Growth and Viability of *Bifidobacterium bifidum* in Cheddar Cheese

**ABSTRACT**

*Bifidobacterium bifidum* (ATCC 15696) was grown in MRS broth containing cysteine-HCl at 37°C, and the cells were harvested by centrifuging at 1300 x g for 15 min at 4°C. Equal volumes of the cell slurry and a 2.5% solution of κ-carrageenan were mixed and transferred by drops into a solution of .3 M KCl at 20°C under an atmosphere of nitrogen. The gelled beads were separated, frozen, and lyophilized immediately. This preparation and a commercial powder preparation were added to Cheddar cheese curd at milling as two treatments. Treatments did not affect cheese composition. Soluble protein increased during ripening at 7°C but without differences between treatments; SDS-PAGE patterns of proteolysis were also similar. Lactic acid content of cheeses increased during ripening, but differences between treatments were minor. Acetic acid and ethanol, common metabolites of bifidobacteria, were not detected during ripening. Bifidobacteria remained viable and increased in numbers in cheese during this 24-wk study but did not affect the flavor, flavor intensity, texture, or appearance of the cheese compared with that of the control. (Key words: bifidobacteria, Cheddar cheese, ripening)

**INTRODUCTION**

Bifidobacteria are the natural predominant microflora in the gut of breast-fed infants and offer resistance to enteropathogens. Reduction of cholesterol in serum, activation of the immune system, and anticarcinogenic activity are suggested health benefits attributed to bifidobacteria (31). Populations of bifidobacteria in feces typically decline after weaning. Dairy products have been used as a medium to reintroduce a viable population into the gastrointestinal tract of children and adults (17, 19, 32).

To exert a positive influence, a fermented dairy product has to meet certain basic criteria: the organism used for fermentation must be a normal intestinal inhabitant that can survive passage through the upper digestive tract, must be capable of colonizing in the intestine, must be metabolically active to produce beneficial effects, and must remain viable in the food carrier up to consumption (18). In some cases, bifidobacteria meet all but the last of these criteria and may be ideal candidates for use in fermented products. Fermented products should contain ≥10^6 cfu/ml to be effective and should be consumed regularly (22), or consumption needs to be ≥100 g/d of product with 10^5 to 10^6 cfu/ml (15). The most popular dairy products for the delivery of viable bifidobacteria are fluid milk, cultured milk, cultured buttermilk, yogurt, and powder preparations (17, 19). Advantage is often taken of the symbiotic or synergistic relationship between bifidobacteria and lactobacilli in development of new products (12, 13, 14, 18).

Viability of bifidobacteria in products over a long shelf-life at refrigeration temperatures is not satisfactory (33). The low pH of fermented milks and the aerobic conditions of production and packaging are not conducive to their survival; e.g., in one study (23), bifidobacteria did...
not survive 2 wk of storage in a frozen, low pH, cultured milk, but significant numbers remained viable up to 8 wk in a pH-controlled frozen dessert during storage (~29°C). However, bifidobacteria were able to survive and to grow after being frozen in yogurt mix and could resist .45% bile salts, but not .01N HCl, for 2 h (16). Reuter (32) observed a decrease in colony-forming units of 3 to 4 log cycles of bifidobacteria during storage in yogurt-like products.

The objectives of this study were to explore the possibility of introducing bifidobacteria in Cheddar cheese, to study their viability during long-term ripening and storage, and to evaluate their effects on cheese flavor and texture.

**MATERIALS AND METHODS**

**Cultures**

A lyophilized culture of *Bifidobacterium bifidum* 15696 was obtained from the American Type Culture Collection, (ATCC, Rockville, MD), rehydrated in modified lactobacilli MRS media (Difco Laboratories, Detroit, MI) containing .05% L-cysteine-HCl (Sigma Chemical Co., St. Louis, MO), and incubated for 24 h at 37°C without agitation. A commercial powder preparation of *B. bifidum* was obtained (Chr. Hansen's Lab., Inc., Milwaukee, WI) and stored in a desiccator at 4°C.

