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The Effect of Exopolysaccharide-Producing Cultures on the Moisture Retention and Functional Properties of Low Fat Mozzarella Cheese

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THE EFFECT OF EXOPOLYSACCHARIDE-PRODUCING CULTURES ON THE

MOISTURE RETENTION AND FUNCTIONAL PROPERTIES OF

LOW FAT MOZZARELLA CHEESE

by

David B. Perry

A thesis submitted in partial fulfillment
of the requirements for the degree

of

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ABSTRACT

The Effect of Exopolysaccharide-Producing Cultures on the Moisture

Retention and Functional Properties of Low Fat Mozzarella Cheese

by

David B. Perry, Master of Science

Utah State University, 1999

Major Professor: Dr. Donald J. McMahon
Department: Nutrition and Food Sciences

Low fat Mozzarella cheese was made using exopolysaccharide-producing starter cultures consisting of single strains of *Streptococcus thermophilus* MR-1C and *Lactobacillus delbrueckii* ssp. *bulgaricus* MR-1R with or without the addition of mesophilic exopolysaccharide-producing adjunct mixed culture consisting of *Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris*. A control cheese was made using a non-exopolysaccharide-producing starter culture consisting of *S. thermophilus* TA061 and *Lactobacillus helveticus* LH100. Cheeses were analyzed for moisture, melt, fat, and protein. Cheeses made with the addition of the mesophilic exopolysaccharide-producing adjunct culture showed significant differences in moisture, but not in melting properties when compared to cheeses made without adjunct culture. Cheeses made with both the exopolysaccharide-producing starter and exopolysaccharide-producing adjunct cultures showed a 4% increase in moisture, but the use of the exopolysaccharide-producing starter

cultures alone produced a 3% increase in moisture over the control cheese. Melt also increased in these cheeses as moisture increased.

The same cultures were used to determine the effects on moisture when the cheesemaking procedure was scaled up from 10-kg vats to using 454-kg horizontal blade double-O vats, and hand stretching was replaced by an Alfa Laval cooker stretcher machine. Cheese made using the exopolysaccharide-producing cultures showed a 2% increase in moisture over cheese made using non-exopolysaccharide-producing cultures. All of the cheeses made in the double-O vats showed a decrease in moisture compared to cheeses made in the 10-kg stainless steel vats. Cheeses with elevated moisture levels showed increased melt.

(61 pages)

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David B. Perry

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LIST OF ABBREVIATIONS

EPS = exopolysaccharide

CHAPTER 1

GENERAL INTRODUCTION

The removal of fat from Mozzarella cheese can result in low moisture cheese, making it tough and rubbery causing an adverse affect on the functional properties of the cheese. Higher levels of moisture are needed in low fat Mozzarella cheese to provide sufficient melt when the cheese is baked on a pizza. However, the moisture content of the cheese must be increased without causing any "weeping" or loss of moisture over 28 d of storage. If the moisture content in the cheese is too high, it will be difficult to shred after proteolysis has taken place during the aging of the cheese. Different strains of lactic acid bacteria produce exopolysaccharide (EPS) material and maintain it as a capsule or release it into the surrounding medium. Cultures that maintain the EPS as a capsule are able to bind moisture and prevent syneresis in the production of different dairy products.

The purpose of this study is to determine if EPS-producing starter and adjunct cultures can be used to increase moisture levels in low fat (6% fat) Mozzarella cheese. An additional query was whether or not increased moisture levels would affect the meltability of the cheese.

The objectives of this study were:

1. To determine if EPS-producing starter and adjunct cultures can be used to increase moisture levels in low fat (6% fat) Mozzarella cheese.

2. To determine if such changes in moisture levels of the low fat Mozzarella cheese improve melting properties of the cheese.

CHAPTER 2

LITERATURE REVIEW

LOW FAT MOZZARELLA CHEESE

Consumer demands for reduced-fat and low fat food products have created a market for the research and production of dairy products with lower fat content (26, 33, 37). Due to the increased popularity of Mozzarella cheese, and its use on pizza, it has been targeted for the low fat cheese market. Mozzarella cheese production (which totaled 2.62 billion pounds) accounted for 79% of the production of Italian-type cheeses in 1994 and showed a 5% increase in production over 1993. The pizza industry has proved to be a major factor in the increase of Mozzarella cheese production and has, therefore, required cheese makers to produce Mozzarella with functional properties suitable for pizza. Increasing competition between rival pizza companies has fostered a need to supply new ideas and concepts to be used in the promotion of new pizza products. Ever-increasing nutrition awareness by consumers has created a segment in the Mozzarella market for reduced-fat and low fat products. Low-moisture part-skim Mozzarella is the cheese frequently made in the US for use on pizzas and is lower in fat than many other common cheese varieties, such as Cheddar. This makes Mozzarella cheese a good target for creation of reduce or low fat products. Although the demand is high for lower fat cheeses, the window of consumer acceptance is very narrow. For low fat products to succeed, they must sufficiently mimic flavor and functionality of their full-fat cheese counterparts. Tunick et al. (37) found low-moisture, low fat Mozzarella cheese was too

hard and lacked in sufficient melt when compared to a full-fat Mozzarella cheese unless it was aged for 6 wk. Low fat Mozzarella cheese for pizza should exhibit good shredding, melting, and stretching properties, and be free from any off-flavors or textural defects (24).

MOISTURE IN MOZZARELLA CHEESE

In low fat Mozzarella, there are fewer fat globules, which allows the casein strands to become more compact as the curd is forming (27). Without sufficient fat globules available to form fat and water columns in the cheese during stretching, the protein network becomes more compact with less available space for water, making it difficult to retain moisture in the final cheese product. Decreased moisture levels in low-fat Mozzarella cheese results in a cheese with a tough, rubbery texture, along with poor melting and stretching properties (20, 22, 28, 29).

