Effect of Exopolysaccharide-Producing Cultures on Moisture Retention in Low Fat Mozzarella Cheese[1]

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ABSTRACT

Low fat (6%) Mozzarella cheese was made in 10-L vats using an exopolysaccharide-producing starter culture consisting of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. A control cheese was made using strains of *S. thermophilus* and *Lactobacillus helveticus* that did not produce exopolysaccharide. Both starter cultures were also used with the addition of a mesophilic exopolysaccharide-producing adjunct culture consisting of *Lactococcus lactis* ssp. *lactis* and *L. lactis* ssp. *cremoris* strains. Moisture content of the cheese was measured at d 1, and melt was measured at 1, 7, 14, and 28 d of storage at 4°C. Analysis of variance showed significant differences in moisture and melting properties between cheeses made with or without exopolysaccharide-producing starter cultures. Cheeses made with the addition of the adjunct culture showed significant differences in moisture, but not in melting properties. The moisture content of cheeses made with both the exopolysaccharide-producing starter and the adjunct cultures increased 4%, and the use of the exopolysaccharide-producing starter cultures alone increased moisture content 3% over that of the control cheese. Melt also increased in cheeses as moisture content increased.

(**Key words**: Mozzarella cheese, low fat, moisture, polysaccharide, capsule)

Abbreviation key: **EPS** = exopolysaccharide-producing.

INTRODUCTION

Removal of fat from Mozzarella cheese affects several physical properties of the cheese (15). For low fat Mozzarella cheese, fewer fat globules allow increased coalescence of the casein strands in the curd as it is cooked and stretched (18). This process results in a shrinkage of the curd so that less space is available for entrapment of serum in the curd. Thus, more syneresis occurs, and the serum is expelled as whey. Such low fat cheese tends to become tough, rubbery, and have poor stretching properties (12, 13, 17, 20).

The manufacturing procedures of reduced-fat and low fat Mozzarella cheese have been modified in an attempt to increase the moisture content of the cheese. Merrill et al. (16) found that elevated pasteurization temperatures, preacidification of milk, larger cutting knives, and lower cooking temperatures helped to increase the moisture retention of reduced-fat Mozzarella cheese. Fat replacers in low fat Mozzarella cheese have been effective in retaining moisture; however, the melting and stretching properties of the cheese may still be inadequate (14). Such fat replacers included those based on microparticulation of proteins as well as those derived from insoluble polysaccharides (such as cellulose) and polysaccharides (such as starch) that function to bind water and thus increase the moisture content of the cheese.

A wide variety of exocellular polysaccharides are produced by lactic acid bacteria, including many of the thermophilic organisms (7, 11). Cerning et al. (4, 5) found that certain strains of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*, when grown in skim milk, produce exopolysaccharides that are primarily composed of glucose and galactose monomers. The exopolysaccharide-producing (**EPS**) trait of these strains can be very unstable, and the composition of the exopolysaccharide varies with environmental factors and the available carbon sources (1, 6, 8). Because they can absorb water and retard whey expulsion, such EPS cultures have been used to improve rheological behavior and texture in fermented dairy foods such as yogurt (3, 22).

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TABLE 1. Composition of low fat Mozzarella cheese made using exopolysaccharide-producing (EPS) starter cultures and control (non-EPS) starter cultures (n = 3).

Vat	Starter	Adjunct	Moisture		Fat		Protein	
			(%)					
			$\bar{X}$	SEM	$\bar{X}$	SEM	$\bar{X}$	SEM
1	Non-EPS	. . .[1]	58.2	0.50	6.3	0.33	26.3	1.70
2	EPS	. . .	61.0	0.47	6.2	0.20	25.9	0.63
3	Non-EPS	EPS	60.9	0.12	6.2	0.20	26.5	0.60
4	EPS	EPS	62.2	0.38	6.4	0.20	24.7	0.63

[1]No adjunct culture.