**Immobilization of Bifidobacteria**

The rejuvenated culture of *B. bifidum* was subcultured in increasing quantities of modified lactobacilli MRS broth in sterile culture flasks at 37°C without shaking (8). Cells were harvested by centrifugation at 1300 × g for 10 min in a refrigerated centrifuge (Sorvall RC-5B; DuPont Instruments, Wilmington, DE) at 5°C. The supernatant broth was decanted, and the cells were resuspended in the residual broth by a vortex mixer. Harvested cells were pooled, and the cell slurry was added to an equal weight of 3% κ-carrageenan (Sigma Chemical Co.) solution in sterile distilled water. The mixed solution was drawn into a sterile syringe and added by drops into a solution of 3 M KCl at 20°C in a 500-ml Erlenmeyer flask with a side arm. The side arm of the flask was connected to a nitrogen cylinder to supply a gentle stream of nitrogen, which helped maintain an anaerobic atmosphere and develop a positive pressure in the flask, preventing adventitious contamination during the immobilization process. The entire arrangement was placed on a magnetic stir plate and stirred gently.

The mixture of carrageenan and cell slurry gelled into small beads on contact with the cold KCl solution. The gelled beads were allowed to settle, separated from the rest of the solution by decanting, transferred to a sterile lyophilizer jar, immediately frozen in a freezing mixture of dry ice and ethyl alcohol, and lyophilized in a freeze dryer (Virtis, Gardiner, NY). The resulting immobilized, freeze-dried powder was transferred to sterile Petri plates and stored at 4°C in a desiccator until use.

**Cheese Manufacture**

Raw whole milk from the South Dakota State University dairy farm was pasteurized in 40-L cans at 63°C for 30 min and cooled to 4°C in an ice bath. Five replicates of Cheddar cheese were manufactured (21) with 100 kg of milk per replicate in 250-kg cheese vats (Kusel 4MX Model 65®; Kusel Equipment Co., Watertown, WI). Direct-vat-set, freeze-dried mesophilic lactic starter culture (EZ 100; Mar- schall Products, Rhône-Poulenc, Madison, WI) was added at the rate of 5 g/100 kg of milk at 31°C and allowed to ripen for approximately 45 min. Double-strength calf rennet (Marshall Products, Rhône-Poulenc) was added to the cheese milk at the rate of 20 ml/100 kg. Curd formed in approximately 30 min and was cut with .65-cm wire knives. After a 15-min healing period, the temperature of the curd and whey mixture was raised to 37 to 38°C in 30 min and then maintained at that temperature for an additional 30 min. Whey was drained, and curd was cheddared to pH 5.2 before milling.

The milled curd was weighed and divided into three equal portions. One portion of the curd (approximately 3.5 kg) was salted (2%) and packed into hoops. The other two portions were also similarly salted. Additionally, the commercial bifidobacteria preparation was added to one portion, and the immobilized, freeze-dried preparation to the other, prior to thorough mixing and packing into hoops. The
rate of addition of either preparation was calculated to introduce approximately the same number of viable cells \((1 \times 10^6 \text{ cfu/g})\) of bifidobacteria in the cheese.

The hoops were pressed overnight \((2.46 \text{ kg/cm}^2)\), weighed, and packed in oxygen barrier Cryovac® bags (Cryovac® Division, W. R. Grace and Co., Duncan, SC) and heat-sealed with Multivac® vacuum packaging equipment (Koch, Kansas City, MO). The three cheese treatments were control cheese, cheese with commercial bifidobacteria preparation (CBP), and cheese with immobilized, freeze-dried bifidobacteria preparation (IBP). Cheeses were ripened at 6 to 7°C for 6 mo.

**Cheese Composition**

Moisture in cheese was determined by an Ohaus MB200 moisture balance (Ohaus Corp., Florham Park, NJ) (9). Total protein was analyzed by the macro-Kjeldahl method (3) and fat by the Mojonnier method (4). Ash in cheese was determined by heating the sample in a muffle furnace at 100°C for 1 h, 200°C for 2 h, and then at 550°C overnight (7). Salt was determined by a sodium electrode with a Coming 150 analyzer (Corning Medical, Medfield, MA) (20).

