Modification of microstructure and texture of rennet curd by using a capsule-forming non-ropy lactic culture

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Summary. Microstructural and textural characteristics of rennet curd made from rehydrated non-fat dried milk standardized to 40 g fat/l were compared with those of non-fat curd made with capsule-forming non-ropy lactic culture. Microstructure examination using confocal scanning laser microscopy indicated that the presence of a capsule-forming culture produced an open casein network with large pores. Distribution of fat globules was observed within the undisturbed casein structure after staining with a fluorescent lipophilic dye which was added to the milk before coagulation. Bacterial capsules and milk fat reduced curd tension and firmness of rennet curd to similar levels.

There is a substantial interest in developing low-fat cheeses that are similar to existing types, but reduction of fat results in cheese products with undesirable texture. Cheddar cheese made from low-fat milk was described as a brick-like mass of casein (Le Roux & Abbott, 1962) and was over-firm at 160 g fat/kg (Banks *et al.* 1989). Low-fat Mozzarella cheese is harder and less meltable than full-fat Mozzarella (Tunick *et al.* 1991). As the fat level decreased, Feta cheese became firmer, shorter and harder (Katsiari & Voutsinas, 1994). The firm texture of low-fat cheese is due to the dominance of the protein matrix (Mistry & Anderson, 1993). Fat globules produce weak spots in the casein network by interfering with linkage formation between casein micelles (Maxcy *et al.* 1955; Green *et al.* 1983; Kebary & Morris, 1990). This results in less structural matrix per unit cross sectional area (Emmons *et al.* 1980). Producing acceptable texture in low-fat cheese requires duplicating the structural role of milk fat (Mistry & Anderson, 1993). Different means have been used to maintain desirable texture in low-fat cheese, such as increasing moisture levels in the non-fat matter (Emmons *et al.* 1980), enhancement of protein degradation (Muir *et al.* 1992) and replacing some of the fat with a filler material that does not contribute to either structure formation or undesirable flavour (Jameson, 1990).

Some strains of lactic acid bacteria produce capsules, which are a polymeric matrix of high water content surrounding the cells (Hassan *et al.* 1995*a*). These capsules are distinguished from ropy polysaccharides in that they are of a discrete size and are not liberated into the growth medium. Ropy cultures may produce capsular as well as ropy polysaccharide, but capsule-producing cells do not necessarily produce ropy polysaccharides (Hassan *et al.* 1995*a*). Both bacterial

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capsules and milk fat produce milk gels with a spongy structure (Mistry & Anderson, 1993; Hassan *et al.* 1995*b*). Bacterial capsules might have the ability to mimic the effect of fat on curd texture because they could decrease the dominance of the structural matrix and hold the water needed in low-fat products. Because the textural properties of finished cheese are governed by structural characteristics of cheese curd, we compared the textural characteristics of rennet curd made with full fat milk and with capsule-producing non-ropy culture as a fat substitute.

MATERIALS AND METHODS

Preparation of cell concentrate

Two different strains of *Streptococcus thermophilus* were used in this study, capsule-forming non-ropy S3855 (Rhone-Poulenc Dairy Ingredients, Madison, WI 53711) and non-capsule-forming non-ropy S4 (isolated from retail yogurt using the procedure of Matalon & Sandine (1986)). Elliker's broth (50 ml; Difco, Detroit, MI 48232) was inoculated with the test strain at 10 ml/l and incubated at 37 °C overnight. Cells of both cultures were washed three times with 50 ml Elliker's broth by centrifugation at 250 ***g*** for 30 min and the pellets were resuspended in 5 ml Elliker's broth. This procedure removed preformed capsule from culture S3855.

