Probiotic viability and storage stability of yogurts and fermented milks prepared with several mixtures of lactic acid bacteria

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ABSTRACT

Currently, the food industry wants to expand the range of probiotic yogurts but each probiotic bacteria offers different and specific health benefits. Little information exists on the influence of probiotic strains on physicochemical properties and sensory characteristics of yogurts and fermented milks. Six probiotic yogurts or fermented milks and 1 control yogurt were prepared, and we evaluated several physicochemical properties (pH, titratable acidity, texture, color, and syneresis), microbial viability of starter cultures (Lactobacillus delbrueckii ssp. bulgaricus and Streptococcus thermophilus) and probiotics (Lactobacillus acidophilus, Lactobacillus casei, and Lactobacillus reuteri) during fermentation and storage (35 d at 5°C), as well as sensory preference among them. Decreases in pH (0.17 to 0.50 units) and increases in titratable acidity (0.09 to 0.29%) were observed during storage. Only the yogurt with S. thermophilus, L. delbrueckii ssp. bulgaricus, and L. reuteri differed in firmness. No differences in adhesiveness were determined among the tested yogurts, fermented milks, and the control. Syneresis was in the range of 45 to 58%. No changes in color during storage were observed and no color differences were detected among the evaluated fermented milk products. Counts of S. thermophilus decreased from 1.8 to 3.5 log during storage. Counts of L. delbrueckii ssp. bulgaricus also decreased in probiotic yogurts and varied from 30 to 50% of initial population. Probiotic bacteria also lost viability throughout storage, although the 3 probiotic fermented milks maintained counts ≥10⁷ cfu/mL for 3 wk. Probiotic bacteria had variable viability in yogurts, maintaining counts of L. acidophilus ≥10⁷ cfu/mL for 35 d, of L. casei for 7 d, and of L. reuteri for 14 d. We found no significant sensory preference among the 6 probiotic yogurts and fermented milks or the control. However, the yogurt and fermented milk made with L. casei were better accepted. This study presents relevant information on physicochemical, sensory, and microbial properties of probiotic yogurts and fermented milks, which could guide the dairy industry in developing new probiotic products.

Key words: probiotic yogurt, fermented milk, microbial viability, storage stability

INTRODUCTION

Microbial food cultures have 2 main roles: in food processing and in product development. The first role of microbial food cultures in foods is technological, which refers to their role in food fermentation processes. Starter cultures are regularly used to initiate and control fermentation processes, and no health benefit claims are commonly associated with the presence of these microorganisms in food. The second role of microbial food cultures in foods is functional, which refers to the perceived ability of certain live microbes to impart health benefits to the consumer. Health benefit claims characterize this use and those microorganisms are called “probiotics.” According to the FAO/WHO (2002), probiotics are defined as “live microorganisms, which when administered in adequate amounts confer a health benefit on the host.” Habitually, microorganisms useful for technological transformations are not the same as those needed to impart healthful attributes. However, it is possible that one microbe could serve both purposes or that a single final food product could include microbial cultures of both types.

The main commercial probiotic foods are dairy products, due to the buffering capacity of milk that ensures the survival of probiotics during fermentation and storage (Chandan, 1999). The most widespread dairy probiotic products are yogurts and fermented milks. Yogurt can include both types of microbial food cultures, starters and probiotics, so many different microbial combinations can be used. Yogurt was introduced in the American diet during the 1940s and was well accepted as a good source of calcium. Traditionally, yogurt is manufactured from milk by adding starter cultures; it can be made with skim or low-fat milk, flavorings, sweeteners, and fruit preparations (Katz, 2001). The Codex Alimentarius defines yogurt as milk fermented by mixing cultures of Streptococcus...
thermophilus and any Lactobacillus species (Codex Alimentarius, 2003). Today, it is very common to find in the market yogurt and fermented milks containing Lactobacillus acidophilus, Lactobacillus casei, bifidobacteria, or combinations thereof. The development of the characteristic flavor and texture in yogurt depends on numerous variables, of which the most significant are the type of starter culture and time and temperature of fermentation, which in turn determine the final pH of the product (Tamine and Robinson, 1999). The lack of proteolytic activity in some probiotic bacteria (Klaver et al., 1993) can extend the fermentation time during yogurt production. Some authors report the feasibility of adding probiotics once the product is fermented (Gilliland et al., 2002), but other authors report that postacidification could compromise probiotic viability.

Probiotic bacteria provide health benefits for consumers, while in the product they may develop different patterns of flavor and texture; each mixture of microbial cultures may result in a specific product. The probiotic included in the formulation of a fermented dairy product may or may not affect the development of particular sensory attributes. Probiotic cultures do not tend to modify the sensory attributes of the products to which they are added (Champagne et al., 2005). In some cases, yogurts fermented with L. delbrueckii ssp. bulgaricus can be evaluated by consumers as being too acidic. Therefore, probiotic cultures have been selected to develop preferred flavors, as is the case of known ABT cultures (L. acidophilus, Bifidobacterium, and S. thermophilus; Saarela et al., 2000). However, the most important factors in fermented dairy products containing probiotics are to ensure viability of the probiotics and to determine the sensory changes and physical properties changes that may occur, especially if a mixture of lactic acid bacteria (LAB) is used as starter or probiotic. Few reports have characterized yogurt or fermented milk made with Lactobacillus reuteri or have compared various probiotic products in respect to a wide variety of properties. Most studies focus on specific areas; for example, structural or sensory properties or microbial viability.

The aim of this work was to evaluate viability of starter and probiotic bacteria during fermentation and storage, and evaluate several physicochemical properties and sensory preference of 6 probiotic yogurts or fermented milks, each made with a different combination of LAB.

