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Improvement of Texture and Structure of Reduced-Fat Cheddar Cheese by Exopolysaccharide-Producing Lactococci

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ABSTRACT

The objective of this study was to evaluate the effect of capsular and ropy exopolysaccharide (EPS)-producing strains of *Lactococcus lactis* ssp. *cremoris* on textural and microstructural attributes during ripening of 50%-reduced-fat Cheddar cheese. Cheeses were manufactured with added capsule- or ropy-forming strains individually or in combination. For comparison, reduced-fat cheese with or without lecithin added at 0.2% (wt/vol) to cheese milk and full-fat cheeses were made using EPS-nonproducing starter, and all cheeses were ripened at 7°C for 6 mo. Exopolysaccharide-producing strains increased cheese moisture retention by 3.6 to 4.8% and cheese yield by 0.28 to 1.19 kg/100 kg compared with control cheese, whereas lecithin-containing cheese retained 1.4% higher moisture and had 0.37 kg/100 kg higher yield over the control cheese. Texture profile analyses for 0-d-old cheeses revealed that cheeses with EPS-producing strains had less firm, springy, and cohesive texture but were more brittle than control cheeses. However, these effects became less pronounced after 6 mo of ripening. Using transmission electron microscopy, fresh and aged cheeses with added EPS-producing strains showed a less compact protein matrix through which larger whey pockets were dispersed compared with control cheese. The numerical analysis of transmission electron microscopy images showed that the area in the cheese matrix occupied by protein was smaller in cheeses with added EPS-producing strains than in control cheese. On the other hand, lecithin had little impact on both cheese texture and microstructure; after 6 mo, cheese containing lecithin showed a texture profile very close to that of control reduced-fat cheese. The protein-occupied area in the cheese matrix did not appear to be significantly affected by lecithin addition. Exopolysaccharide-producing strains could contribute to the modification of cheese texture and microstructure and thus modify the functional properties of reduced-fat Cheddar cheese.
Key words: exopolysaccharide, reduced-fat Cheddar cheese, texture, microstructure

INTRODUCTION

During the past few decades, consumption of low-fat products has grown steadily because of consumer awareness about health concerns related to decreasing the risks associated with obesity, atherosclerosis, coronary heart disease, and elevated blood pressure. In the dairy industry, manufacturers are developing several varieties of reduced-, low-, and fat-free products. However, these products are often characterized by inadequate flavor, poor textural quality, and lower keeping quality (Olson and Johnson, 1990; Muir et al., 1992; O'Donnell, 1993).

Textural attributes are believed to be important criteria in determining the identity and quality of a cheese and its consumer acceptability. The texture and fracture properties of a cheese are largely determined by the nature and arrangement of its structural network. In full-fat Cheddar cheese, structure is usually described as a continuous protein matrix in which fat globules and residual whey are dispersed (Ustunol et al., 1995). When fat decreases, the protein matrix becomes closer with less fat globule dispersion leading to a more compact structure that affects cheese texture characteristics and overall acceptability (Ustunol et al., 1995). This effect seems to be proportionally correlated with the amount of fat removed. Beal and Mittal (2000) reported that hardness, gumminess, and chewiness increased linearly, and cohesiveness and springiness decreased nonlinearly with fat content decrease in Cheddar cheese.

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Due to increased consumer demand for low- and reduced-fat Cheddar cheeses, several studies have attempted to improve both textural and flavor attributes of these cheeses to resemble more closely those of full-fat cheese (Muir et al., 1992; Drake et al., 1996b). Increasing moisture content to a level beyond that of full-fat cheese is thought to be useful to correct the textural defects associated with fat reduction (Mistry, 2001). This can be accomplished through modification of cheese-making procedures or by addition of emulsifying and thickening agents. As fat is removed from cheese milk, cheese-making procedures that are usually used for manufacturing full-fat cheese should be modified to meet the requirements to increase moisture retention and decrease acid accumulation in the resultant cheeses to correct textural and flavor defects associated with fat removal. Several technological modifications have been proposed in previous studies to improve low-fat Cheddar cheese characteristics. These modifications include reducing cooking time and temperature (Banks et al., 1989), employing a high pH range (between 5.6 and 5.8) at milling (Kosikowski and Mistry, 1997), and washing cheese curd with cold water (22°C) to help retain moisture, remove excess lactose, and solubilize calcium, which helps soften cheese texture (Chen and Johnson, 1996). The use of thickening agents has been reported to improve the textural attributes of reduced-fat Cheddar cheese (Drake et al., 1996a,b; Fenelon and Guinee, 1997). However, these agents can interfere with the authentic cheese flavor and may adversely affect cheese aroma and flavors by developing undesirable off-flavor such as sour and oxidative flavors (Drake et al., 1996b).

Exopolysaccharide (**EPS**)-producing cheese starter cultures could be another alternative for increasing moisture retention in reduced-fat Cheddar cheese. We have recently reported on the effects of EPS capsule- and ropy-forming strains of *Lactococcus lactis* ssp. *cremoris* and their combination on 50%-reduced-fat Cheddar cheese production and whey composition and viscosity (Dabour et al., 2005a). Cheeses made with capsular or ropy strains or their combination retained 3.6 to 4.8% higher moisture over control cheese, and both strains had very little impact on whey composition and viscosity, which aids in the further use of the resultant whey. In the present study, the objective was to evaluate the impact of incorporating such strains either individually or in combination, compared with 0.2% (wt/vol) lecithin added to cheese milk, on the textural and microstructural attributes during ripening of 50%-reduced-fat Cheddar cheese.

MATERIALS AND METHODS

Bacterial Strains and Growth Conditions

Exopolysaccharide nonproducing strains *Lactococcus lactis* ssp. *lactis* RBL259 (*L. lactis* RBL259), *Lactococcus lactis* ssp. *lactis* RBL133 (*L. lactis* RBL133), and *Lactococcus lactis* ssp. *cremoris* RBL132 (*L. cremoris* RBL132) were obtained from the Canadian Research Network on Lactic Acid Bacteria (NLAB) culture collection (STELA Dairy Research Centre, Université Laval, Quebec, QC, Canada). Exopolysaccharide capsule-forming *Lactococcus lactis* ssp. *cremoris* SMQ-461 (*L. cremoris* SMQ-461), a raw milk isolate, was provided by Sylvain Moineau (Department of Biochemistry and Microbiology, Université Laval, Quebec, Canada). Exopolysaccharide ropy-forming strain *Lactococcus lactis* ssp. *cremoris* JRF-1 (*L. cremoris* JRF-1), isolated from retail cultured buttermilk, was provided by Joseph Frank and Ashraf Hassan (Department of Food Science and Technology, University of Georgia, Athens). All strains were genetically identified and phenotypically characterized as described previously (Dabour et al., 2005a).

Pure bacterial cultures were maintained in 20% glycerol stock at –80°C. They were cultivated in M17 broth medium at pH 7.1 (Quelab, Montreal, Canada) supplemented with 0.5% (wt/vol) glucose (**GM17**) and incubated overnight at 30°C (Terzaghi and Sandine, 1975). Before beginning the experiments, each bacterial strain was subcultured at least twice (1%, vol/vol) into the indicated media at 24-h intervals.

Cheese-Making Procedure

The cheese mixed culture consisting of EPS-nonproducing *L. lactis* (RBL259), *L. lactis* (RBL133), and *L. cremoris* (RBL132) at a ratio of 0.67:0.33:1.0 (vol/vol/vol), respectively, was previously selected for the production of reduced-fat Cheddar cheese (Dabour et al., 2005a). For the application of EPS-producing strains, *L. cremoris* (RBL132) was replaced by either capsular-EPS-producing *L. cremoris* (SMQ-461) or ropy-EPS-producing *L. cremoris* (JRF-1) or a mixture of both strains (1:1) at the same ratio. Before cheese making, lactococcal strains were individually subcultured at least 3 times (1% vol/vol) in sterilized skim milk at 24-h intervals. The 6 experimental cheeses are as follows: 1) Control cheese made with full-fat milk with EPS-nonproducing starter (**CFF**); 2) reduced-fat cheese with EPS-nonproducing starter (**CLF**); 3) reduced-fat cheese with capsular EPS-producing strain (**SMQ**); 4) reduced-fat cheese with ropy EPS-producing strain (**JRF**); 5) reduced-fat cheese with capsular and ropy EPS-producing strains (1:1 mix; **SMJF**); and 6) cheese with 0.2% lecithin added, with EPS-nonproducing starter (**LEC**). The experimental cheeses and strains used are provided in Table 1.