Modifications in the make procedures of reduced-fat and low fat Mozzarella cheese have been aimed at retaining more moisture in the cheese. Merrill et al. (25) found that elevated pasteurization temperatures, milk pre-acidification, larger cutting knives, and lower cook temperatures helped retain moisture in reduced-fat (10% fat) Mozzarella cheese. The use of fat replacers in low fat Mozzarella cheese has also proven effective in retaining moisture; however, the melt and stretch properties may still be inadequate (23, 26). In addition, some of these processing modifications may have adverse effects on the curd quality during cheesemaking, leading to inconsistencies in the final cheese product and to decreased cheese yield.

STARTER CULTURES

By using EPS-producing starter and adjunct cultures, a cheesemaker could increase cheese moisture without compromising curd integrity throughout the cheese-making process. The most commonly used starter cultures for the production of Mozzarella cheese are *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. *Lactobacillus helveticus* is often used in place of *L. delbrueckii* ssp. *bulgaricus* particularly to ferment residual galactose in the cheese. A symbiotic relationship exists between these cultures, making them very useful for the production of different cheese and yogurt products. *S. thermophilus* promotes the initial acid drive, producing lactic acid and decreasing curd pH. *S. thermophilus* also produces formic acid and carbon dioxide, which stimulate the growth of the *L. delbrueckii ssp. bulgaricus* and *L. helveticus* (2, 10, 31, 36). *L. delbrueckii ssp. bulgaricus* is more proteolytic and produces peptides and amino acids from the milk caseins, which stimulate the growth of *S. thermophilus* (17, 18, 21, 30, 31, 36).

S. thermophilus could be used alone to provide the needed acid drive; however, *S. thermophilus* provides little proteolysis in comparison with *L. delbrueckii ssp. bulgaricus* or *L. helveticus* (16, 35). Proteolysis is very important during aging of the Mozzarella cheese to provide proper texture and melting characteristics.

EXOPOLYSACCHARIDE-PRODUCING STARTER CULTURES

Various exocellular polysaccharides or exopolysaccharides (EPS) are produced by lactic acid bacteria, including many of the thermophilic organisms (4, 7, 8). Cerning et al. (5, 6) found that certain strains of *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus* grown in skim milk were capable of producing an EPS composed primarily of glucose and galactose monomers. The EPS-producing trait of these bacterial strains can be very unstable, and the composition and yield of the EPS produced may differ depending on environmental factors and carbon sources available (9, 11, 12, 19, 34). Some cultures produce a capsule of polysaccharide material that is associated with the outer surface of the cell wall, while others release their polysaccharide material into the surrounding environment (13, 14). Cultures that produce exopolysaccharides have been used to improve rheological behavior and texture in fermented dairy products because EPS can bind free water and slow whey separation (1, 15, 32). EPS-producing starter cultures have been especially useful to improve the texture and viscosity of fermented yogurt products in France and the Netherlands, where the use of stabilizers is prohibited (3).

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CHAPTER 3

EFFECT OF EXOPOLYSACCHARIDE-PRODUCING CULTURES ON MOISTURE RETENTION IN LOW FAT MOZZARELLA CHEESE[1]

ABSTRACT

Ten-liter vats of low fat (6% fat) Mozzarella cheese were made using an exopolysaccharide-producing starter culture consisting of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. A control cheese was made using single strains of *S. thermophilus* and *Lactobacillus helveticus* that did not produce exopolysaccharide. Both starter cultures were used with the addition of a mesophilic EPS-producing adjunct culture consisting of *Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris* strains. Moisture 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 EPS-producing starter cultures. Cheeses made with the addition of the mesophilic EPS-producing adjunct culture showed significant differences in moisture, but not in melting properties. Cheeses made with both the EPS-producing starter and EPS-producing adjunct cultures showed a 4% increase in moisture. The use of the EPS-producing starter cultures alone produced a 3% increase in moisture over the control cheese. Melt also increased in cheeses as moisture increased.

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 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 and rubbery and has 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 moisture of the cheese. Merrill et al. (16) found that elevated pasteurization temperatures, milk pre-acidification, larger cutting knives, and lower cooking temperatures helped to increase moisture retention in reduced-fat Mozzarella cheese. Fat replacers in low fat Mozzarella cheese have been effective in retaining moisture; however, the melt and stretch properties of these cheeses may still be inadequate (14).

Various 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* grown in skim milk produce exopolysaccharides primarily composed of glucose and galactose monomers. The EPS-producing trait of these strains can be very unstable, and the composition of the exopolysaccharide varies with environmental factors and the carbon sources available (1, 6, 8). The EPS-producing cultures have been used to improve

rheological behavior and texture in fermented dairy products because they can imbibe water and retard whey expulsion (3, 22).

The objective of this study was to determine whether EPS-starter and EPS-adjunct cultures could be used to retain more moisture in a low fat (6% fat) Mozzarella cheese. In addition, we wanted to determine whether differences in moisture could affect the melting property of the cheese.

MATERIALS AND METHODS

Milk and Cultures

Skim milk and cream from the G.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. Direct-vat set lyophilized cultures, consisting of *S. thermophilus* TA061 and *Lactobacillus helveticus* LH100 (Rhodia, Marschall Products, Madison, WI), 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 Sure Set XL® internal pH-controlled medium (Gist-brocades, Millville, UT) to pH 4.4 one day prior to use and stored at 4°C. An EPS-producing direct-vat set frozen pellet adjunct culture DSG-HB (Chr. Hansen's Laboratory, Milwaukee, WI), consisting of *Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris* strains, was weighed into sterile test tubes and stored at -70°C.

Manufacturing Procedure

Four stainless steel vats (34 x 22 x 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 milk in vats 1 (control) and 3 was inoculated with the non EPS-producing *S. thermophilus* TA061 (1.0 g and 0.75 g) and *L. helveticus* LH100 (1.0 g and 0.75 g). The milk in vats 2 and 4 was inoculated with the EPS-producing starter cultures, *S. thermophilus* MR-1C and *L. delbrueckii* ssp. *bulgaricus* MR-1R (75 and 50 ml, respectively). In preliminary experiments, manufacturing time had an effect on cheese moisture, so starter culture levels were selected to provide comparable acid development and manufacturing times (from cutting to stretching) corresponding to industry practices (150 min ± 10 min).