Preparation of rennet curd

Non-fat dried milk (low-heat NDM, Mid-American Dairymen, Springfield, MO 65802) was rehydrated (110 g/l deionized water) and kept overnight at 4 °C to allow protein hydration. For full fat curd, milk was standardized to 40 g fat/l by using cream (400 g/l) from raw milk (Animal and Dairy Science Department, University of Georgia). Milk (95 ml) was warmed to 30 °C and 5 ml cell concentrate (from either capsule-forming or non-capsule-forming culture) was added. $CaCl_2$ solution (450 g/l) was added (0·07 ml/l milk) to the inoculated milk which was then incubated at 30 °C. Culture S3855 reformed its capsule during this incubation. After a pH of 6·0 was attained ($\sim$ 1·5 h), milk was treated with 2 ml/l of a 1 : 10 dilution of single strength milk clotting enzyme solution from *Mucor miehei* (Chr. Hansen's Laboratory Inc., Milwaukee, WI 53214). The mixture (80 ml) was immediately measured into 100 ml beakers and incubated at 31 °C for 40 min to form a coagulum. The samples were cooled and kept at 4 °C overnight before texture analysis. Additional samples were prepared for microstructure examination according to the method of Hassan *et al.* (1995*b*). Portions (7 ml) of the renneted milk were dispensed into a glass chamber, covered with a coverslip and incubated upside down at 31 °C for 20 or 40 min for gel formation. The chambers were then cooled for 30 min before microstructure examination.

The distribution of fat globules in curd made from raw milk (39 g fat/l; Animal and Dairy Science Department, University of Georgia) was visualized by adding Nile red (Sigma Chemical Co., St. Louis, MO 63178) solution (0·4 g/l) to the milk (0·7 ml/l milk) to stain fat globules $\sim$ 30 min before adding rennet as previously described. This curd contained the non-capsule-forming culture.

Confocal scanning laser microscopy

An MTC-600 confocal scanning laser microscope (Bio-Rad Laboratories Ltd, Hemel Hempstead HP2 7TD, UK) with a 60 × oil immersion objective (numerical aperture, 1·4) and an Ar–Kr laser operating in the reflectance mode was used to

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examine the rennet curd microstructure by the procedure described by Hassan *et al.* (1995*b*). Stained fat globules were examined using fluorescence mode with 568 nm excitation wave length.

Texture analysis

Curd tension and firmness were determined by the method of Mohamed & Morris (1987) using an Instron Universal testing machine model 1122 (Instron Corporation, Canton, MA 02021) with a 2 kg compression load cell. The stainless steel wire network penetrated the gel at a speed of 20 mm/min, and it penetrated 35 mm below the surface of the coagulum. Total energy for penetration was calculated as the area under the force–distance curve and converted to kg/mm. Results were collected on duplicate samples prepared at two different times from the same lot of NDM.

Statistical analysis

Results were tested by analysis of variance (SAS, 1985) with two replications. When effects were significant, least significant differences were determined. A significance level of 0·05 was used for all analyses.

RESULTS

A non-ropy *Str. thermophilus* culture that produces a 5 μm capsule when grown in milk was used in this study. This culture resynthesized its capsule (removed by washing) after incubation at 30 °C for $\sim$ 2 h as reported by Hassan *et al.* (1995*a*). A cell concentrate ($0{\cdot}7 \times 10^8$ cfu) was added to the milk to provide a high concentration of capsule material without excess acidification.

Microstructure

Fig. 1 shows the microstructure of non-fat rennet curd made with capsule-forming and non-capsule-forming cultures. At 20 min after adding rennet, curd made with capsule-forming culture exhibited large pores in which bacterial cells could be seen. These pores were not found in curd made with non-capsule-forming culture. Rennet curd made with the latter culture exhibited a more continuous and better defined network than did curd made with capsule-forming culture. At this stage of coagulation, rennet curd made with either culture showed large casein aggregates. At 40 min after adding rennet, both types of curd exhibited a well defined casein network with more porous structure and thinner areas of casein aggregates compared with that at 20 min after adding rennet (Fig. 1).

Fig. 2 shows the distribution of fat globules within undisturbed rennet curd made using raw milk of 39 g fat/l and incubated for 40 min. Large variations were observed in the size of the fat globules, which exhibited little clumping. Some fat globules, especially the larger ones, appeared to fit in structural pores while others were distributed within casein network aggregates and strands. The presence of fat globules resulted in a discontinuous structure.