Whole fat (3.25%), partially-skimmed (2%), and skim milk homogenized using 2-stage pressures of 17.25 and 3.43 MPa, respectively, and pasteurized at 72°C for

Table 1. Cheese treatments and the ratios of their corresponding mixed cultures

Strains and lecithin	Cheese[1]					
	CFF	CLF	SMQ	JRF	SMJF	LEC
Lactococcus lactis ssp. *lactis* RBL259[2]	0.67	0.67	0.67	0.67	0.67	0.67
L. lactis ssp. *lactis* RBL133[3]	0.33	0.33	0.33	0.33	0.33	0.33
L. lactis ssp. *cremoris* RBL132[4]	1.0	1.0	—	—	—	1.0
L. lactis ssp. *cemoris* SMQ-461[5]	—	—	1.0	—	0.5	—
L. lactis ssp. *cemoris* JRF-1[6]	—	—	—	1.0	0.5	—
Lecithin	—	—	—	—	—	+[7]

[1]The 6 experimental cheeses are as follows: CFF = control cheese made with full-fat milk with EPS-nonproducing starter; CLF = reduced-fat cheese with EPS-nonproducing starter; SMQ = reduced-fat cheese with capsular EPS-producing strain; JRF = reduced-fat cheese with ropy EPS-producing strain; SMJF = reduced-fat cheese with capsular and ropy EPS-producing strains (1:1 mix); and LEC = cheese with 0.2% lecithin added, with EPS-nonproducing starter. The CFF cheese was made from full-fat (3.25%) milk, and other cheeses were made from milk containing 1.7% fat. Cheese starter mixture was added to cheese milk at a level of 1.5% (vol/vol) with a subspecies ratio of 1:1 between *L. lactis* and *L. cremoris*.

[2]High acid-producing, EPS-nonproducing strain.

[3]Cheddar-temperature profile sensitive, EPS-nonproducing strain.

[4]Moderate acid-producing, EPS-nonproducing strain.

[5]Capsular-EPS forming strain.

[6]Ropy-EPS forming strain (Dabour et al., 2005a).

[7]Lecithin was added to cheese milk at level of 0.2% (wt/vol).

16 s were obtained from Natrel Inc. (Longueuil, QC, Canada). Before cheese making, cheese milk was standardized to a constant fat content of 1.7% by adding partially skimmed milk with 2% fat. Fat content of standardized cheese milk was determined using the Babcock test as described by Bradley et al. (1993). Reduced-fat Cheddar cheese was made using computer-controlled cheese equipment (INRA, Poligny, France) equipped with four 10-L vats and pH meters (Accumet model 7; Fisher Scientific Ltd., Nepean, ON, Canada) according to the method described below, which was adapted from those described previously (Banks et al., 1989; Anderson et al., 1993). Milk was warmed to 31°C, inoculated with the desired cheese mixed starter cultures at 1.5% (vol/vol) and allowed to ripen until the pH dropped to 6.5 (approximately 60 min). In lecithin-containing trials, 0.2% (wt/vol) lecithin (L-α-phosphatidylcholine from dried egg yolk, P5394; Sigma Chemical Co., St. Louis, MO) was added to cheese milk 15 min before renneting to ensure its even distribution in the coagulum. Calcium chloride was added to all cheese milk trials at 0.02% (wt/vol). Milk was coagulated at 31°C for 45 min using 0.2 mL/L of bovine rennet (230 international milk clotting units/mL) obtained from Chr. Hansen (Milwaukee, WI). The coagulum was cut and cooked for 45 min. The cooking temperature was raised to 35°C in 30 min and the curds were held at 35°C for 15 min. Whey was then drained and curds were subjected to the cheddaring process for approximately 90 min at 35°C. When the pH of whey reached 5.5 ± 0.05, the curd was milled, dry salted (2.5%, wt/wt), transferred into a round mold, and pressed (14.71 N/cm^2) overnight. Following pressing, the cheese was cut, vacuum-packed in plastic film (4-mil thickness, Winpax Co., Winnipeg, MB, Canada), and ripened at 7°C for 6 mo.

For comparison, full-fat Cheddar cheese was made from 3.25% fat homogenized and pasteurized milk using conventional manufacturing procedures described by Benech et al. (2003). Milk (10 L) was warmed to 32°C and inoculated with 1.5% (vol/vol) starter culture (Table 1), then ripened until the pH reached 6.5 (approximately 45 min). Calcium chloride (0.02%, wt/vol) was added and milk was coagulated during 45 min using 0.2 mL/L of bovine rennet (230 international milk clotting units/mL). The coagulum was cut and the temperature was gradually raised to 39°C (0.2°C/min). Whey was drained and the curd was subjected to the cheddaring process for 120 min at 36°C, until the pH reached 5.3 ± 0.05, milled, dry-salted (2.0%, wt/wt), transferred into a round mold, and pressed (14.71 N/cm^2) overnight. Cheese was then cut, vacuum packed, and ripened at 7°C for 6 mo.

Cheese Composition

Moisture, total nitrogen, and ash contents of cheese samples were determined in triplicate (AOAC, 1990). The fat content of cheese samples was determined by the Babcock-fat test described by Bartels et al. (1987). Cheese pH was measured using a Spear Tip combination electrode (VWR Scientific, Montreal, QC, Canada). The cheese yield was expressed as the mass ratio be-

tween the curd obtained after the pressing stage and the weight of milk.

Water-Soluble Nitrogen Determination

Fat-free cheese homogenates were prepared according to the method of Kuchroo and Fox (1982) to follow the evolution of cheese proteolysis. Water-soluble nitrogen (**WSN**) of the fat-free homogenate was determined by the Kjeldahl method (International Dairy Federation, 1993).

Microbiological Analyses

Cheese samples (10 g) were homogenized for 3 min with 90 mL of a sterile 2% sodium citrate solution in a Laboratory Blender 80 Stomacher (Seward Medical, London, UK) and serially diluted 10-fold using sodium citrate. Appropriate dilutions were spread on plate count agar (Difco Laboratories, Detroit, MI) and incubated aerobically at 30°C for 48 h to determine the total viable bacterial count. Total viable lactococci were enumerated on GM17 agar medium (Quelab), and incubated aerobically at 30°C for 48 h. To distinguish between EPS-producing and EPS-nonproducing lactococci, GM17 agar medium containing 80 mg/L ruthenium red (**RRM17**) and 5% skim milk powder was used (Dabour et al., 2005a). Ruthenium red (Sigma) was sterilized through a 0.45-μm filter and was added to the molten GM17 agar medium just before pouring it into the plates. Serial dilutions of cheese samples were plated on RRM17 agar media and incubated aerobically at 30°C for 48 h. Following incubation, EPS-producing colonies appear rosy white, whereas EPS-nonproducing colonies are red.

Textural Profile Analysis

Texture profile analysis (**TPA**) was performed on cheese samples using the double compression test (TA-XT2 Texture Analyzer, Texture Technologies Corp., Scarsdale, NY). Ten cylindrical portions (1 cm high and 1 cm in diameter) were removed from the interior of the cheese with a cork borer and held in sealed containers at room temperature for 1 h before testing. Samples were double compressed to 80% of their original height at a compression speed of 2 cm/min. The following parameters were evaluated by TPA according to the definitions given by the International Dairy Federation (1991): Hardness is the force required to attain a given deformation; fracturability is the force at which the material fractures; springiness or elasticity is the rate at which a deformed material goes back to its undeformed condition after the deforming force is removed; and cohesiveness is defined as the quantity simulating the strength of the internal bonds making up the body of the product.

The above textural parameters were determined using Texture Expert software (version 1.22, Stable Micro Systems Ltd., Haslemere, UK). The value of the peak force of the first compression (bite) is the measure of hardness (in newtons, N). The force recorded at the yield point during the first bite is the measure of fracturability (N). The ratio between areas under peak force of the second bite to that of the first bite is the measure of cohesiveness (no dimension). The measure of springiness (no dimension) is the ratio of the distance taken to reach the force peak during the second bite to the distance elapsed to reach the peak force during the first bite.

Analysis of Cheese Microstructure by Transmission Electron Microscopy

For microstructural studies, samples (0.8 to 1.0 mm^3) cut from cheese blocks were immersed overnight at 4°C in a fixative mixture containing 0.05% (vol/vol) glutaraldehyde (Marivac Ltd., Halifax, NS, Canada), 2.5% (wt/vol) paraformaldehyde, 2 m*M* calcium chloride, 1% (wt/vol) sucrose, and 0.15% (wt/vol) ruthenium red (Sigma) in 0.1 *M* sodium cacodylate buffer, pH 7.2 (Dabour et al., 2005b). After thorough washing in cacodylate buffer containing 0.15% ruthenium red, cheese samples were postfixed overnight with 1% (wt/vol) osmium tetroxide (JBS-CHEM, Dorval, PQ, Canada) in the same buffer at 4°C, dehydrated in a graded ethanol series, and embedded in Epon 812 (Polyscience Inc., Warrington, PA). Thin sections (0.7 μm), cut from the Epon-embedded material using glass knives, were mounted on glass slides and stained with 1% aqueous toluidine blue before examination with a Zeiss Axioscope microscope (Carl Zeiss Canada, Don Mills, ON, Canada). Ultrathin sections (0.1 μm) were collected on Formvar-coated nickel grids and were contrasted with uranyl acetate and lead citrate for direct examination with a JEOL 1230 EX electron microscope (JEOL, Tokyo, Japan) at 80 kV. An average of 3 grids from each cheese sample containing 3 to 4 ultrathin sections were examined, and approximately 3 to 5 fields from each ultrathin section were subjected to image analysis.