After 40 min of ripening, 10 g of the mesophilic EPS mixed-adjunct culture DSG-HB was added to the milk in vats 3 and 4. After an additional 5 min of ripening, 0.75 ml of double- strength Chymax® (Chr. Hansen's Laboratory 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 rennet addition. After cutting, the curd in each vat was allowed to heal for 15 min, followed by 15 min of gentle agitation. Curd was then heated to a cook temperature of 39°C. When 39°C was reached (curd pH was still 6.0), 5 kg of whey was drained from each vat. The curd was gently stirred at 39°C in the remaining whey every 5 min until the final curd pH reached 5.35, when the remaining whey was 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 5.2, the curd was hand stretched in a 5% brine at 82°C for 2 min. The stretched curd

was then put into stainless steel molds (9 x 9 x 9 cm) and cooled in an 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 loss in weight (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 the tube

test method modified with a higher cooking temperature (110°C) for 1 h (19).

Analyses of variance were run separately for the dependent variables, moisture,

and melt, based on three independent replicates of each treatment. A split-plot design was

used for melt with cultures as the whole-plot effect and storage time as the split-plot

effect. A randomized block design was used to analyze moisture. Correlations, means,

and analyses of variance were calculated using Jmp™ software (10).

Microscopy

Samples of cheese were cut into 3 mm x 3 mm x 10 mm pieces using a sterile

blade, immersed in 2% glutaraldehyde in a 0.85% saline buffer, and then stored at 5°C.

The cheese samples were 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, GA) where confocal imaging was used to determine the size of the EPS

capsules formed by each culture (9).

RESULTS AND DISCUSSION

Cheese Composition

All cheeses had similar fat percentages (6.0 to 6.4%) that were appropriate for a low fat cheese and had a d 1 pH of 5.27 ± 0.03 (Table 1). The higher moisture cheeses contained slightly less protein on a wet basis. Addition of the EPS-producing starter and EPS-producing adjunct cultures significantly increased ($P < 0.001$) moisture retention in the cheese. The EPS-producing starter culture increased moisture by 3%. Addition of the EPS-producing adjunct culture with the non-EPS-producing starter cultures increased cheese moisture by nearly 2%. Using the EPS-producing starter culture with addition of the EPS-producing adjunct culture increased cheese moisture by 4%. No whey leakage was observed in any of the cheeses over 28 d of storage at 4°C.

Cheese Melt

The EPS-producing starter culture significantly increased cheese melt, but the EPS-producing adjunct culture did not (Table 2). Cheese made with the EPS-producing

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

starter culture (without the EPS-producing adjunct) had the next highest melting values at all days of storage. Adding the EPS-producing adjunct culture to the control (non-EPS-producing) starter culture did not increase the meltability of the cheese. Storage time also significantly affected cheese melting (Table 2). The cheese with the most melt was made using the EPS-producing starter culture plus the EPS-producing adjunct culture (Figure 1). In general, cheese melt increased with storage time, and cheese with higher moisture had greater melt (Figure 2). All cheeses showed an increase in melting properties between d 1 and d 7. Cheeses with EPS-producing starter cultures decreased in melt between d 14 and d 28. Cheeses with no EPS-producing starter cultures showed little change in melt (Figure 1). The EPS-producing starter culture increased cheese moisture and cheese melt, but addition of an EPS-producing adjunct culture with a non-EPS-producing starter culture increased cheese moisture and did not affect cheese melt. These changes were

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^{NS1}
Starter cultures (S)	1	174.80	21.93^{**}
Adjunct cultures (A)	1	20.20	2.53^{NS}
S x A	1	14.08	1.77^{NS}
Error A	6	7.97	
Time (T)	3	8.44	11.22^{***}
S x T	3	3.88	4.41^{*}
A x T	3	0.62	0.70^{NS}
S x A x T	3	0.34	0.39^{NS}
Error B	24	0.88	
Corrected total	47		

[1]$P > 0.05$, $^{*}P \le 0.05$, $^{**}P \le 0.01$, $^{***}P \le .001$.
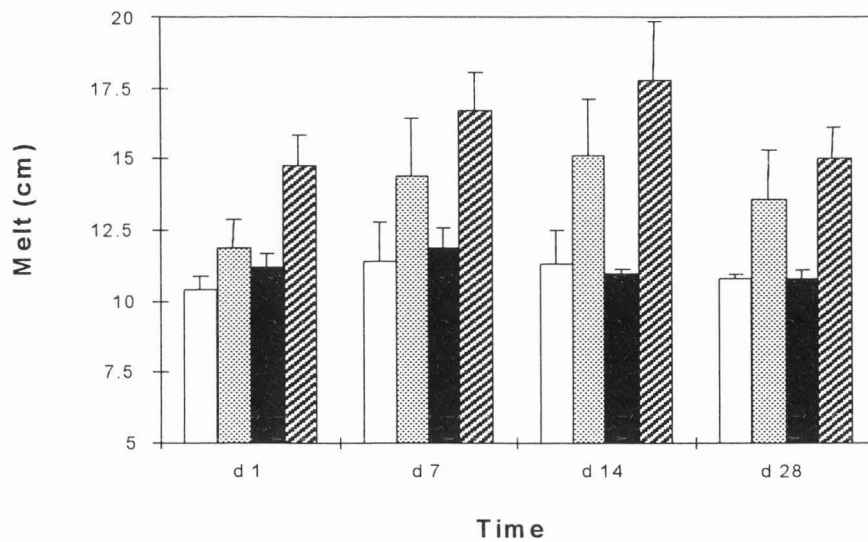
Figure 1. Effect of exopolysaccharide (EPS)-producing cultures on meltability of low fat Mozzarella cheese during 28 d of storage at 4°C. Cultures used were non-EPS-producing starter (open bar), EPS-producing starter (light bar), non-EPS-producing starter plus EPS-producing adjunct (dark bar), and EPS-producing starter plus EPS-producing adjunct (striped bar). Error bars represent individual standard errors for each mean (n = 3).
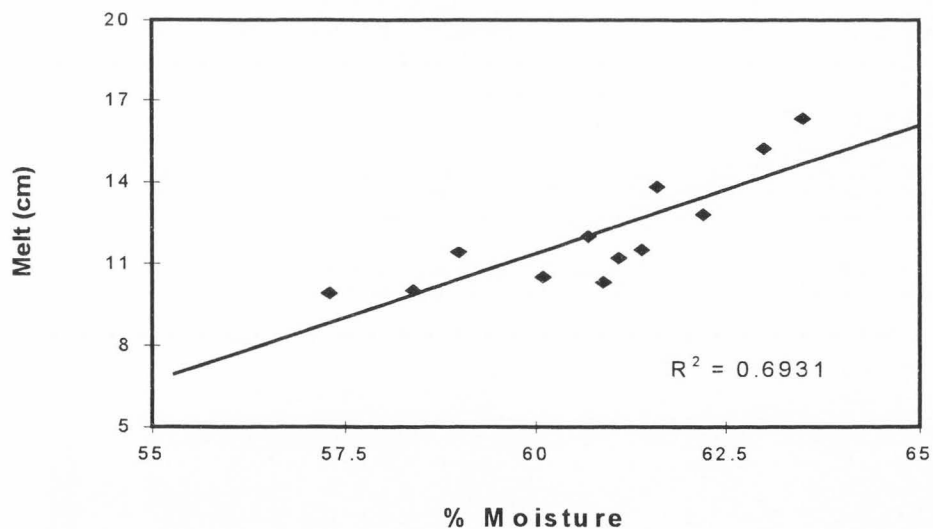
Figure 2. Correlation between cheese moisture content and cheese meltability on d 1 of low fat Mozzarella cheese made using exopolysaccharide (EPS)-producing and non-EPS-producing cultures.

