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Application of Exopolysaccharide-Producing Cultures in Reduced-Fat Cheddar Cheese: Composition and Proteolysis*

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ABSTRACT

Proteolysis during ripening of reduced fat Cheddar cheeses made with different exopolysaccharide (EPS)-producing and nonproducing cultures was studied. A ropy strain of *Lactococcus lactis* ssp. *cremoris* (JFR1) and capsule-forming nonropy and moderately ropy strains of *Streptococcus thermophilus* were used in making reduced-fat Cheddar cheese. Commercial Cheddar starter was used in making full-fat cheese. Results showed that the actual yield of cheese made with JFR1 was higher than that of all other reduced-fat cheeses. Cheese made with JFR1 contained higher moisture, moisture in the nonfat substance, and residual coagulant activity than all other reduced-fat cheeses. Proteolysis, as determined by PAGE and the level of water-soluble nitrogen, was also higher in cheese made with JFR1 than in all other cheeses. The HPLC analysis showed a significant increase in hydrophobic peptides (causing bitterness) during storage of cheese made with JFR1. Cheese made with the capsule-forming nonropy adjunct of *S. thermophilus*, which contained lower moisture and moisture in the nonfat substance levels and lower chymosin activity than did cheese made with JFR1, accumulated less hydrophobic peptides. In conclusion, some EPS-producing cultures produced reduced-fat Cheddar cheese with moisture in the nonfat substance similar to that in its full-fat counterpart without the need for modifying the standard cheese-making protocol. Such cultures might accumulate hydrophobic (bitter) peptides if they do not contain the system able to hydrolyze them. For making high quality reduced-fat Cheddar cheese, EPS-producing cultures should be used in conjunction with debittering strains.
(**Key words:** reduced-fat Cheddar cheese, proteolysis, exopolysaccharide-producing cultures)

Abbreviation key: EPS = exopolysaccharide, **FFC** = full-fat control cheese, **MNFS** = moisture in the nonfat substance, **RF-3534** = reduced-fat cheese made with the moderate ropy strain *Streptococcus thermophilus* CHCC 3534, **RF-5842** = reduced-fat cheese made with the EPS-negative genetic variant *Streptococcus thermophilus* CHCC 5842, **RFC** = reduced-fat control cheese, **RF-JFR1** = reduced-fat cheese made with the ropy culture *Lactococcus lactis* ssp. *cremoris* JFR1, **RF-Slab** = reduced-fat cheese made with the capsule-forming nonropy strain *Streptococcus thermophilus* Slab, **WSN** = water-soluble nitrogen.

INTRODUCTION

Proteolysis during ripening plays a major role in the development of cheese texture and flavor. A well-balanced breakdown of casein into small peptides and amino acids is necessary for the development of an acceptable Cheddar cheese flavor (Singh et al., 2003). These products contribute directly to flavor (Visser, 1993) or act as precursors for the production of flavor components.

Low- and reduced-fat cheeses are gaining popularity due to consumer awareness of the health benefits of low-fat diets. Low-fat cheeses typically have poor body, flavor, and functional properties (Mistry, 2001). The firm/rubbery texture of low-fat cheeses is due to the inadequate breakdown of casein (Mistry and Kasperson, 1998). To overcome rigidity in reduced-fat cheese, moisture level is increased by lowering cooking temperatures or draining whey at higher pH. However, such conditions result in a lower retention of chymosin and a negative influence on both flavor and texture of cheese (Mistry, 2001).

Exopolysaccharide (**EPS**)-producing cultures have been used in making fermented dairy products to improve their rheological properties, prevent syneresis, and replace stabilizers (Hassan et al., 1996; Adapa

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and Schmidt, 1998; Broadbent et al., 2003; Hassan et al., 2004). In addition, these cultures increase water retention and improve melting properties of low-fat Mozzarella cheese (Perry et al., 1998; Broadbent et al., 2003). In a current research project, several EPS-producing cultures have been used in making reduced-fat Cheddar cheese. These cultures produced reduced-fat cheese with physical characteristics similar to those of its full-fat counterpart (Awad et al., 2005). However, cheese made with some EPS-producing cultures developed bitterness after a few months of ripening. The objective of this work was to monitor proteolysis during ripening of reduced-fat Cheddar cheeses made with different EPS-producing and nonproducing cultures and to study relationships among moisture levels, chymosin activity, production of free amino acids, and accumulation of bitter peptides.

MATERIALS AND METHODS

Cultures

An EPS-producing moderate ropy *Streptococcus thermophilus* CHCC 3534 and its EPS-negative genetic variant *Streptococcus thermophilus* CHCC 5842 (Chr. Hansen, Hørsholm, Denmark), a capsule-forming nonropy *Streptococcus thermophilus* (Slab; Hassan et al., 1995), and a ropy *Lactococcus lactic* spp. *cremoris* (JFR1; Hassan et al., 2003) were used in this study. None of the EPS-producing cultures used in this study increased the viscosity of Cheddar cheese whey (data not shown). All strains were maintained at –80°C in 11% sterile reconstituted skim milk supplemented with 20% (vol/vol) glycerol. Lactococci and streptococci were grown in M17 broth (Becton Dickinson and Co., Sparks, MD), supplemented with 0.5% (wt/vol) lactose and incubated overnight at 32°C (for lactococci) or 39°C (for streptococci). Each strain was subcultured (1% vol/vol) 3 times and then transferred to 11% reconstituted skim milk for overnight incubation to produce the cheese starter culture. Commercial direct to vat set Cheddar culture (DVS 850) was obtained from Chr. Hansen (Milwaukee, WI).