The images obtained by JEOL 1230 were uploaded into Gatan Digital Micrograph (version 3.7.0), converted to digital images, with pixels in the grayscale from 0 to 250 (from black to white), and analyzed using Gatan software (Gatan Inc., Pleasanton, CA) according to the method described by Pastorino et al. (2003b). During image analysis, brightness and contrast were adjusted to 50%. By applying threshold function, image gray pixels were converted either to white or black pix-

Table 2. Cheese yield and composition on the day of manufacture[1]

Parameters	Cheese[2]					
	CFF	CLF	SMQ	JRF	SMJF	LEC
Yield, kg/100 kg)	11.73 ± 0.21[a]	9.48 ± 0.17[d]	9.76 ± 0.03[c]	10.67 ± 0.14[b]	10.48 ± 0.14[b]	9.85 ± 0.01[bc]
Fat,[3] %	30.75 ± 0.35[a]	15.50 ± 0.03[b]	15.00 ± 0.25[b]	15.00 ± 0.00[b]	14.75 ± 0.35[b]	15.25 ± 0.35[b]
Protein, %	23.77 ± 0.57[d]	28.63 ± 0.23[a]	27.06 ± 0.38[b]	25.26 ± 0.66[c]	25.60 ± 0.11[c]	27.13 ± 0.26[b]
Ash, %	2.85 ± 0.09[d]	3.40 ± 0.13[a]	3.16 ± 0.10[b]	2.80 ± 0.10[d]	3.04 ± 0.11[c]	3.24 ± 0.15[b]

[a–d]Superscript letters following numbers in the same row denote significant differences ($P \leq 0.05$).

[1]Samples were taken after pressing. Data are means ± standard deviation.

[2]The 6 experimental cheeses are as follows: CFF = control cheese made with full-fat milk with EPS-nonproducing starter; CLF = reduced-fat cheese with EPS-nonproducing starter; SMQ = reduced-fat cheese with capsular EPS-producing strain; JRF = reduced-fat cheese with ropy EPS-producing strain; SMJF = reduced-fat cheese with capsular and ropy EPS-producing strains (1:1 mix); and LEC = cheese with 0.2% lecithin added, with EPS-nonproducing starter.

[3]Wet weight basis determination.

els, which corresponded to areas of the micrograph occupied by fat/serum pockets and protein matrix, respectively. Pixels with gray values lower than the threshold level were converted to black and those with gray values higher than the threshold level were converted to white. Thresholding was then stopped and the image analyzed by choosing particle analysis from the analysis menu, which compiles the particle frequency according to the categories chosen. The area of cheese matrix occupied by fat and serum pockets (white area) and protein matrix (dark area) was determined and expressed as percentage of the total area.

Statistical Analyses

All cheese treatments were carried out in duplicate using the same lot of milk; all analyses were done in triplicate. Statistical analyses were performed with Statgraphics plus 4.1 (Manugistics Inc., Rockville, MD). Significant differences between treatments were tested by ANOVA. Treatment comparisons were performed using Fisher's least-significant differences (LSD) test, with a P-value of ≤ 0.05 considered significant.

RESULTS AND DISCUSSION

Chemical Composition and Yield

The mean chemical composition and yield values (Table 2) for full-fat (CFF) and 50% reduced-fat (CLF) cheeses are similar to those reported previously by Benech et al. (2003), Mistry and Kasperson (1998), and Olabi and Barbano (2002). Reduced-fat cheeses with added EPS-producing strains had significantly higher yield compared with CLF cheese, which could be attributed mainly to the increased moisture retention in these cheeses (Table 3). The ropy JRF-1 strain appeared to significantly increase cheese yield more than the capsular SMQ-461 strain, which may result from the higher accessibility of the released EPS (ropy) to bind water molecules than that of cell-attached EPS (capsular). Similarly, the application of the ropy strain of *Streptococcus thermophilus* MTC360 in low-fat Mozzarella cheese production resulted in 11.3% cheese yield vs. 10.83% for cheese with the capsular strain *Strep. thermophilus* MR-1C and 10.13% for cheese made with the EPS-nonproducing strain *Strep. thermophilus* TAO61 (Petersen et al., 2000). Using transmission electron microscopy, we previously demonstrated that EPS released by the JRF-1 ropy strain into the Cheddar cheese matrix was arranged to form a network-like structure in the residual whey pockets at the fat/casein interface, and this structure may help retain more water molecules than capsule-attached EPS (Dabour et al., 2005b). On the other hand, cheese made with both EPS-producing strains (SMJF) had a significantly higher yield than did SMQ cheese, but slightly lower ($P > 0.05$) than JRF cheese.

The addition of 0.2% lecithin (LEC cheese) resulted in significant increase in cheese yield compared with CLF cheese. The cheese yield of 9.85 kg/100 kg reported in this study is very close to the yield of 9.7 kg/100 kg reported by Drake et al. (1996b) for 33%-reduced-fat Cheddar cheese containing 0.2% lecithin. Yield values reported for LEC did not significantly differ from that of SMQ, but were significantly lower than those of JRF and SMJF.

Generally, cheeses with added EPS-producing strains or lecithin had significantly lower protein and ash contents compared with control cheese. However, fat content did not significantly differ among cheeses (Table 2).

Changes in Moisture and pH

The mean moisture content of CFF (39.51%) and CLF (49.26%) cheeses on the day of manufacture are in

Table 3. Changes in moisture, pH and water-soluble nitrogen (means ± SD) during ripening of experimental full- and reduced-fat Cheddar cheeses

Cheese[1]	Cheese age, mo	Moisture, %	pH	WSN/TN,[2] %
CFF	0	39.5 ± 0.10^{o}	5.05 ± 0.07^{c}	5.43 ± 0.22^{n}
	1	39.19 ± 0.15^{p}	4.97 ± 0.03^{e}	10.10 ± 0.38^{j}
	3	38.89 ± 0.24^{q}	4.95 ± 0.04^{f}	18.95 ± 0.14^{e}
	6	38.62 ± 0.10^{r}	4.95 ± 0.07^{f}	20.42 ± 0.14^{c}
CLF	0	49.26 ± 0.11^{k}	5.00 ± 0.03^{d}	5.14 ± 0.17^{n}
	1	48.78 ± 0.37^{l}	4.90 ± 0.04^{h}	9.61 ± 0.05^{k}
	3	48.25 ± 0.39^{m}	4.98 ± 0.03^{e}	17.95 ± 0.03^{f}
	6	47.76 ± 0.85^{n}	5.00 ± 0.02^{d}	19.62 ± 0.07^{d}
SMQ	0	52.90 ± 0.36^{b}	5.10 ± 0.05^{a,b}	5.05 ± 0.26^{n}
	1	51.95 ± 0.10^{d}	4.95 ± 0.04^{f}	10.11 ± 0.15^{j}
	3	49.89 ± 0.16^{i}	4.83 ± 0.03^{j}	18.20 ± 0.20^{f}
	6	48.04 ± 0.15^{m}	4.90 ± 0.01^{h}	20.32 ± 0.27^{c}
JRF	0	54.06 ± 0.39^{a}	4.88 ± 0.03^{i}	6.59 ± 0.17^{l}
	1	53.98 ± 0.10^{a}	4.83 ± 0.04^{j}	13.04 ± 0.09^{h}
	3	52.68 ± 0.10^{c}	4.80 ± 0.03^{k}	20.30 ± 0.02^{c}
	6	51.17 ± 0.10^{e}	4.73 ± 0.04^{m}	23.46 ± 0.35^{a}
SMJF	0	52.90 ± 0.73^{b}	4.93 ± 0.03^{g}	6.14 ± 0.19^{m}
	1	51.51 ± 0.35^{d}	4.75 ± 0.04^{l}	11.56 ± 0.39^{i}
	3	49.76 ± 0.15^{j}	4.75 ± 0.05^{l}	19.56 ± 0.19^{d}
	6	48.17 ± 0.10^{m}	4.70 ± 0.03^{n}	21.71 ± 0.18^{b}
LEC	0	50.69 ± 0.13^{f}	5.15 ± 0.07^{a}	5.12 ± 0.25^{n}
	1	50.21 ± 0.18^{h}	4.98 ± 0.04^{e}	9.68 ± 0.09^{k}
	3	50.09 ± 0.12^{h}	4.93 ± 0.03^{g}	17.18 ± 0.10^{g}
	6	50.13 ± 0.10^{h}	5.00 ± 0.05^{d}	19.75 ± 0.05^{d}

[a–r]Superscript letters following numbers in the same column denote significant differences ($P \leq 0.05$).

[1]The 6 experimental cheeses are as follows: CFF = control cheese made with full-fat milk with EPS-nonproducing starter; CLF = reduced-fat cheese with EPS-nonproducing starter; SMQ = reduced-fat cheese with capsular EPS-producing strain; JRF = reduced-fat cheese with ropy EPS-producing strain; SMJF = reduced-fat cheese with capsular and ropy EPS-producing strains (1:1 mix); and LEC = cheese with 0.2% lecithin added, with EPS-nonproducing starter.