probably related to the manner in which different cultures bind the exopolysaccharides they produce.

After the EPS is produced, some cultures release the EPS into the surrounding environment while in others it remains attached to the cell wall as a capsule. When the EPS adjunct cultures were grown in milk, no EPS capsule was observed (data not shown) suggesting that cultures maintaining their exopolysaccharide as a capsule are better suited for use in cheese manufacturing to retain moisture. In contrast, those cultures that release their EPS into the medium are better suited for use in manufacturing yogurt.

Cheese Microstructure

Because the EPS-producing adjunct did not influence cheese melting the same as the EPS-producing starter culture did, the EPS adjunct and the EPS-producing starter cultures have different effects on the cheese microstructure.

Confocal imaging pictures of the EPS-producing starter cultures show clearing zones around the bacteria as a result of capsule formation (Figure 3). The *S. thermophilus* MR-1C portion of the EPS-producing starter culture produced the largest capsule, which measured 3 μm from the cell wall to the outer edge of the capsule (Figure 3a). The rod portion of the EPS-producing starter culture produced a capsule measuring only 1 μm (Figure 3b). The non-EPS-producing starter cultures and the EPS-producing adjunct culture showed no clearing zones around the surface of the bacteria, demonstrating no capsular formation (Figure 3c). Studies of Mozzarella cheese microstructure by Oberg et al. (18) show that much of the water in the cheese is contained in columns surrounded by
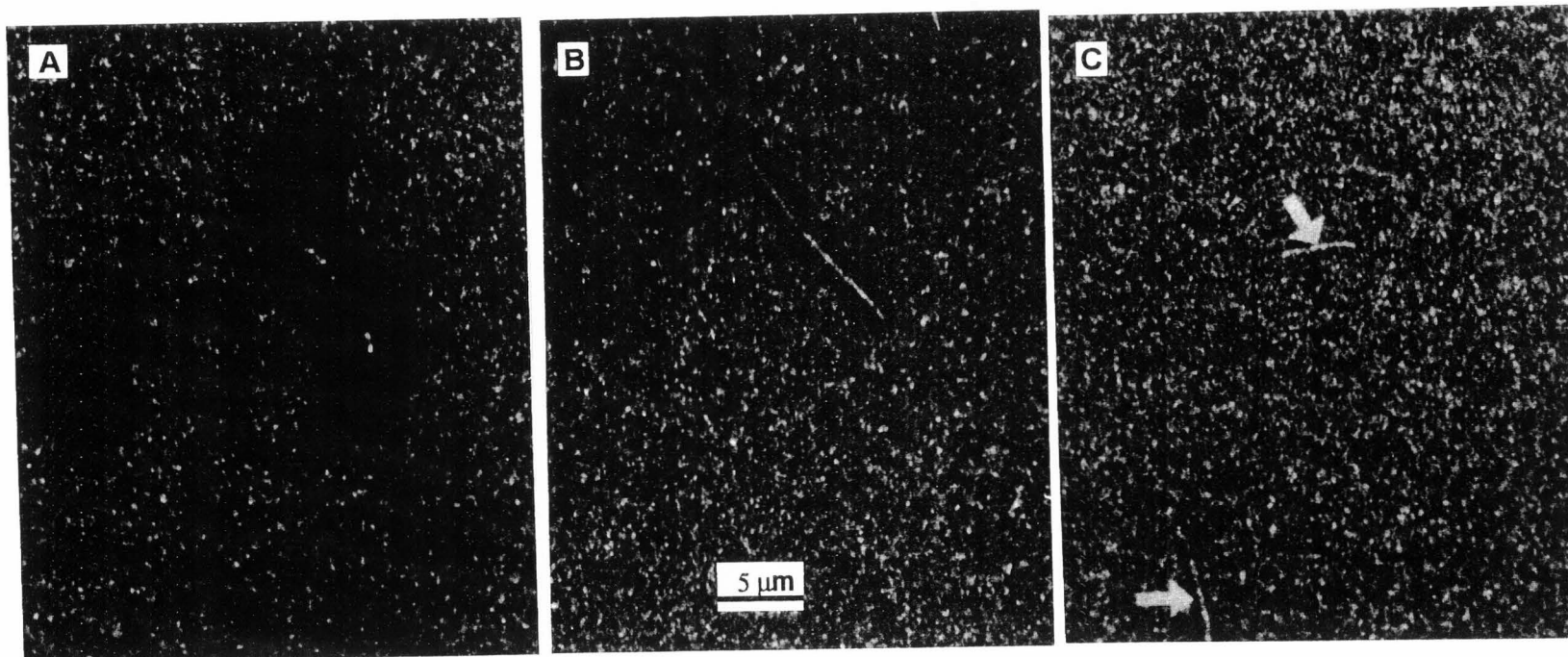
Figure 3. Confocal micrographs of *Streptococcus thermophilus* MR-1C (A), *Lactobacillus delbrueckii* ssp. *bulgaricus* (B), and *Lactobacillus helveticus* LH100 (C) grown in skim milk. Arrows (C) show bacteria that are difficult to observe when no capsule is present.

the protein network. Removal of fat causes these columns to become narrower, reducing space for water retention in the cheese curd. In addition to binding free water, the production of EPS material may help to hold the protein network apart, producing larger columns capable of entrapping more water.