Texture analysis

Table 1 shows the textural characteristics of rennet curds. The presence of either bacterial capsules or fat globules reduced curd tension and firmness. Rennet curd made with non-capsule-forming culture and no fat (control) had the highest curd tension and firmness. In addition, the total energy required for the test cell to

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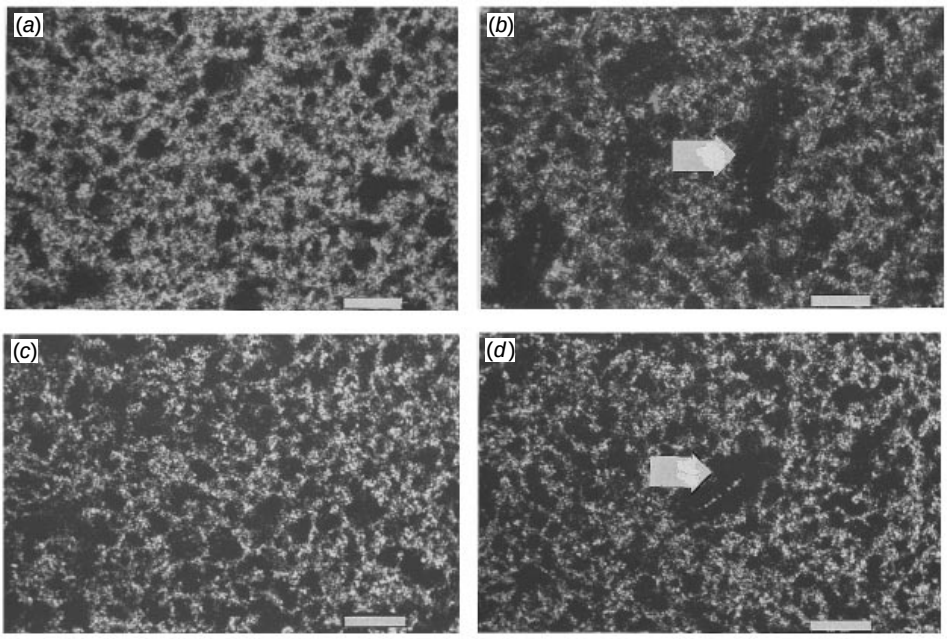


Fig. 1. Optical thin sections of non-fat milk curd made with (*a*, *c*) non-capsule-forming and (*b*, *d*) capsule-forming *Streptococcus thermophilus* cultures (*a*, *b*) 20 min and (*c*, *d*) 40 min after adding rennet. Light areas indicate the protein structure; dark areas are soluble components including capsular material in (*b*) and (*d*). Arrows point to pores associated with encapsulated bacteria. Sections were obtained 15–20 μm beneath the cover slip. Scale bar, 10 μm.

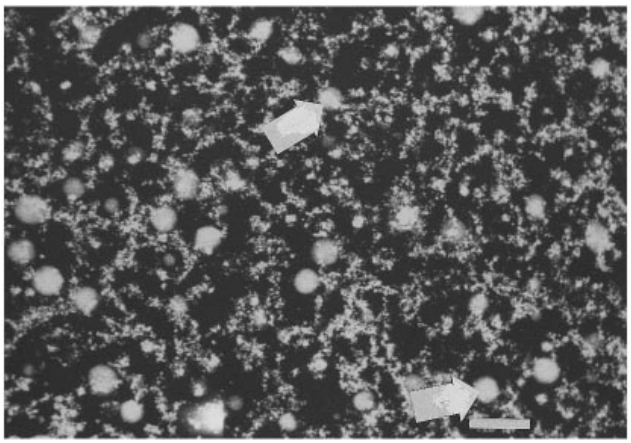


Fig. 2. Distribution of fat globules within undisturbed casein network prepared from raw milk containing a non-capsule-forming culture showing the presence of fat globules in structural pores and within casein aggregates. The micrograph was obtained by merging image of labelled fat globules in the fluorescence mode and that of the casein network obtained in the reflectance mode. Light areas indicate protein structure and fat globules, dark areas indicate water-soluble components. Arrows point to fat globules. Scale bar, 10 μm.

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Table 1. *Textural characteristics of non-fat rennet curd made with non-capsule-forming* (Str. thermophilus *S4*) *and capsule-forming* (Str. thermophilus *3855*) *cultures and full fat rennet curd*

(Values are means of two replicates)

Sample	Curd tension, kg	Firmness, kg	Total energy, kg/mm
Non-capsule-forming	0·105[a]	0·102[a]	3·21[a]
Capsule-forming	0·056[b]	0·055[b]	1·72[b]
Full fat	0·056[b]	0·052[b]	1·80[b]

[a, b] Means within the same column without a common superscript were significantly different: $P < 0{\cdot}05$.

penetrate 35 mm into the curd made with non-capsule-forming culture was almost twice that for the curd made from full fat milk or with capsule-forming bacteria.