**Proteolysis**

Soluble protein in cheese was extracted with Sharp’s solution (21), and nitrogen in the extract was estimated by the macro-Kjeldahl method (3) and converted to protein using 6.38 as the factor. Proteolysis was also monitored (2, 5) using a Bio-Rad Protean II slab cell® (Bio-Rad, Richmond, CA) on a 10 to 20% continuous gradient separating gel and continuous running buffer (pH 8.3) of Tris (3%), glycine (1.44%), and 1% SDS (5). Gels were stained in 1% Coomassie blue solution (40% methanol and 10% glacial acetic acid) for 18 to 20 h; destained (50% distilled water, 40% methanol, and 10% glacial acetic acid) for 4 to 5 h; preserved in a solution containing 67% distilled water, 30% methanol, and 3% glycerol; and photographed.

**Metabolic Activity**

Production of lactic acid, acetic acid, and ethanol was determined by HPLC (6). Cheese (5 g) was blended with 25 ml of .01N sulfuric acid and stirred with a magnetic stirrer for 1 h. The slurry was centrifuged (Sorvall RC-5B refrigerated superspeed centrifuge; DuPont Instruments) at 5000 \(\times g\) for 10 min at 10°C. The supernatant was then filtered (number 1 filter paper; Whatman Int. Ltd., Maidstone, England). The filtrate was filtered through a .20-μm sterile membrane filter (Chrom Tech Inc., Apple Valley, MN) into HPLC vials and stored at 4°C.

Analysis was performed on an HPLC system (Spectra Physics Inc., San Jose, CA) using a refractive index detector with a SP 8875 autosampler, Chromjet SP 4400 integrator, and a HPX-87H column (Bio-Rad) operated at 65°C and a flow rate of .6 ml/min. The mobile phase was .01N H2SO4. The HPLC system was calibrated between .5 and 5.0 g/L for lactic and acetic acids and ethanol.

**Microbiological Profile**

Total aerobic, anaerobic, and selective bifidobacteria were counted in the cheese samples at 6-wk intervals. Cheeses were sampled aseptically into sterile sample bags and stored at 4°C until plated. Cheese (11 g) was diluted in 99 ml of 2% sodium citrate at 45°C (26). The sample was macerated in a stomacher laboratory blender (model 400-BA 7021; Seward Medical Ltd., London, England) for 2 min at high speed in stomacher bags to obtain a slurry for the first dilution, and subsequent serial dilutions were in .1% peptone solution. Appropriate dilutions were pour plated.

Total aerobic counts were determined on all purpose Tween agar (Difco Laboratories). Plates were incubated aerobically at 32°C for 24 h. Total anaerobic counts were determined on lactobacilli MRS agar after 48 h of incubation at 37°C using gas pak anaerobic systems [BBL, Cockeysville, MD (now Becton Dickinson Microbiology Systems, Cockeysville, MD)].

Bifidobacteria were enumerated according to the method of Wijsman et al. (36). A mixture of antibiotics, including 2 g of neomycin sulfate, 4 g of paromomycin sulfate, .3 g of nalidixic acid, and 60 g of lithium chloride (NPNL; Sigma Chemical Co.), was prepared in 1 L of distilled water, filter-sterilized (.22 μm), and stored at 4°C until use. The mixture of
antibiotics (5 ml) was added to 100 ml of lactobacilli MRS agar prior to plating. Cysteine-HCl was added at the rate of 0.05% to decrease the redox potential of the medium. Plates were incubated at 37°C for 48 to 72 h anaerobically as previously described.