[2]Water-soluble nitrogen/total nitrogen.

agreement with values reported previously for full- and 50%-reduced-fat cheeses, respectively (Mistry, 2001; Kheadr et al., 2002). The moisture content of lecithin-containing cheese on the day of manufacture did not differ significantly from that for SMQ cheese, but was significantly lower compared with JRF and SMJF cheeses (Table 3). In general, moisture content determined on the day of manufacture in JRF, SMJF, LEC, and SMQ cheeses were, respectively, 4.80, 3.64, 1.43, and 1.32% higher than that for CLF cheese. For EPS-containing cheeses, moisture retention was the highest in cheeses with the added ropy JRF-1 strain (JRF) compared with cheeses with either added capsular SMQ-461 strain (SMQ) alone or the ropy/capsular strain mixture (SMJF; Table 3). Similarly, higher water retention by EPS ropy-producing streptococci in low-fat Mozzarella cheese compared with EPS capsular-forming streptococci has been reported by Petersen et al. (2000). The ropy *Strep. thermophilus* MTC360 strain has been reported to increase moisture retention in Mozzarella cheese by 6.1 vs. 2.7% resulting from the use of the capsule-forming *Strep. thermophilus* MR-1C strain compared with control cheese made with EPS-nonproducing starter culture.

Although cheeses were vacuum packed, there were significant decreases in moisture content for all cheeses as ripening progressed. A very fine water layer on the cheese surface was observed on cheese blocks after opening the vacuum-packed plastics. The highest decrease in moisture content was observed for SMJF cheese, in which moisture at 6 mo decreased by 4.73% compared with moisture values for the same cheese on the day of manufacture. Moisture decreases of 0.56, 1.50, 2.54, and 2.89% were observed for 6-mo-old LEC, CLF, SMQ, and JRF cheeses, respectively. The lowest moisture decrease noted during ripening (for LEC) may indicate stability of its water-holding capacity over ripening time. Water losses in cheeses with added EPS-forming strains might be related to reduction in the stability of the EPS/water complex in cheese during ripening, perhaps due to EPS degradation. This possibility raises the necessity of developing a method for measuring the EPS in cheese and following its stability during ripening. Other chemical changes that take

place during ripening, including structural and pH changes, might also be responsible for water losses.

Although reduced-fat cheese production was stopped at the relatively high pH value of 5.5 ± 0.05, pH values of 0-d-old cheeses (samples taken immediately after cheese pressing) were lower than pH 5.5 by approximately 0.35 to 0.6 pH units, indicating that acid production continued during cheese pressing (Table 3). The highest shift in pH was observed in cheeses with the added ropy JRF-1 strain, which may be attributed partly to the acidification capacity of this EPS-producing strain or to the elevated moisture content. In a previous study, both ropy JRF-1 and capsular SMQ-461 strains showed high and moderate acidification capacity, respectively, during skim milk fermentation and Cheddar cheese production (Dabour et al., 2005a). This study showed that the time required to complete the cheese-making procedure (i.e., attain pH 5.5) for SMQ, SMJF, and JRF cheeses was 41, 66, and 91 min less, respectively, than that required for the control (CLF) cheese. In addition, increasing the moisture level in cheese, as caused by EPS-producing strains, results in increased lactose retention, and thus, bacterial activity leading to greater acid accumulation in cheese (Visser, 1993; Walstra et al., 1993).

Upon aging, pH continued to decrease in cheeses with the added ropy JRF-1 strain alone or mixed with the SMQ-461 strain. The SMQ cheese showed significantly lower pH values at 3 and 6 mo compared with CLF cheese. However, lecithin did not appear to affect pH as much as the EPS-producing strains did, as LEC showed pH values either slightly higher or similar to those for CLF throughout ripening.

Water-Soluble Nitrogen

On the day of manufacture, there were no significant differences in WSN content among cheeses except for those with the added *L. cremoris* JRF-1 ropy strain (JRF and SMJF; Table 3). The WSN contents of 0-d-old SMJF and JRF cheeses were 19.5 and 28.0% higher, respectively, than that of control CLF cheese. The increased WSN content in cheeses with added *L. cremoris* JRF-1 could be attributed to the elevated moisture content determined in these cheeses or to the proteolytic and autolytic activities of the added JRF-1 strain. Elevated moisture content in cheese was previously reported to induce cheese proteolysis (Visser, 1993). The proteolytic activity of lactococcal strains used for cheese production in the present study was assessed individually in reconstituted skim milk using *o*-phthaldialdehyde reagent, and the JRF-1 strain showed the highest activity to liberate free amino acids (Dabour et al., 2005a). In a previous study, we reported on the autolytic activity of the JRF-1 strain in 0-d-old Cheddar cheese, as visualized by transmission electron microscopy, where this strain appeared to be extensively autolysed with massive degradation and hydrolysis of the cell wall (Dabour et al., 2005b). This activity may also contribute to cheese proteolysis and induce the formation of water-soluble nitrogen in the form of small peptides and amino acids.

Upon aging, the amount of WSN increased significantly in all cheeses with increasing ripening time. This is consistent with previous studies (Kheadr et al., 2000; Sallami et al., 2004) and can be attributed to continued degradation of casein to low molecular weight water-soluble peptides and amino acids by residual coagulant and proteolytic activity of the cheese starter culture. Six-month-old SMQ, SMJF, and JRF cheeses accumulated, respectively, 3.6, 10.7, and 19.6% higher WSN compared with CLF cheeses. On the other hand, lecithin did not appear to affect cheese WSN content, as LEC cheese did not show significant differences in its WSN content compared with those determined in CLF cheese throughout the 6 mo of ripening, except that a lower content was found at 3 mo.

Viable Cell Enumeration and Localization in the Cheese Matrix

The total viable bacterial counts did not significantly differ among fresh cheese samples and 1-mo-old cheeses (Figure 1A). However, significant reductions in total viable counts became evident at 3 and 6 mo. The total viable lactococcal counts did not significantly differ among 0-d-old cheese samples (Figure 1B). However, there was a gradual decline in viable lactococci as ripening progressed, resulting in an approximately 2 to 3 log reduction after 6 mo. Similar results have been reported previously by Wilkinson et al. (1994) and Sallami et al. (2004), who showed that lactococcal populations reached a maximum during or shortly after Cheddar cheese manufacture and decreased gradually as cheese age progressed. Significant reductions in total viable lactococci were observed for JRF cheese at 1 mo and for SMQ and SMJF cheeses at 3 and 6 mo compared with CLF. Reductions in viable lactococcal counts in JRF were significantly higher compared with the reductions determined for SMQ and SMJF cheeses.

No significant differences were found in counts of EPS-producing strains among fresh-pressed cheese blocks SMQ, JRF, and SMJF (Figure 1C). Upon aging, however, there was a significant reduction in viability of EPS-producing strains, which was more remarkable for the ropy strain than for the capsular strain. For mo 1 and 3, counts of EPS-producing strains were not significantly different between JRF and SMJF cheeses,

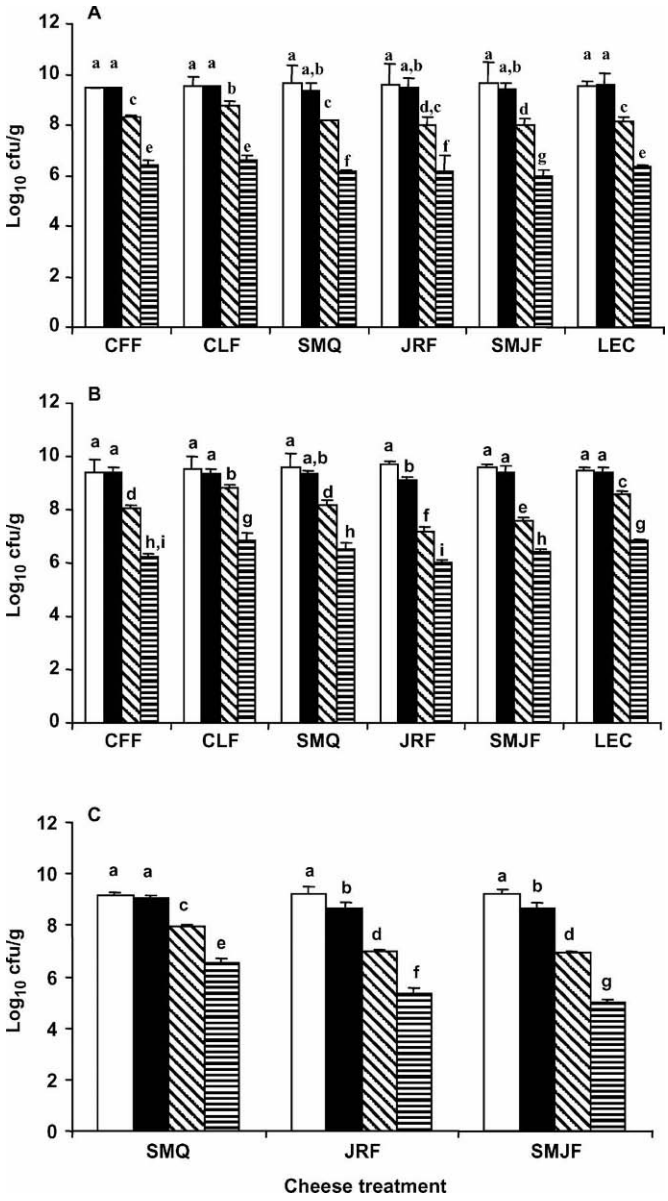

Figure 1. Changes in total bacterial (A), lactococcal viable counts (B), and exopolysaccharide (EPS)-producing viable counts (C) during ripening of experimental cheeses. Cheese samples were analyzed after pressing (white bars) and at 1 mo (black bars), 3 mo (diagonal striped bars), and 6 mo (horizontal striped bars) of ripening at 7°C. Means without common letters are significantly different ($P < 0.05$). Cheeses: CFF and CLF = full- and reduced-fat cheeses made with EPS-nonproducing starter culture, respectively; SMQ, JRF, and SMJF = cheeses made with starter culture containing capsular, ropy, or capsular/ropy combination of strains, respectively, and LEC = cheese made with lecithin (0.2% wt/vol) added to cheese milk with the EPS-nonproducing starter culture.

