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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 $\geq 10^7$ cfu/mL for 3 wk. Probiotic bacteria had variable viability in yogurts, maintaining counts of *L. acidophilus* $\geq 10^7$ 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*

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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.