Microstructure

The microstructure of the bifidobacteria preparations and Cheddar cheese was examined by scanning electron microscopy. Clean aluminum stubs were applied with double-sided scotch tape. Powder samples of bifidobacteria were applied to the sticky surface, and the excess sample was dusted off with a gentle stream of air. Cheese samples were prepared as described previously (27). Prepared samples of powder and cheese were sputter-coated (Hummer IV® sputter coater; Technics Electron Microscopy Systems Inc., Munich, Germany) under a gold and palladium target to a 20-nm thickness and viewed on a scanning electron microscope [IS1 Super IIIA, International Scientific Instruments, Inc., Glen Ellyn, IL (now Top Con Technologies, Inc., Pleasanton, LA)] operated at 15 kV. Samples were photographed on a Type 55 Polaroid 50 ASA film (Polaroid Corp., Cambridge, MA).

Sensory Evaluation

An experienced taste panel of eight judges evaluated the cheeses at 6-wk intervals for flavor, texture, and appearance on a 10-point hedonic scale (1 = poor to 10 = excellent). Cheddar cheese flavor intensity was also evaluated on a 10-point hedonic scale (1 = low to 10 = high intensity).

Statistical Analysis

The general linear models procedure of SAS (34) was used to analyze the data. Least squares means analysis was performed to determine differences in means at P < .05 or P < .01. A split-plot technique was used for analysis of data using TRT as main plots and age as subplots.

RESULTS

Microstructure of the two powder preparations of bifidobacteria, examined by scanning electron microscopy, showed the presence of bifidobacteria as individual and exposed cells in the commercial preparation or as fixed cells in the immobilized, freeze-dried preparation (Figure 1). Bifidobacteria counts in the two preparations were $3.0 \times 10^{10}$ and $3.0 \times 10^7$ cfu/g, respectively.

Cheese Composition

Moisture or protein did not differ (P > .05) among the three treatments (Table 1). Fat content in IBP was lower (P < .05) than in the control and CBP, although the reason was not apparent. All cheeses were legal for moisture and for fat in the dry cheese. Ash contents of the control and CBP were lower (P < .05) than those of IBP, probably because of the use of $\kappa$-carrageenan, which contains cations of K, Ca, and Na.

Proteolysis

Soluble protein was unaffected by treatment but, as expected, was affected by age (P < .05) (Table 2). Soluble protein, averaged across all treatments, increased from 2.29% at 1 wk to 6.07% at 24 wk. Proteolysis during ripening was also monitored by SDS-PAGE. Figure 2 shows representative electrophoresis patterns in the three cheeses at 24 wk. No apparent differences in the electrophoretic patterns were observed in cheeses at any age.

TABLE 1. Composition1 of Cheddar cheese with added bifidobacteria.

<table>
<thead>
<tr>
<th>Component</th>
<th>Control</th>
<th>CBP</th>
<th>IBP</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>37.4a</td>
<td>37.9a</td>
<td>38.0a</td>
<td>.20</td>
</tr>
<tr>
<td>Protein</td>
<td>23.4a</td>
<td>23.1a</td>
<td>23.0a</td>
<td>.14</td>
</tr>
<tr>
<td>Fat</td>
<td>33.2a</td>
<td>33.1a</td>
<td>32.5b</td>
<td>.12</td>
</tr>
<tr>
<td>Ash</td>
<td>3.1a</td>
<td>3.1a</td>
<td>3.3b</td>
<td>.05</td>
</tr>
<tr>
<td>Salt</td>
<td>1.32a</td>
<td>1.04b</td>
<td>1.28a</td>
<td>.06</td>
</tr>
</tbody>
</table>

1Means in rows with like superscripts do not differ (P > .05).

2Mean of five replicates.

Figure 1. Scanning electron micrographs showing bifidobacteria in a commercial preparation (a) and an immobilized freeze-dried preparation (b). The cells were immobilized in κ-carrageenan. Arrows show bifidobacteria.
TABLE 2. Soluble protein\(^1\) in Cheddar cheese with added bifidobacteria.