Cheese Making

Raw milk was obtained from the Dairy Research and Training Facility at South Dakota State University. Three replicates of Cheddar cheese were manufactured from standardized (reduced-fat, 2% or full-fat, 3.6%) pasteurized milk (heated to 63°C for 30 min and cooled to 31°C). Cheese milk (100 kg) was assigned to 2 double-O cheese vats (Kusel Equipment Co., Watertown, WI). The following 6 treatments of cheese were made: 1) **FFC** = full-fat cheese made using the commercial Cheddar starter culture (DVS 850; 0.015% wt/wt); 2) **RFC** = reduced-fat cheese made using the commercial Cheddar starter culture (DVS 850; 0.015% wt/wt); 3) **RF-JFR1** = reduced-fat cheese made with the ropy strain *Lactococcus lactis* spp. *cremoris* JFR1 (2% vol/wt); 4) **RF-Slab** = reduced-fat cheese made with a capsule-forming nonropy *Streptococcus thermophilus* (0.4% vol/wt) plus the commercial culture (0.011% wt/wt); 5) **RF-3534** = reduced-fat cheese made with EPS-producing *Streptococcus thermophilus* CHCC 3534 (0.4% vol/wt) plus the commercial culture (0.011 % wt/wt); and 6) **RF-5842** = reduced-fat cheese made with the EPS-negative genetic variant of CHCC 3534 (*Streptococcus thermophilus* CHCC 5842; 0.4% vol/wt) plus the commercial culture (0.011% wt/wt). The inoculum size was selected, based on the preliminary experiment data, to give the same acidification rate and cheese making time (5 h) in all treatments.

Cultures were added at 31°C to milk that was then ripened for 1 h. A 0.01% (vol/wt) chymosin (Chymax, Chr. Hansen) was added to clot milk in 30 min. The coagulum was cut and cooked to 39°C over 30 min and held at this temperature for 30 more minutes. After whey drainage, the curd was cheddared and then milled when the pH reached 5.4. Curd was salted at 1.7% in 3 equal applications over 15 min. The curd was hooped in rectangular blocks, pressed overnight at 2.5 kg/cm^2, vacuum-packed, and ripened at 4°C for 6 mo.

Chemical Composition of Cheese

Cheese (1 d old) was analyzed for moisture by the oven method (method 926.08; AOAC, 2003), salt by chloride analyzer (model 926, Nelson Jameson Inc., Marshfield, WI), fat by Mojonnier (method 933.05; AOAC, 2003), and total protein by macro-Kjeldahl (method 920.123; AOAC, 2003). The pH was measured in a slurry prepared by macerating 20 g of grated cheese in 20 mL of deionized water.

Cheese yield was expressed as the ratio mass between the curd obtained after pressing stage and the weight of milk.

Microbiological Analyses

Cheese samples (10 g) were homogenized for 4 min with 90 mL of a sterile 2% sodium citrate solution in a laboratory blender 80 Stomacher (Seward 400, London, UK) and serially diluted using sterile 0.05% peptone. Appropriate dilutions were plated on lactose M17 agar. Duplicate dishes were incubated at 25 or 45°C for enumeration of lactococci and streptococci, respectively.

Determination of Residual Chymosin Activity

The residual coagulant activity was determined by the method described by Hurley et al. (1999) with some modifications. The HPLC analysis was conducted using a Varian Prostar composed of 2 Prostar pumps and photo diode array detector. The peptides resulting from hydrolysis of the substrate by chymosin were separated on an RP-C18-Lichrospher analytical column (250 × 4.6 mm, 5 μm; Perkin Elmer, Norwalk, CT) equilibrated with solvent A (0.1% trifluoroacetic acid in water) and solvent B (0.1% trifluoroacetic acid in acetonitrile). A gradient was generated by increasing the concentration of solvent B as follows: 15 to 50% solvent B over 20 min, 50% solvent B for 5 min, 50 to 95% solvent B over 3 min, maintaining at 95% solvent B for 2 min, and finally returning to equilibration conditions over 3 min. The flow rate was 1 mL/min. The photo diode array detector was used to monitor analyses at 300 nm. Integration of HPLC spectra was achieved using Star Software (Varian Chromatography Systems, Walnut Creek, CA). The concentration of residual coagulant activity was expressed as rennet activity units per kilogram of cheese using a standard curve of chymosin activity.

Proteolysis Assessments

Water-soluble nitrogen and free amino acids determination. Water-soluble nitrogen (**WSN**) in the fat-free cheese homogenates was determined according to the method developed by Kuchroo and Fox (1982). Free amino groups were measured using the cadmium-ninhydrin method described by Folkertsma and Fox (1992).

Gel Electrophoresis Analysis

Urea-PAGE was conducted on whole cheese samples using a Protean II vertical slab gel unit (BioRad Laboratories, Hercules, CA) as described by Andrews (1983). Bands were scanned and quantified using a computerized densitometer and Image Quant 3.3 software (GE Healthcare, Sunnyvale, CA).

Reverse Phase-HPLC Analysis

The profile of peptides in water-soluble extract (Kuchroo and Fox, 1982) were separated using the C18-Lichrospher analytical column (250 × 4.6 mm, 5 μm; Perkin Elmer). Samples were eluted with a 4-step linear gradient over a period of 75 min according to Awad et al. (1999). Separation was conducted at 21°C and peptides were monitored at 214 and 280 nm. The areas of the chromatograms were divided into 2 regions according to the peptide elution time: hydrophilic peptides (retention time <35 min) and hydrophobic peptides (retention time >35 min). These peak areas of the different regions were statistically analyzed to evaluate the influence of the EPS-producing cultures on the relative amounts of peptides.