**MATERIALS AND METHODS**

**Yogurt Preparation**

The starter culture was obtained from a lyophilized commercial mixture (YO-MIX, Danisco, Madison, WI), which contains a mixture of S. thermophilus and L. delbrueckii ssp. bulgaricus. One gram of the powder was poured into 100 mL of de Man, Rogosa and Sharpe (MRS) broth (Difco, Sparks, MD), incubated at 42°C for 24 h. Then, samples were plated on Streptococcus thermophilus (ST) agar (Sigma-Aldrich, St. Louis, MO) to distinguish the 2 strains; yellow colonies corresponded to the typical morphology of S. thermophilus, whereas white colonies correspond to L. delbrueckii ssp. bulgaricus. Isolated strains were cultured again and stored at −18°C until use. Lactobacillus acidophilus NRRL B-4495, L. casei NRRL B-1922, and L. reuteri NRRL B-14171 were provided in lyophilized form by the USDA (Agricultural Research Service, Peoria, IL). These strains were activated and routinely subcultured in MRS broth under anaerobic conditions at 37°C. Seven culture mixtures were prepared to produce the same number of yogurts or fermented milks, as follows: (1) S. thermophilus and L. delbrueckii ssp. bulgaricus; (2) S. thermophilus and L. acidophilus; (3) S. thermophilus and L. casei; (4) S. thermophilus and L. reuteri; (5) S. thermophilus, L. delbrueckii ssp. bulgaricus, and L. acidophilus; (6) S. thermophilus, L. delbrueckii ssp. bulgaricus, and L. casei; and (7) S. thermophilus, L. delbrueckii ssp. bulgaricus and L. reuteri. The 4 lactobacilli were cultivated during 16 h in 100 mL of MRS broth at 37°C; S. thermophilus was grown in 7 flasks containing 100 mL of trypticase soy broth for 16 h at 37°C. Cells were harvested by centrifugation (8,000 × g, 10 min at 4°C), and washed once with sterilized 0.1 M sodium phosphate buffer pH 7.0; the culture biomass was used as inoculum. Each starter culture mixture was inoculated in 2.5 L of pasteurized whole milk (33 g/L of fat, 31 g/L of protein, 5 g/L of vitamin D, and 666 mg/L of retinol equivalents) with 0.1 g of each microorganism according to the mixtures defined above. For instance, the total quantity of microorganisms was 0.2 g for mixtures of 2 microorganisms and 0.3 g for mixtures of 3 microorganisms. The inoculated milk was distributed in 24 portions of 100 mL each in sterile glass bottles and 40 portions of 2 mL each in sterile Eppendorf tubes. Every container was incubated at 42°C until a pH of 4.5 was reached. Then, all containers were cooled to 5°C for 24 h. After this cooling time, the storage time was considered equal to 0 d. During storage (at 5°C) every 7 d until 35 d, 3 bottles of 100 mL and 3 tubes of 2 mL were taken to determine texture, color, syneresis, titratable acidity, pH, and viability of starter bacteria and probiotics. After 21 d of storage, samples were also evaluated for sensory profile. The study was conducted for 35 d to simulate the shelf life of commercial yogurt. The study was conducted in duplicate.
**Determination of Physicochemical Properties**

pH was measured by electrode immersion with a pH meter (UB-10, Denver Instrument, Bohemia, NY). The percentage titratable acidity was determined by titration of 10 g of yogurt or fermented milk with 0.1 N NaOH using phenolphthalein as indicator. Results were expressed as grams of lactic acid per 100 g of fermented milk product. These determinations were performed in triplicate.

A texture analyzer TA.XT2 (Stable Micro Systems, Godalming, UK) was utilized for a penetration test. Two minutes after removing the sample from the refrigerator, the test was carried out directly in a 100-mL sample jar, performed in triplicate. A 60° cone probe (27 mm in diameter, 25 mm in height and weight of 18.144 g) was moved at a test speed of 10 mm/s from the yogurt surface until a distance of 35 mm within the sample was reached; this test quantifies the gel strength (firmness) by the positive area of the graph of force (N) versus time (s), which indicates the strength to break it. At the same time, the test allowed us to determine product adhesiveness by the negative area of the curve obtained when the cone returns to its original position.

To determine syneresis, 3 tubes with 2 mL of yogurt or fermented milk (for each of the studied formulations) were weighed and placed in a centrifuge (Denver Instrument Co.). Tubes were centrifuged at 2,000 × g for 5 min and the separated serum was then weighed. Syneresis was calculated using the following equation:

\[ \text{Syneresis} \, (\%) = \frac{(W_s)(100)}{W_y}, \]

where \( W_s \) was the supernatant weight after centrifugation and \( W_y \) was the weight of the yogurt or fermented milk in the tube.

Color of yogurts and fermented milks was measured in 20-mL samples in triplicate, using the Color System 05 (BYK-Gardner Inc., Columbia, MD), which was calibrated to measure reflectance, using the CIELAB scale for measuring the parameters lightness (\( L^* \)), red-green color (\( a^* \)), and yellow-blue color (\( b^* \)). The difference in brightness (\( \Delta L^* \)), chrome (\( \Delta C_{ab}^* \)), hue (\( \Delta h_{ab}^* \)), and their respective directions (\( S_L \), \( S_C \), and \( S_H \)) define the lengths of the semi-axes of the tolerance ellipsoid at the position of the standard in the CIELAB space in each of the 3 directions (\( S_L \) = lightness, \( S_C \) = chrome, and \( S_H \) = hue; Kim et al., 2011). The terms \( S_L \), \( S_C \), \( S_H \), and the perceptibility of color differences (\( \Delta E \)) were calculated according to the equations reported by Marcus (1998). The terms \( S_L \), \( S_C \), and \( S_H \) and the perceptibility of color differences (\( \Delta E \)) also were calculated with the equations described by Marcus (1998). It includes 2 parameters: lightness weighting (l) and chrome weighting (c), allowing us to weight the difference based on the ratio of l to c:

\[ \Delta E = \left( \frac{\Delta L^*}{L^*_0} \right)^2 + \left( \frac{\Delta C_{ab}^*}{c^*_0} \right)^2 + \left( \frac{\Delta h_{ab}^*}{h^*_0} \right)^2 \],

where \( l \) takes the value of 2.0 and \( c \) the value of 1.0, values suggested for several products in the most common industries. A perceptibility of color differences was detected if \( \Delta E > 1.57 \) (Marcus, 1998).

Two commercial brands of natural yogurts (CY1 and CY2) were also analyzed (pH, titratable acidity, firmness, adhesiveness, syneresis, and microbial viability of starter cultures) in the first week of the study to compare them with studied yogurts and fermented milks.

