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Application of Exopolysaccharide-Producing Cultures in Reduced-Fat Cheddar Cheese: Cryo-Scanning Electron Microscopy Observations*

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ABSTRACT

The microstructure of reduced- and full-fat Cheddar cheeses made with exopolysaccharide (EPS)-producing and nonproducing cultures was observed using cryo-scanning electron microscopy. Fully hydrated cheese samples were rapidly frozen in liquid nitrogen slush (–207°C) and observed in their frozen hydrated state without the need for fat extraction. Different EPS-producing cultures were used in making reduced-fat Cheddar cheese. Full-fat cheese was made with a commercial EPS-nonproducing starter culture. The cryo-scanning electron micrographs showed that fat globules in the fully hydrated cheese were surrounded by cavities. Serum channels and pores in the protein network were clearly observed. Young (1-wk-old) full-fat cheese contained wide and long fat serum channels, which were formed because of fat coalescence. Such channels were not observed in the reduced-fat cheese. Young reduced-fat cheese made with EPS-nonproducing cultures contained fewer and larger pores than did reduced-fat cheese made with a ropy strain of *Lactococcus lactis* ssp. *cremoris* (JFR1), which had higher moisture levels. A 3-dimensional network of EPS was observed in large pores in cheese made with JFR1. Major changes in the size and distribution of pores within the structure of the protein network were observed in all reduced-fat cheeses, except that made with JFR1, as they aged. Changes in porosity were less pronounced in both the full-fat and the reduced-fat cheeses made with JFR1.
(**Key words:** Cheddar, exopolysaccharides, cryo-scanning electron microscopy, microstructure)

Abbreviation key: EPS = exopolysaccharide, **FFC** = full-fat control cheese, **RF-JFR1** = reduced-fat cheese made with the ropy culture *Lactococcus lactis* ssp. *cremoris* JFR1, **SEM** = scanning electron microscopy.

INTRODUCTION

Scanning electron microscopy (**SEM**) is a useful tool for providing information on microstructure of dairy products, which assists researchers in understanding factors affecting functional, sensory, and physical properties. This technique has been used to study microstructure of different types of cheese such as Cheddar (Metzger and Mistry, 1995), Mozzarella (McMahon et al., 1999), soft cheese (Guerzoni et al., 1999), cream cheese (Sainani et al., 2004), and process cheese (Raval and Mistry, 1999). Cryo-SEM is recommended for studying the microstructure of samples containing high moisture levels (Serp et al., 2002a,b; Hassan et al., 2003). Unlike conventional SEM, the cryo-SEM technique does not require chemical fixation or fat extraction that might induce artifacts. Cheese is a 3-dimensional protein network in which moisture and fat are trapped; therefore, cryo-SEM is valuable when observation of different cheese components is desirable.

Exopolysaccharides (**EPS**)-producing cultures have been used to modify texture of fermented dairy products (Hassan et al., 1996; Hassan and Frank, 1997; Perry et al., 1997, 1998), but the mechanism by which EPS affects the textural and functional properties of dairy products is not fully understood. Part of the problem was the unavailability of an appropriate microscopy technique. Exopolysaccharides contain about 95% water, and sample preparation before observation by conventional electron microscopy changes the initial structure and distribution of EPS (Kalab, 1993; Serp et al., 2002a,b). Hassan et al. (2003) used cryo-SEM to visualize the microstructure of soft cheeses and milk fermented with different EPS-producing and nonproducing cultures. Their images showed that EPS and milk fat were visible in pores within the protein network. Exopolysaccharides appeared as a fully hydrated network segregated from the protein network.

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Table 1. Cultures used in this study.

Strain	Description	Source/Reference
Streptococcus thermophilus CHCC 3534	Moderately ropy	Chr. Hansen, Denmark
Streptococcus thermophilus CHCC 5842	Exopolysaccharide-negative genetic variant of CHCC3534	Chr. Hansen, Denmark
Lactococcus lactic spp. *cremoris* JFR1	Highly ropy	Hassan et al., 2003
Streptococcus thermophilus Slab	Capsule-forming nonropy	Hassan et al., 1995a

In a companion study, both full-fat cheese and reduced-fat cheese made with a ropy strain of *Lactococcus lactis* spp. *cremoris* JFR1 had similar textural and melting characteristics (Awad et al., 2005b). In addition, changes in their physical properties during ripening followed the same pattern (Awad et al., 2005b). The physical and functional properties of cheese are governed by its microstructure (Kalab et al., 1987; Pastorino et al., 2003). Therefore, cheese microstructure might explain why this particular strain of *Lactococcus lactis* spp. *cremoris* (JFR1) produced reduced-fat cheese with characteristics similar to those of its full-fat counterpart. The objectives of this work were to 1) study microstructure of Cheddar cheese in its fully hydrated state using cryo-SEM, 2) observe microstructural changes in full- and reduced-fat Cheddar cheeses manufacturing with EPS-producing cultures and, 3) relate microscopic observations to the previously reported textural, functional, and viscoelastic properties of reduced- and full-fat Cheddar cheeses made with different cultures (Awad et al., 2005b; Hassan et al., 2005).

MATERIALS AND METHODS

Cultures

Table 1 shows cultures used in this study. Bacterial 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 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 Lab (Milwaukee, WI).

Cheese Making

Raw milk was obtained from the Dairy Research and Training Facility at South Dakota State University. Cheddar cheese was manufactured from standardized (2% reduced fat or 3.6% full fat) pasteurized (63°C for 30 min and cooled to 31°C) milk. The following 6 cheese treatments were made: 1) **FFC** = full-fat cheese made using the commercial Cheddar starter culture (DVS 850; 0.015% wt/wt); 2) 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) reduced-fat cheese made with a capsule-forming nonropy *Streptococcus thermophilus* (0.4% vol/wt) plus the commercial culture (0.011% wt/wt); 5) 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) 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 experimental data, to give the same acidification rate and cheese-making time in all treatments. Cheddar cheese was made as described by Awad et al. (2005a).

Cryo-SEM

Two cheese samples from 1 replication were examined in this study. Several microscopic fields were observed and representative images have been selected. Samples (1 cm × 1 cm) were mounted onto holders and pumped into liquid nitrogen slush at –207°C. Frozen specimens were transferred under vacuum into an attached preparation chamber where they were fractured using a gold scalpel blade. Fractured surfaces were etched at –80°C for 15 min and coated with 300 Å of sputtered gold. The specimens were transferred under vacuum onto the cold stage where they were maintained at –95°C, and imaged using SEM (Leo Electron Microscopy Inc., Thornwood, NY) at 4 kV.

RESULTS AND DISCUSSION

Figure 1 shows the microstructure of young (1 wk old) full- and reduced-fat Cheddar cheeses made with EPS-producing and nonproducing cultures. Fat globules in all cheeses were surrounded by cavities. Such zones do not seem to be formed as a result of differences in thermal expansion/contraction between fat and wa-