Statistical Analyses

Data reported are the average of 3 measurements per replicate. Cheeses were made 3 times. The SAS statistical analysis software package (SAS Institute, 1999) was used for ANOVA using the GLM procedure. Differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Cheese Composition

The composition of Cheddar cheese made with EPS-producing and nonproducing cultures is summarized in Table 1. In agreement with previous findings (Fenelon and Guinee, 1999; Guinee et al., 2000), decreasing the fat content of cheese milk resulted in an increase in cheese moisture and protein and a decrease in cheese yield, level of fat in DM, moisture in the nonfat substance (**MNFS**), and salt-in-moisture. Cheese made with the EPS-producing culture JFR1 had higher ($P < 0.05$) moisture and yield than did all other reduced-fat cheeses. Cheese made with the capsule-forming nonropy culture (Slab) had higher ($P < 0.05$) moisture and yield than those made with the moderately ropy culture of *S. thermophilus* (RF-3534) and its EPS-negative variant (RF-5842). The yield of RF-JFR1 was 7.8% higher than that of cheeses made with the EPS-nonproducing culture (RF-5842). Fat in DM was similar ($P > 0.05$) among all reduced-fat cheeses. The MNFS was higher ($P < 0.05$) in RF-JFR1 than in all other reduced-fat cheeses. It is always the objective of the cheese manufacturer to produce reduced-fat cheese with similar MNFS to that of the full-fat counterpart. This requires modifications in cheese-making protocol that are always associated with textural and flavor defects. Interestingly, the use of JFR1 produced reduced-fat cheese with similar MNFS to that of the full-fat type without the need for modifying the cheese-making procedure used in making the full-fat type.

No significant differences in pH were found among all cheeses after 1 d of manufacture (Figure 1). A drop in pH of all cheeses was observed during the first month of ripening. The relatively high MNFS in FFC and RF-JFR1 might have induced excessive growth of starter organisms, resulting in the most significant reduction in pH. Under our cheese-making conditions, viscosity of the whey was not affected by using the

Table 1. Chemical composition (%), actual yield, and residual coagulant activity of full-fat and reduced-fat Cheddar cheese.

	Composition[1]									
Treatment[2]	Protein	Fat	Moisture	Yield	Salt	Fat in DM	SM	MNFS	RCA	RCA/g of protein
FFC	25.1^{c}	32.1^{a}	39.7^{d}	10.4^{a}	1.5^{a}	53.2^{a}	3.7^{a}	58.5^{a}	18.4^{d}	0.07^{b}
RFC	32.2^{a}	20.2^{b}	42.6^{c}	8.5^{d}	1.4^{a}	35.2^{b}	3.4^{b}	53.4^{c}	20.2^{c}	0.06^{b}
RF-JFR1	28.5^{b}	18.9^{c}	47.3^{a}	9.1^{b}	1.5^{a}	35.9^{b}	3.1^{c}	58.4^{a}	25.5^{a}	0.09^{a}
RF-Slab	30.5^{a}	19.9^{b}	44.0^{b}	8.8^{c}	1.4^{a}	35.5^{b}	3.3^{b}	54.9^{b}	21.5^{b}	0.07^{b}
RF-3534	30.9^{a}	20.1^{b}	43.4^{c}	8.6^{d}	1.5^{a}	35.5^{b}	3.4^{b}	54.3^{bc}	20.5^{bc}	0.07^{b}
RF-5842	32.3^{a}	20.0^{b}	42.7^{c}	8.5^{d}	1.4^{a}	35.0^{b}	3.3^{b}	53.4^{c}	20.2^{c}	0.06^{b}

$^{a–d}$Means within the same column with different subscriptions are significantly different ($P < 0.05$).

[1]SM = Salt-in-moisture, MNFS = moisture in nonfat substance, RCA = residual coagulant activity, rennet activity units/kg of cheese.

[2]FFC = Full-fat control; RFC = reduced-fat control; RF-JFR1 = reduced-fat cheese made with the ropy culture *Lactococcus lactis* ssp. *cremoris* JFR1; RF-Slab = reduced-fat cheese made with the capsule-forming nonropy strain *Streptococcus thermophilus* Slab; RF-3534 = reduced-fat cheese made with the moderate ropy strain *Streptococcus thermophilus* CHCC 3534; and RF-5842 = reduced-fat cheese made with the exopolysaccharide-negative genetic variant *Streptococcus thermophilus* CHCC 5842.

ropy culture JFR1. Preliminary experiments showed that ropiness produced by JFR1 increased as the growth temperature decreased. The cooking temperature used in this study (39°C) was much higher than the optimum temperature for production of ropiness (around 28°C). This might explain why the viscosity of the whey was slightly increased when Cheddar cheese curd was cooked at a relatively lower temperature (35°C; Dabour et al., 2005).

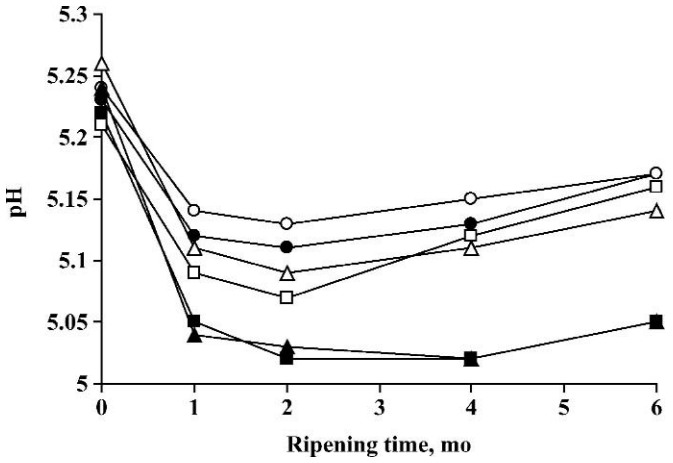

Figure 1. Changes in pH of full-fat and reduced-fat Cheddar cheeses during ripening. ▲ = FFC (Full-fat control); △ = RFC (reduced-fat control); ■ = RF-JFR1 (reduced-fat cheese made with the ropy culture *Lactococcus lactis* ssp. *cremoris* JFR1); □ = RF-Slab (reduced-fat cheese made with the capsule-forming nonropy strain *Streptococcus thermophilus* Slab); ● = RF-3534 (reduced-fat cheese made with the moderate ropy strain *Streptococcus thermophilus* CHCC 3534); and ○ = RF-5842 (reduced-fat cheese made with the exopolysaccharide-negative genetic variant *Streptococcus thermophilus* CHCC 5842).