The objectives of this study were to determine whether EPS starter or EPS adjunct cultures could be used to retain more moisture in a low fat (6%) Mozzarella cheese and, in addition, to determine whether differences in moisture content could affect the melting properties of the cheese.

MATERIALS AND METHODS

Milk and Cultures

Skim milk and cream from the Gary H. Richardson Dairy Products Laboratory (Utah State University, Logan) were pasteurized at 80°C for 29 s and then cooled overnight at 4°C. Skim milk was standardized to 0.6% fat using cream of known fat content. Lyophilized cultures (Rhône-Poulenc, Marschall Products, Madison, WI) that can be added directly to the vat, consisting of *S. thermophilus* TA061 and *Lactobacillus helveticus* LH100, were weighed into separate sterile test tubes and stored at 4°C until used. An EPS mixed-pair starter culture consisting of *S. thermophilus* MR-1C and *L. delbrueckii* ssp. *bulgaricus* MR-1R (from the Department of Microbiology culture bank at Weber State University, Ogden, UT) was grown in medium with internal pH control (Sure Set XL®; Waterford Foods, Millville, UT) to pH 4.4 1 d prior to use and stored at 4°C. An EPS-producing adjunct culture DSG-HB as a direct-to-vat set frozen pellet (Chr. Hansen's Laboratory, Milwaukee, WI), consisting of *Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris*, was weighed into sterile test tubes and stored at –70°C.

In preliminary experiments, manufacturing time was observed to affect cheese moisture. Therefore, starter culture inocula were selected to provide acid development and manufacturing times (from cutting to stretching) corresponding to industry practices (150 min ± 10 min).

Manufacturing Procedure

Four stainless steel vats (34 × 22 × 22 cm) were each filled with 10 kg of standardized milk and preacidified to pH 6.0 using acetic acid diluted 1:10 (vol/vol) with distilled water. The milk in each vat was heated in a water bath to 34°C. The milks in vats 1 (control) and 3 were inoculated with a non-EPS starter culture of *S. thermophilus* TA061 and *L. helveticus* LH100 (1.0 g and 0.75 g, respectively). The milk in vats 2 and 4 was inoculated with the mixed-pair EPS starter culture of *S. thermophilus* MR-1C and *L. delbrueckii* ssp. *bulgaricus* MR-1R (75 and 50 ml, respectively).

After 40 min of ripening, 10 g of the mesophilic EPS adjunct culture DSG-HB were added to the milk in vats 3 and 4. After an additional 5 min of ripening, 0.75 ml of double-strength fermentation-derived chymosin (Chymax®; Pfizer Inc., Milwaukee, WI), diluted in 10 ml of distilled water, was added to each vat. Curd was cut into 1.9-cm cubes 20 min after

TABLE 2. Analysis of variance for melt of low fat Mozzarella cheese as a function of starter culture and adjunct culture during 28 d of storage at 4°C.

Source of variation	df	MS	*F*
Replicates	2	40.17	5.04^{NS}[1]
Starter culture (S)	1	174.80	21.93**
Adjunct culture (A)	1	20.20	2.53^{NS}
S × A	1	14.08	1.77^{NS}
Error A	6	7.97	
Time (T)	3	8.44	11.22***
S × T	3	3.88	4.41*
A × T	3	0.62	0.70^{NS}
S × A × T	3	0.34	0.39^{NS}
Error B	24	0.88	
Corrected total	47		

[1]$P > 0.05$.

*$P \leq 0.05$.