Scanning electron micrographs of cheese made with EPS-producing starter cultures show the bacteria are covered with remnants of the dehydrated EPS capsule, giving them a "fuzzy" appearance (Figure 4a). Unless physically restrained, the EPS capsule collapses as the cheese samples are dehydrated during preparation for electron microscopy. When hydrated, the capsular material would be large enough to fill the void spaces in the protein matrix and lessen coalescence of the protein strands. This can be seen in the top micrograph of Figure 4a (upper right-hand corner) where EPS material formed a bridge between the two protein strands, blocking their coalescence and forming a serum cavity. Scanning electron micrographs of cheese made with the non-EPS-producing starter cultures show bacteria with a smoother surface (Figure 4b). The bacteria in the cheese made with the EPS-producing starter culture and EPS-producing adjunct culture were also covered with dehydrated EPS material. In comparison to micrographs of the EPS-producing starter culture alone, the bacteria in cheese made with the EPS-producing starter culture and the EPS-producing adjunct culture were more often present as clumps rather than individual chains. These bacteria appeared to form aggregates, which may be a result of the adjunct cultures releasing EPS material. Presumably, these clumps consisted of the *S. thermophilus* as well as the lactococci, but we were unable to differentiate the two organisms in the micrographs. Another
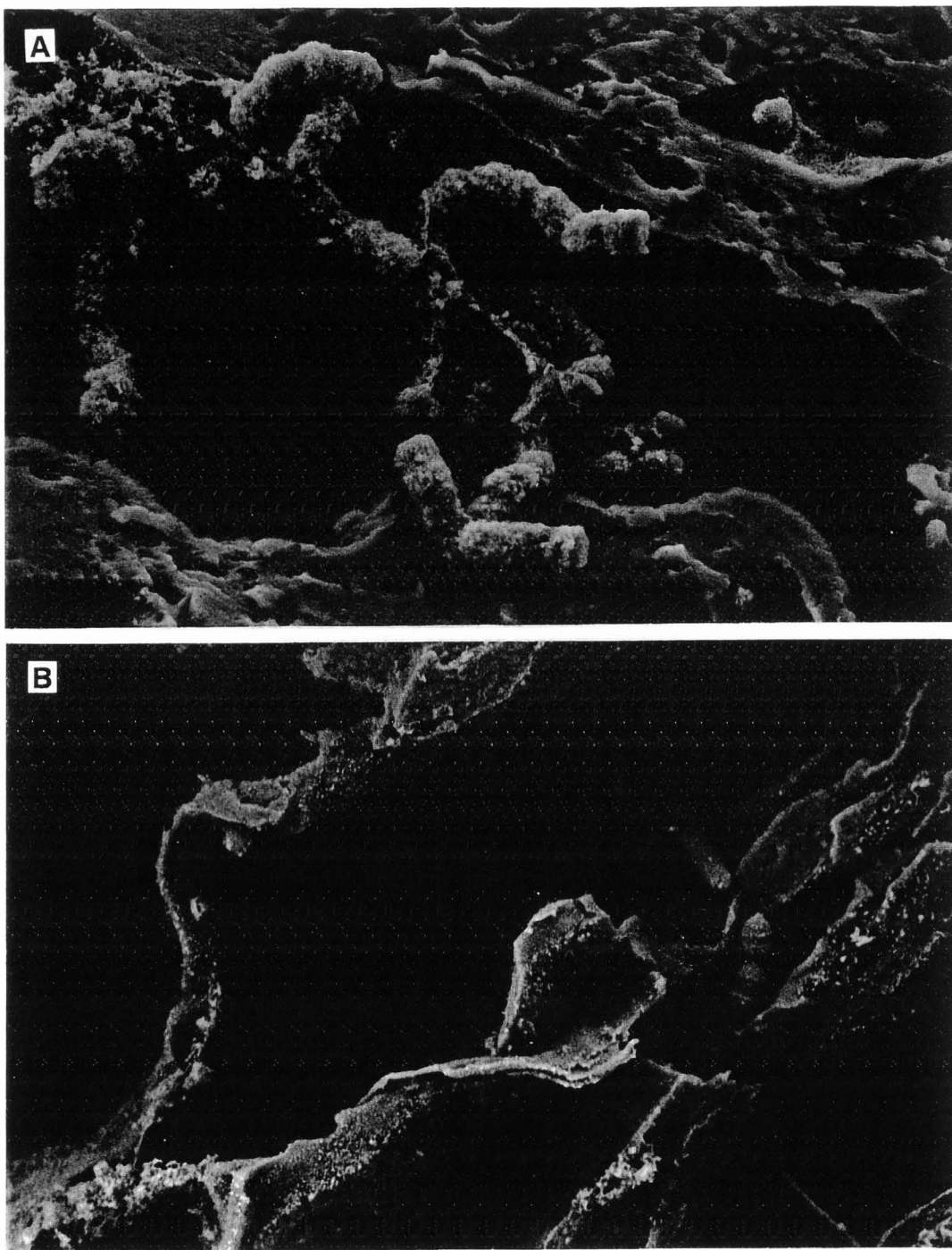
Figure 4. Scanning electron micrographs of low fat Mozzarella cheese manufactured using exopolysaccharide (EPS)-producing starter cultures *Streptococcus thermophilus* MR-1C and *Lactobacillus delbrueckii* ssp. *bulgaricus* MR-1R (A), and non-EPS starter cultures *S. thermophilus* TA061 and *Lactobacillus helveticus* LH100 (B).

consequence of the cultures producing EPS is that a greater number of bacteria were retained in d 1 cheese (10^8 versus 10^7 cfu / g). Presumably the "sticky"nature of the EPS increases retention of the bacterial cells in the cheese curd during manufacture. We also observed the same trend in the electron micrographs with more cells apparent in the EPS cheese. This could also be a function of non-EPS-producing cells being more easily lost when the samples are freeze-fractured.