**Determination of Microbial Viability**

One milliliter of yogurt or fermented milk was diluted in 9 mL of sterile peptone (Merck, Darmstadt, Germany) water (0.1%), and appropriate dilutions were plated on ST agar for \( S. \) thermophillus counts (Dave and Shah, 1996); on MRS agar (Difco BD, Sparks, MD) for enumeration of total lactobacilli; on maltose agar [composition: 10 g of tryptone, 10 g of meat extract, 5 g of yeast extract, 1 g of Tween 80, 2.6 g of \( K_2 \)HPO4, 5 g of sodium acetate, 2 g of triammonium citrate, 0.2 g of MgSO4·7H2O, 0.05 g of MnSO4·4H2O, 12 g of bacteriological agar, and 1 L of distilled water; after sterilization at 121°C for 15 min, 10 mL of filter-sterilized 20% maltose was added to 90 mL of basal agar (2% final concentration) just before pouring the agar medium] for \( L. \) acidophilus counts (Thammaraj and Shah,2003); on \( Lactobacillus \) casei (LC) agar (composition: 10 g of bacteriological peptone, 1 g of yeast extract, 4 g of meat extract, 2 g of KH2PO4, 3 g of sodium acetate, 1 g of triammonium citrate, 0.2 g of MgSO4·7H2O, 0.05 g of MnSO4·4H2O, 1 g of acid casein hydrolysate, 1 g of Tween 80, 12 g of bacteriological agar, and 1 L of distilled water) for \( L. \) casei counts (Ravula and Shah, 1998); and on MRS agar with 0.1% bile salts for \( L. \) reuteri counts. Ingredients used for agar formulation were of reagent or microbiological grade. Inoculated plates were incubated aerobically at 37°C for 24 ± 3 h for ST agar and anaerobically for 72 h for MRS, maltose, and 0.1% bile salts agar. Inoculated plates with LC agar were incubated aerobically at 27°C for 7 d. The counts for \( L. \) delbrueckii ssp. bulgaricus in probiotic yogurts were made by subtracting, from the MRS agar total count, the number of colony-forming units of probiotics obtained from selective media. All bacterial counts were conducted in triplicate.
**Statistical Analysis**

Statistical analysis of the data was performed by ANOVA and Tukey’s mean comparison tests ($P \leq 0.05$) using the Minitab statistical package (version 16; Minitab Inc., State College, PA) to identify significant differences between the evaluated responses.

**Determination of Sensory Characteristics**

An incomplete balanced paired comparison design (IBPCD) was utilized to determine yogurt and fermented milk preference. This comparison design was selected because a large number of equally important samples needed to be analyzed. Scheffé (1952) developed a general model for studies of consumer preference studies (Gacula and Singh, 1984). The IBPCD can compare treatments or products ($n = 7$) by a preference test adapted from the Scheffé model with a 4-point modified scale ($1 = $ slightly prefer B to C, $2 = $ moderately prefer B to C, $3 = $ greatly prefer B to C, and $0 = $ for no preference). The IBPCD parameters were as follows: $k = 2$ (number of judges that compared each pair), $r = 2$ (pairs compared by each judge), $b^* = 42$ (number of comparisons), $\lambda^* = 2$ (pairs that are compared by any 2 judges); tt required 21 untrained panelists ($b = 21$). Analysis of variance was performed in Minitab 16 and estimated differences were evaluated with the general lineal model procedure using $P = 0.05$.

**RESULTS AND DISCUSSION**

**Fermentation Times During Yogurt or Fermented Milk Preparation**

Table 1 presents fermentation times (to reach pH 4.5) for the prepared yogurts and fermented milks (initial pH of milk was 6.74). Probiotic yogurt fermented with $S$. thermophilus-$L$. delbrueckii ssp. bulgaricus-$L$. acidophilus required the shortest fermentation time, followed by the control yogurt ($S$. thermophilus-$L$. delbrueckii ssp. bulgaricus), and the $S$. thermophilus-$L$. acidophilus fermented milk product. Mixtures of $S$. thermophilus-$L$. delbrueckii ssp. bulgaricus-$L$. casei and $S$. thermophilus-$L$. delbrueckii ssp. bulgaricus-$L$. reuteri had intermediate times between the control yogurt and their corresponding fermented milks ($S$. thermophilus-$L$. casei and $S$. thermophilus-$L$. reuteri). Long fermentation times (14 and 12 h, respectively) were observed in the mixtures $S$. thermophilus-$L$. delbrueckii ssp. bulgaricus-$L$. casei and $S$. thermophilus-$L$. delbrueckii ssp. bulgaricus-$L$. reuteri, which could be due to the possible inhibition of the starter cultures in the presence of probiotic bacteria. Vinderola et al. (2002) reported that probiotic bacteria delay the growth of starter cultures. They observed that $L$. casei slows the growth of $S$. thermophilus and $L$. delbrueckii ssp. bulgaricus in milk. A similar behavior could occur with $L$. reuteri, but very few papers report its growth in milk and its interaction with starter cultures. Products with $S$. thermophilus-$L$. casei and $S$. thermophilus-$L$. reuteri required more than 14 h to reach the desired pH. Variability in fermentation time could be due to differences in the ability of lactic acid bacteria to grow and fermenting milk. Similar results were reported by Dave and Shah (1997a, 1998). They made yogurts with $S$. thermophilus-$L$. delbrueckii ssp. bulgaricus-$L$. acidophilus-bifidobacteria and $S$. thermophilus-$L$. acidophilus-bifidobacteria; these culture mixtures needed 5 and 12 h, to reach pH of 4.5, respectively. Sodini et al. (2002) reported a range of fermentation times from 5.1 to 13.2 h for a mix of $S$. thermophilus-$L$. acidophilus LA5 in different milk bases. The addition of $L$. delbrueckii ssp. bulgaricus in probiotic yogurts reduced about 46% the fermentation time (Sodini et al., 2002). Other researchers have also reported that probiotic bacteria have a poor acidification performance in milk compared with common yogurt starter cultures (Donkor et al., 2007; Damin et al., 2008). Fermented milks that did not include $L$. delbrueckii ssp. bulgaricus had lesser pH changes during storage; milks with $L$. acidophilus, $L$. casei, and $L$. reuteri being those that presented less postacidification.

