March 1994

# Isolation of Galactose-Fermenting Thermophilic Cultures and Their Use in the Manufacture of Low Browning Mozzarella Cheese

Kamla K. Mukhurjee
*University of Nebraska - Lincoln*

Robert W. Hutkins
*University of Nebraska - Lincoln*, rhutkins1@unl.edu

# Isolation of Galactose-Fermenting Thermophilic Cultures and Their Use in the Manufacture of Low Browning Mozzarella Cheese[1]

KAMLA K. MUKHERJEE and ROBERT W. HUTKINS
Department of Food Science and Technology
University of Nebraska-Lincoln
Lincoln 68583-0919

## ABSTRACT

The objectives of this study were to isolate galactose-fermenting, galactose-nonreleasing strains of *Streptococcus* and *Lactobacillus* and to use these strains as starter cultures in the manufacture of low browning Mozzarella cheese. Four *Streptococcus* isolates having the desirable phenotype, combined with a *Lactobacillus helveticus* strain, were acceptable as starter cultures based on activity tests. Fifteen vats of Mozzarella cheese were produced, in triplicate, representing four experimental treatments (made with galactose-nonreleasing strains) and one control (made with a galactose-fermenting, galactose-releasing *Streptococcus*). Analyses were performed on cheese after 5 and 28 d of refrigerated storage. The lactose and galactose content of cheese remained constant or decreased over the 28-d testing period for all experimental treatments. The galactose content in the control cheese increased over the testing period and was significantly higher by d 28 than in the test cheeses. Browning was greatest on d 28 and at the more severe baking conditions. Control cheese browned more than experimental cheeses did. Melt behavior and free oil formation were generally not affected by treatment. The results indicated that the galactose-fermenting, galactose-nonreleasing strains could be used to make low browning Mozzarella cheese.

(**Key words**: galactose-fermenting, thermophilic cultures, Mozzarella cheese, browning)

**Abbreviation key**: **Gal$^+$** = galactose-fermenting, **Rel$^+$** = galactose-releasing, **Rel$^-$** = galactose-nonreleasing.

## INTRODUCTION

Fueled by the continued popularity of pizza and other Italian specialty foods, consumption of Mozzarella cheese has increased dramatically in the last 15 yr. Mozzarella is now second only to Cheddar cheese in economic importance in the US (12), accounting for over 25% of the total US production of cheese (19). Nearly 1 billion kg of Mozzarella are now produced annually in the US, and increased demand is expected to continue (12).

Over 70% of the Mozzarella cheese produced in the US is used for pizza (7). Mozzarella is used primarily for its unique functional properties in the melted state. According to Kindstedt (7), important functional properties include meltability, stretchability, free oil formation, and browning. Large institutional buyers of Mozzarella want a uniform product and often set rigid standards for those functional properties. Consequently, manufacturers are under considerable pressure to produce cheese with consistent functional characteristics.

One manufacturing variable that has a profound effect on the finished cheese is the selection of the starter culture. The lactose-fermenting abilities of the two thermophilic lactic acid bacteria used to make Mozzarella cheese, *Streptococcus salivarius* ssp. *thermophilus* and *Lactobacillus helveticus* (or *Lactobacillus delbrueckii* ssp. *bulgaricus*), are particularly important in determining the browning potential of the cooked cheese. Because most strains ferment only the glucose portion of lactose and release the galactose

Received June 7, 1993.
Accepted March 14, 1994.
[1]Published as Paper Number 10398, Journal Series Nebraska Agricultural Experiment Station, Lincoln 68583-0919.

portion into the medium or curd (5, 13, 15, 17, 18), Mozzarella cheese cooked at a high temperature (e.g., during pizza baking) may brown beyond that color tolerated by pizza manufacturers. Consequently, thermophilic starter cultures are needed that ferment lactose without releasing galactose. Several researchers (2, 4, 6, 12) have suggested that Mozzarella cheese made with galactose-fermenting (**Gal$^+$**) strains of *S. salivarius* ssp. *thermophilus* have reduced capacity for browning. However, even most Gal$^+$ strains examined still release galactose into the curd as long as lactose is present in the medium (5, 17, 18).

The objectives of this study were to isolate strains of *S. salivarius* ssp. *thermophilus* that would ferment the galactose portion of lactose without releasing it first into the surrounding medium. The isolated strains were then used as starter cultures in Mozzarella cheese manufacture, and the cheese functional properties, especially browning, were analyzed.

## MATERIALS AND METHODS

### Isolation of Gal$^+$ Strains

Strains of *Streptococcus* spp. and *Lactobacillus* spp. were isolated from imported Swiss, Emmenthaler, Gruyère, Parmesan, Bal Paese, and Jarlsburg cheeses; from commercial yogurt samples; and from laboratory stock cultures maintained at the Department of Food Science and Technology, University of Nebraska-Lincoln. Samples were prepared by stomaching (Stomacher 400; Tekmar Company, Cincinnati, OH) 11 g of cheese or yogurt with 99 ml of sterile phosphate buffer solution for 2 min. Dilutions were spread or streaked onto Elliker-galactose agar (3) containing 1.0% galactose and 40 mg/L of bromcresol purple. Active stock cultures were streaked directly onto the Elliker-galactose plates. After 24 to 48 h of anaerobic incubation (model 1025; Forma Scientific, Inc., Marietta, OH) at 42°C, colonies that formed a yellow zone were considered Gal$^+$. Isolated cultures were classified based on Gram reaction, morphology, catalase reaction, sugar fermentation patterns, and temperature optima. Membrane fatty acid analyses were performed by Analytical Services, Inc. (Essex Junction, VT).