but were significantly lower than those determined in SMQ cheese. At 6 mo, there were significant differences in the counts of EPS-producing strains among the 3 EPS cheese types. Compared with the viable counts (approximately 10^9 cfu/g) determined in freshly pressed SMQ and JRF cheeses, the counts of capsular SMQ-461 and ropy JRF-1 strains after 1 mo decreased, respectively, by 0.2 and 0.9 log cycles cfu/g, whereas reductions of 2.5 and 4 log cycles cfu/g were determined after 6 mo. These reductions correspond to those previously reported for total lactococcal counts and might be related to the reduced survivability of the ropy JRF-1 strain in the cheese matrix during ripening. In a previous study, we reported on the autolytic ability of both capsular SMQ-461 and ropy JRF-1 strains as determined by transmission electron microscopy in freshly pressed cheese blocks made with these strains (Dabour et al., 2005b). Cells of the ropy JRF-1 strain showed higher autolysis with massive destruction and disappearance of large fragments of the cell wall accompanied by either complete or partial disappearance of the cytoplasmic content. Autolysis of the SMQ-461 capsular strain appeared to be more regular, resulting from the formation of small pores in the cell wall, having a diameter of approximately 40 to 50 nm, followed by a regular release of the cytoplasmic content.

The micrographs in Figure 2 show the EPS-producing strains in the cheese matrix at the beginning and after 6 mo of ripening. The EPS-producing strains are generally localized in the whey pockets at the fat/casein interface, where water and nutrients are accessible, and very few were seen embedded in the protein matrix, as observed previously (Oberg et al., 1993). As was reported by Dabour et al. (2005b), the EPS released by the ropy JRF-1 strain into cheese whey pockets appeared to form a network-like structure (Figure 2, b and c). In Feta and Karish cheeses, using cryoscanning electron microscopy, Hassan et al. (2003) also reported the formation of a network-like structure formed by EPS released from *L. cremoris* JRF-1, appearing as large masses of dense filaments and occupying the spaces among the casein micelles. After 6 mo, this structure was still visualized, but it became less dense (Figure 2d). The EPS layer produced by the capsule-forming strain could be easily seen at the beginning and after 6 mo of ripening (Figure 2, e and f).

Textural Characteristics

The changes in rheological parameters were determined by the texture profile analyzer in terms of hardness, fracturability, springiness, and cohesiveness during ripening of experimental cheeses (Table 4). The manufacturing procedure used for the production of reduced-fat cheese in this study was not the same as for full-fat cheese. The low temperature profile and the higher pH at salting used for reduced-fat cheese manu-

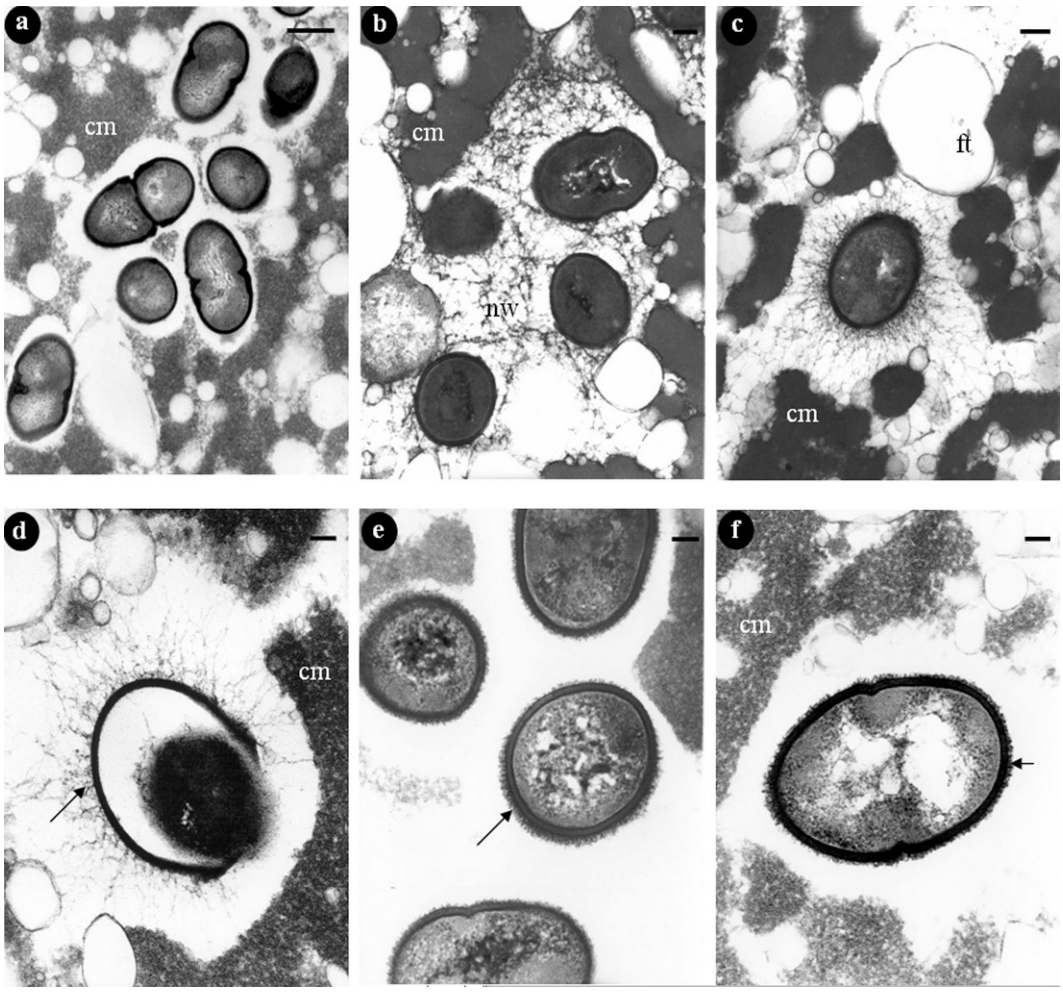

Figure 2. Transmission electron micrographs showing exopolysaccharide (EPS)-producing lactococci in reduced-fat Cheddar cheese matrix at the beginning and after 6 mo of ripening. Panels show EPS-nonproducing lactococci in 0-d-old CLF cheese (A); and ropy strain *Lactococcus lactis* ssp. *cremoris* JRF-1 present in residual whey pockets in 0-d-old (B, C) and 6-mo-old (D) JRF cheese matrix. CLF = reduced-fat cheese made with EPS-nonproducing starter culture; JRF = cheese made with starter culture containing ropy strain JRF-1; cm = casein matrix, ft = fat globule, and nw = EPS network-like structure; arrows point to cell-attached EPS. Bars indicate 500 nm (a), 200 nm (b, c), and 100 nm (d, e, f).

facturing aimed to increase moisture content and reduce acid accumulation to correct the textural defects resulting from fat removal. Even with these modifications, values for the 4 rheological parameters for CLF cheese remained significantly different from those reported for CFF cheese throughout the ripening period. These results are in agreement with those of Beal and Mittal (2000), who found that as fat in Cheddar cheese decreased, hardness, springiness, and cohesiveness increased.

The use of EPS-producing strains modified the textural characteristics of experimental reduced-fat Cheddar cheeses as determined by TPA. The values for the 4 textural parameters were all significantly lower than those determined for CLF cheese on the day of manufacture. The JRF cheese exhibited significantly lower values for hardness, fracturability, and springiness, but slightly higher values for cohesiveness compared with SMQ cheese. The values for TPA hardness and fracturability on the day of manufacture were in the order CLF > SMQ > SMJF > JRF. However, values for springiness were in the order CLF > SMQ > JRF> SMJF. Lecithin also affected the textural characteristics of reduced-fat cheese. On the day of manufacture, LEC cheese had significantly lower values for hardness, fracturability, and cohesiveness, but higher springiness values compared with CLF cheese. Lecithin addition resulted in lower TPA hardness, fracturability, and cohesiveness, but not as low as the values obtained with cheeses containing EPS-producing strains.