Residual Chymosin Activity

The residual chymosin activity in cheeses at d 1 of manufacture is given in Table 1. The activity was 18.4 rennet activity units/kg in full-fat Cheddar cheese and was in the range (11.1 to 20.1) found by Hurley et al. (1999) in commercial Cheddar cheese. The chymosin activity in reduced-fat Cheddar cheeses ranged from 20.2 to 25.5 rennet activity units/kg, which was significantly higher than that found in the full-fat type. The amount of coagulant retained in cheese depends on several factors, such as the pH of curd at cutting and whey drainage, cooking temperature, and final moisture content of cheese (Fox and McSweeney, 1996). Because the same procedure was used in making reduced- and full-fat cheeses and there were no significant differences in the amount of rennet used or the pH at d 1 of manufacture among all treatments, the differences in residual chymosin activity could be related only to differences in the moisture level.

Viability of Starter in Cheese During Ripening

The mean population of starter cultures is shown in Table 2. Numbers of mesophilic bacteria at d 1 of manufacture were similar ($P > 0.05$) in all cheeses. However, after 1 and 2 mo of ripening, counts of lactococci were higher in RF-JFR1 than in all other cheeses. The high moisture and low salt-in-moisture in RF-JFR1 might have increased survival of lactococci during the first 60 d of ripening. A gradual decline in numbers of lactococci was seen after 60 d of ripening in all cheeses, resulting in about a 2-log reduction after 6 mo. The results were in agreement with those reported by Sallami et al. (2004), who found a decline

Table 2. Changes in lactococci and streptococci viable counts in full-fat and reduced-fat Cheddar cheese during ripening.[1]

Treatment	Ripening time, d 1	30	60	120	180
Lactococci	Log cfu/g of cheese				
FFC	$8.4^{a,A}$	$8.0^{b,A}$	$7.7^{b,B}$	$6.4^{c,C}$	$6.0^{b,D}$
RFC	$8.4^{a,A}$	$8.1^{b,A}$	$7.8^{b,B}$	$6.6^{b,C}$	$6.2^{a,D}$
RF-JFR1	$8.5^{a,A}$	$8.6^{a,A}$	$8.2^{a,B}$	$6.5^{b,C}$	$6.0^{b,D}$
RF-Slab	$8.3^{a,A}$	$8.2^{b,A}$	$7.8^{b,B}$	$6.9^{a,C}$	$6.1^{ab,D}$
RF-3534	$8.3^{a,A}$	$8.2^{b,A}$	$7.9^{b,B}$	$6.8^{a,C}$	$6.1^{b,D}$
RF-5842	$8.2^{a,A}$	$8.1^{b,A}$	$7.8^{b,B}$	$6.7^{b,C}$	$6.3^{a,D}$
Streptococci					
RF-Slab	$8.1^{a,A}$	$7.1^{b,B}$	$6.7^{b,C}$	$6.7^{b,D}$	$6.6^{b,D}$
RF-3534	$8.2^{a,A}$	$8.1^{a,A}$	$7.0^{a,B}$	$7.0^{a,B}$	$6.8^{ab,C}$
RF-5842	$8.0^{a,A}$	$8.0^{a,A}$	$7.0^{a,B}$	$7.0^{a,B}$	$7.0^{a,B}$

^{a,b,c}Means within the same column with different subscriptions are significantly different ($P < 0.05$).

$^{A–D}$Means within the same row with different subscriptions are significantly different ($P < 0.05$).

[1]FFC = Full-fat control; RFC = reduced-fat control; RF-JFR1 = reduced-fat cheese made with the ropy culture *Lactococcus lactis* ssp. *cremoris* JFR1; RF-Slab = reduced-fat cheese made with the capsule-forming nonropy strain *Streptococcus thermophilus* Slab; RF-3534 = reduced-fat cheese made with the moderate ropy strain *Streptococcus thermophilus* CHCC 3534; and RF-5842 = reduced-fat cheese made with the exopolysaccharide-negative genetic variant *Streptococcus thermophilus* CHCC 5842.

in the viable counts of lactococci from approximately 10^9 at d 1 to 10^7 cfu/g after 6 mo of Cheddar cheese ripening. Midje et al. (2000) also reported that populations of starter cultures reached a maximum during Cheddar cheese manufacture and decreased thereafter.

There were no significant differences in the counts of *S. thermophilus* in freshly pressed cheese blocks made with these adjuncts. However, a sharp decline in their numbers was noted between 30 and 60 d of ripening, with Slab showing the greatest reduction in numbers.

Proteolysis During Ripening

WSN. The concentration of nitrogen soluble in water expressed as either a percentage of total nitrogen or grams of N/100 grams of cheese increased in all cheeses during ripening (Table 3). Among 6-mo-old cheeses, the WSN as a percentage of total N was highest in RF-JFR1 followed by FFC and RF-Slab, whereas the lowest levels were found in reduced-fat cheeses made with no EPS. The level of WSN as a percentage of total N increased with increasing MNFS and the ratio of the residual chymosin activity to cheese proteins (Table 1). Such factors were reported by other researchers to affect WSN as a percentage of total N (Fenelon et al., 2000). The soluble nitrogen expressed as grams of N/100 grams of cheese was higher in RF-JFR1 than in all other cheeses, which were not different.