<table>
<thead>
<tr>
<th>Age (wk)</th>
<th>Soluble protein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.29(^a)</td>
</tr>
<tr>
<td>6</td>
<td>3.23(^b)</td>
</tr>
<tr>
<td>12</td>
<td>4.32(^c)</td>
</tr>
<tr>
<td>18</td>
<td>5.61(^d)</td>
</tr>
<tr>
<td>24</td>
<td>6.07(^e)</td>
</tr>
<tr>
<td>SE</td>
<td>.04</td>
</tr>
</tbody>
</table>

\(^{a,b,c,d,e}\) Means in columns with like superscripts do not differ (\(P > .05\)).

\(^1\) Least squares means across all treatments of five replicates.

Metabolic Activity

The metabolic activity of the microorganisms in cheese was monitored by estimation of the metabolic products, lactic and acetic acids, and ethanol. Lactic acid production during ripening is shown in Table 3. Control cheese contained 1.07% lactic acid at 1 wk and a maximum of 1.55% after 24 wk; CBP showed a maximum lactic acid concentration of 1.54% at 18 wk before declining to 1.44% at 24 wk. The trend was similar for IBP. At 1 and 12 wk, lactic acid did not differ (\(P > .05\)) among TRT, but, at 6, 18, and 24 wk, differences were significant (\(P < .05\)). However, no trend was
TABLE 3. Lactic acid \(^1\) production in Cheddar cheese with added bifidobacteria.

<table>
<thead>
<tr>
<th>Age (wk)</th>
<th>Treatment</th>
<th>Control</th>
<th>CBP</th>
<th>IBP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.07%</td>
<td>1.08%</td>
<td>1.01%</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1.24%</td>
<td>1.33%</td>
<td>0.90%</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>1.16%</td>
<td>1.06%</td>
<td>1.11%</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>1.28%</td>
<td>1.54%</td>
<td>1.52%</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>1.55%</td>
<td>1.44%</td>
<td>1.28%</td>
<td></td>
</tr>
</tbody>
</table>

\(^{a,b}\)Means in rows with like superscripts do not differ (\(P > .05\)).

\(^{A,B,C,D}\)Means in columns with like superscripts do not differ (\(P > .05\)).

\(^{1}\)Mean of five replicates; standard error of least squares means = 0.8.

\(^{2}\)CBP = Cheese with commercial and bifidobacteria preparation, and IBP = cheese with immobilized bifidobacteria preparation.

apparent, and the differences seemed to be random. Acetic acid and ethanol were not detected in any of the cheeses during ripening.

Microbiological Profile

Aerobic and anaerobic counts of Cheddar cheeses during ripening were unaffected by treatment and are presented in Table 4 as least squares means over all treatments. Aerobic counts at 1 wk were >10\(^7\) cfu/g because the highest dilution plated had too many colonies to count. Thereafter, counts remained in the range of 2.4 x 10\(^5\) to 2.9 x 10\(^6\) cfu/g until 18 wk and dropped (\(P < .05\)) to 1.2 x 10\(^5\) cfu/g at 24 wk. Anaerobic counts increased slightly but not significantly (\(P > .05\)) between 6 and 18 wk of ripening.

Counts (3 or 4 log cycles) on MRS-NPNL agar were low in control cheese during 1, 12, and 18 wk (Table 5). At 6 and 24 wk, colonies in the dilutions plated were too few to count. At 1 wk, CBP had 3.3 x 10\(^6\) cfu of bifidobacteria/g of cheese, which increased to a maximum of 1.7 x 10\(^7\) cfu/g at 18 wk; IBP had 2.6 x 10\(^5\) cfu of bifidobacteria/g of cheese at 1 wk, which gradually increased over ripening to a maximum of 2.6 x 10\(^7\) cfu/g at 24 wk.

Scanning electron microscopy of IBP showed beads from the immobilized preparation added to the cheese (Figure 3). At 24 wk, the cluster of bifidobacteria was relatively intact and undispersed. The immobilization matrix used, \(\kappa\)-carrageenan, is a food-grade polysaccharide extracted from seaweed, which appeared to have held the entrapped cells together, even after 24 wk without degradation. The bifidobacteria cells were viable, as confirmed by plating on a selective medium. Such clusters were not observed in CBP.