**$P \leq 0.01$.

***$P \leq 0.001$.

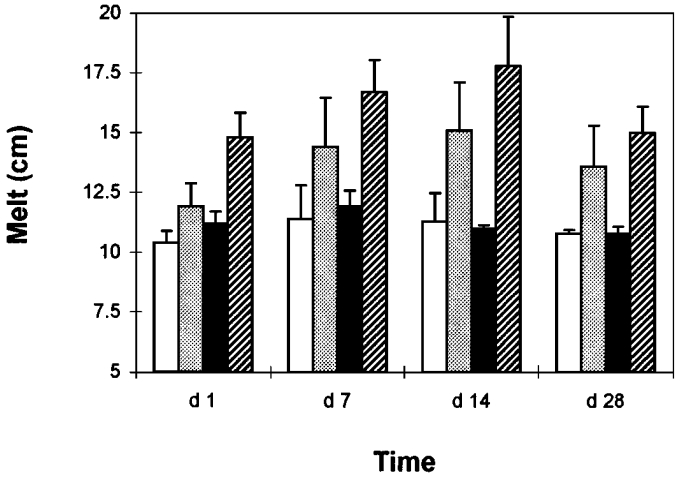


Figure 1. Effect of exopolysaccharide-producing (EPS) cultures on the meltability of low fat Mozzarella cheese during 28 d of storage at 4°C. Cultures used were non-EPS starter (open bar), EPS starter (light bar), non-EPS starter plus EPS adjunct (dark bar), and EPS starter plus EPS adjunct (striped bar). Error bars represent individual standard errors for each mean (n = 3).

rennet addition. After cutting, the curd in each vat was allowed to heal for 15 min and then gently agitated for 15 min. Curd was then heated to a cooking temperature of 39°C. When 39°C was reached (pH was 6.0), 5 kg of whey were drained from each vat. The curd was gently stirred at 39°C in the remaining whey every 5 min until the curd pH reached 5.35, and the whey were drained. Ten minutes following the final drain, the curd was salted by dry-stirring 1.0% (wt/wt) salt in each vat. At curd pH of 5.2, the curd was hand-stretched in a 5% brine at 82°C, then put into stainless steel molds (9 × 9 × 9 cm), and cooled in a ice bath for 1 h. Blocks of cheese were vacuum-packaged and stored at 4°C.

Cheese Analysis

After 1 d of storage, cheese was analyzed for moisture using a vacuum oven, and moisture was determined as weight loss (2). Fat content was determined using a modified Babcock method (21). Protein was determined by measuring total nitrogen using the Kjeldahl method (2). Melt was determined at 1, 7, 14, and 28 d using a tube test method modified with a higher heating temperature (110°C) for 1 h (19).

Analyses of variance were run separately for the dependent variables, moisture, and meltability, based on three independent replicates of each treatment. A split-plot design was used for meltability; cultures were the whole-plot effect, and storage time was the split-plot effect. A randomized block design was used to analyze moisture content. Correlations, means, and analyses of variance were calculated using JMP™ software (10).

Microscopy

Samples of cheese were cut into pieces (3 mm × 3 mm × 10 mm) using a sterile blade, were immersed in 2% glutaraldehyde in a 0.85% saline buffer, and were stored at 5°C. The cheese samples were then prepared for scanning electron microscopy using the method of Oberg et al. (18). Samples of the starter cultures were sent to Joseph Frank (University of Georgia, Athens) for confocal microscopy imaging to determine presence and size of any polysaccharide capsules formed by the cultures (9).

RESULTS AND DISCUSSION

Cheese Composition

On d 1, fat percentages (6.0 to 6.4%) of all cheeses were similar and were appropriate for a low fat cheese (Table 1), and pH was 5.27 ± 0.03. Addition of the EPS starter culture or the EPS adjunct culture significantly increased ($P < 0.001$) cheese moisture retention. Cheese made with EPS adjunct culture (vat 3), EPS starter culture (vat 2), or both EPS starter and adjunct cultures (vat 4) had 2, 3, and 4% more moisture, respectively, than did the control