**pH and Titratable Acidity in Fermented Products During Storage**

pH values of yogurts and fermented milks decreased during storage due to postacidification. Yogurts that included $L$. delbrueckii ssp. bulgaricus presented the highest acidity values. Figure 1 shows the tendency of yogurt pH to decrease during storage. Declining pH can be attributed to the residual activity of microorganisms. Significant differences ($P < 0.05$) in the pH of yogurts during storage were detected. Dave and Shah (1997a) obtained pH values of 4.16 and 4.40 after 35 d of storage (5°C) in probiotic yogurts, when the initial pH values were 4.33 and 4.61, respectively. Gilliland et al. (2002) obtained pH values of 4.1 and 4.2 at the end of 35 d of refrigerated (5°C) storage of yogurt-type products fermented with $S$. thermophilus-$L$. acidophilus-bifidobacteria or $L$. casei; their initial pH values were 4.7 and 4.8, respectively. Gueimonde et al. (2004) analyzed 14 commercial fermented milks and observed pH values around 3.9 to 4.2. Our results are similar to these reports, corroborating the residual acidification during storage. Postacidification depends on the LAB used for yogurt fermentation. Figure 2 represents the trend in product acidity during storage. Greater increases were
found for yogurts that included *L. delbrueckii* ssp. *bulgaricus*. Initial values varied from 0.72 to 0.74% and after 35 d of storage from 0.82 to 1.04%. Acidity in fermented milks without *L. delbrueckii* ssp. *bulgaricus* varied from 0.67 to 0.79% and from 0.74 to 0.83% at the end of the storage. Acidity was significantly different (*P* < 0.05) during storage for those yogurts that included *L. delbrueckii* ssp. *bulgaricus* in their fermentation. Similar to pH changes, observed acidity changes could be attributed to the residual activity of the LAB. The ANOVA demonstrated that no major (*P* > 0.05) differences in acidity occurred in yogurts without *L. delbrueckii* ssp. *bulgaricus*. Commercial yogurts (Table 2) had acidity values of 0.74 and 0.88%, respectively. Only one commercial yogurt fulfilled the requirements of the Mexican Ministry of Health that establishes a range of 0.85 to 1.8% for titratable acidity. Observed acidity values were similar to those reported for 14 commercial fermented milks that varied from 0.79 to 1.16% (Gueimonde et al., 2004). Dave and Shah (1997a) noted initial acidity in its probiotic yogurts and fermented milk of 0.68 and 0.77%, respectively; they also observed increments after 5 d at 4°C to values of 0.82 to 0.84%, and constant values were observed (30 d) thereafter. Donkor et al. (2007) observed production of acetic and lactic acids in milk fermented by *L. acidophilus* and *L. casei* associated with yogurt bacteria during 28 d of cold storage. Korbekandi et al. (2008) reported similar results in yogurts with *L. casei*.

### Firmness and Adhesiveness in Fermented Products During Refrigerated Storage

Different trends for firmness were observed for the probiotic yogurts and fermented milks (Table 3). Yogurts containing *L. delbrueckii* ssp. *bulgaricus* and *L. reuteri* or *L. acidophilus* increased in firmness during 35 d of storage. Yogurt with *L. casei*—*S. thermophillus*—*L. delbrueckii* ssp. *bulgaricus* and control yogurt (*S. thermophillus* and *L. delbrueckii* ssp. *bulgaricus*) did not present a clear tendency regarding firmness. The values for 2 commercial yogurts (Table 2) were smaller than those for tested yogurts with *L. delbrueckii* ssp. *bulgaricus*. Firmness of fermented dairy products was significantly different (*P* < 0.05) during storage. This parameter was important for sensory evaluation: firmness values around 5.88 N were considered to be “creamy” compared with a yogurt with higher firmness (8.34 N, yogurt made with *L. reuteri*). A firmness value around 55.20 N was perceived as a soft gel. These values (5.20 and 5.88 N) coincide with those obtained in commercial yogurts (Table 2). Therefore, it is possible to make probiotic yogurts or fermented milks with firmness similar to that of commercial products (which are well accepted by consumers). Akalin et al. (2012) reported lower values for firmness (1.76–2.16 N) than those observed in this work, although they evaluated

<table>
<thead>
<tr>
<th>Microorganism mixture</th>
<th>Fermentation time (h)</th>
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<tbody>
<tr>
<td><em>Streptococcus thermophilus</em> + <em>Lactobacillus bulgaricus</em></td>
<td>7 ± 0.08</td>
</tr>
<tr>
<td><em>S. thermophilus</em> + <em>Lactobacillus acidophilus</em></td>
<td>8 ± 0.06</td>
</tr>
<tr>
<td><em>S. thermophilus</em> + <em>Lactobacillus casei</em></td>
<td>15 ± 0.11</td>
</tr>
<tr>
<td><em>S. thermophilus</em> + <em>L. bulgaricus</em> + <em>L. acidophilus</em></td>
<td>14.5 ± 0.10</td>
</tr>
<tr>
<td><em>S. thermophilus</em> + <em>L. bulgaricus</em> + <em>L. casei</em></td>
<td>6.5 ± 0.09</td>
</tr>
<tr>
<td><em>S. thermophilus</em> + <em>L. bulgaricus</em> + <em>L. reuteri</em></td>
<td>12 ± 0.10</td>
</tr>
</tbody>
</table>

**Figure 1.** pH changes during storage (5°C) of probiotic yogurts and fermented milks: ○ *Streptococcus thermophilus*—*Lactobacillus delbrueckii* ssp. *bulgaricus*; □ *S. thermophilus*—*L. delbrueckii* ssp. *bulgaricus*—*L. acidophilus*; ◊ *S. thermophilus*—*L. delbrueckii* ssp. *bulgaricus*—*L. casei*; ▲ *S. thermophilus*—*L. delbrueckii* ssp. *bulgaricus*—*L. reuteri*; ● *S. thermophilus*—*L. acidophilus*; ▼ *S. thermophilus*—*L. casei*; and ♦ *S. thermophilus*—*L. reuteri*.  

probiotic yogurts with added nonfat milk solids. Damin et al. (2008) reported firmness values of 0.64 to 0.93 N for yogurts prepared with *S. thermophilus-L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus-L. acidophilus*, values lower than those determined in the present study. González-Martínez et al. (2002) reported that yogurt supplemented with whey protein has more elasticity and viscosity, thereby increasing its gel strength (firmness). The differences can be attributed to the type of strains used.

Table 4 presents adhesiveness of the studied products; yogurts with *L. delbrueckii* ssp. *bulgaricus* and *L. reuteri* or *L. casei* increased adhesiveness during storage, and adhesiveness was significantly different between products during storage. Adhesiveness is strongly linked to firmness. In the current study, larger firmness values were generally associated with low adhesion values (Tables 3 and 4). Consumers prefer fermented dairy products with adhesiveness values of −0.024 to −0.049 N·s, and they describe them as soft. Gels with adhesiveness of −0.073 to −0.147 N·s are perceived as consistent but less acceptable. Furthermore, the adhesiveness of commercial yogurts was −0.070 and −0.196 N·s (Table 2). Magenis et al. (2006) reported similar adhesiveness, −0.053 and −0.127 N·s, for 2 yogurts made from a mixture of milk and whey retentate. Hilali et al. (2011) observed similar adhesiveness values for sheep milk yogurt.