Table 3. Evolution of water-soluble nitrogen (WSN) as a percentage of the total nitrogen (WSN%TN) or cheese weight (WSN%Ch) in full-fat and reduced-fat cheeses during ripening.[1]

Treatment	Ripening time, d 1	30	60	120	180
WSN%TN					
FFC	$4.2^{b,E}$	$10.3^{a,D}$	$12.8^{b,C}$	$19.3^{b,B}$	$23.7^{b,A}$
RFC	$4.0^{cd,E}$	$8.3^{c,D}$	$10.4^{d,C}$	$14.9^{d,B}$	$18.4^{e,A}$
RF-JFR1	$4.7^{a,E}$	$11.1^{a,D}$	$14.2^{a,C}$	$20.7^{a,B}$	$24.8^{a,A}$
RF-Slab	$4.1^{b,E}$	$9.0^{b,D}$	$11.4^{c,C}$	$17.0^{c,B}$	$20.8^{c,A}$
RF-3534	$4.1^{bc,E}$	$8.7^{b,D}$	$11.1^{cd,C}$	$16.3^{c,B}$	$20.2^{d,A}$
RF-5842	$3.6^{d,E}$	$7.9^{c,D}$	$9.8^{e,C}$	$15.4^{d,B}$	$19.0^{e,A}$
WSN%Ch					
FFC	$0.17^{b,E}$	$0.40^{b,D}$	$0.50^{c,C}$	$0.76^{b,B}$	$0.92^{c,A}$
RFC	$0.20^{a,E}$	$0.42^{b,D}$	$0.53^{bc,C}$	$0.75^{b,B}$	$0.93^{bc,A}$
RF-JFR1	$0.21^{a,E}$	$0.49^{a,D}$	$0.63^{a,C}$	$0.92^{a,B}$	$1.11^{a,A}$
RF-Slab	$0.20^{a,E}$	$0.43^{b,D}$	$0.55^{b,C}$	$0.81^{b,B}$	$1.00^{b,A}$
RF-3534	$0.20^{a,E}$	$0.42^{b,D}$	$0.54^{b,C}$	$0.79^{b,B}$	$0.98^{b,A}$
RF-5842	$0.18^{b,E}$	$0.40^{b,D}$	$0.50^{c,C}$	$0.78^{b,B}$	$0.96^{b,A}$

$^{a–e}$Means within the same column with different subscriptions are significantly different ($P < 0.05$).

$^{A–E}$Means within the same row with different subscriptions are significantly different ($P < 0.05$).

[1]FFC = Full-fat control; RFC = reduced-fat control; RF-JFR1 = reduced-fat cheese made with the ropy culture *Lactococcus lactis* ssp. *cremoris* JFR1; RF-Slab = reduced-fat cheese made with the capsule-forming nonropy strain *Streptococcus thermophilus* Slab; RF-3534 = reduced-fat cheese made with the moderate ropy strain *Streptococcus thermophilus* CHCC 3534; and RF-5842 = reduced-fat cheese made with the exopolysaccharide-negative genetic variant *Streptococcus thermophilus* CHCC 5842.

Free Amino Groups

Free amino groups (expressed as milligrams of leucine per gram of cheese) at different ripening stages are presented in Table 4. The level of free amino groups significantly increased as ripening progressed. However, their release occurred at a much slower rate in RF-JFR1 than in all other cheeses. The Slab culture produced higher levels ($P < 0.05$) of free amino groups than all other cultures used in this study. Starter peptidases are mainly responsible for the production of free amino acids in cheese (Visser, 1977), and rennet and plasmin are not able to contribute to the further degradation of the larger peptides in the soluble nitrogen fraction (Berg and Exterkate, 1993). The high level of free amino acids produced by Slab along with the decline in their numbers during the first few weeks and the relatively sharp increase in pH of cheese made with this culture after 2 mo of ripening might indicate extensive autolysis.

Urea-PAGE

Urea-PAGE electrophoretograms of the various cheese samples were applied to the gel based on fixed

Table 4. Levels of free amino group (mg of leucine/g of cheese) in full-fat and reduced-fat Cheddar cheeses during ripening.[1]

Treatment	Ripening time, d				
	1	30	60	120	180
FFC	$0.71^{b,E}$	$0.98^{b,D}$	$2.01^{a,C}$	$2.65^{b,B}$	$3.59^{b,A}$
RFC	$0.74^{ab,E}$	$1.21^{a,D}$	$2.13^{a,C}$	$2.80^{b,B}$	$3.66^{b,A}$
RF-JFR1	$0.58^{c,E}$	$0.68^{c,D}$	$0.83^{c,C}$	$1.22^{c,B}$	$1.64^{c,A}$
RF-Slab	$0.77^{a,E}$	$1.19^{a,D}$	$2.08^{a,C}$	$3.06^{a,B}$	$3.89^{a,A}$
RF-3534	$0.70^{b,E}$	$0.99^{b,D}$	$1.87^{b,C}$	$2.66^{b,B}$	$3.57^{b,A}$
RF-5842	$0.67^{b,E}$	$0.83^{b,D}$	$1.65^{b,C}$	$2.49^{b,B}$	$3.48^{b,A}$

[a,b,c]Means within the same column with different subscriptions are significantly different ($P < 0.05$).

[A–E]Means within the same row with different subscriptions are significantly different ($P < 0.05$).

[1]FFC = Full-fat control; RFC = reduced-fat control; RF-JFR1 = reduced-fat cheese made with the ropy culture *Lactococcus lactis* ssp. *cremoris* JFR1; RF-Slab = reduced-fat cheese made with the capsule-forming nonropy strain *Streptococcus thermophilus* Slab; RF-3534 = reduced-fat cheese made with the moderate ropy strain *Streptococcus thermophilus* CHCC 3534; and RF-5842 = reduced-fat cheese made with the exopolysaccharide-negative genetic variant *Streptococcus thermophilus* CHCC 5842.