DISCUSSION

Determining curd microstructure at cutting time is difficult because artifacts arise during sampling and sample preparation. Samples of milk gel cannot be taken without surface syneresis, which obscures the real structure of the sample (Lagoueyte *et al*. 1994). In addition, when studying the microstructure of cheese using electron microscopy it is necessary to extract fat prior to critical point drying to avoid obscuring the protein matrix and charging, which lead to artifacts (Kalab, 1977, 1981). The confocal scanning laser microscope permits direct observation of samples in their fully hydrated state. In this study milk was coagulated in a sample holder that allowed the undisturbed coagulum to be viewed. Therefore, confocal images should accurately reflect the physical properties of the sample. We have previously studied yogurt microstructure using confocal scanning electron microscopy (Hassan *et al*. 1995*b*).

The structural differences observed between rennet curd made with capsule-forming and non-capsule-forming cultures can be related to the presence of the bacterial capsules within the gel structure. The observations of Hassan *et al*. (1995*a*, *b*) indicate that chains of capsule-forming streptococci are enclosed in one large capsule. Chains of streptococci are only partly visible within the capsules shown in Fig. 1 because the micrographs were obtained using unstained samples. These capsules cannot be penetrated by casein and therefore interrupt the protein network (Hassan *et al*. 1995*a*, *b*). During the early stages of coagulation, the bacterial capsules direct the casein network around them, causing large pores to be formed (arrow in Fig. 1*b*). Hassan *et al*. (1995*b*) reported that bacterial capsules acted as 'nuclei' for the formation of these pores. The large pores produce the discontinuous gel structure observed in curd made with capsule-forming culture (Fig. 1*b*). On the other hand, curd made with non-capsule-forming culture exhibited a more continuous structure, indicating that an even coagulation took place within the network. After treatment with rennet for 40 min the large areas of casein aggregates observed in both types of rennet curd after 20 min became thinner and were connected with finer bridges. This corresponds to contraction within the protein aggregates, i.e. the pores within clusters and strands decreased, and as a result the porosity of the overall structure increased. This contraction of the casein network occurred in the same manner in curd made with both cultures. Lagoueyte *et al*. (1994) assumed that there are intermicellar porosity (spaces between micelles), small extramicellar porosity (spaces

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between micelles aggregated in strands and clusters) and the great extramicellar porosity which is delimited by the coagulum framework. Although rennet curd made with capsule-forming culture had a more continuous network after treatment with rennet for 40 min compared with 20 min, it still exhibited a more uneven structural network containing larger pores than did curd made with non-capsule-forming culture.

Unlike bacterial capsules, which have a large consistent size (Hassan *et al.* 1995*a*), fat globules are smaller and of varied sizes, so each contributes in a different way to the microstructure of the curd. Individual fat globules, even large ones, displaced smaller areas of the gel than did capsule-forming bacteria, which are usually in chains. Full fat curd did not exhibit the large pores observed in curd made with capsule-forming culture. In addition, small fat globules fitted within some small protein strands and aggregates, unlike the much larger bacterial capsules.

Results obtained from Instron analysis indicated that fat and bacterial capsules had a similar effect on the texture. The large pores associated with gel-containing capsule-forming bacteria increased the porosity of the curd, which was reflected by low curd tension and firmness. However, fat globules, which did not cause such an open structure, could still reduce firmness by preventing close contact between micelle strands and aggregates, thus reducing the number and strength of links between them. These links determine the rigidity of the gel (Green *et al.* 1978).

In conclusion, milk fat and capsule-forming starter culture produced similar effects on the texture of rennet curd. Bacterial capsules and large fat globules produced large pores within the gel structure. Small fat globules could fit in smaller pores and reduce the bond strength within the casein structure. The similarity of the effect of milk fat and bacterial capsules on the textural properties of rennet curd in addition to the possible role of bacterial capsules in stabilizing the aqueous phase indicated the possibility of using capsule-forming cultures as a partial fat substitute for some cheese varieties.

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