<table>
<thead>
<tr>
<th>Test</th>
<th>Commercial yogurt 1</th>
<th>Commercial yogurt 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>4.44 ± 0.01</td>
<td>4.48 ± 0.03</td>
</tr>
<tr>
<td>Titratable acidity (%)</td>
<td>0.88 ± 0.023</td>
<td>0.74 ± 0.011</td>
</tr>
<tr>
<td>Firmness (N)</td>
<td>5.34 ± 0.12</td>
<td>5.79 ± 0.17</td>
</tr>
<tr>
<td>Adhesiveness (N·s)</td>
<td>−0.070 ± 0.009</td>
<td>−0.195 ± 0.008</td>
</tr>
<tr>
<td>Syneresis (%)</td>
<td>32.65 ± 1.63</td>
<td>34.62 ± 0.94</td>
</tr>
<tr>
<td><em>Lactobacillus delbrueckii</em> ssp. <em>bulgaricus</em> (log_{10} cfu/mL)</td>
<td>8.10 ± 0.28</td>
<td>8.10 ± 0.19</td>
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</table>

**Table 2.** Physicochemical and microbial characterization (means ± SD) of 2 commercial natural yogurts

**Syneresis**

Syneresis is the separation of the liquid phase from the gel and it is an undesirable feature in yogurt. Whey loss measures the level of collapsed gel and is an indicator for poor quality and stability. Syneresis may be spontaneous or may occur only when the gel is mechanically disrupted by cutting, agitating, or being subjected to a centrifugal force. Furthermore, several adjuvants, solids, and stabilizers were added to milk before fermentation took place to diminish syneresis. Figure 3 presents the trends for syneresis in the studied yogurts and fermented milks. Commercial yogurts (Table 2) presented less syneresis than probiotic yogurts. Probiotic yogurts had values of syneresis >40%, which may be due to the absence of additional solids. The presence of nonfat milk solids (whey proteins) increases the interaction between the proteins that form the 3-dimensional network of the gel, thereby increasing the compactness of its microstructure and reducing syneresis. Moreover, probiotic bacteria grow slowly in milk because of the lack of proteolytic enzymes; the latter contribute poor textural characteristics to the product, including syneresis (Dave and Shah, 1998). Amatayakul et al. (2006) reported syneresis values >45% in yogurts with 9 and 14% total solids and fermented with exopolysaccharide-producing starter cultures. González-Martínez et al. (2002) reported syneresis ranges from 23 to 36 ± 5%.

**Table 3.** Perceptibility of color differences (dimensionless) of yogurts and fermented milks during storage at 5°C (all values were <1.57, no differences were detected)

<table>
<thead>
<tr>
<th>Culture mixture1</th>
<th>Time (d)</th>
<th>St-Lb</th>
<th>St-Lb-La</th>
<th>St-Lb-Lc</th>
<th>St-Lb-Lr</th>
<th>St-La</th>
<th>St-Lc</th>
<th>St-Lr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>0.13</td>
<td>0.13</td>
<td>0.28</td>
<td>0.33</td>
<td>0.34</td>
<td>0.11</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>0.03</td>
<td>0.39</td>
<td>0.53</td>
<td>0.34</td>
<td>0.04</td>
<td>0.11</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>0.08</td>
<td>0.20</td>
<td>0.25</td>
<td>0.28</td>
<td>0.07</td>
<td>0.13</td>
<td>0.13</td>
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<tr>
<td></td>
<td>21</td>
<td>0.17</td>
<td>0.08</td>
<td>0.37</td>
<td>0.22</td>
<td>0.03</td>
<td>0.10</td>
<td>0.08</td>
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<tr>
<td></td>
<td>28</td>
<td>0.08</td>
<td>0.06</td>
<td>0.15</td>
<td>0.14</td>
<td>0.04</td>
<td>0.12</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>0.21</td>
<td>0.13</td>
<td>0.31</td>
<td>0.36</td>
<td>0.13</td>
<td>0.10</td>
<td>0.10</td>
</tr>
</tbody>
</table>

1St-Lb = *Streptococcus thermophilus* + *Lactobacillus bulgaricus*; St-La = *S. thermophilus* + *Lactobacillus acidophilus*; St-Lc = *S. thermophilus* + *Lactobacillus casei*; St-Lr = *S. thermophilus* + *Lactobacillus reuteri*; St-Lb-La = *S. thermophilus* + *Lactobacillus bulgaricus* + *L. acidophilus*; St-Lb-Lc = *S. thermophilus* + *L. bulgaricus* + *L. casei*; St-Lb-Lr = *S. thermophilus* + *L. bulgaricus* + *L. reuteri.*
in yogurts supplemented with whey protein. Less syneresis was observed during storage (6–10%) for yogurts fermented with *L. delbrueckii* ssp. *bulgaricus*. After 14 d of storage, no significant differences (*P* > 0.05) were observed among yogurts or fermented milks prepared with *S. thermophilus*—*L. delbrueckii* ssp. *bulgaricus*, *S. thermophilus*—*L. delbrueckii* ssp. *bulgaricus*—*Lactobacillus acidophilus*, *S. thermophilus*—*L. delbrueckii* ssp. *bulgaricus*—*Lactobacillus casei*, *S. thermophilus*—*L. acidophilus*, or with *S. thermophilus*—*L. reuteri*. Bakirci and Kavaz (2008) reported lower syneresis values for in a sweet banana yogurt (16–21.4%) due to banana fiber.

**Color**

Color is an important attribute in food; it is the first characteristic perceived by the consumers and thus often influences the consumer’s preference. Few reports describe the color of fermented dairy products. An objective report is necessary to clarify whether color differences could be present during fermentation with different starter strains and probiotics. The color parameters L*, a*, and b* of yogurts and fermented milks remained almost constant during storage. Luminosity values ranged from 83.12 to 84.58 for tested products. Hue (h*) values varied from −2.19 to −2.73, and b* ranged from 6.87 to 8.04. Calculations of the perceptibility of color differences (ΔE) are presented in Table 5; all values were <1.57 (reference value), so no perceptible differences existed in the color of studied products. Color differences were expected because each combination of strains could generate a unique profile of color, texture, and flavor. Because no differences were observed, studied yogurts and fermented milks were equally accepted at first view.