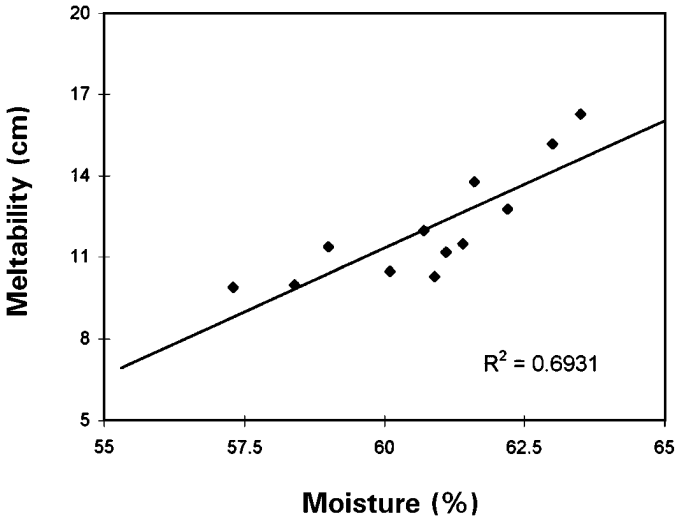


Figure 2. Correlation between cheese moisture content and cheese meltability on d 1 of low fat Mozzarella cheese made using exopolysaccharide-producing (EPS) and non-EPS cultures.

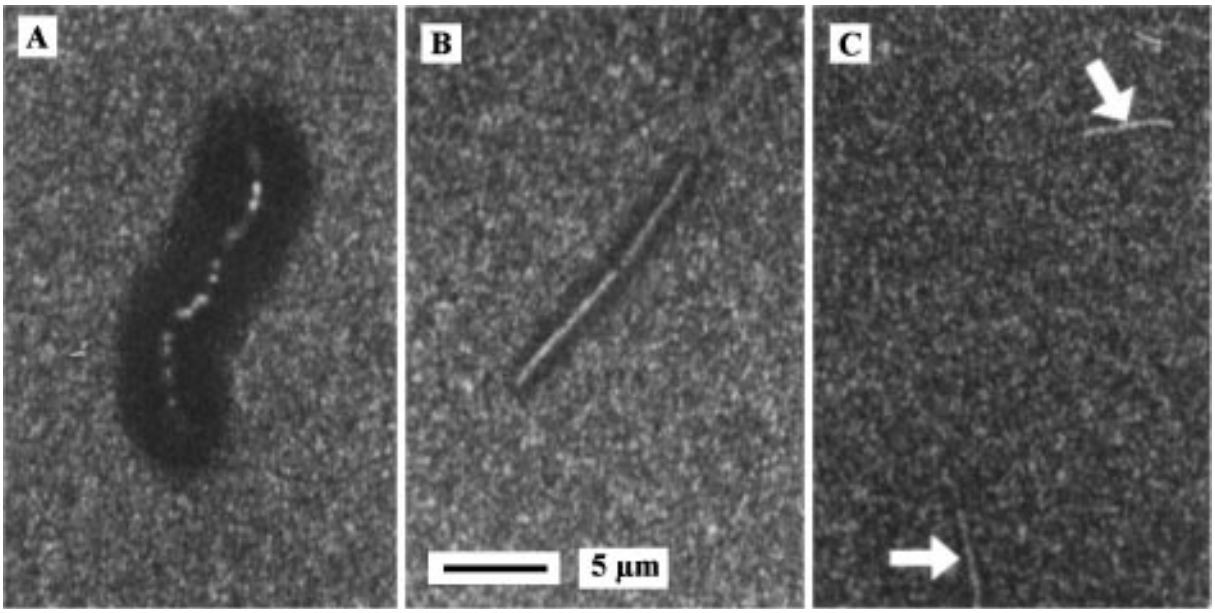


Figure 3. Confocal micrographs of *Streptococcus thermophilus* MR-1C (A), *Lactobacillus delbrueckii* ssp. *bulgaricus* MR-1R (B), and *Lactobacillus helveticus* LH100 (C) grown in skim milk. Arrows (C) show bacteria that are difficult to observe when no capsule is present.

cheese (vat 1) made with non-EPS cultures (Table 1). Even at the highest moisture percentage (62.2%), no whey leakage was observed during 28 d of storage, showing that the moisture was stabilized within the cheese matrix.

