L. casei; (#) S. thermophilus.

Figure 2. Changes in the microbial flora of AC-Milk during storage at 4°C. A: soy milk; B: cow’s milk; (●) total lactic flora; (▲) L. acidophilus; (■) L. casei.

Figure 3. Survival of L. acidophilus in soy milk during storage at 4°C. (●) soy milk; (△) cow’s milk.
TABLE 1. Changes in the pH value of fermented milks (ACT, AC, AC2) during storage at 4°C.

<table>
<thead>
<tr>
<th>Storage (d)</th>
<th>ACT A</th>
<th>ACT B</th>
<th>AC A</th>
<th>AC B</th>
<th>AC2 A</th>
<th>AC2 B</th>
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<tbody>
<tr>
<td>1</td>
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<td>6.0</td>
<td>6.2</td>
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<td>5.8</td>
<td>5.6</td>
<td>5.9</td>
<td>5.9</td>
<td>5.6</td>
</tr>
<tr>
<td>14</td>
<td>5.0</td>
<td>5.5</td>
<td>5.4</td>
<td>5.7</td>
<td>5.4</td>
<td>5.6</td>
</tr>
<tr>
<td>21</td>
<td>4.9</td>
<td>5.2</td>
<td>4.9</td>
<td>5.4</td>
<td>5.1</td>
<td>5.5</td>
</tr>
</tbody>
</table>

A: soy milk; B: cow milk.

TABLE 2. Lactic acid production during shelf life of ACT, AC, AC2 Milks.

<table>
<thead>
<tr>
<th>Storage (d)</th>
<th>ACT A</th>
<th>ACT B</th>
<th>AC A</th>
<th>AC B</th>
<th>AC2 A</th>
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<tbody>
<tr>
<td>1</td>
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<td>0.30</td>
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<td>7</td>
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<td>21</td>
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<td>0.35</td>
<td>0.42</td>
<td>0.24</td>
<td>0.36</td>
<td>0.32</td>
</tr>
</tbody>
</table>

A: soy milk; B: cow milk.

TABLE 3. Proteolysis in fermented milks (ACT, AC, AC2) during storage at 4°C.

<table>
<thead>
<tr>
<th>Storage (d)</th>
<th>ACT A</th>
<th>ACT B</th>
<th>AC A</th>
<th>AC B</th>
<th>AC2 A</th>
<th>AC2 B</th>
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</thead>
<tbody>
<tr>
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<td>1.75</td>
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<td>1.14</td>
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<tr>
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<td>2.52</td>
<td>1.78</td>
<td>1.84</td>
<td>1.73</td>
</tr>
</tbody>
</table>

A: soy milk; B: cow milk.

storage period considered (Table 1). For this product, the loss in viability was of a greater magnitude than was the loss of activity, i.e., acid production. These results are in agreement with those reported by other authors, who claim that growth depends upon fermentation, but fermentation can proceed in absence of the former (1,5).

The microorganisms in AC-Milk and AC2-Milk showed a similar pattern of metabolic activity, despite the lower survival rate found for L. acidophilus in the latter product. In light of the results obtained in the present study, it is feasible to use soy milk as substrate to prepare mild-fermented products containing L. acidophilus and L. casei and maintain them under refrigeration for up to 1 month without appreciable loss of viability and activity. The presence of L. casei markedly improved the taste of the fermented soy milk, because of its ability to produce aroma compounds (4).

Since both microorganisms remained viable and active during storage, this kind of soy-milk product could serve as an efficient source of lactase for lactose malabsorbers.

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REFERENCES