**Microbial Viability of Starter Cultures During Storage**

Figures 4 and 5 present *S. thermophilus* counts for tested fermented products during storage. Initial inocula were log$_{10}$ 7.69 ± 0.26 cfu/mL; at the end of the fermentation (at pH = 4.5), counts varied from log$_{10}$ 9.48 ± 0.14 cfu/mL to log$_{10}$ 10.34 ± 0.36 cfu/mL for tested fermented products. Constant counts were observed after 14 d of storage; therefore, the presence of probiotic lactic acid bacteria did not suppress *S. thermophilus* viability. Dave and Shah (1997a) reported increases of 15 to 20% in *S. thermophilus* during storage of probiotic yogurts. On the other hand, after 21 d, *S. thermophilus* counts declined from 7 to 33%, but counts were still >10$^{7}$ cfu/mL at the end of the storage. Dave and Shah (1997b) observed decreasing counts of *S. thermophilus* from 50 to 70% after 35 d of storage in probiotic yogurts. Gueimonde et al. (2004), in their study of 14 commercial fermented milks, reported counts from 10$^{7}$ to 10$^{9}$ cfu/mL after 30 d of storage.

**Figure 2.** Titratable acidity changes during storage (5°C) of probiotic yogurts and fermented milks: ○ *Streptococcus thermophilus*—*Lactobacillus delbrueckii* ssp. *bulgaricus*; □ *S. thermophilus*—*L. delbrueckii* ssp. *bulgaricus*—*Lactobacillus acidophilus*; ◊ *S. thermophilus*—*L. delbrueckii* ssp. *bulgaricus*—*Lactobacillus casei*; ▲ *S. thermophilus*—*L. delbrueckii* ssp. *bulgaricus*—*Lactobacillus reuteri*; ● *S. thermophilus*—*L. acidophilus*; ▼ *S. thermophilus*—*L. casei*; and ♦ *S. thermophilus*—*L. reuteri*.

**Figure 3.** Syneresis changes during storage (5°C) of probiotic yogurts and fermented milks: ○ *Streptococcus thermophilus*—*Lactobacillus delbrueckii* ssp. *bulgaricus*; □ *S. thermophilus*—*L. delbrueckii* ssp. *bulgaricus*—*Lactobacillus acidophilus*; ◊ *S. thermophilus*—*L. delbrueckii* ssp. *bulgaricus*—*Lactobacillus casei*; ▲ *S. thermophilus*—*L. delbrueckii* ssp. *bulgaricus*—*Lactobacillus reuteri*; ● *S. thermophilus*—*L. acidophilus*; ▼ *S. thermophilus*—*L. casei*; and ♦ *S. thermophilus*—*L. reuteri*. 
**Table 4.** Firmness (N; means ± SD) of yogurts and fermented milks during storage at 5°C

<table>
<thead>
<tr>
<th>Time (d)</th>
<th>Culture mixture¹</th>
<th>St-Lb</th>
<th>St-Lb-La</th>
<th>St-Lb-Lc</th>
<th>St-Lb-Lr</th>
<th>St-La</th>
<th>St-Lc</th>
<th>St-Lr</th>
</tr>
</thead>
<tbody>
<tr>
<td>End of fermentation</td>
<td>St-Lb = S. thermophilus + L. bulgaricus; St-La = S. thermophilus + L. acidophilus; St-Lc = S. thermophilus + L. casei; St-Lr = S. thermophilus + L. reuteri; St-Lb-La = S. thermophilus + L. bulgaricus + L. acidophilus; St-Lb-Lc = S. thermophilus + L. bulgaricus + L. casei; St-Lb-Lr = S. thermophilus + L. bulgaricus + L. reuteri.</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>4.08 ± 0.24⁴</td>
<td>3.37 ± 0.25⁵</td>
<td>2.73 ± 0.22⁶</td>
<td>2.63 ± 0.18⁷</td>
<td>4.29 ± 0.14⁸</td>
<td>3.59 ± 0.25⁹</td>
<td>3.06 ± 0.26⁹</td>
<td></td>
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</tr>
<tr>
<td>7.53 ± 0.20²⁴</td>
<td>4.63 ± 0.20²⁵</td>
<td>5.22 ± 0.25²⁶</td>
<td>6.20 ± 0.15²⁷</td>
<td>4.74 ± 0.08²⁸</td>
<td>6.59 ± 0.22²⁹</td>
<td>5.98 ± 0.15²⁹</td>
<td></td>
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</tr>
<tr>
<td>6.75 ± 0.07³⁴</td>
<td>5.51 ± 0.16³⁵</td>
<td>6.58 ± 0.15³⁶</td>
<td>6.95 ± 0.25³⁷</td>
<td>5.00 ± 0.20³⁸</td>
<td>6.50 ± 0.22³⁹</td>
<td>6.59 ± 0.21³⁹</td>
<td></td>
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<tr>
<td>6.72 ± 0.15³⁴</td>
<td>5.97 ± 0.14³⁵</td>
<td>5.19 ± 0.16³⁶</td>
<td>8.12 ± 0.17³⁷</td>
<td>4.88 ± 0.10³⁸</td>
<td>5.98 ± 0.28³⁹</td>
<td>6.37 ± 0.20³⁹</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹Means within a column with different superscripts were significantly different (P < 0.05).
²Means within a row with different superscripts were significantly different (P < 0.05).

