Microstructure and Rheology of Yogurt Made with Cultures Differing Only in Their Ability to Produce Exopolysaccharides

A. N. Hassan,^{*,1} R. Ipsen, T. Janzen,[†] and K. B. Qvist^{*}

^{*}Center for Advanced Food Studies, Department of Dairy and Food Science,
The Royal Veterinary and Agricultural University, Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark

[†]Chr. Hansen A/S, Boege Alle 10-12, DK-2970 Hoersholm, Denmark

ABSTRACT

Yogurt was made using an exopolysaccharide-producing strain of *Streptococcus thermophilus* and its genetic variant that only differed from the mother strain in its inability to produce exopolysaccharides. The microstructure was investigated using confocal scanning laser microscopy, allowing observation of fully hydrated yogurt and the distribution of exopolysaccharide within the protein network. Yogurt made with the exopolysaccharide-producing culture exhibited increased consistency coefficients, but lower flow behavior index, yield stress, viscoelastic moduli and phase angle values than did yogurt made with the culture unable to produce exopolysaccharide. The exopolysaccharides, when present, were found in pores in the gel network separate from the aggregated protein. These effects could be explained by the incompatibility of the exopolysaccharides with the protein aggregates in the milk.

Stirring affected the yogurt made with exopolysaccharide differently from yogurt without exopolysaccharide, as it did not exhibit immediate syneresis, although the structural breakdown was increased. The shear-induced microstructure in a yogurt made with exopolysaccharide-producing culture was shown to consist of compartmentalized protein aggregates between channels containing exopolysaccharide, hindering syneresis as well as the buildup of structure after stirring. (**Key words:** yogurt, exopolysaccharides, rheology, confocal laser scanning microscopy)

Abbreviation key: δ = the phase angle, where $\tan(\delta) = G''/G'$; ΔA = % difference in area under the curves for the upward and downward shear rate sweep; A_{up} = area under the shear stress vs. shear rate curve at increasing shear rates (upward flow curve); **CLSM** = confocal laser scanning microscopy; **EPS** = exopolysaccharide; **EPS⁺** = exopolysaccharide-producing bacterial

strain; **EPS⁻** = bacterial strain not producing exopolysaccharide; **G'** = the elastic modulus; **G''** = the viscous modulus.

INTRODUCTION

Some bacterial strains used in cultures for manufacture of yogurt or other fermented milks are known to produce polysaccharides outside the cell wall, called exopolysaccharides (**EPS**). The use of such strains modifies the physical properties of fermented milk (Hassan et al., 1996; Bouzar et al., 1997; Hassan et al., 2001b). EPS can either be attached to the bacterial cells as capsules or found as unattached material in the growth medium. Strains have been found that produce both capsular and unattached EPS (Hassan et al., 1995), and the chemical composition of the capsular and the unattached EPS produced by a given strain can be either similar or varied between the two types (Ariga et al., 1992; Hassan et al., 2001c). We have found a ropy strain of *Lactobacillus delbruekii* ssp. *bulgaricus* (CHCC 2164; Chr. Hansen A/S, DK-2970 Hørsholm, Denmark) that produces only unattached EPS, but at present there seems to be no reported findings on strains producing only capsular EPS. Capsular EPS does not result in ropiness in fermented milks, nor does production of unattached EPS automatically ensure ropy characteristics because some nonropy strains are known to produce significant amounts of EPS (van Marle and Zoon, 1995).

No relation has been established between the amount of EPS produced and the physical properties of fermented milk, but EPS produced by different strains vary significantly in the structural characteristics. Factors affecting the function of EPS in fermented milk include monosaccharide composition, charge, linkage types, branching, molecular weight, and the ability to interact with milk protein (Kleerebezem et al., 1999; Duboc and Mollet, 2001; Ruas-Madiedo et al., 2002).

Two fundamental problems arise when studying the function of EPS in fermented milks: 1) it is difficult to obtain a control culture that possesses similar characteristics, aside from production of EPS, and 2) conven-

Received August 14, 2002.