Table 5. Levels of β- and α_{S1}-caseins and α_{S1}-1 caseins in relation to the corresponding casein fraction at d 1 of manufacture in full-fat and reduced-fat Cheddar cheeses during ripening.[1]

Treatment	Ripening time, d				
	1	30	60	120	180
$\%\beta$-caseins/β-caseins					
FFC	100	$91.3^{ab,A}$	$88.7^{a,B}$	$87.8^{a,C}$	$87.7^{a,D}$
RFC	100	$92.3^{ab,A}$	$89.1^{a,B}$	$87.9^{a,C}$	$86.4^{a,D}$
RF-JFR1	100	$83.1^{c,A}$	$81.3^{c,B}$	$77.1^{c,C}$	$69.9^{d,D}$
RF-Slab	100	$88.7^{b,A}$	$85.5^{b,B}$	$82.9^{b,C}$	$76.6^{c,D}$
RF-3534	100	$91.6^{ab,A}$	$89.9^{a,B}$	$86.7^{a,C}$	$83.4^{b,D}$
RF-5842	100	$93.8^{a,A}$	$89.5^{a,B}$	$88.9^{a,C}$	$87.6^{a,D}$
$\%\alpha_{S1}$-caseins /α_{S1}-caseins					
FFC	100	$63.0^{a,A}$	$43.7^{a,B}$	$31.2^{a,C}$	$13.4^{a,D}$
RFC	100	$62.0^{a,A}$	$45.9^{a,B}$	$30.6^{a,C}$	$12.7^{ab,D}$
RF-JFR1	100	$51.6^{c,A}$	$35.7^{c,B}$	$18.0^{d,C}$	$6.9^{d,D}$
RF-Slab	100	$57.8^{b,A}$	$38.5^{b,B}$	$22.8^{c,C}$	$11.1^{c,D}$
RF-3534	100	$62.6^{a,A}$	$43.3^{a,B}$	$28.6^{b,C}$	$11.4^{bc,D}$
RF-5842	100	$63.1^{a,A}$	$45.9^{a,B}$	$32.5^{a,C}$	$13.6^{a,D}$
$\%\alpha_{S1}$-1 caseins/α_{S1}-caseins					
FFC	$18.0^{a,C}$	$47.3^{c,B}$	$63.8^{b,A}$	$61.0^{a,A}$	$46.4^{b,B}$
RFC	$6.3^{c,E}$	$39.9^{d,D}$	$58.5^{bc,A}$	$53.8^{b,B}$	$47.7^{b,C}$
RF-JFR1	$11.0^{b,E}$	$67.5^{a,B}$	$72.7^{a,A}$	$61.2^{a,C}$	$38.5^{c,D}$
RF-Slab	$2.6^{d,D}$	$33.8^{e,C}$	$64.7^{b,A}$	$64.0^{a,A}$	$53.4^{a,B}$
RF-3534	$2.9^{d,D}$	$55.7^{b,B}$	$59.0^{bc,A}$	$53.7^{b,B}$	$50.0^{b,C}$
RF-5842	$15.7^{ab,D}$	$43.6^{cd,C}$	$57.1^{c,A}$	$53.7^{b,B}$	$43.1^{bc,C}$

[a–e]Means within the same column with different subscriptions are significantly different ($P < 0.05$).

[A–E]Means within the same row with different subscriptions are significantly different ($P < 0.05$).

[1]FFC = Full-fat control; RFC = reduced-fat control; RF-JFR1 = reduced-fat cheese made with the ropy culture *Lactococcus lactis* ssp. *cremoris* JFR1; RF-Slab = reduced-fat cheese made with the capsule-forming nonropy strain *Streptococcus thermophilus* Slab; RF-3534 = reduced-fat cheese made with the moderate ropy strain *Streptococcus thermophilus* CHCC 3534; and RF-5842 = reduced-fat cheese made with the exopolysaccharide-negative genetic variant *Streptococcus thermophilus* CHCC 5842.

weight of cheese protein. Urea-PAGE was followed by densitometry analysis to quantify the degradation of α_{S1}- and β-caseins. The values of individual casein fractions were expressed as a percentage of the values of the corresponding casein in the same cheese at d 1. The relative proportions of the residual β- and α_{S1}-caseins and the level of α_{S1}-1-casein as a percentage of the corresponding α_{S1}-caseins at d 1 are shown in Table 5. Generally, the trend observed with urea-PAGE was consistent with that for WSN data, with the overall level of proteolysis in RF-JFR1 being higher than in all other cheeses. The α_{S1}-casein was hydrolyzed more extensively than β-casein during ripening. After 6 mo of ripening, the α_{S1}-casein was almost completely degraded in all cheeses. The intensity of the band corresponding to β-casein decreased slightly throughout ripening with a concomitant increase in the bands corresponding to the γ-caseins (data not shown). Cheese RF-JRF1 showed the most significant reduction in the level of β-casein (Table 5). Peptides produced by chymosin action on β-casein have been widely reported as the major cause of bitterness in cheeses (Lemieux and Simard, 1992; Broadbent et al., 1998). No differences in the levels of γ-caseins were found among all cheeses (data not shown).

The level of α_{S1}-1 casein (f24-199) during the 2 mo of ripening was higher in RF-JFR1 than in all other cheeses, which could be due to its higher residual chymosin activity and moisture content. The concentration of α_{S1}-1 casein (f24-199) decreased after 60 d of ripening in all cheeses. It was reported earlier that the relationship between the amounts of α_{S1}-casein and α_{S1}-1 peptides in cheese is very weak, probably because α_{S1}-casein can be hydrolyzed to other products of low molecular weight (less than 11 kD; Marcos et al., 1979). In addition, α_{S1}-1 casein undergoes further degradation by rennet or other proteases (Grappin et al., 1985).