**Table 5.** Adhesiveness (N·s; means ± SD) on yogurts and fermented milks during storage at 5°C

<table>
<thead>
<tr>
<th>Time (d)</th>
<th>Culture mixture¹</th>
<th>St-Lb</th>
<th>St-Lb-La</th>
<th>St-Lb-Lc</th>
<th>St-Lb-Lr</th>
<th>St-La</th>
<th>St-Lc</th>
<th>St-Lr</th>
</tr>
</thead>
<tbody>
<tr>
<td>End of fermentation</td>
<td>St-Lb = S. thermophilus + L. bulgaricus; St-La = S. thermophilus + L. acidophilus; St-Lc = S. thermophilus + L. casei; St-Lr = S. thermophilus + L. reuteri; St-Lb-La = S. thermophilus + L. bulgaricus + L. acidophilus; St-Lb-Lc = S. thermophilus + L. bulgaricus + L. casei; St-Lb-Lr = S. thermophilus + L. bulgaricus + L. reuteri.</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>−0.003 ± 0.0002¹⁴</td>
<td>0.001 ± 0.0004¹⁵</td>
<td>−0.002 ± 0.0001¹⁶</td>
<td>0.015 ± 0.0006¹⁷</td>
<td>−0.003 ± 0.0005¹⁸</td>
<td>−0.009 ± 0.0011¹⁹</td>
<td>−0.001 ± 0.0000¹²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>−0.094 ± 0.0024¹⁴</td>
<td>−0.001 ± 0.0006¹⁵</td>
<td>0.012 ± 0.0017¹⁶</td>
<td>−0.025 ± 0.0017¹⁷</td>
<td>−0.0003 ± 0.0015¹⁸</td>
<td>−0.030 ± 0.0005¹⁹</td>
<td>−0.019 ± 0.0000¹²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>−0.048 ± 0.0031¹⁴</td>
<td>−0.042 ± 0.0037¹⁵</td>
<td>−0.015 ± 0.0019¹⁶</td>
<td>−0.070 ± 0.0020¹⁷</td>
<td>−0.0005 ± 0.0014¹⁸</td>
<td>−0.062 ± 0.0024¹⁹</td>
<td>−0.043 ± 0.0003¹²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>−0.097 ± 0.0043¹⁴</td>
<td>−0.021 ± 0.0015¹⁵</td>
<td>−0.049 ± 0.0025¹⁶</td>
<td>−0.112 ± 0.0019¹⁷</td>
<td>−0.002 ± 0.0005¹⁸</td>
<td>−0.060 ± 0.0002¹⁹</td>
<td>−0.042 ± 0.0005¹²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>−0.083 ± 0.0027¹⁴</td>
<td>−0.001 ± 0.0003¹⁵</td>
<td>−0.078 ± 0.0036¹⁶</td>
<td>−0.080 ± 0.0021¹⁷</td>
<td>−0.0003 ± 0.0008¹⁸</td>
<td>−0.058 ± 0.0010¹⁹</td>
<td>−0.057 ± 0.0001¹²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>−0.131 ± 0.0058¹⁴</td>
<td>−0.054 ± 0.0030¹⁵</td>
<td>−0.054 ± 0.0017¹⁶</td>
<td>−0.089 ± 0.0010¹⁷</td>
<td>−0.008 ± 0.0008¹⁸</td>
<td>−0.047 ± 0.0003¹⁹</td>
<td>−0.039 ± 0.0022¹²</td>
<td></td>
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</tr>
<tr>
<td>−0.074 ± 0.0016¹⁴</td>
<td>−0.025 ± 0.0028¹⁵</td>
<td>−0.052 ± 0.0026¹⁶</td>
<td>−0.147 ± 0.0036¹⁷</td>
<td>−0.0020 ± 0.0010³⁸</td>
<td>−0.056 ± 0.0020³⁹</td>
<td>−0.049 ± 0.0006³⁰</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹Means within a column with different superscripts were significantly different (P < 0.05).
²Means within a row with different superscripts were significantly different (P < 0.05).
Counts from the current study were very similar to or higher than those reported by Gueimonde et al. (2004). Minor decreases were observed in our case due to the type of strain used. Counts of the commercial yogurts (Table 2) had values similar to those of tested products.

*Lactobacillus delbrueckii* ssp. *bulgaricus* initial counts varied from $3 \times 10^6$ to $2.5 \times 10^7$ cfu/mL; the fermentation process increased culture counts by 2 to 3.2 log and the differences are probably due to the initial inoculum and the fermentation time required for each tested yogurt. Yogurt with *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* maintained counts of this starter culture. Otherwise, *L. delbrueckii* ssp. *bulgaricus* decreased about 30 to 50% in probiotic yogurts (Figure 4). Dave and Shah (1997a) reported declines of almost

![Figure 4](image_url)

*Figure 4.* Viability (log cfu/mL) of *Streptococcus thermophilus* (gray), *Lactobacillus delbrueckii* ssp. *bulgaricus* (black), and probiotic bacteria (white) *Lactobacillus acidophilus* (panel a), *Lactobacillus casei* (panel b), and *Lactobacillus reuteri* (panel c), or no probiotic (panel d) on yogurts during storage at 5°C. For the same microorganism, different letters or numbers indicate significant differences ($P < 0.05$).
50% of the population after 5 d of storage, and declines in probiotic yogurts continued during the 35 d of storage. Moreover, those authors, in other studies, reported counts below 10⁵ cfu/mL for \textit{L. delbrueckii} ssp. \textit{bulgaricus} in probiotic yogurts stored for 35 d (Dave and Shah, 1997b,c). In the current work, the probiotic yogurt that included \textit{S. thermophilus}, \textit{L. delbrueckii} ssp. \textit{bulgaricus}, and \textit{L. reuteri} had counts <10⁵ cfu/mL. It is common to observe decreasing counts of \textit{L. delbrueckii} ssp. \textit{bulgaricus} in probiotic yogurts. This may be due to the secretion of inhibition metabolites (e.g., bacteriocins or reuterin) produced by probiotics that may affect species of the same genus. Viable counts for the commercial yogurts tested (Table 2) were 10⁸ cfu/mL for \textit{L. delbrueckii} ssp. \textit{bulgaricus}; these values were similar to the values obtained in our experimental yogurts.

\textbf{Figure 5.} Viability (log cfu/mL) of \textit{Streptococcus thermophilus} (solid) and probiotic bacteria (striped) \textit{Lactobacillus acidophilus} (panel a), \textit{Lactobacillus casei} (panel b), and \textit{Lactobacillus reuteri} (panel c) on fermented milks during storage at 5°C. For the same microorganism, different letters indicate significant differences ($P < 0.05$).
Microbial Viability of Probiotics During Storage