The Gal$^+$ strains were further characterized based on their growth in milk and their ability to utilize the galactose derived from intracellular lactose hydrolysis. Each Gal$^+$ strain was grown in sterile (autoclaved 12 min, 121°C) reconstituted 10% NDM for 12 h at 42°C. Periodically, 5-ml portions of growth medium were aseptically removed, and the pH (Corning pH Meter 145; Corning, NY) was measured. Samples were centrifuged (model J2-21; Beckman Instruments, Fullerton, CA) at 48,400 × *g* for 20 min to remove milk solids and cells. The supernatants were then assayed enzymatically for lactose and galactose using enzyme kits (catalog number 176303; Boehringer Mannheim, Indianapolis, IN).

### Culture Activity Tests

Culture activity tests, based on typical Mozzarella manufacturing procedures (9), were performed to select starter culture strains with adequate manufacturing times. In these small laboratory cheese-making trials, .02 g of calcium chloride was added to a 250-ml beaker containing 100 ml of whole pasteurized milk. The milk was inoculated with 2% Gal$^+$ strains (*Streptococcus* sp. paired with a suitable *Lactobacillus* sp. in a 1:1 [vol/vol] ratio) and incubated for 30 min in a 32°C water bath. Rennet was added (.02 ml), and coagulation occurred in 30 to 35 min. The curd was cut with a laboratory spatula. The cut curd was heated to 42°C in 30 min with continuous stirring, cooked at 42°C for an additional 45 min, drained through cheese cloth, and cheddared. The temperature of the cheddaring curd was maintained at 42°C in an incubator (Precision Scientific Co., Chicago, IL). The pH of the curd was periodically measured. Acceptable starter culture pairs reduced the curd pH to 5.2 or less within 3 h of cheddaring.

### Starter Culture Preparation

Strains suitable as cheese-making cultures were maintained on Elliker slants containing .5% galactose. Prior to starter culture preparation, *Streptococcus* spp. and *Lactobacillus* spp. strains were propagated individually in sterile (autoclaved 12 min) reconstituted 10% low heat NDM (Mid American Dairymen, Inc., Springfield, MO) containing .5% food-grade yeast extract (Red Star, Juneau, WI). The latter was also used as a bulk starter medium, but was steamed for 60 min rather than autoclaved. Media were inoculated (1%) with either *Streptococcus* sp. or *Lactobacillus* sp. and incubated for 13 to 15 h at 42°C.

### Mozzarella Manufacture

Low moisture Mozzarella cheese was manufactured at the University of Nebraska-Lincoln Dairy Pilot Plant using a combination of previously described procedures (4, 9, 12). Raw milk was pasteurized for 23 s at 74.4°C and then cooled to 32°C. Fat was adjusted to 3% by the addition of pasteurized skim milk. Two percent (vol/vol) of starter culture (1% *Streptococcus* sp. and 1% *Lactobacillus* sp.) was added to 181 kg of milk. The milk was ripened for 30 min. Single-strength calf rennet (Chr. Hansen's Laboratory, Inc., Milwaukee, WI) was diluted (1:10) in cold water and added to the milk at the rate of 84 ml/45 kg of milk. The milk coagulated in 30 to 35 min. Curd was cut with .95-cm knives, held for 10 min, and heated to 42°C in 45 min with constant stirring. Temperature and stirring were maintained during cooking for an additional 45 min. After draining, curd slabs were cheddared at 42°C until the curd pH reached 5.2. The curd was milled manually, stretched in a Mozzarella cooker-stretcher (model Supreme 640; Stainless Steel Fabricating, Inc., Columbus, WI) in 82°C water (approximate curd temperature was 55°C) and formed into 60-cm logs having a 13.5-cm diameter. The molded cheese was placed in cold water (10°C) to firm the curd and then brined in a saturated sodium chloride solution for 20 to 22 h at 4°C. After removal from the brine, the cheese was briefly air dried, vacuum packaged in 200- to 400-g blocks, and stored at 4 to 6°C for 28 d.

Five strains of *Streptococcus* sp. were used in the cheese-making trials. Four $Gal^+$, galactose-nonreleasing ($Rel^-$) strains (KK-1, KK-2, KK-3, KK-5), and one $Gal^+$, galactose-releasing ($Rel^+$) strain (KK-4) were used; the latter strain served as a control. Each *Streptococcus* sp. was paired with the same $Gal^+$ *L. helveticus* strain (JM-31). These five streptococci-lactobacilli pairs are referred to as treatments KK-1, KK-2, KK-3, KK-5, and KK-4, respectively. Treatment KK-4 served as the control. In independent trials, triplicate batches of cheese were made with each of the five treatments.

### Cheese Sampling and Analyses

Samples were taken at various stages during the cheese-making process, including samples from the pasteurized milk, curd at cutting, curd at draining, curd after cheddaring, and curd after brining. Samples were frozen at –20°C in Whirl-Pak bags (Nasco, Fort Atkinson, WI) for subsequent proteolysis and sugar analyses. Samples of the aged cheese were taken on d 5 and 28. Three to four of the vacuum packages (or enough to give a total sample weight of approximately 1500 g) were used on each day. Aged samples were ground in a food processor until a particle size of ≤5 mm was attained. Samples were tested on the same day for melt, free oil, and browning. A portion of each sample was frozen (–20) in Whirl-Pak bags for subsequent proteolysis and sugar analyses.

Milk, curd, and cheese samples for sugar analysis were removed during Mozzarella cheese manufacture and were prepared according to the recommended procedures as described in the Boehringer Mannheim kit (Indianapolis, IN). Lactose and galactose concentrations were determined enzymatically for all milk, curd, and cheese samples (Boehringer Mannheim kit). Samples for cheese proteolysis were prepared and assayed using the TCA-trinitrobenzenesulfonic acid method (1, 16).