The moisture content of the low fat cheeses may have been too high for optimal shredability. All cheeses were too soft and gummy to shred after 28 d of storage. Cheeses with the highest moisture contents became difficult to shred by d 7.

Cheese Melt

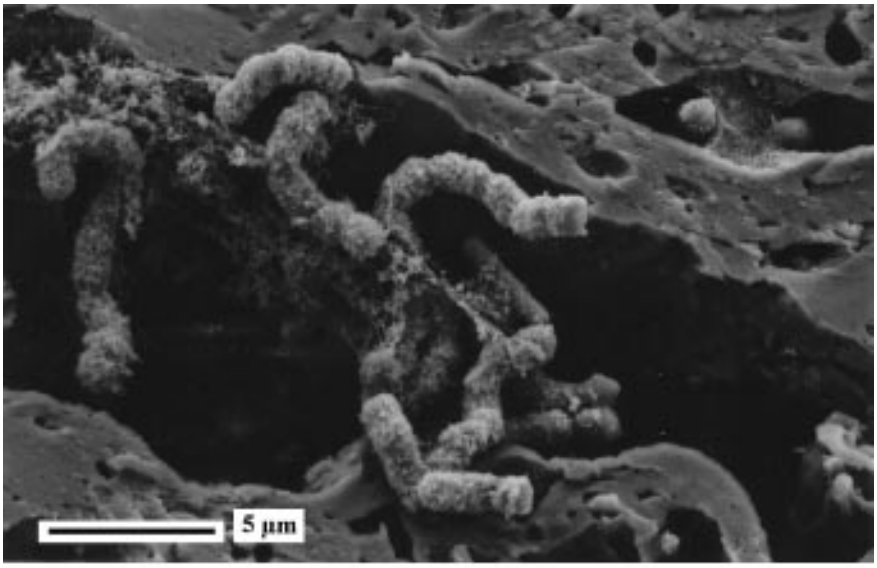
Use of EPS starter culture and storage time (Table 2) affected ($P < 0.05$) cheese melting, but use of the EPS adjunct culture did not ($P > 0.05$). The interaction between starter culture and storage time was also significant. Meltability of cheese made with the non-EPS starter cultures did not increase during the 28 d of ripening (Figure 1). In contrast, cheese made with EPS starter culture increased in meltability from d 1 to d 14 and then decreased in meltability from d 14 to d 28. The cheese with the highest meltability, at all days of storage, was made using the EPS starter culture plus the EPS adjunct culture. Addition of the EPS adjunct culture to the control (non-EPS) starter culture did not increase meltability of the cheese.

In general, cheese with higher moisture content had higher meltability (Figure 2). Application of this result for Mozzarella cheese would suggest that increasing moisture content is a crucial strategy for the manufacture of a low fat Mozzarella cheese that will melt adequately when baked on a pizza.

Formation of Exopolysaccharide Capsules

Although use of the EPS starter culture increased cheese moisture contents and cheese meltability, use of the EPS adjunct culture plus the non-EPS starter culture increased cheese moisture contents but did not improve cheese meltability. This difference in functional performance of EPS cultures can be related