Table 4. Changes in textural characteristics and protein-occupied area (mean ± SD) in cheese matrix during ripening of experimental full- and reduced-fat Cheddar cheeses

Cheese[1]	Cheese age (mo)	Textural characteristics[2]				Protein-occupied area,[3] %
		Hardness, N	Fracturability, N	Springiness	Cohesiveness	
CFF	0	27.2 ± 1.9^{i}	25.8 ± 2.4^{b}	0.598 ± 0.051^{e}	0.278 ± 0.025^{a}	46.3 ± 3.4^{g}
	1	26.0 ± 2.5^{ij}	14.6 ± 1.4^{ijk}	0.281 ± 0.088^{n}	0.182 ± 0.023^{o}	ND[4]
	3	31.9 ± 2.2^{fg}	14.6 ± 1.9^{ijk}	0.233 ± 0.047^{s}	0.194 ± 0.011^{l}	ND
	6	33.8 ± 3.2^{de}	15.7 ± 1.3^{hi}	0.216 ± 0.041^{u}	0.180 ± 0.006^{p}	57.4 ± 3.6^{cd}
CLF	0	37.8 ± 3.6^{b}	29.2 ± 2.7^{a}	0.726 ± 0.060^{b}	0.272 ± 0.029^{b}	58.0 ± 2.5^{cd}
	1	33.8 ± 3.2^{de}	23.5 ± 2.6^{c}	0.386 ± 0.038^{g}	0.236 ± 0.012^{e}	ND
	3	35.9 ± 1.8^{c}	17.5 ± 2.6^{def}	0.277 ± 0.05^{o}	0.203 ± 0.010^{k}	ND
	6	38.0 ± 3.3^{b}	17.9 ± 2.0^{de}	0.260 ± 0.027^{p}	0.187 ± 0.008^{n}	67.3 ± 2.9^{a}
SMQ	0	32.4 ± 2.4^{ef}	23.6 ± 2.4^{c}	0.708 ± 0.061^{c}	0.229 ± 0.021^{f}	55.6 ± 3.0^{de}
	1	30.6 ± 2.9^{gh}	17.1 ± 1.4^{efg}	0.331 ± 0.039^{j}	0.212 ± 0.008^{h}	ND
	3	34.9 ± 2.6^{cd}	18.2 ± 2.5^{de}	0.277 ± 0.041^{o}	0.210 ± 0.018^{i}	ND
	6	39.2 ± 2.7^{ab}	18.7 ± 2.0^{d}	0.243 ± 0.037^{q}	0.207 ± 0.013^{j}	63.2 ± 2.6^{ab}
JRF	0	24.0 ± 2.8^{jk}	10.0 ± 0.4^{l}	0.646 ± 0.044^{d}	0.242 ± 0.022^{d}	52.6 ± 2.6^{ef}
	1	20.7 ± 1.8^{l}	10.2 ± 1.5^{l}	0.337 ± 0.047^{i}	0.195 ± 0.011^{l}	ND
	3	26.6 ± 2.6^{i}	11.3 ± 0.9^{l}	0.225 ± 0.032^{t}	0.194 ± 0.008^{l}	ND
	6	32.3 ± 2.9^{efg}	13.3 ± 1.4^{k}	0.213 ± 0.037^{v}	0.186 ± 0.009^{n}	58.8 ± 2.0^{cd}
SMJF	0	24.7 ± 2.4^{k}	17.7 ± 1.0^{de}	0.538 ± 0.077^{f}	0.249 ± 0.017^{c}	50.7 ± 2.9^{fg}
	1	23.2 ± 1.7^{k}	13.3 ± 1.0^{k}	0.300 ± 0.044^{l}	0.194 ± 0.013^{l}	ND
	3	35.4 ± 2.6^{c}	14.1 ± 1.7^{jk}	0.285 ± 0.071^{m}	0.190 ± 0.015^{m}	ND
	6	40.5 ± 3.2^{a}	15.3 ± 1.5^{hij}	0.259 ± 0.048^{p}	0.167 ± 0.014^{q}	60.8 ± 1.7^{bc}
LEC	0	33.2 ± 2.1^{ef}	24.9 ± 2.0^{b}	0.804 ± 0.045^{a}	0.237 ± 0.018^{e}	57.1 ± 2.4^{cd}
	1	24.3 ± 0.4^{k}	24.0 ± 2.8^{bc}	0.369 ± 0.054^{h}	0.219 ± 0.011^{g}	ND
	3	29.9 ± 1.7^{fgh}	16.0 ± 0.9^{gh}	0.304 ± 0.037^{k}	0.213 ± 0.006^{h}	ND
	6	38.5 ± 3.4^{b}	16.2 ± 1.3^{fgh}	0.238 ± 0.043^{r}	0.189 ± 0.013^{m}	66.6 ± 1.8^{a}

$^{a–v}$Superscript letters following numbers in the same column denote significant differences ($P \leq 0.05$).

[1]The 6 experimental cheeses are as follows: CFF = control cheese made with full-fat milk with EPS-nonproducing starter; CLF = reduced-fat cheese with EPS-nonproducing starter; SMQ = reduced-fat cheese with capsular EPS-producing strain; JRF = reduced-fat cheese with ropy EPS-producing strain; SMJF = reduced-fat cheese with capsular and ropy EPS-producing strains (1:1 mix); and LEC = cheese with 0.2% lecithin added, with EPS-nonproducing starter.

[2]Determined by texture profile analyzer.

[3]Determined through image analysis of transmission electron micrographs (see materials and methods).

[4]ND = Not determined.

Major changes in the rheological parameters occurred during the first month of ripening, when all experimental cheeses exhibited significant decreases in values for each rheological parameter. Indeed, texture development of Cheddar cheese during ripening takes place in 2 distinct phases (Lawrence et al., 1987). During the first 2 wk of ripening, α_{s1}-casein is broken down to α_{s1}-1 casein, which reduces the rubbery texture of the cheese. The second stage takes place during the remainder of the ripening period, when textural changes are slowed down and depend on proteolysis of the protein by residual coagulant enzymes and proteases released by starter bacteria and cheese secondary microflora (Fox, 1989). On the other hand, the changes of each rheological parameter during the remainder of ripening were quite different. From the third month, values for hardness and fracturability started to increase until the end of ripening, whereas springiness and cohesiveness continued to decrease.

At 6 mo, texture data revealed that CLF cheese was more firm, springy, and cohesive, but less brittle compared with CFF cheese. Among EPS-containing cheeses, JRF had the lowest values for each rheological parameter at 6 mo. The SMQ cheese was more firm and cohesive, but less brittle and springy than CLF. The SMJF cheese appeared to be more firm but less brittle and cohesive than CLF cheese. These results indicate that the application of EPS-producing strains for reduced-fat Cheddar production can contribute to modifying the textural properties of the resultant cheeses. The form of EPS may have a remarkable role in modifying cheese textural properties because ropy EPS, unlike the capsular form, may induce the formation of a less compact texture, which is undesirable in old Cheddar cheese. Old Cheddar cheese is usually characterized by harder, and less cohesive, adhesive, gummy, springy, and chewy texture compared with unripened cheese (Beal and Mittal, 2000).

Six-month-old LEC cheese showed slightly higher values for hardness and cohesiveness but lower values for fracturability and springiness compared with CLF cheese. Drake et al. (1996b) used the TA-XT2 texture analyzer to evaluate the firmness of 33% reduced-fat Cheddar cheese with added 0.2% (wt/wt) lecithin after 3 mo and reported that cheese firmness did not significantly differ between cheese with added lecithin and the control cheese.

Cheddar cheese texture is highly determined by the pH of the cheese curd at the whey drainage step, cheese moisture content, and the extent of proteolysis during ripening. The increased moisture content of cheeses with added EPS-producing strains has influenced their textural characteristics. Slight differences in moisture in Cheddar cheese have been shown to cause major differences in rheological properties (De Jong, 1978). On the day of manufacture, the water molecules entrapped in the cheese matrix are accumulated principally in pockets surrounding the bacterial cells or attached to the bacterial cell surface. During ripening, casein is broken down and protein-protein interactions increase, leading to increased protein matrix strength and contraction of the protein matrix, which induces water expulsion from the cheese matrix. This may explain the increased values for hardness and fracturability between 3 and 6 mo of ripening and moisture loss observed for experimental cheeses. The increased hardening of cheese could be due to the increase in free amino acids. Creamer and Olson (1982) attributed increased cheese hardness as ripening progressed to increased concentration of free amino acids and small peptides generated from casein hydrolysis, which have the ability to bind free water molecules and consequently increase the strength of the casein matrix and thus increase the resistance of cheese to deformation. In addition, low pH of cheese was also reported to increase cheese hardness (Lawrence et al., 1987).