Cheese with too much moisture may cause shredding equipment to clog up and not work properly. 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 moistures became difficult to shred by d 7.

CONCLUSIONS

We demonstrated that EPS-producing cultures can be useful in increasing moisture retention of low fat Mozzarella cheese. A similar effect may be observed in low-moisture, part-skim Mozzarella cheese. Increasing the moisture content of low fat Mozzarella cheese can improve melting properties. The EPS-producing cultures could be used as an alternative for manufacturing low fat cheeses without fat replacers. Further work is needed to determine the optimal moisture for low fat Mozzarella cheese to provide good meltability without becoming too soft to shred upon aging.

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CHAPTER 4

MANUFACTURE OF LOW FAT MOZZARELLA CHEESE USING EXOPOLYSACCHARIDE-PRODUCING STARTER CULTURES[2]

ABSTRACT

Exopolysaccharide-producing starter cultures, consisting of single strains of *Streptococcus thermophilus* MR-1C and *Lactobacillus delbrueckii* ssp. *bulgaricus* MR-1R, were used to make three replicates of low fat (6% fat) Mozzarella cheese. Our aim was to determine if observations made using small (22-lb capacity) vats with hand-stretching of curd, could be replicated using pilot-scale (1000-lb capacity) vertical blade double-O vats with mechanical cooking and stretching of the curd. A control cheese was made using starter cultures, *S. thermophilus* TA061 and *Lactobacillus helveticus* LH100, that do not produce exopolysaccharides. Cheese was measured for moisture content and meltability at d 1. Cheese made with the exopolysaccharide-producing starter cultures had a 2% higher moisture content and exhibited slightly higher meltability. Because of changes in the low fat cheese manufacturing procedure necessary when using the mechanized vats, the cheeses made in the double-O vats were slightly lower in moisture than cheeses previously made in the hand-stirred laboratory-scale vats.

INTRODUCTION

Because of its continued popularity, Mozzarella cheese has been targeted for the low-fat and nonfat cheese market. The pizza industry has played a major role in the increased production of Mozzarella cheese; therefore, the majority of Mozzarella cheese produced must have functional properties suitable for pizza production. Mozzarella cheese for pizza should exhibit good shredding, melting, and stretching properties and be free of off-flavors or textural defects (6).

The removal of fat in low fat Mozzarella cheese can result in cheese that is low in moisture, giving the cheese poor melting and stretching properties (8). There are various strategies that have been used to increase moisture content of lower-fat Mozzarella cheeses. Merrill et al. (9) used higher pasteurization conditions (175°F for 29 s) to denature some of the whey proteins and increase water-holding capacity of the cheese curd. They also used pre-acidification of the milk to pH 6.0 to shorten the make time, cut the curd into larger pieces than normal, and minimized stirring to reduce curd syneresis. Such modifications are effective on a laboratory scale but are not always practical for application by cheese manufacturers. The use of fat replacers has also been suggested. McMahon et al. (7) reported on how microparticulated additives can be used to increase cheese moisture content. Another approach (14) is to take advantage of the natural properties of some strains of cheese starter cultures to help retain more moisture in the cheese.

Exopolysaccharide (EPS)-producing lactic acid bacteria (commonly called ropy or slime-producing cultures) have been used in fermented dairy products, such as yogurt and

buttermilk, to improve product rheology and slow syneresis by binding free water (1, 3, 4, 5). We have used EPS-producing strains of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* to produce low fat Mozzarella cheese in small-scale hand-stirred vats (12). Unlike ropy cultures which release the exopolysaccharides they produce into the surrounding medium, the EPS-producing cultures that are suitable for cheesemaking maintain the polysaccharide around the bacterial cells. This capsule of hydrated EPS effectively increases the size of the bacteria, up to 5 mm more in diameter. Thus, not only is more moisture being retained around the bacteria, but in making low fat Mozzarella cheese, these EPS-producing cultures can also help increase cheese moisture content by blocking some of the protein matrix fusion that takes place during cooking and stretching (12). Cheese made in the small hand-stirred vats using the EPS-producing cultures retained 3% more moisture (61% versus 58%, respectively) than a control cheese made using non-EPS-producing cultures, and the cheese made using EPS-producing cultures also had improved meltability (12).

The object of this study was to determine if increased cheese moisture levels could still be obtained using the EPS-producing starter cultures when the cheese manufacturing procedure (9, 10, 12) for making lower-fat Mozzarella cheese was scaled up to better represent commercial practice. Instead of using the small 22-lb (10 kg) vats with hand stirring and hand stretching of the curd, cheese was made in 1000-lb (454-kg) double-O vats with mechanical cooking and stretching of the curd. We also wanted to know how changes made during scale-up of the cheesemaking procedure affected the meltability of the cheese.

MATERIALS AND METHODS

Starter Cultures

Starter cultures from the Department of Microbiology culture bank at Weber State

University (Ogden, UT) were grown separately in Sure Set XL$^{®}$ internal pH-controlled

medium (Gist-brocades, Millville, UT). Cultures were grown at 42°C (108°F) to a pH of

4.4 one day prior to cheesemaking and kept at 6°C (43°F) until used. Both coccus and

rod portions of the starters were added separately to the milk in each vat. The milk in vat

1 was inoculated with 4.4 lb (2.0 L) each of the non-EPS *S. thermophilus* TA061 and *L.*

helveticus LH100. The milk in vat 2 was inoculated with 6.6 lb (3.0 L) each of the EPS-

producing *S. thermophilus* MR-1C and *L. delbrueckii* ssp. *bulgaricus* MR-1R. Starter

culture inoculum levels were selected to provide similar manufacturing times between the

vats.