Figure 4. Scanning electron micrographs of low fat Mozzarella cheese manufactured using exopolysaccharide-producing (EPS) starter cultures *Streptococcus thermophilus* MR-1C and *Lactobacillus delbrueckii* ssp. *bulgaricus* MR-1R (top), non-EPS starter cultures *S. thermophilus* TA061 and *Lactobacillus helveticus* LH100 (middle), and EPS starter cultures (*S. thermophilus* MR-1C and *L. delbrueckii* ssp. *bulgaricus* MR-1R) combined with an EPS adjunct culture (*Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris*) (bottom).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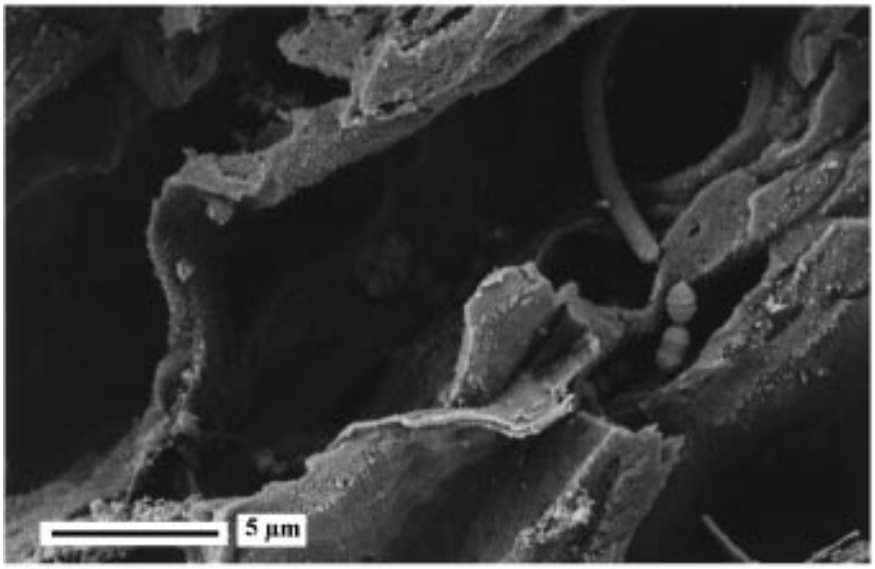
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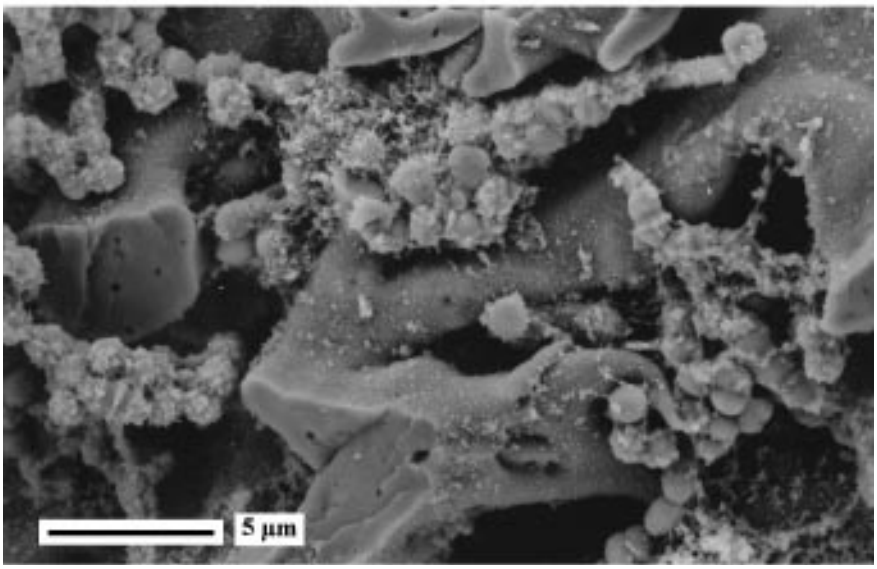
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to the manner in which they produce exopolysaccharides. After the exopolysaccharide is produced, some cultures release the exopolysaccharide into the surrounding environment, but, in other cultures, exopolysaccharide remains attached to the cell wall.

When the EPS starter cultures were grown in milk, an exopolysaccharide capsule surrounded each cell (Figure 3). Confocal scanning micrographs of the EPS starter cultures showed clear zones around the bacteria that were indicative of capsule formation from exopolysaccharide material. This anatomical trait was not observed for the EPS adjunct cultures or the non-EPS cultures, suggesting that cultures maintaining their exopolysaccharide as a capsule (such as the EPS starter cultures) are better suited for use in the manufacture of low fat cheeses than those that release their exopolysaccharides into the surrounding environment.