A modified version of the predictive test described by Johnson and Olson (6) was used to measure the color of baked Mozzarella cheese. Forty-gram samples of grated cheese were placed on disposable aluminum pan liners (12-cm diameter; Baxter Scientific Products, McGaw Park, IL). The samples were patted down by hand to form a flat surface and held at 22°C for 2 to 3 h prior to baking. The cheese pans were placed on a baking tray and baked (Lincoln Impinger III oven; Lincoln Foodservice Products, Inc., Fort Wayne, IN) at various temperatures for various times (Table 1). Each sample was run in duplicate for each

TABLE 1. Time and temperature combinations for cooked color test.

| Cooking temperature | Cooking time |
|---|---|
| (°C) | (min) |
| 232 | 2.00 |
| 260 | 1.25 |
| 288 | 1.00 |
| 307 | 1.00 |
| 307 | 1.25 |
| 307 | 1.50 |

temperature and time combination. Samples were cooled to room temperature before browning was measured. The cooked color of the cooled samples was analyzed using a Hunter Tristimulus Colorimeter (model D25-9; Reston, VA). Three color indices were measured, L* (white to black), a* (red to green), and b* (yellow to blue). Each index was measured at the center and at four points (90° angles) around the circumference of the cheese pan. The mean for each index was calculated from the five measurements. The final measurement for each index is stated as an average of the duplicate samples.

Meltability was measured according to procedures described by Oberg et al. (12). The assays were performed in duplicate, and the results stated are the mean of these duplicate measurements. Formation of free oil was measured using the method of Kindstedt and Rippe (8). Percentage of free oil was expressed as the mean of duplicate determinations.

### Statistical Analyses

General linear models analysis, least squares means, and contrasts were run using SAS software (14). Tests for significance were conducted at the 5% level.

## RESULTS

### Isolation of Gal$^+$ Strains

Dilutions from 23 samples of cheese, yogurt, and laboratory stock cultures were plated on Elliker agar-galactose. Selection of the Gal$^+$ phenotype was based on the appearance of a distinct yellow zone surrounding isolated colonies. Although several thousand colonies grew on this nonselective medium, only 13 of the approximately 50 Gal$^+$ isolates were selected for further study (Table 1). Based on cell morphology, Gram reaction, catalase reaction, and temperature optima, 8 of the Gal$^+$ isolates were classified initially as *Streptococcus*, and 5 were classified as *Lactobacillus* sp. Sugar fermentation patterns of the streptococci suggested that these strains were more closely related to the enterococci. However, membrane fatty acid analyses revealed poor correlation with *Enterococcus* sp. and greater correlation with the type strain, *S. salivarius* ssp. *thermophilus* ATCC 19258 (data not shown). Therefore, we concluded that these isolates were thermophilic streptococci of undetermined species. Strains KK-6 and KK-7 grew only anaerobically, and strain KK-8 stopped growing on galactose after several transfers. Consequently, these latter three cultures were eliminated as suitable Mozzarella starter strains. The other 5 isolates were maintained on Elliker slants containing .5% galactose as the carbohydrate source.

The Gal$^+$ isolates just described were grown in reconstituted NDM and were evaluated for their ability to utilize lactose without releasing galactose. At various times, 5-ml samples were removed and centrifuged to remove cells. The cell-free medium was enzymatically assayed for lactose and galactose content. Strains were considered Rel$^-$ if more galactose was present in the growth medium at the beginning of the growth curve than at the end. In contrast, growth medium from Rel$^+$ strains contained less galactose at the beginning than at the end of the fermentation. Fermentation curves for strains KK-2 (Rel$^-$) and KK-4 (Rel$^+$) are shown in Figure 1, and Table 2 shows the Rel phenotype for each of the other strains. Strains KK-1, KK-2, KK-3, and KK-5 were Rel$^-$; i.e., net galactose concentration decreased during incubation. The galactose content in culture medium with KK-4, however, increased by 30%; this strain was considered to be Rel$^+$. Because nearly .2% lactose was fermented during the incubation period (data not shown), the latter strain had nonetheless fermented much of the available galactose and had released only a portion of the galactose. None of the strains released free glucose into the medium (data not shown).

Of the *Lactobacillus* strains, JM-21 and JM-31 were also characterized as partial Rel$^+$. Because *Lactobacillus* strains JM-32, JM-41, and JM-42 released stoichiometric amounts of galactose (i.e., none of the available galactose was fermented), they were eliminated as suitable low browning Mozzarella starter strains. None of the strains released glucose during growth in milk.

A percentage comparison of the final galactose content in the growth medium for each strain showed that milk fermented by the four Rel$^+$ strains, KK-4, JM-32, JM-41, and JM-42, contained up to 10 times more galactose than milk fermented by the Rel$^-$ strains, KK-1, KK-

2, KK-3, and KK-5 (data not shown). Medium from the Rel$^+$ strains contained .06 to .22% galactose (g/100 ml of milk) after 12 h of incubation, but growth medium from the Rel$^-$ strains never contained more than .05% galactose during the 12-h incubation.