Increased cheese moisture content in Cheddar cheese has been associated with decreased values for fracturability determined by TPA (Kheadr et al., 2002 and 2003). In addition to moisture, casein breakdown and cheese pH have also been reported to reduce cheese fracturing force (Kheadr et al., 2000; Creamer et al., 1982). This may explain lower values for fracturability reported for JRF and SMJF cheeses, which showed the highest WSN content and the lowest pH values among experimental cheeses. The rapid decrease in fracturing force reported in this study during the initial 4 wk of maturation was previously reported by Creamer et al. (1982). The increased fracturing force during the last 3 mo of ripening in our experimental cheeses was similar to that reported by Luyten and van Vliet (1996) for mature Gouda cheese and ascribed to moisture loss during ripening.

The breakdown of the protein network caused by proteolysis is related to decreased cheese elasticity (Creamer and Olson, 1982; Tunick et al., 1990; Hort and Le Grys, 2001). This may explain the lowest springiness values determined for JRF cheese, which also generated the highest WSN content among reduced-fat experimental cheeses. The decrease in cheese springiness during ripening has been reported previously for Cheddar and other cheeses, and is attributed to the release of calcium ions from monocalcium and dicalcium para κ-caseinate molecules and to the hydrolysis of these molecules during ripening (Fox, 1989; Maifreni et al., 2002).

The significant reduction in cohesiveness values reported for all cheeses as ripening progressed could be attributed to water losses and increased proteolysis. Cheese cohesiveness was shown to decrease as cheese moisture content decreased (Pastorino et al., 2003b). Cheese cohesiveness is inversely related to cheese proteolysis, with a trend of decreasing with increasing proteolysis (Lane et al., 1997). In addition, the lower pH of cheese, as for cheeses with the added JRF-1 strain, may cause further reductions in TPA-cohesiveness values. Decreases in the pH of the cheese curd are correlated with gradual dissociation of the casein micelles into small aggregates (Roefs et al., 1985). At pH below 4.8 (JRF and SMJF cheeses at 3 and 6 mo), casein micelles lose their integrity and cohesion (Lawrence et al., 1987).

Cheese Microstructure

The microstructural characteristics of experimental cheeses as visualized by transmission electron microscopy were remarkably different between the day of manufacture and after ripening. On the day of the manufacture, the structure of full-fat cheese consisted of protein matrix interrupted by fat globules and residual whey pockets (Figure 3a). These structural characteristics are typical of those in previous studies of fresh Cheddar cheese (Mistry and Anderson, 1993; Kheadr et al., 2000). On the contrary, CLF cheese exhibited a more extended protein matrix in which few fat globules and substantially larger whey pockets were dispersed (Figure 3b). The overall structure of this cheese seems to be less compact and dense compared with CFF cheese. Drake et al. (1996b) described the structure of reduced-fat Cheddar cheese as a stretched protein matrix with few fat globules scattered between that might be responsible for firmer characteristics compared with full-fat Cheddar.

The numerical analysis of electron microscopy images of 0-d-old full-fat cheese showed that protein occupied

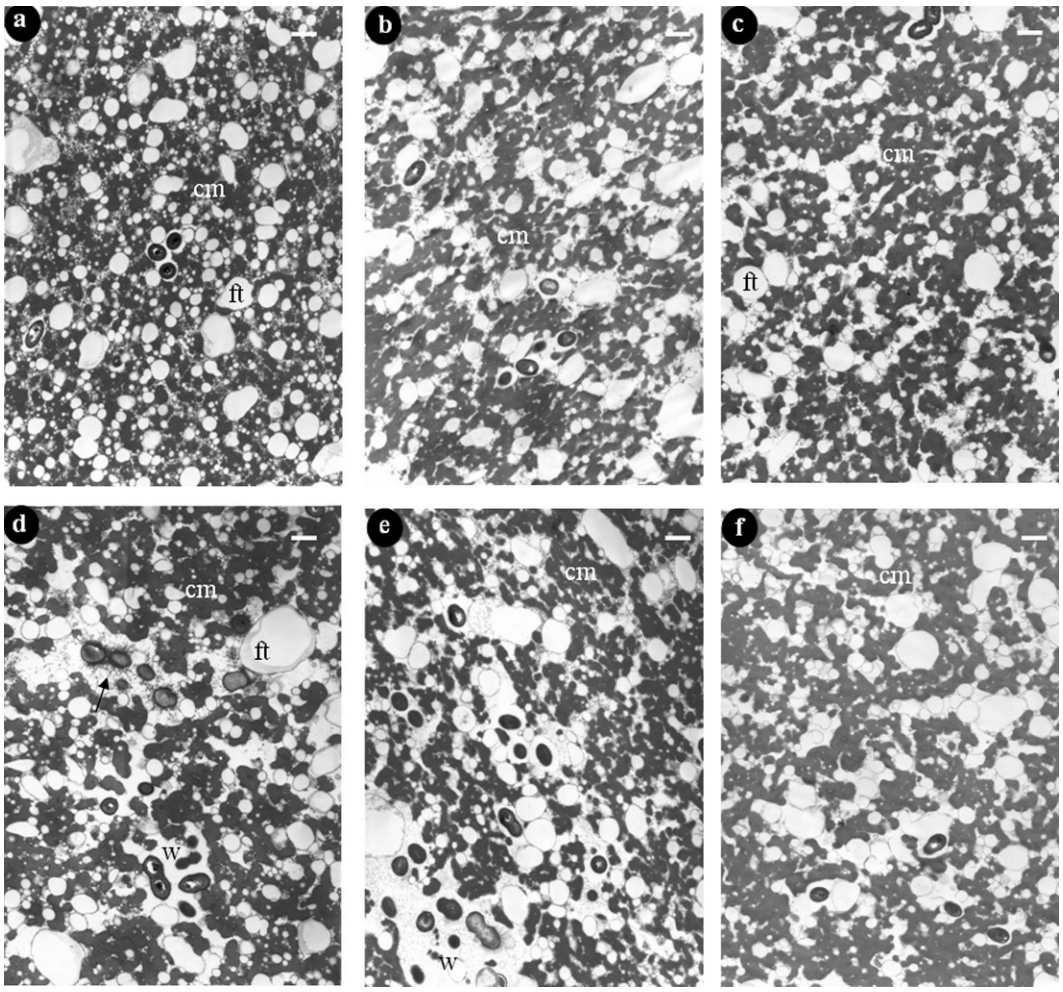

Figure 3. Transmission electron micrographs of full- and reduced-fat Cheddar cheeses at the beginning of ripening (samples taken immediately after cheese pressing). A and B: Full-fat and reduced-fat cheeses made with exopolysaccharide (EPS)-nonproducing starter culture, respectively; C, D, and E: Reduced-fat cheeses made with EPS capsule-forming *Lactococcus lactis* ssp. *cremoris* SMQ-641, ropy-producing *Lactococcus lactis* ssp. *cremoris*, or their combination, respectively; F: Reduced-fat cheese made with 0.2% (wt/vol) lecithin added to cheese milk. Arrows point to bacterial EPS; cm = casein matrix, w = whey pockets, and ft = fat globules; bars indicate 1 μm.

46.3% of the total cheese matrix area, whereas fat and whey occupied the remainder (53.7%; Table 4). These values are very different from those reported by Pastorino et al. (2003a,b), who analyzed digitized images obtained by scanning electron microscopy for 6-wk-old full-fat Cheddar cheese and found that protein and fat/serum occupy 82 and 18% of the cheese matrix, respectively. The differences in area distribution reported in this study and those reported by Pastorino et al. (2003a,b) may have various reasons. The use of organic solvents for defatting cheese samples during preparation for scanning electron microscopy, may have an influence on protein matrix arrangement and may have led to the formation of a more compact matrix. On the other hand, cheeses in this study were made from homogenized milk and consequently, the area occupied by fat globules is thus expected to be higher than that reported by Pastorino et al. (2003a,b) for cheeses made from nonhomogenized milk.

Protein occupied area in 0-d-old CLF cheese was 58% of the total matrix area, which was significantly higher than that reported for CFF cheese. This is in agreement with the previous finding reported by Ustunol et al. (1995), who described the microstructure of reduced-fat Cheddar cheese as a continuous protein network in which few fat globules were dispersed throughout. The higher area occupied by protein in CLF cheese might be responsible for higher TPA firmness, fracturability, and springiness values compared with CFF cheese.

The use of EPS-producing strains resulted in remarkable changes in the microstructure of fresh cheeses (Figure 3 a, b, and c). The protein matrix in these cheeses

was composed of casein aggregates that were irregular in shape and size and granular in appearance. Compared with the CLF cheese, the protein matrix in EPS-containing cheeses was less extended and more open, and larger whey pockets could be seen dispersed throughout. In accordance with the higher moisture retention determined in cheeses with the added ropy JRF-1 strain, large whey pockets of up to 3.5 μm could be seen in both JRF and SMJF cheeses. However, smaller pockets of up to 1.9 μm were dispersed throughout the matrix of SMQ cheese. These pockets were larger than those observed (1 to 1.5 μm) for CLF cheese. This may explain the lower values for TPA hardness and fracturability reported for 0-d-old cheeses containing these organisms compared with control cheeses, indicating that EPS-producing strains can improve the textural characteristics of reduced-fat Cheddar cheese. To improve the textural attributes of reduced-fat Cheddar, it has been suggested that the protein matrix must be broken down to a greater extent than in full-fat cheese (Banks et al., 1989), or moisture content must be increased to levels beyond those in full-fat cheeses (Mistry, 2001). Exopolysaccharide-producing strains can accomplish both goals to improve the texture of reduced-fat cheeses, as they can increase moisture retention, through the water-holding capacity of the EPS, leading to the formation of larger whey pockets that break up the protein matrix.