Figures 4 and 5 present changes in probiotic counts during fermentation and storage of probiotic yogurts and fermented milks. Inocula counts varied from $1 \times 10^6$ to $3.3 \times 10^5$ cfu/mL. After fermentation, counts reached values that varied from $2 \times 10^5$ to $2 \times 10^6$ cfu/mL for the probiotic strains. Five of 6 probiotic bacteria reached populations $>10^6$ cfu/mL (the count was $<10^5$ cfu/mL only in the yogurt prepared with S. thermophilus-L. delbrueckii ssp. bulgaricus-L. reuteri), the level required by the National Yogurt Association (NYA, McLean, VA), in probiotic yogurts at the manufacturing step and for products labeled “live and active cultures” (Talwalkar and Kailasapathy, 2004). Both yogurt and fermented milk that contained L. acidophilus decreased by 1.5 to 1 log, respectively, during storage but remained at the level recommended by FAO/WHO (2002) ($>10^7$ cfu/mL) to have beneficial effects as a probiotic. Similar reports observed losses around 2 log of L. acidophilus in yogurt after 28 d of storage (Mortazavian et al., 2007; Damin et al., 2008). Lactobacillus delbrueckii ssp. bulgaricus influenced depletion of L. acidophilus due to the effect of postacidification (Shah, 2000; Damin et al., 2008). Other authors addressed an antagonistic effect against probiotics of L. delbrueckii ssp. bulgaricus due to hydrogen peroxide production, which can partially damage the probiotic cells (Dave and Shah, 1997a). Gilliland et al. (2002) reported probiotic counts $>10^6$ cfu/mL in fermented milks after 35 d of storage. Overall, the viability of probiotic bacteria depends on strain type, storage conditions, and culture mixture. Lactobacillus casei presented the greatest loss of viability (decrease 4 to 5 log) during storage. Yogurt with L. delbrueckii ssp. bulgaricus maintained counts of $10^7$ for 7 d, whereas fermented milk maintained recommended levels of L. casei for 3 wk (FAO/WHO, 2002); again, the presence of L. delbrueckii ssp. bulgaricus promoted loss of viability. Gilliland et al. (2002) reported reductions in L. casei of 1 log in fermented milks, maintaining populations of $10^5$ to $10^6$ cfu/mL. In general, few studies report constant counts of L. casei in yogurts after 21 to 28 d of storage (Nighswonger et al., 1996; Korbekandi et al., 2008). Lactobacillus reuteri presented reductions of 2 and 3.5 log during storage for the yogurt and fermented milk, respectively. Similar counts ($10^6$) of L. reuteri were reported in milk (Hekmat et al., 2009) and in a whey fermented beverage ($10^7$; Hernandez-Mendoza et al., 2007) after 28 d of refrigerated storage. Lactobacillus reuteri had good stability and viability in fermented milk products in the current study; the recommended levels of probiotic bacteria ($10^7$ cfu/mL; FAO/WHO, 2002) remained for 14 and 21 d. Daily dietary intake of L. reuteri is important because it is a natural commensal bacteria. Yogurts that contain L. reuteri should be developed and promoted by the food industry. Microbial viability of the 3 fermented milks slowly decreased as pH was reduced and acidity was increased; however, they maintained counts of $\geq10^5$ cfu/mL during the first 21 d with any of the probiotics (L. acidophilus, L. casei, or L. reuteri). In the last 2 wk of storage, a gradual decline in the probiotic population occurred, which was independent of pH, because the pH was constant. Therefore, this decline may be due to the cessation of metabolic activity of bacteria due to long storage.

Sensory Attributes

The preference test used for the sensory evaluation had a 4-point scale (1 = slightly prefer B to C, 2 = moderately prefer B to C, 3 = greatly prefer B to C, and 0 = no preference) and was conducted with 21 untrained judges. The ANOVA revealed no significant differences ($P > 0.05$) in preferences among yogurts and fermented milks. The results indicate good acceptability of the different probiotic yogurts developed. Consumers did not identify texture or flavor differences among yogurts; therefore, probiotic yogurts can be modified using different culture mixtures without sensory complaints. Similar results were reported by Hekmat and Reid (2006) when they conducted consumer taste panel evaluations to compare sensory properties of probiotic and standard yogurts; the appearance, flavor, texture, and overall quality of probiotic 1% fat yogurt were comparable and similar to that of standard 1% fat yogurt.

According to the judges’ preferences of the products, it was possible to infer some relationships between instrumental and sensory measurements. We detected no significant differences ($P \geq 0.05$) among yogurts and fermented milks. However, the yogurt containing L. acidophilus was considered creamier (−0.024 N·s, 5.93 N, and 4.3 for adhesiveness, firmness, and pH, respectively) and less acidic than the yogurt containing L. reuteri (−0.147 N·s, 8.34 N, and 4.11 for adhesiveness, firmness, and pH, respectively). Furthermore, yogurt containing L. casei (pH 4.11) was better perceived because it was less acidic than the control yogurt (pH 3.96). Milk fermented with L. acidophilus had a mild flavor and aroma compared with yogurt containing L. reuteri. The fermented milk containing L. reuteri (−0.049 N·s, 6.19 N, and 4.27 for adhesiveness, firmness, and pH, respectively) was better perceived, being softer than yogurt with L. reuteri (−0.147 N·s, 8.34 N, and 4.11 for adhesiveness, firmness, and pH, respectively). The control yogurt (S. thermophilus-L. delbrueckii ssp. bulgaricus) was the least preferred compared with the
probiotic yogurts or fermented milks; this was probably due to its high acidity and common aroma. In general, probiotic yogurt and fermented milk containing L. casei were better accepted by judges than were the other yogurts and fermented milks. In this particular case, with adhesiveness of −0.054 N, sand firmness of 6.27–6.37 N, judges described products with L. casei as creamy, and preferred them over other probiotic yogurts and fermented milks. Milk fermented with L. acidophilus and yogurt containing L. acidophilus were moderately preferred over other yogurts and fermented milks for their taste and light aroma. We were able to establish a descending order of preference according to the total points assigned to the evaluation of products, as follows: S. thermophilus-L. bulgaricus-L. casei > S. thermophilus-L. acidophilus > S. thermophilus-L. reuteri > S. thermophilus-L. bulgaricus-L. acidophilus > S. thermophilus-L. bulgaricus-L. reuteri > S. thermophilus-L. bulgaricus. The yogurt containing L. casei was the best perceived product, followed by fermented milks, which might be because fermented milks have a milder flavor. Furthermore, the comments from the judges compared with instrumental values (adhesiveness, firmness, and pH) for each product agreed in terms of acidity and texture perception.

CONCLUSIONS

Probiotic fermented milks can be formulated using L. acidophilus, L. casei, or L. reuteri in combination with S. thermophilus; or probiotic yogurts combining S. thermophilus, L. bulgaricus, and probiotics without affecting their physicochemical and sensory properties. Viability of L. acidophilus during refrigerated storage was greater than that of L. casei or L. reuteri. According to the sensory evaluation, yogurt and fermented milk containing L. casei were the most accepted; thus, these products could be successfully introduced commercially.

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