### Activity Tests

Activity tests were performed with the isolated strains to determine whether they would produce cheese in an adequate manufacturing time. Steps such as rennet addition, cutting, cooking, draining, and cheddaring were included in the activity test procedure. Acceptable cheese-making performance was based on the ability of the cultures to lower the curd pH to 5.2 or less within a 3-h period. Strains KK-1, KK-2, KK-3, KK-4, and KK-5 were each paired with *Lactobacillus* strains JM-21 or JM-31. Several different inocula concentrations were used to determine whether the starter culture concentration affected the rate of acid production. Table 3 shows the mean curd pH

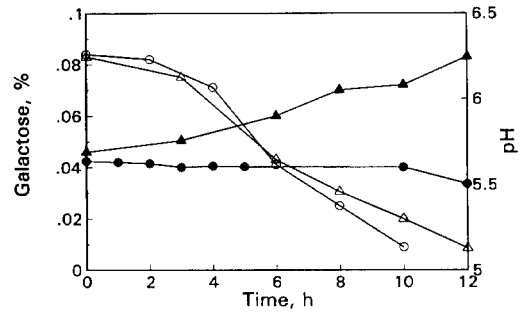


Figure 1. Galactose release (g/100 ml of milk) by *Streptococcus* sp. KK-2 (●) and KK-4 (▲) during growth in reconstituted NDM. The medium pH is shown for KK-2 (○) and KK-4 (△), respectively.

TABLE 2. Galactose-fermenting thermophilic cultures.

| Isolate | Presumptive identification | Source | Phenotype[1] |
|---|---|---|---|
| KK-1 | *Streptococcus* sp. | Emmenthaler (imported) | – |
| KK-2 | *Streptococcus* sp. | Yogurt (plain lowfat) | – |
| KK-3 | *Streptococcus* sp. | Gruyère (imported) | – |
| KK-4 | *Streptococcus* sp. | Parmesan (imported) | + |
| KK-5 | *Streptococcus* sp. | Lab Culture Collection | – |
| KK-6 | *Streptococcus* sp. | Bel Paese (imported) | + |
| KK-7 | *Streptococcus* sp. | Jarlsburg (imported) | ND[2] |
| KK-8 | *Streptococcus* sp. | Yogurt (strawberry) | ND |
| JM-21 | *Lactobacillus delbrueckii* ssp. *bulgaricus* | Lab Culture Collection | – |
| JM-31 | *Lactobacillus helveticus* | Lab Culture Collection | – |
| JM-32 | *L. helveticus* | Lab Culture Collection | + |
| JM-41 | *Lactobacillus* sp. | Swiss (domestic) | + |
| JM-42 | *Lactobacillus* sp. | Yogurt (imported, plain) | + |

[1]+ = Galactose-releasing; – = galactose-nonreleasing.

[2]ND = Not determined.

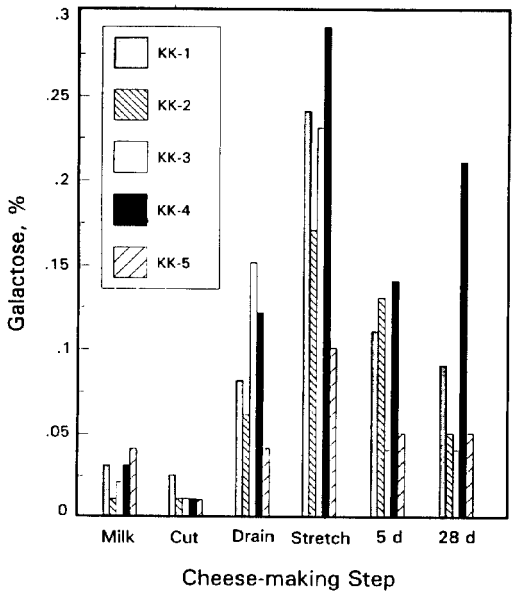


Figure 2. Galactose release during cheese making.

after 3 h for each pair of cultures. The curd pH was reduced to approximately pH 5.2 within 3 h only when strain JM-31 was used as the lactobacilli. When strain JM-21 was used, the curd pH remained above pH 5.6. Inocula, which ranged from 2 to 4%, had no appreciable effect on the rate of acid formation in the curd during the 3-h cheddaring period.

### Sugar Analysis of Cheese

Cheese samples from all five treatments (four experimental and one control) were enzymatically assayed for lactose and galactose concentrations. Mean lactose percentages (wt/wt) in curd after cutting ranged from 2.01 to 2.68% but declined rapidly, such that, after cheddaring and brining, no more than .25% lactose remained in any of the treatments. Results of the galactose analysis for samples taken during cheese making and after 5 and 28 d of storage are shown in Figure 2. As expected, the galactose content in the milk and curd at cutting were all <.05% (wt/wt) for all treatments. During cheddaring, the galactose concentration in the control treatment, which contained the $Gal^+Rel^+$ strain, increased nearly 10-fold to as high as .297%. However, the galactose content for experimental treatments ($Gal^+$, $Rel^-$ *Streptococcus* strains) also increased, although less than that of the control. After 28 d of aging, the galactose concentrations in the experimental treatments had decreased to <.1%. In contrast, galactose in the control cheese remained high (about .2%) and was significantly ($P < .05$) higher than all of the experimental treatments.

### Cheese Proteolysis

Proteolysis, expressed as the amount of free glycine released per gram of cheese, increased during aging for each treatment, although most of this increase occurred only during the first 5 d of aging (Figure 3). On d 0 (after brining), values ranged from 4 to 13 μmol of glycine/g of curd, and, on d 28, values ranged from 19 to 25 μmol of glycine/g of curd. No significant differences existed between experimental and control treatments.

TABLE 3. Activity tests for five strains of *Streptococcus* paired with *Lactobacillus* strains JM-21 and JM-31.

| *Streptococcus* strain | Curd pH after 3 h of cheddaring at 42°C | | | |
|---|---|---|---|---|
| | JM-21[1] | JM-21[2] | JM-31[3] | JM-31[4] |
| KK-1 | 5.63 | 5.65 | 5.17 | 5.17 |
| KK-2 | 5.75 | 5.79 | 5.23 | 5.16 |
| KK-3 | 5.83 | 5.80 | 5.19 | 5.19 |
| KK-4 | 5.79 | 5.80 | 5.10 | 5.18 |
| KK-5 | 5.81 | 5.81 | 5.24 | 5.15 |

[1]1% lactobacilli and 1% streptococci.