Sensory Evaluation

Effect of treatment on sensory criteria was insignificant. Least squares means of sensory evaluation scores for flavor, flavor intensity,
body and texture, and appearance as means across all treatments are shown in Table 6. Flavor scores for all treatments increased with age and peaked at 12 to 18 wk. Flavor intensity scores increased continuously up to 18 wk and remained constant thereafter. As expected, body and texture improved ($P < .05$) with age; maximum scores were attained at 24 wk. Appearance improved after 12 wk of ripening.

**DISCUSSION**

Bifidobacteria in the commercial preparation were present as free or exposed cells, but, in the immobilized preparation, a distinct envelope of carrageenan surrounded a mass of cells of bifidobacteria. The process of immobilization would probably shield the cells to a certain extent but would also restrict the free or uniform distribution in cheese. Microencapsulation of bifidobacteria was successfully used by Rao et al. (30) to maintain higher viability during passage through the gastrointestinal tract to the large intestine.

The addition of bifidobacteria to Cheddar cheese, as a free flowing powder, CBP, or as an immobilized preparation, IBP, did not change cheese composition compared with the control cheese. The increase in the ash content in IBP could possibly be due to inorganic salts from $\kappa$-carrageenan in the immobilized preparation. However, the rate of addition of the bifidobacteria preparation was too low for a fundamental change in composition.

Aerobic counts followed a predictable pattern during ripening; counts remained steady through the first 18 wk and then declined at 24 wk. The presence of bifidobacteria in the cheese did not affect the normal aerobic microflora of Cheddar cheese. Although bifidobacteria exhibit antibacterial activity (1), they do not inhibit Gram-positive lactobacilli or lactococci. Anaerobic counts on MRS agar varied widely in the fresh cheeses but were in the normal range for Cheddar cheese (29). The anaerobic counts increased up to 12 wk and remained steady in the $10^8$ log cycle throughout the remainder of the study.

Figure 3. Cluster of cells in an intact bead of immobilized preparation of bifidobacteria (black arrow) added to Cheddar cheese. Open arrow shows cheese matrix.
TABLE 6. Sensory evaluation of Cheddar cheese with added bifidobacteria.

<table>
<thead>
<tr>
<th>Age</th>
<th>1 wk</th>
<th>6 wk</th>
<th>12 wk</th>
<th>18 wk</th>
<th>24 wk</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavor</td>
<td>6.7a</td>
<td>7.2ab</td>
<td>7.5c</td>
<td>7.4ab</td>
<td>7.0b</td>
<td>.11</td>
</tr>
<tr>
<td>Flavor intensity</td>
<td>3.36</td>
<td>4.1c</td>
<td>5.8b</td>
<td>6.4a</td>
<td>6.7a</td>
<td>.12</td>
</tr>
<tr>
<td>Body and texture</td>
<td>6.36</td>
<td>7.1c</td>
<td>6.8b</td>
<td>7.4a</td>
<td>7.6a</td>
<td>.08</td>
</tr>
<tr>
<td>Appearance</td>
<td>7.6b</td>
<td>7.9a</td>
<td>8.2a</td>
<td>8.3a</td>
<td>8.1a</td>
<td>.09</td>
</tr>
</tbody>
</table>

a,b,c,d Means in rows with like superscripts do not differ (P > .05).

1Least squares means across all treatments of five replicates and five judges.

2Ten-point scale (1 = poor to 10 = excellent).

3Ten-point scale (1 = low intensity to 10 = high intensity).

The CBP contained more bifidobacteria than the IBP at 1 wk (3.3 x 10⁶ and 5.0 x 10⁵ cfu/g, respectively), probably because of the uneven distribution of the immobilized preparation in the cheese compared with the powder, which was better distributed in the cheese curd. Approximately 10⁶ cfu/g of bifidobacteria were added to the cheese curd for CBP and IBP. Counts of bifidobacteria in the fresh cheese (1 wk) approached this theoretical value.