Cheese Manufacture

Skim milk was pasteurized at 80°C (175.5°F) for 29 s and cooled to 13°C (55°F)

in the G. H. Richardson Dairy Products Laboratory at Utah State University, Logan.

Three replicates of low fat Mozzarella cheese were made using two 1000-lb capacity,

open top, vertical-blade double-O vats (Damrow DEC International, Fond DuLac, WI).

Each vat was filled with 800 lb (363 kg) of pasteurized skim milk, which was then

standardized to 0.6% fat using pasteurized cream of known fat content. Standardized

milk was preacidified to pH 6.3 using acetic acid diluted 1:10 (vol./vol.) with distilled

water, and the milk was then heated to 34°C (93°F). After inoculation, the cultures were

allowed to ripen for 10 min. Ripened milk was set at 93°F using 1 oz (27.2 ml) of double-strength Chymax® (Chr. Hansen's Laboratory, Milwaukee, WI) diluted in 10 oz (270 ml) of distilled water.

Curd was cut 25 min after rennet addition using a medium cut speed for 8 to 10 revolutions of the knives. Directly after cutting, the knives were reversed and set on low agitation to provide healing time for the curd. After 15 min of slow agitation, the speed was gradually increased to a high speed over a 15-min period. The curd was then heated to a cooking temperature of 102°F (39°C) over a 10-min period with continued agitation. When a curd pH of 5.4 was reached, the whey was completely drained from the vat, and the curd moved to the side of the vat. The curd was then salted with 1.0% (wt/wt) salt in each vat and the curd stirred manually. After salting, the curd pH had dropped to 5.2 and run through an Alfa-Laval Cooker/Stretcher (Tetra-Pak, Greenwood, IN). The circulating water in the cooker/stretcher had 5% salt added and was maintained at 180°F (82°C). As the molten cheese emerged from the cooker/stretcher, it was put in 4 x 15 x 4 in (10 x 38 x 10 cm) stainless steel molds and cooled in an ice bath for 1 h. Blocks of cheese were vacuum-packaged and stored at 40°F (4°C).

Cheese Analysis

After 1 d of storage, cheese was analyzed for composition and meltability. Moisture content was measured using a vacuum oven method (14). Meltability was determined using the tube test method (13) using an oven temperature of 230°F (110°C) for 1 h. Fat content was measured using a modified Babcock method (2), and pH was measured using a glass electrode. Differences between moisture content and meltability of

cheeses made using EPS and non-EPS cultures were analyzed using Student's t test. Significance was declared at $P \leq 0.05$, and tendencies at $0.10 \geq P \geq 0.05$.

RESULTS AND DISCUSSION

All cheese had fat percentages in the range (6.2 to 6.3%) required for low fat cheese. They also had comparable manufacturing times (180 ± 5 min), and had d 1 pH of 5.22 ± 0.03. Use of the EPS-producing starter cultures for making cheese significantly increased cheese moisture and had a tended to increase cheese meltability (Table 3). In comparison to the 3% moisture increase observed when cheese had been made with EPS-producing cultures in the hand-stirred (22-lb) vats (14), the increase in moisture for cheese made using EPS-producing cultures in the 1000-lb double-O vats was only 2%. All of the cheeses made in the double-O vats (using either EPS-producing or non-EPS-producing cultures) were lower in moisture than the respective cheeses made in the smaller vats. This was expected, because in the small hand-stirred vats the cheese curd was stirred only intermittently, and it was relatively easy to keep the curd particles separated. However,

TABLE 3. Mean moisture contents, pH, meltability, and manufacturing time (from cut to stretching) for three replicates of low fat Mozzarella cheese made with exopolysaccharide (EPS)-producing starter cultures and non-EPS-producing starter cultures.

Starter Culture	Moisture contents (%)		pH		Meltability (cm)		Manufacturing time (min)	
	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE
EPS	57.1	0.35	5.22	0.02	10.6	0.5	183	2
non-EPS	55.3	0.15	5.22	0.01	9.5	0.1	177	3

in the double-O vats, the larger volume of curd made it more difficult to keep the curd suspended in the whey; therefore, more vigorous and constant agitation was required to keep the curd from matting together on the bottom of the vat.

Another problem encountered when the experiments were scaled up from 22-lb batches to 800-lb batches was the effect of milk preacidification on the properties of the cheese curd. Previously, we had preacidified the milk to pH 6.0 when making lower fat Mozzarella cheeses (9, 10, 14). In preliminary cheesemaking trials with the double-O vats, however, it was observed that if the milk were preacidified to pH 6.0, the curd became quite sticky and would adhere to the agitator blades as the curd was being stirred. The same effect was observed for milk preacidified to either pH 6.1 or 6.2.

When milk is coagulated at its normal pH (6.6 to 6.7), there is a time delay after the initial aggregation has occurred before the casein micelles start to fuse together. This allows time for the surface of the curd particles to heal, and a thin skin is formed that acts as a semi-permeable membrane controlling movement of components (e.g., water, lactose, lactic acid) in and out of the curd particles (12). At a milk pH of 6.0, the process of casein micelle destabilization (initiated by renneting of the milk) has been accelerated to the extent that casein micelle fusion is actively taking place when the curd is cut (10). Consequently, with the agitation required to prevent curd settling in the double-O vats, the low fat curd particles do not heal or form a nonadhesive skin properly; instead, they stick to each other and to the agitator blades. By preacidifying the milk only to pH 6.3, this problem could be sufficiently reduced (provided constant agitation was maintained) to

allow the low fat cheese to be manufactured without excessive sticking of the curd to the agitator blades or the bottom of the vat.