[2]2% lactobacilli and 2% streptococci.

[3]1.5% lactobacilli and 1.5% streptococci.

[4]2% lactobacilli and 1.5% streptococci.

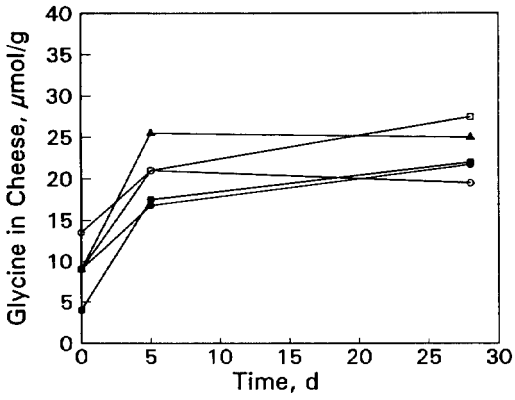


Figure 3. Proteolysis during cheese making using starter cultures KK-1 (●), KK-2 (■), KK-3 (▲), KK-4 (○), and KK-5 (□).

### Cheese Browning

Browning was measured by baking cheese samples (Lincoln Impinger III conveyer oven). Several baking parameters were established. Cheese samples were cooked at time and temperature combinations ranging from 232 to 307°C for 1 to 2 min. The cooled samples were then analyzed with a Hunterlab colorimeter for three color indices using the L* a* b* scale.

Few differences were significant between either the cheese treatments or cooking conditions based on Hunter b* values (yellow to blue; Table 4). The b* value was least affected by cooking severity, and most values did not change significantly between treatments. However, treatments KK-1, KK-2, and KK-3 had significantly ($P < .05$) lower b* values than the control at the lower temperature (5-d cheese), and KK-2 and KK-5 had significantly ($P < .05$) higher values than the control at 28 d. In all cases, b* values remained between 18 and 22.

In contrast, differences in a* values (red to green) became more positive as the severity of the cooking conditions increased, indicating a more intense red color for cooked cheese (Table 5). Significant ($P < .05$) color differences between experimental and control treatments were observed on both 5- and 28-d cheese, but the most pronounced differences occurred with the 28-d cheeses at the more severe baking conditions (307°C for 1.5 min).

Results were similar when L* values (light to dark) were compared (Table 6). The L* values from the experimental cheeses were significantly ($P < .05$) higher (less dark) than those for control cheese for many of the conditions tested. Differences were especially pronounced at the higher cooking temperatures used in this study. Hunter L* values also decreased significantly ($P < .05$) during aging, indicating an increased tendency for aged samples to brown. Hunter values for treatment KK-4 (control) decreased the most during storage, from 62.7 to 67.5 on d 5 to 52.3 to 63.9 on d 28. Treatment KK-1 L* values changed the least, from 63.8 to 69.1 on d 5 to 61.9 to

TABLE 4. Values of the Hunterlab b* (yellow to blue) index of Mozzarella cheese for each treatment on d 5 and 28.[1]

| Temperature | Time | KK-1 | KK-2 | KK-3 | KK-4 | KK-5 |
|---|---|---|---|---|---|---|
| (°C) | (min) | | | d 5 | | |
| 232 | 2.00 | 18.3[a] | 18.7[a] | 18.3[a] | 20.5[b] | 19.2[ab] |
| 260 | 1.25 | 19.2 | 19.9 | 19.4 | 20.5 | 19.6 |
| 288 | 1.00 | 20.0 | 20.0 | 19.8 | 20.4 | 20.2 |
| 307 | 1.00 | 19.7 | 20.1 | 19.8 | 20.6 | 20.2 |
| 307 | 1.25 | 20.2 | 19.8 | 19.9 | 20.6 | 19.2 |
| | | | | d 28 | | |
| 232 | 2.00 | 19.5 | 19.8 | 20.3 | 20.3 | 19.6 |
| 260 | 1.25 | 19.7 | 19.7 | 20.3 | 19.3 | 20.2 |
| 288 | 1.00 | 19.7 | 20.4 | 20.7 | 20.1 | 20.6 |
| 307 | 1.00 | 20.3 | 20.6 | 20.9 | 20.6 | 20.9 |
| 307 | 1.25 | 20.8[ab] | 21.6[a] | 20.6[ab] | 20.5[b] | 21.7[a] |
| 307 | 1.50 | 21.2 | 21.8 | 21.5 | 20.2 | 21.3 |

[a,b]Means in the same row followed by different superscript letters differ ($P < .05$). Means in rows with no superscripts do not differ ($P > .05$).

[1]Standard error = .52.

TABLE 5. Values of the Hunterlab a* (red to green) index of Mozzarella cheese for each treatment on d 5 and 28.[1]