Streptococcus thermophilus MR-1C produced the largest capsule, measuring 3 μm from the cell wall to the outer edge of the capsule. *Lactobacillus delbrueckii* ssp. *bulgaricus* MR-1R had a capsule measuring 1 μm. The non-EPS starter cultures (*S. thermophilus* TA061 and *L. helveticus* LH100) and the EPS adjunct culture (*L. lactis* ssp. *lactis* and *L. lactis* ssp. *cremoris*) did not produce capsules, as demonstrated by the lack of clear zones around the surface of their cells.

Cheese Microstructure

The differences in capsule formation between the EPS starter cultures and the EPS adjunct cultures also influenced their effect on cheese microstructure. In scanning electron micrographs of cheese made with EPS starter cultures, the bacteria appeared to be covered with remnants of the dehydrated exopolysaccharide capsule, giving them a fuzzy appearance (Figure 4, top photograph). This appearance is typical of polysaccharide gels observed using scanning electron microscopy. Unless the gel is physically restrained, it collapses during the dehydration step of sample preparation.

Based upon measurements of capsule size, obtained using confocal scanning microscopy, the hydrated capsular material would have been large enough to fill the void spaces in the protein matrix. Oberg et al. (18) showed that much of the water in Mozzarella cheese is contained in the columns of serum and fat that separate the protein fibers formed during cooking and stretching of the cheese curd. During stretching, the protein matrix fuses together into fibers, and the fat globules become closely packed between the fibers preventing further fusion of the protein matrix. If Mozzarella cheese is made with reduced levels of fat, more fusion of the protein matrix can occur, and the columns of serum and fat become smaller, resulting in less space for water to be retained in the cheese curd.

Using a capsule-forming EPS culture for the manufacture of low fat Mozzarella facilitated the formation of serum channels in the cheese (Figure 4, top photograph). The presence of polysaccharides around the two bacterial cells (Figure 4, upper right of top photograph) acted as a bridge between fibers in the protein matrix, blocking coalescence of the proteins and forming a serum cavity. This increased openness of the cheese microstructure explains why the EPS starter culture was more effective at increasing moisture retention in the cheese than the EPS adjunct culture. In addition to binding free water, the polysaccharide gel capsules that formed around the EPS starter culture bacteria produced more columns containing serum and fat.

In contrast to the EPS starter cultures, cells of the non-EPS starter cultures had comparatively smoother surfaces (Figure 4, middle photograph). The bacteria in the cheese made using the EPS starter culture combined with the EPS adjunct culture were covered with dehydrated EPS material (Figure 4, bottom photograph). Compared with bacteria in cheese that had been made using the EPS starter culture alone (vat 2), the bacteria in cheese made with the EPS starter culture and the EPS adjunct culture (vat 4) were present more often as aggregates than as individual chains. It may be that the released exopolysaccharide (produced by the adjunct cultures) interacts with the exopolysaccharides of the starter culture capsules. These large aggregates of bacteria would further promote openness in the cheese matrix and act to provide maximum moisture retention and cheese melting properties.

Another consequence of the EPS cultures is a 10-fold difference in bacterial numbers in cheese on d 1 (10^8 cfu/g vs. 10^7 cfu/g when using non-EPS cultures). Presumably, the sticky nature of the exopolysaccharides increases retention of the bacterial cells in the cheese curd during whey drainage. The electron micrographs also showed that more cells were apparent in the EPS cheese.

CONCLUSIONS

We demonstrated that EPS cultures can be useful to increase moisture retention in low fat Mozzarella cheese. A similar effect may be observed in low

moisture, part-skim Mozzarella cheese. Increasing the moisture of low fat Mozzarella cheese can improve its melting properties. The EPS cultures could be used as an alternative for producing low fat cheeses without fat replacers. Exopolysaccharide-producing cultures that form attached capsules are more effective for increasing cheese moisture content than are EPS cultures that do not form capsules. Further work is needed to determine the optimal moisture for low fat Mozzarella cheese for good meltability without becoming too soft to shred upon aging and to adapt this process to commercial scale.

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