Changes in Peptide Profile During Cheese Ripening

The reverse phase-HPLC chromatograms of water-soluble peptides from cheeses during ripening are shown in Figure 2, panels A to F. The chromatograms were divided into 6 zones (I, II, III, IV, V, and VI), each of which contained one peak or more. Similar to previous findings for Cheddar cheese (Fenelon et al., 2000), the chromatograms for all cheeses showed age-related changes in the area and distribution of different peaks. This trend is consistent with the increase in WSN in all cheeses and suggests the progressive breakdown of casein by residual coagulant, plasmin, and microbial proteinases, resulting in the formation of peptides of different molecular masses and free amino

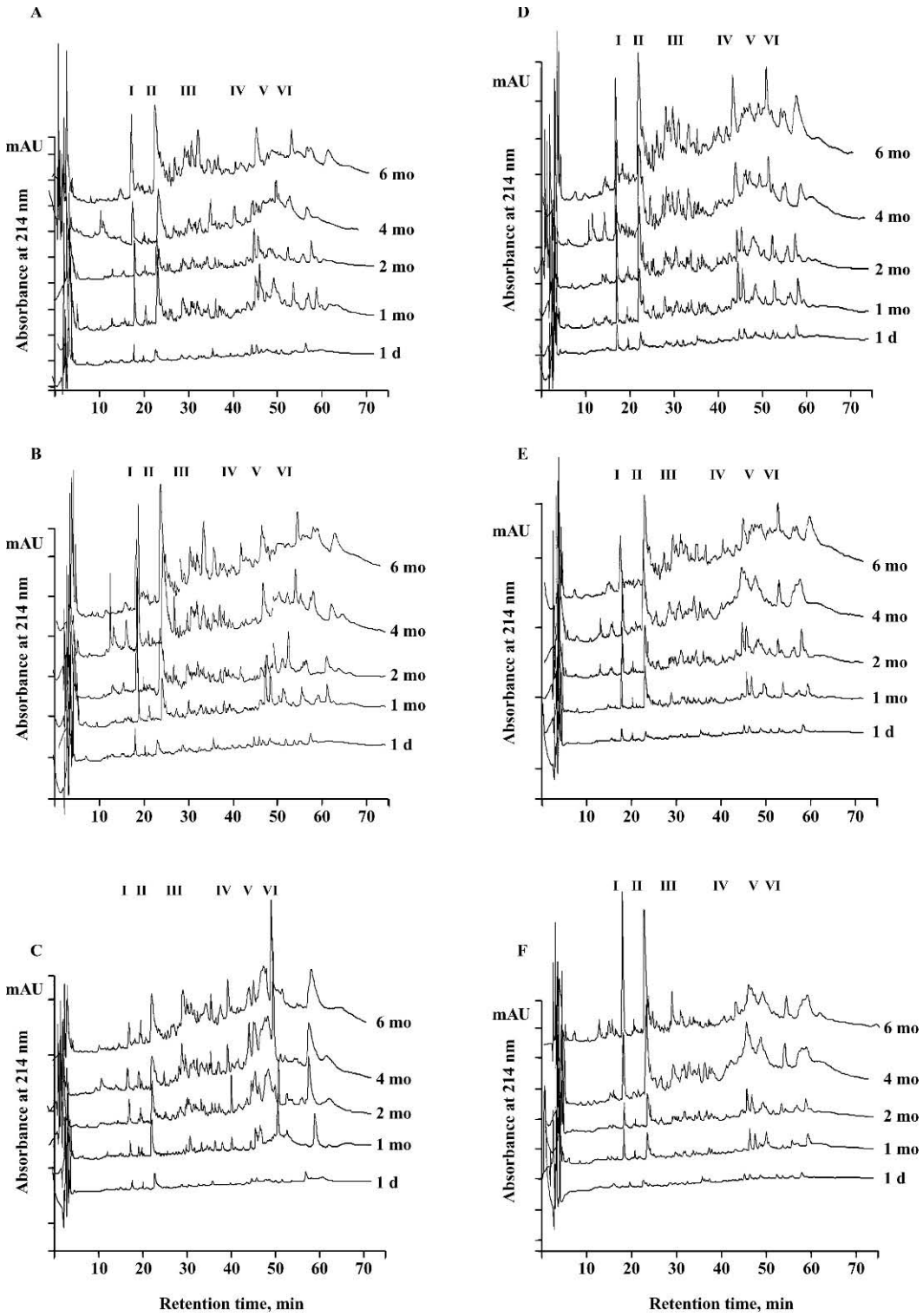

Figure 2. Reverse phase-HPLC chromatograms of water-soluble nitrogen (WSN) extract from A) FFC (full-fat control), B) RFC (reduced-fat control), C) RF-JFR1 (reduced-fat Cheddar cheese made with ropy *Lactococcus lactis* spp *cremoris* JFR1), D) RF-Slab (reduced-fat Cheddar cheese made with capsule-forming nonropy *Streptococcus thermophilus* Slab, E) RF-3534 (reduced-fat Cheddar cheese made with the exopolysaccharide (EPS)-producing moderate ropy *Streptococcus thermophilus* CHCC 3534), and F) RF-5842 (reduced-fat Cheddar cheese made EPS-nonproducing culture *Streptococcus thermophilus* CH 5842) at 0 (1 d), 1, 2, 4, and 6 mo of ripening. The chromatograms were compiled from the mean data taken from 3 replicate trials.

acids. The total peaks area, and in particular, that of the early eluting peaks increased during storage. The late eluting peaks increased during the first 4 mo of ripening and decreased thereafter. The results indicated the accumulation of new proteolytic products and increasing contribution of bacterial enzymes with ripening time.

Major differences were found between the peptide profiles of the WSN of RF-JFR1 (which contained the highest moisture and chymosin activity levels) and those from all other cheeses. Peaks I and II were present at relatively low concentrations in RF-JFR1 (Figure 2C), suggesting that peptides corresponding to these peaks were produced by proteinases from commercial culture used in all cheeses but RF-JFR1. The retention times of peaks I and II were very similar to those of α_{S1}-casein (f 1-9) and α_{S1}-casein (f 1-13) produced in Cheddar cheese by proteinases from *Lactococcus* (Singh et al., 1994).