| Temperature | Time | KK-1 | KK-2 | KK-3 | KK-4 | KK-5 |
|---|---|---|---|---|---|---|
| (°C) | (min) | | | d 5 | | |
| 232 | 2.00 | $-2.9^{ab}$ | $-2.7^{ab}$ | $-3.5^{a}$ | $1.7^{b}$ | $3.4^{b}$ |
| 260 | 1.25 | $-3.3^{a}$ | $-3.6^{a}$ | $-3.6^{a}$ | $-2.1^{b}$ | $-4.0^{a}$ |
| 288 | 1.00 | -2.9 | -3.4 | -3.1 | -1.9 | -3.4 |
| 307 | 1.00 | $-2.3^{a}$ | $-2.9^{a}$ | $-3.4^{a}$ | $-.5^{b}$ | $-2.9^{a}$ |
| 307 | 1.15 | -.9 | -1.5 | -2.0 | -.5 | -2.2 |
| | | | | d 28 | | |
| 232 | 2.00 | $-3.5^{a}$ | $-3.0^{ab}$ | $-3.7^{a}$ | $-1.5^{b}$ | $-1.8^{b}$ |
| 260 | 1.25 | -4.2 | -3.8 | -4.4 | -3.7 | -4.2 |
| 288 | 1.00 | -3.9 | -3.3 | -4.4 | -2.9 | -4.1 |
| 307 | 1.00 | $-3.2^{a}$ | $-2.4^{ab}$ | $-3.7^{a}$ | $-1.2^{b}$ | $-3.4^{a}$ |
| 307 | 1.25 | $-2.2^{a}$ | $.3^{bc}$ | $-1.1^{ab}$ | $.7^{c}$ | $-2.3^{a}$ |
| 307 | 1.50 | $-1.7^{a}$ | $1.6^{b}$ | $-1.7^{a}$ | $3.7^{c}$ | $1.3^{b}$ |

[a,b,c]Means in same row followed by different superscripts differ ($P < .05$). Means in rows with no superscripts do not differ ($P > .05$).

[1]Standard error = .62.

65.7 on d 28. Differences were generally not significant between d 5 and d 28 for either the a* or b* indices (data not shown).

### Melt and Free Oil Formation

The melt test was performed by packing grated cheese into the end of a glass tube, which was then heated horizontally in a 100°C oven for 60 min. Melt was measured as millimeters of cheese flow. Melt values for each of the five treatments are given in Table 7. Similar values for the experimental treatments were obtained for samples collected on d 5 and 28. General linear models analysis showed no significant ($P > .05$) difference in melt between culture pairs or between storage times.

Free oil was measured by a method that uses centrifugal force and standard Babcock equipment to separate free oil from melted cheese (8). Values for free oil percentage in cheese are given in Table 8. Free oil increased

TABLE 6. Mean values of the Hunterlab L* (light to dark) index of Mozzarella cheese for each treatment on d 5 and 28.[1]

| Temperature | Time | KK-1 | KK-2 | KK-3 | KK-4 | KK-5 |
|---|---|---|---|---|---|---|
| (°C) | (min) | | | d 5 | | |
| 232 | 2.00 | $63.8^{ab}$ | $63.7^{ab}$ | $65.9^{ab}$ | $63.6^{a}$ | $66.3^{b}$ |
| 260 | 1.25 | 68.1 | 69.3 | 69.2 | 67.5 | 69.5 |
| 288 | 1.00 | 69.1 | 69.0 | 69.1 | 67.4 | 69.7 |
| 307 | 1.00 | $66.8^{ab}$ | $67.6^{bc}$ | $69.6^{c}$ | $64.5^{a}$ | $68.4^{bc}$ |
| 307 | 1.25 | $64.0^{ab}$ | $62.7^{a}$ | $64.8^{ab}$ | $62.7^{a}$ | $66.1^{b}$ |
| | | | | d 28 | | |
| 232 | 2.00 | $63.7^{b}$ | $63.1^{ab}$ | $63.7^{b}$ | $60.9^{a}$ | $64.1^{bc}$ |
| 260 | 1.25 | 65.7 | 65.0 | 65.3 | 63.9 | 66.2 |
| 288 | 1.00 | $64.8^{ab}$ | $64.3^{ab}$ | $65.1^{ab}$ | $63.8^{a}$ | $66.6^{b}$ |
| 307 | 1.00 | $64.4^{b}$ | $63.5^{ab}$ | $64.5^{b}$ | $61.6^{a}$ | $65.4^{bc}$ |
| 370 | 1.25 | $62.3^{bd}$ | $59.2^{a}$ | $63.1^{cd}$ | $57.5^{a}$ | $63.3^{d}$ |
| 307 | 1.50 | $61.9^{c}$ | $57.2^{b}$ | $61.4^{c}$ | $52.3^{a}$ | $61.1^{c}$ |

[a,b,c,d]Means in same row followed by different superscripts differ ($P < .05$). Means in rows with no superscripts do not differ ($P > .05$).

[1]Standard error = .94.

TABLE 7. Mean melt values of Mozzarella cheese after aging 5 and 28 d.[1]

| Treatment | d 5 | | d 28 | |
|---|---|---|---|---|
| | (mm) | | | |
| | $\bar{X}$ | SD | $\bar{X}$ | SD |
| KK-1 | 156.0 | 39.0 | 161.7 | 29.9 |
| KK-2 | 155.2 | 13.9 | 146.8 | 14.9 |
| KK-3 | 132.8 | 52.3 | 127.5 | 21.2 |
| KK-4 | 96.0 | 29.7 | 135.8 | 8.4 |
| KK-5 | 124.3 | 33.4 | 127.3 | 35.1 |

[1]Means of replicate tests from each of three trials. None of the means within rows or columns differed significantly ($P > .05$).

TABLE 8. Mean free oil formation in Mozzarella cheese after aging 5 and 28 d.[1,2]

| Treatment | d 5 | d 28 |
|---|---|---|
| | (%) | |
| KK-1 | 12.5[a] | 14.0 |
| KK-2 | 12.3[a] | 14.4 |
| KK-3 | 12.9[a] | 14.8 |
| KK-4 | 12.7[a] | 14.8 |
| KK-5 | 12.5[a] | 14.0 |

[a]Mean on d 5 differs significantly from that on d 28 ($P < .05$).

[1]Means of replicate tests from each of three trials.

[2]Standard error = .93.

between d 5 and 28 for all treatments. Analysis using general linear models found no significant ($P > .05$) difference in free oil between treatments. However, free oil was significantly ($P < .05$) affected by storage time.