The previous problem demonstrates the differences that can occur when changes are made from one type of vat to another. Nevertheless, the same overall effect of increased cheese moisture content observed when cheese was made using the EPS-producing cultures in small hand-stirred vats was observed when cheese was made using mechanized equipment more representative of commercial manufacturing practices. When cheese was manufactured in the double-O vats, constant agitation of the curd was required after cutting to prevent the curd from sticking to the knives and to the bottom of the vat. This constant agitation caused more moisture to be lost from the cheese curd than occurred in the 22-lb vats where stirring was minimal. In addition, the milk used in the double-O vats was preacidified only to a pH 6.3, rather than pH 6.0 for cheese made in the 22-lb vats. By reducing the level of milk preacidification before renneting, the time until draining was increased, and by scaling up from 22 lb to 800 lb, the larger volume of whey to be drained meant that it took longer to drain the curd (this would be even further exacerbated when making cheese in commercial volume vats, e.g., 40,000 lb of milk). Consequently, when cheese was made in the double-O vats, the overall manufacturing time was 30 min longer than for cheese made in the small hand-stirred vats.

We had previously observed that when making cheese in the small vats there was a strong correlation between manufacturing time and cheese moisture (14). By shortening the manufacturing time, more moisture could be retained in the cheese. Given the increased manufacturing times when cheese was made in the double-O vats as compared

to the hand-stirred vats, more syneresis of whey from the curd was expected and, hence, a slightly drier cheese. However, the increase in cheese moisture obtained when using the EPS-producing cultures appears to be independent of overall moisture content. This suggests that EPS-producing cultures could be used by a cheese manufacturer to increase cheese moisture content irrespective of the moisture content of the cheese being made using non-EPS-producing starter cultures.

When cheese was made in the 22-lb vats, a 14% increase in cheese meltability was observed when the EPS-producing cultures were used. With the double-O vats, the increase in meltability of the cheese made with use of the EPS-producing cultures was 12%. This slight decrease reflects having only a 2% rather than 3% increase in moisture content. The influence of cheese moisture on meltability was also demonstrated by the observation that all of the cheeses manufactured in the double-O vats melted slightly less (mean = 10.1 cm) than the cheeses made in the 22-lb vats (mean = 11.3 cm). This reduced meltability corresponds to the cheeses from the double-O vats having lower moisture contents.

A concern that may be raised about using EPS-producing cultures for making cheese is that the EPS produced by the cultures may end up in the whey rather than in the cheese curd. This may be a problem if a ropy or slime-producing culture is used as suggested by Nauth and Hayashi (11), because the EPS produced by the culture is released from the bacterial cells into the surrounding media. However, for *S. thermophilus* MR-1C and *L. delbrueckii* ssp. *bulgaricus* MR-1R, the EPS remains bound as a capsule around the bacterial cells, and only minute amounts of EPS could be

recovered from a cell-free extract of the starter cultures (unpublished data, Debra Low

and Jeffrey Broadbent, Utah State University). Furthermore, there were no differences

observed in viscosity or appearance of the whey produced using either the EPS-producing

or non-EPS-producing starter cultures. Using *S. thermophilus* MR-1C or *L. delbrueckii*

ssp. *bulgaricus* MR-1R in cheesemaking would, therefore, not be expected to cause any

problems in whey processing.

CONCLUSIONS

Scaling up the size of the cheese manufacturing process using 1000-lb capacity

double-O vats required that some changes be made to the low fat Mozzarella cheese

manufacturing procedure to avoid curd sticking to the agitator blades and vat bottom.

While the procedure used did not exactly duplicate how cheese is made in large 50,000-lb

capacity vats, it provided a useful intermediate step up from the 22-lb capacity vats for

testing the efficacy of using EPS-producing cultures to increase cheese moisture content.

Using a starter culture that formed an exopolysaccharide capsule allowed a low fat

Mozzarella cheese to be manufactured that had a 2% higher moisture content and

increased meltability than a corresponding low fat cheese made using non-EPS-producing

starter cultures. The EPS-producing cultures, thus, provide a simple method, apart from

changing manufacturing procedures and times, for increasing moisture in Mozzarella

cheese.

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CHAPTER 5

GENERAL SUMMARY

From this work we were able to determine that EPS-producing starter and adjunct

cultures were useful in making 6% low fat Mozzarella with increased moisture content.

Low fat Mozzarella with higher moisture content correlated with increased melt over 28 d

of storage at 4°C. There were no problems observed in the manufacturing procedure used

to produce low fat Mozzarella when using EPS-producing cultures. When scaling up the

manufacturing procedure to a pilot-scale using 454-kg double-O vats, the EPS-producing

starter cultures still provided increased moisture content in low fat Mozzarella cheese

when compared to low fat Mozzarella cheese made using non-EPS-producing starter

cultures. Using scanning electron microscopy and confocal microscopy imaging, we were

able to determine that the EPS-producing starter culture maintained its exopolysaccharide

material as a capsule. When used in low fat Mozzarella cheese, it is important that the

EPS-producing starter cultures maintain the exopolysaccharide material as a capsule,

maintaining more space between the protein matrix of the cheese to hold water. When

exopolysaccharide material is released from the cultures into the surrounding medium (i.e.,

milk or whey), undesirable effects (slimy texture) may result in the finished cheese

product. With this research we have effectively demonstrated that EPS-producing starter

cultures provide increased moisture, which results in increased melt without having to

modify manufacturing procedures.

APPENDIXES

APPENDIX A

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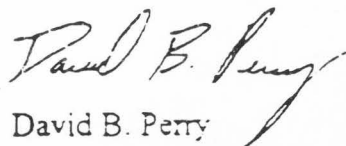<u>Dear Journal of Dairy Science:</u>

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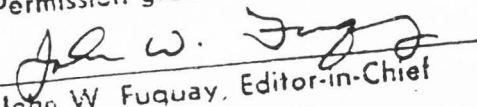
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> Effects of Exopolysaccharide-Producing Cultures on Moisture Retention in Low Fat Mozzarella Cheese. D. B. Perry, D. J McMahon, and C. J. Oberg. 1997. J. Dairy Sci. 80:799-805.

> Manufacture of Low Fat Mozzarella Cheese Using Exopolysaccharide-Producing Starter Cultures. D. B. Perry, D. J. McMahon, C. J. Oberg. 1998. J. Dairy Sci. 81:563-566.

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APPENDIX C

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