At all stages of storage, the reverse phase-HPLC profile for the WSN of RF-JFR1 differed markedly from those of all other cheeses. The WSN of RF-JFR1 showed the greatest number and widest distribution of late-eluting peaks (retention time >45 min), denoted collectively as zones IV, V, and VI. Such peaks correspond to high molecular weight and hydrophobic peptides (Lemieux and Simard, 1992). The larger area of late-eluting peaks in RF-JFR1 (Figure 2C) might indicate accumulation of peptides produced by rennet (as indicated by the high level of WSN found in this cheese), which were not degraded further to peptides of lower hydrophobicity and molecular mass, due to the absence or weak starter culture peptidases (Lemieux and Simard, 1992; Awad et al., 2000). At the same time, the WSN of RF-JFR1 showed a lower concentration of early-eluting peaks, especially the peak with retention time of 18 min, denoted collectively as zone I. The total peak area in zones I and II increased during storage in all cheeses except RF-JFR1, which contained the lowest ratio of hydrophilic to hydrophobic peptides (Table 6). The WSN of RF-Slab (Figure 2D) showed a high number and wide distribution of the early and middle-eluting peaks (retention time < 35 min), which probably correspond to low molecular weight and hydrophilic peptides (Lemieux and Simard, 1992). The total area of late-eluting peaks (zones V and VI) and the ratio of hydrophobic to hydrophilic peptides were generally smaller in RF-Slab than in all other reduced-fat cheeses made with EPS-producing cultures after 60 d of ripening (Table 6). Cheese RF-JFR1 contained the highest levels of the late-eluting peptides and lowest ratio of hydrophilic to hydrophobic peptides among all cheeses. Bitter peptides are rich in hydrophobic amino acids residues (Lemieux and Simard, 1992) and exhibit higher retention times on reverse phase-HPLC columns (Cliffe and Law, 1990; Awad et al., 1999). In contrast, Lee and Warthesen (1996) found little correlation between retention time on the reverse phase-HPLC column and peptide hydrophobicity or suspected bitterness. The high residual chymosin activity and moisture content and low salt-in-moisture in RF-JFR1 might have increased the level of the hydrophobic peptides. Cell envelope proteinases from JFR1 could have accumulated bitter peptides (Broadbent et al., 2002).

Table 6. Ratio of hydrophilic to hydrophobic peptides separated by reverse phase-HPLC and percentage of hydrophobic peptides of total peptides in full-fat and reduced-fat Cheddar cheeses during ripening.

	Ripening time, d				
Treatment	1	30	60	120	180
Ratio of hydrophilic to hydrophobic peptides					
FFC	$1.9^{a,A}$	$0.8^{b,D}$	$1.2^{a,C}$	$1.5^{a,B}$	$1.9^{ab,A}$
RFC	$1.4^{b,B}$	$1.0^{ab,C}$	$1.1^{b,C}$	$1.3^{b,B}$	$1.8^{b,A}$
RF-JFR1	$1.2^{c,A}$	$0.5^{c,B}$	$0.5^{d,B}$	$0.4^{d,C}$	$0.5^{d,B}$
RF-Slab1	$1.2^{c,C}$	$1.2^{a,C}$	$0.8^{c,D}$	$1.5^{a,B}$	$2.0^{a,A}$
RF-3534	$1.0^{c,B}$	$1.2^{a,A}$	$0.8^{c,C}$	$1.0^{c,B}$	$0.9^{c,C}$
RF-5842	$1.3^{bc,A}$	$1.3^{a,A}$	$0.8^{c,C}$	$1.0^{c,B}$	$1.0^{c,B}$
% Hydrophobic peptides of total peptides					
FFC	$34.3^{d,D}$	$55.6^{b,A}$	$44.5^{c,B}$	$40.7^{c,C}$	$34.4^{c,D}$
RFC	$41.8^{c,B}$	$50.8^{b,A}$	$47.9^{c,A}$	$44.3^{c,B}$	$36.0^{c,C}$
RF-JFR1	$45.8^{b,C}$	$65.7^{a,B}$	$68.5^{a,B}$	$72.7^{a,A}$	$67.3^{a,B}$
RF-Slab	$46.5^{ab,B}$	$45.1^{c,B}$	$55.3^{b,A}$	$39.6^{c,C}$	$33.2^{c,D}$
RF-3534	$48.9^{a,B}$	$44.7^{c,C}$	$56.0^{b,A}$	$50.7^{b,AB}$	$53.4^{b,A}$
RF-5842	$44.3^{bc,C}$	$43.7^{d,C}$	$56.1^{b,A}$	$51.1^{b,B}$	$51.3^{b,B}$

[a–d]Means within the same column with different subscriptions are significantly different ($P < 0.05$).

[A–D]Means within the same row with different subscriptions are significantly different ($P < 0.05$).

[1]FFC = Full-fat control; RFC = reduced-fat control; RF-JFR1 = reduced-fat cheese made with the ropy culture *Lactococcus lactis* ssp. *cremoris* JFR1; RF-Slab = reduced-fat cheese made with the capsule-forming nonropy strain *Streptococcus thermophilus* Slab; RF-3534 = reduced-fat cheese made with the moderate ropy strain *Streptococcus thermophilus* CHCC 3534; and RF-5842 = reduced-fat cheese made with the exopolysaccharide-negative genetic variant *Streptococcus thermophilus* CHCC 5842.

CONCLUSIONS

A ropy strain of *Lactococcus lactis* ssp. *cremoris* that produced reduced-fat cheese with similar textural characteristics to its full-fat counterpart accumulated high levels of hydrophobic (bitter) peptides. The high level of such peptides was associated with high MNFS and chymosin activity. However, an adjunct culture of capsule-forming nonropy *S. thermophilus* increased the moisture level of reduced-fat cheese without producing high levels of bitter peptides because of its ability to hydrolyze them. This study shows that increasing MNFS in reduced-fat Cheddar cheese to levels similar to those in the full-fat counterpart might result in bitterness due to the increased chymosin activity and

lower salt-in-moisture levels. To produce reduced-fat Cheddar cheese with characteristics similar to those of its full-fat counterpart using EPS-producing cultures, debittering strains and reduction of residual chymosin activity should be considered.

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