## DISCUSSION

Most strains of *S. salivarius* ssp. *thermophilus* do not ferment galactose but instead prefer disaccharides such as lactose and sucrose (5). During the culture isolation process, cheese and yogurt samples were plated on Elliker-galactose medium. Selection was based on the appearance of yellow colonies, which were considered to be $Gal^+$. Although $Gal^-$ strains were also present in the dairy samples, these latter strains were not selected for further study. Of the 8 $Gal^+$ streptococci isolated, 3 were eliminated as being unsuitable for use as Mozzarella starter cultures because of their inability to grow at ambient atmosphere. Of the 5 remaining $Gal^+$ strains, 4 were classified as $Rel^-$, and 1 was classified as a partial releaser, which was surprising, because most research on *S. salivarius* ssp. *thermophilus* metabolism indicated that few $Gal^+$, $Rel^-$ organisms exist, although most work (5, 13, 15, 17, 18) has been done with laboratory strains.

When strains KK-1, KK-2, KK-3, KK-4, and KK-5 and *Lactobacillus* strain JM-31 were transferred individually from Elliker medium into reconstituted NDM and incubated overnight, coagulation occurred. However, consistently good growth in milk did not occur unless .1 to .5% yeast extract was added to the milk. The slow rate of acid production and the lack of coagulation may be attributed to the inability of these strains to degrade casein. *Streptococcus salivarius* ssp. *thermophilus* strains are weakly proteolytic (5, 12) and are usually combined with more proteolytic strains such as *L. bulgaricus* or *L. helveticus* when used as part of a starter culture. All of the *Streptococcus* strains used as starter cultures showed little or no detectable proteolytic ability (data not shown). Other workers (12) have also reported a wide variation in proteolytic ability among thermophilic *Lactobacillus* cultures. The *Lactobacillus* strains used in this study were also weakly proteolytic (data not shown).

Carbohydrate fermentation in Mozzarella cheese ordinarily occurred during the first few hours of manufacture. Hutkins et al. (4) previously reported that the lactose concentration in curd decreased from 95 to <35 μmol/g during the first 5 h of manufacture. During control and experimental treatments in this study, the lactose content also dropped markedly (<6 μmol/g of curd) within the first 24 h of manufacture. After the cheddaring step, Mozzarella curd is stretched in 82°C water, a step thought to effectively end the fermentation. Clearly, however, starter culture activity was not completely inactivated, and both sugar metabolism and proteolysis occurred during storage, thus accounting for the decrease in galactose from d 5 to d 28.

Galactose content of fresh Mozzarella curd (after brining) made from both experimental and control cultures was very low (<.2%). Hutkins et al. (4) found similar results in Mozzarella made with $Gal^+$ *S. salivarius* ssp. *thermophilus* and $Gal^+$ *L. delbrueckii* ssp. *bulgari-*

*cus*. In the current study, the curd galactose content increased initially during the cheddaring step for all treatments, even those containing Rel⁻ streptococci. We suggest that the galactose present in the experimental treatments had been released by the *Lactobacillus* strain, which had a partial releasing phenotype. Also possible is that the behavior of the Rel⁻ strains in reconstituted NDM is different from that of strains grown in the cheese environment. Importantly, the galactose in the control treatment remained high (about .2%) after 28 d but decreased to between .04 and .09 for the experimental treatments. These values are still much lower than the .8% galactose reportedly (4) found in commercial Mozzarella cheese.

The use of both Gal⁺ *Streptococcus* spp. and Gal⁺ *L. helveticus* strains in the starter culture was probably responsible for the low residual galactose in the cheese. Johnson and Olson (6) reported about .1% galactose (5 m*M*) in Mozzarella made with Gal⁺ *S. salivarius* ssp. *thermophilus* and Gal⁺ *L. delbrueckii* ssp. *bulgaricus* and aged for 28 d. The Rel phenotype of the cultures used by these workers is unknown; however, the control cheese made in the current study with strain KK-4 (Gal⁺, Rel⁺) contained a similar percentage (.2%) of galactose after 28 d of aging.

Results of the browning tests revealed that cheese from the control treatment, KK-4, which contained more galactose than the experimental cheese, also browned more than cheese from the experimental treatments. All treatments showed an increase in browning with aging, and significant differences between treatments occurred most often after the 28-d aging period. As time and temperature of baking conditions became more severe, browning also increased, and significant differences usually occurred between the control and the experimental treatments. Significant differences generally did not occur between the four experimental treatments. Storage time, baking conditions, and strain differences had the greatest impact on the a* and L* indices, but the b* index was largely unaffected, which indicates that the L* index, which expresses light to dark color, is a better measure of browning than the b* index, which expresses blue to yellow color. These findings are in contrast to those of Oberg et al. (10, 11, 12), who reported that Hunter b* values were a valid index of browning. Based on the b* index, Oberg et al. (10) found no significant differences in the cook color of Mozzarella cheese stored over 42-d. In another study, however, Oberg et al. (12) reported an increase in cook color over a 28-d period for Mozzarella made with mixed starter cultures of streptococci and lactobacilli. These differences presumably relate to the manner in which the cheeses were cooked. In the reports cited, cheese was cooked in a water bath at 96°C, but, in our studies, cheese was exposed to much higher temperatures in an oven. The latter probably represents the more usual conditions in which Mozzarella cheese would be cooked.

Oberg et al. (11) measured melt for Mozzarella cheese aged over 28 d. Those researchers found that melt increased rapidly during the first 7 d but changed little with additional aging. It is not surprising that Mozzarella samples tested on d 5 and 28 showed no difference in melt values because any changes probably occurred during the first 5 d of aging.