A medium containing a mixture of antibiotics was used for the selective enumeration of bifidobacteria in cheese. This method was suggested for enumeration in fermented dairy products at dilutions >10⁻⁴ (36) but also seemed to be effective for cheese. Counts were low in control cheese at lower dilutions, which can be attributed to anaerobic organisms, such as lactobacilli. No colonies were observed at higher dilutions.

Soluble protein, an index for cheese ripening (24, 25), was within the normal range for ripening Cheddar cheese for all treatments, and no differences occurred (P > .05) among treatments for cheese at any age. An increase in soluble protein reflects progressive breakdown of proteins into smaller fragments. Addition of bifidobacteria did not change the normal proteolytic behavior during cheese ripening. Bifidobacteria possess proteolytic enzymes (10, 11), which had no influence on cheeses in this study, as confirmed by SDS-PAGE. Electrophoretic patterns revealed the progressive proteolysis occurring in cheese over time, but no discernible differences occurred among the treatments for cheeses at any age.

Lactic acid production showed no discernible pattern and no significant (P > .05) treatment differences. Acetic acid or ethanol was not detected in any cheese throughout ripening; minimum detection was .25%. Bifidobacteria produce acetic and lactic acids in a molar ratio of 3:2 (vol/vol) (31). That ratio is typical of the log phase of growth and could vary widely (10, 28). Ventling and Mistry (35) studied the activity of bifidobacteria in ultrafiltered milks of various concentrations and found that, at higher concentrations, the ratio of acetic to lactic acid decreased. Generation times were also longer as concentrations increased. Although that report (35) may not be applicable to the present study, cheese, similar to ultrafiltered milk, is also a concentrated medium. The presence of other competitive microflora (starter and nonstarter lactic acid bacteria) may also have an effect on the metabolic activity of bifidobacteria.

Bifidobacteria produce acetic and lactic acids from lactose via a fructose-6-phosphate shunt pathway (31). The absence of lactose or glucose, the substrates for glycolysis, may also partially explain the lack of normal bifidobacteria metabolism. The cheeses were ripened at 6 to 7°C, which is below the optimal growth temperature of 37°C for bifidobacteria (31); hence, growth was only moderate during 24 wk. Bifidobacteria remained viable in cheese throughout the ripening period. Isolates of bifidobacteria from Cheddar cheese grew well in MRS broth and produced acetic and lactic acids (data not shown).

The lack of vigorous metabolic activity by bifidobacteria is also indicated by the sensory evaluation, which showed no differences among cheeses at any age. Production and accumulation of acetic acid in the cheese would have been reflected in the scores for...
flavor and flavor intensity. A change in the microflora cannot normally be expected to change physical attributes such as texture or appearance unless proteolytic activity that is due to the bacteria is excessive.

CONCLUSIONS

Addition of bifidobacteria as a starter adjunct for a cheese, such as Cheddar, may not be successful considering 1) the aerobic conditions of cheese making, 2) rigorous conditions of cooking and cheddaring, and 3) the presence of rapidly multiplying and metabolically active lactic acid bacteria. These obstacles may be overcome somewhat if bifidobacteria are introduced at a later stage of cheese making, such as at milling or salting. Two methods to introduce bifidobacteria in Cheddar cheese were developed: CBP and an IBP. Both methods were effective, and differences were not remarkable between methods. The immobilized preparation may not have been uniformly dispersed in the cheese, but this factor did not affect the viability of bifidobacteria in Cheddar cheese. The bifidobacteria remained viable in Cheddar cheese up to the end of 24 wk and had no effect on flavor, flavor intensity, texture, or appearance.

Bifidobacteria were not metabolically active; no acetic acid was produced in cheese. Lack of substrates, low temperature ripening, absence of an active log growth phase, and presence of other anaerobic lactic acid bacteria could account for the lack of vigorous metabolic activity.

Cheese containing bifidobacteria did not differ from the control cheese in soluble protein, lactic acid, or normal microflora. The results of this study show that bifidobacteria can be introduced into Cheddar cheese and can maintain their viability for at least 6 mo.

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