The observed structural modifications are in agreement with data obtained by numerical analysis of electron micrographs, which indicate that EPS-producing strains, in a strain-dependent manner, resulted in significant reduction in areas occupied by protein in SMQ, JRF, and SMJF cheeses compared with CLF cheese. The ropy JRF-1 strain was much more effective than the capsular SMQ-461 strain at reducing the protein-occupied area. Protein-occupied areas in 0-d-old SMQ, JRF, and SMJF cheeses were 55.6, 52.6, and 50.7%, respectively.

Compared with EPS-producing strains, the addition of lecithin did not appear to have remarkable influence on structural characteristics, at least for fresh cheese, because LEC cheese had structural features very close to that of CLF cheese. This was evident from image analysis data, which indicated that the protein occupied area in 0-d-old LEC was only slightly lower ($P > 0.05$) than that determined for CLF.

At 6 mo, cheeses had a more compact structure and the casein matrices became denser and lost their integrity compared with fresh cheeses (Figure 4). This was evident from the TPA data, where hardness values for all cheeses increased at 6 mo. However, in cheeses with the added ropy strain, the cheese matrix was less compact, whereas some casein aggregates kept their integrity, and did not fuse completely into the protein matrix (Figure 4 c, d, and e). Loss of integrity, a granular appearance of casein aggregates, and the development of a dense, homogeneous, and compact protein matrix are considered the major structural changes occurring during the ripening process (Stanley and Emmons, 1977; Kheadr et al., 2000).

In confirmation of the development of a more compact structure among experimental cheeses aged 6 mo, the areas occupied by protein in the cheese matrix became significantly higher than those reported for 0-d-old cheeses (Table 4). However, the increase varied according to the cheese treatment. In comparison with values determined at d 0, the protein-occupied area increased by 11.1, 9.3, 7.6, 6.2, 10.1, and 9.5%, respectively, in CFF, CLF, SMQ, JRF, SMJF, and LEC cheeses. This indicates that JRF cheese had the least compact structure followed by SMQ, and LEC cheeses had structural features similar to that of CLF cheese. The highest increase in protein-occupied area of 10.1% determined during 6 mo of ripening (SMJF cheese) may be responsible for the water loss of 4.73% that occurred during the same period in this cheese.

The loss of integrity of the protein matrix and the increased area occupied by protein observed after 6 mo of ripening may help to understand the water loss from EPS-containing cheeses. This loss could be explained, at a structural level, by increased protein–protein interaction during ripening, leading to increased contraction of the protein and firmness of the protein matrix, and release of water molecules from the matrix, which would primarily accumulate in pockets distributed throughout the matrix (Pastorino et al., 2003b). This is known as "microsyneresis" through which the number and strength of protein–protein interactions increases and protein hydration is reduced. Due to the accumulated effect of microsyneresis, expulsion of serum from the cheese blocks (macrosyneresis) would take place, leading to decreased moisture content and weight of cheese.

CONCLUSIONS

This study shows the effect of incorporating EPS-producing ropy or capsular strains of *L. lactis* ssp. *cremoris* or their combination into Cheddar cheese starter culture, and the effect of lecithin on the rheological and microstructural characteristics of reduced-fat Cheddar cheese. In a strain-dependent manner, EPS-producing strains had more influence on cheese texture and microstructure than did lecithin. The ropy *L. lactis* ssp. *cremoris* JRF-1 strain was more effective at increasing mois-

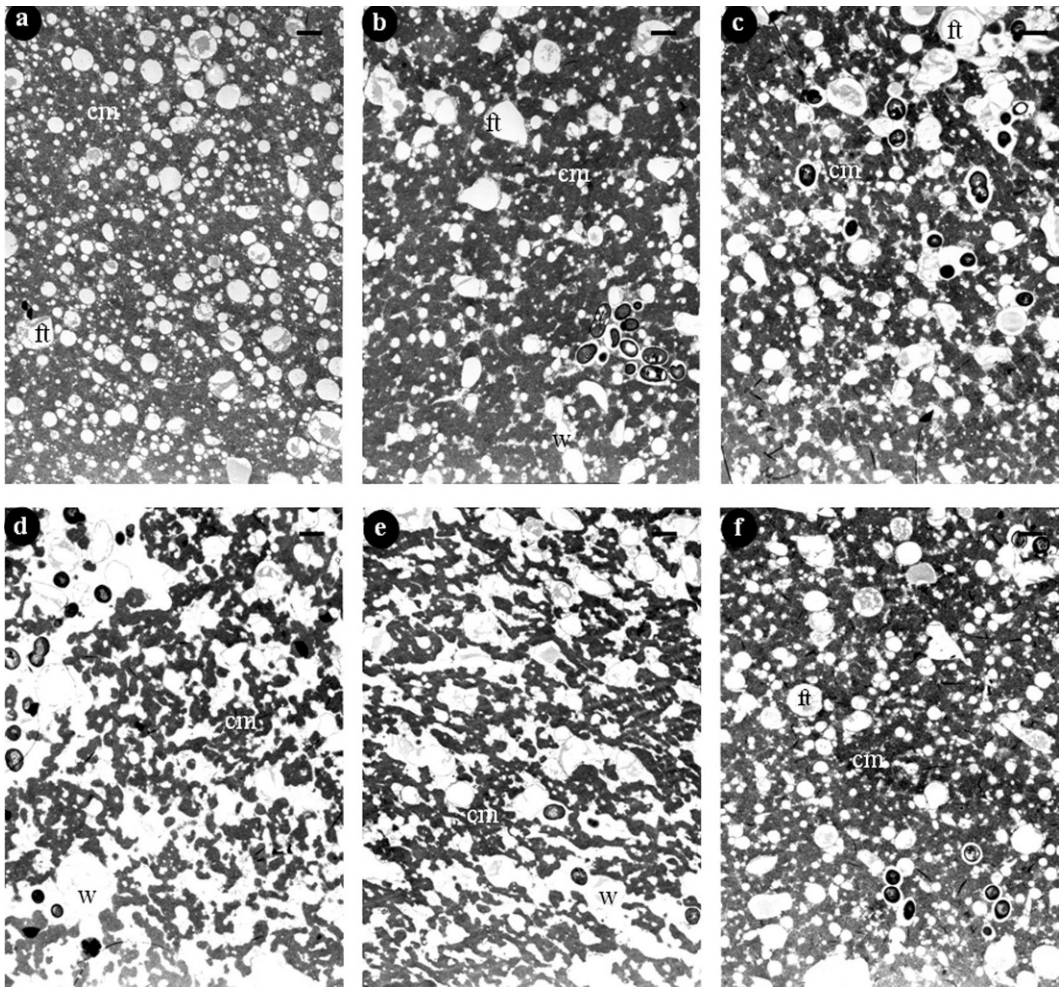

Figure 4. Transmission electron micrographs of full- and reduced-fat Cheddar cheeses at 6 mo of ripening. A and B: Full-fat and reduced-fat cheeses made with exopolysaccharide (EPS)-nonproducing starter culture, respectively; C, D, and E: Reduced-fat cheeses made with EPS capsule-forming *Lactococcus lactis* ssp. *cremoris* SMQ-641, ropy-producing *Lactococcus lactis* ssp. *cremoris*, or their combination, respectively; F: Reduced-fat cheese made with added 0.2% (wt/vol) lecithin to cheese milk. Bars indicate 1 μm; cm = casein matrix, w = whey pockets, and ft = fat globules.

ture retention and modifying the texture and structure of reduced-fat Cheddar cheese compared with the capsular *L. lactis* ssp. *cremoris* SMQ-461 strain. The capsular strain appeared to have better survivability during ripening of Cheddar cheese compared with the ropy strain. Both EPS-producing strains showed that they could help modify and improve textural characteristics particularly in freshly pressed reduced-fat Cheddar cheese. These desirable effects were, however, less pronounced in aged cheeses. The evaluation of factors related to EPS composition and stability in the cheese matrix and survivability of EPS-producing strains during cheese ripening would be of great value in maximizing and prolonging the stabilizing effect of bacterial EPS. The application of ropy or capsular EPS-producing strains in cheese production can be targeted according to cheese type. As the ropy strain resulted in aged cheese with a very open structure and slightly weak texture, it would be more suited for producing reduced-fat unripened or soft cheeses. However, the capsular EPS strain and capsular/ropy mixture could be used for the production of ripened cheese.

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