The percentage of free oil in cheese increased between d 5 and 28 for all treatments. Kindstedt and Rippe (8) also found that free oil in Mozzarella cheese increased with age during refrigerated storage. These researchers also found a mean free oil of 12.8% (SD = 3.8%) in 22 samples of commercial low moisture Mozzarella and a mean free oil of 4.1% (SD = 1.9%) in 22 samples of low moisture, part skim Mozzarella (cheese age unknown but probably varied). Results of Kindstedt and Rippe (8) correspond to the range of free oil, 12.3 to 14.8%, found in this study.

Several strategies have been considered to control browning in Mozzarella cheese. Manufacturers can certainly manipulate cheese-making conditions such that less lactose and galactose remain in the curd (e.g., by washing curds). Selection of suitable nonbrowning cultures (e.g., Gal⁺) has also been suggested. Oberg et al. (12) further suggested that a nonproteolytic Gal⁺ strain of *L. helveticus* would be best for reducing cook color. During aging, nonproteolytic strains would produce fewer amino groups than proteolytic strains and, presumably, less browning. Although proteolysis is certainly important to the results presented here, because all of the *Streptococcus* strains used in this work had similar

proteolytic activity, another factor must be responsible for the differences in brown cook color between treatments. Also, the moisture in all cheese (41 to 43%) was 2 to 4% less than the legal minimum required for low moisture Mozzarella (45%), and these low moisture percentages in Mozzarella cheese may have enhanced browning during cooking. However, no significant moisture differences existed between cheese treatments in this study; thus, we doubt that the low moisture would account for the color differences. Also, final pH for all of the cheeses were the same (5.1 to 5.2), and fat concentrations (on a dry basis) were also the same (46.0 to 46.7%). Only the galactose concentrations were different. We conclude, therefore, that the browning differences between the control and experimental samples of cooked cheese must have been due to the higher levels of galactose released in the control cheese by the action of the $Rel^+$ starter culture.

## ACKNOWLEDGMENTS

We acknowledge the National Dairy Promotion and Research Board for their support of this project and the American Dairy Association of Nebraska for the purchase of the Mozzarella cheese cooker. We thank Laurie Keeler and Monica Metz for cheese-making assistance.

## REFERENCES

1 Alder-Nissen, J. 1979. Determination of the degree of hydrolysis of food protein hydrolysates by trinitrobenzenesulfonic acid. J. Agric. Food Chem. 27:56.

2 Bley, M. E., M. E. Johnson, and N. F. Olson. 1985. Factors affecting nonenzymatic browning of process cheese. J. Dairy Sci. 68:555.

3 Elliker, P. R., A. W. Anderson, and G. Hannesson. 1956. An agar culture medium for lactic acid streptococci and lactobacilli. J. Dairy Sci. 39:1611.

4 Hutkins, R., S. M. Halambeck, and H. A. Morris. 1986. Use of galactose-fermenting *Streptococcus thermophilus* in the manufacture of Swiss, Mozzarella, and short-method Cheddar cheese. J. Dairy Sci. 69:1.

5 Hutkins, R. W., and H. A. Morris. 1987. Carbohydrate metabolism by *Streptococcus thermophilus*: a review. J. Food Prot. 50:876.

6 Johnson, M. E., and N. F. Olson. 1985. Nonenzymatic browning of Mozzarella cheese. J. Dairy Sci. 68:3143.

7 Kindstedt, P. S. 1993. Effect of manufacturing factors, composition, and proteolysis on the functional characteristics of Mozzarella cheese. Crit. Rev. Food Sci. Nutr. 33:167.

8 Kindstedt, P. S., and J. K. Rippe. 1990. Rapid quantitative test for free oil (oiling-off) in melted Mozzarella cheese. J. Dairy Sci. 73:867.

9 Kosikowski, F. V. 1977. Cheese and Fermented Milk Foods. 2nd ed. Edward Brothers, Inc., Ann Arbor, MI.

10 Oberg, C. L., R. K. Merrill, R. J. Brown, and G. H. Richardson. 1992. Effects of freezing, thawing, and shredding on low moisture, part skim Mozzarella cheese. J. Dairy Sci. 75:1161.

11 Oberg, C. J., R. K. Merrill, L. V. Moyes, R. J. Brown, and G. H. Richardson. 1991. Effects of *Lactobacillus helveticus* culture on physical properties of Mozzarella cheese. J. Dairy Sci. 74:4104.

12 Oberg, C. J., A. Wang, L. V. Moyes, R. J. Brown, and G. H. Richardson. 1991. Effects of proteolytic activity of thermolactic cultures on physical properties of Mozzarella cheese. J. Dairy Sci. 74:389.

13 O'Leary, V. S., and J. H. Woychik. 1976. Utilization of lactose, glucose, and galactose by a mixed culture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in milk treated with lactase enzyme. Appl. Environ. Microbiol. 32:89.

14 SAS User's Guide, Version 6. 1989. SAS Inst., Inc., Cary, NC.

15 Somkuti, G. A., and D. H. Steinberg. 1979. Adaptability of *Streptococcus thermophilus* to lactose, glucose, and galactose. J. Food Prot. 42:885.

16 Stratton, J. E. 1990. Histamine production in low-sodium Cheddar cheese. M.S. Thesis, Univ. Nebraska-Lincoln.

17 Thomas, T. D., and V. L. Crow. 1984. Selection of galactose-fermenting *Streptococcus thermophilus* in lactose-limited chemostat cultures. Appl. Environ. Microbiol. 48:186.

18 Tinson, K., A. J. Hillier, and G. R. Jago. 1982. Metabolism of *Streptococcus thermophilus*. 1. Utilization of lactose, glucose, and galactose. Aust. J. Dairy Technol. 38:8.

19 United States Department of Agriculture. 1991. Dairy Products 1990 Summary. USDA, Washington, DC.