Accepted December 23, 2002.

Corresponding author: K. B. Qvist; e-mail: kbq@kvl.dk.

¹Present address: Department of Food Science and Technology, Athens, GA 30602.

Table 1. Culture combinations used for manufacture of yogurt. All culture combinations consisted of equal amounts of a *Streptococcus thermophilus* and a *Lactobacillus delbrueckii* ssp. *bulgaricus* culture.

Culture combination	Culture components	Description of culture component	Acidification rate ¹
A	CHCC 2136	Nonropy, noncapsuleforming <i>S. thermophilus</i>	a
	CHCC 769	Nonropy noncapsuleforming <i>L. delbrueckii</i> ssp. <i>bulgaricus</i>	
B	CHCC 3534	Ropy <i>S. thermophilus</i> producing 3- μ m capsules	b
	CHCC 769	Nonropy, noncapsule-forming <i>L. delbrueckii</i> ssp. <i>bulgaricus</i>	
C	CHCC 5842	Nonropy, noncapsule-forming (the EPS ⁻ variant of CHCC 3534)	b
	CHCC 769	Nonropy noncapsule-forming <i>L. delbrueckii</i> ssp. <i>bulgaricus</i>	
D	CHCC 3541	Ropy <i>S. thermophilus</i> producing 2.5- μ m capsules	a
	CHCC 769	Nonropy noncapsule-forming <i>L. delbrueckii</i> ssp. <i>bulgaricus</i>	

¹Cultures with same letter used the same amount of time to reach pH 4.3 during yogurt manufacture at 40°C.

tional scanning electron microscopy is not suitable for observing EPS, which is highly hydrated (Hassan et al., 2002). In the current study we have overcome the first of these problems by using an EPS-producing mother strain (EPS⁺) and its non-EPS-producing genetic variant (EPS⁻). Aside from lacking the ability to produce EPS, the EPS⁻ variant had all the characteristics of the mother strain. The microstructure of the yogurt was investigated using confocal laser scanning microscopy (CLSM), which allows visualization of fully hydrated specimens, thus avoiding the artifacts associated with conventional scanning electron microscopy (Hassan et al., 2002). Using these tools, our objective was to gain understanding of the role of EPS in yogurt by relating microstructure of yogurts made with EPS⁺ and EPS⁻ cultures to their rheological properties.

MATERIALS AND METHODS

Starter Cultures

The starter culture combinations used in this study and their compositions are listed in Table 1. Cultures were tested for encapsulation according to the method developed by Hassan et al. (1995). The culture CHCC 5842 (Table 1) was isolated as a spontaneous EPS⁻ mutant of CHCC 3534.

Manufacture of Yogurt

Low-heat skim milk powder (ArlaFoods, DK-8260 Viby, Denmark) was reconstituted to 11% (wt/vol) (using distilled water). The resulting reconstituted milk was steamed for 12 min in a steaming chamber, cooled and kept in the refrigerator overnight. The following day it was warmed to 40°C and inoculated with 2% each of *Streptococcus thermophilus* and *L. delbrueckii* ssp. *bulgaricus*, using one of the four culture combinations (A through D, in Table 1). When a pH value of 4.3 was obtained, the fermented milk was kept at 5°C for 24 h, followed by microscopic observation and rheological analysis.

Experimental Design

Each experimental block consisted of four yogurts made from the same batch of reconstituted skim milk and fermented with the four culture combinations (Table 1). This block structure was replicated three times.

Confocal Laser Scanning Microscopy

The microstructure of the protein network in the fermented milk was observed using CLSM in the reflectance mode as described by Hassan et al. (1995). A small piece of each of the unstirred and stirred (gently stirred by spoon 10 times) samples were carefully transferred to chambered coverglasses (Nalge Nunc International Corp., Naperville, IL) and observed using a Leica TCS SP confocal laser scanning system (Leica Microsystems, Heidelberg, Germany) fitted with an inverted Leica DM IRBE microscope and an Ar/Kr laser. Wheat germ agglutinin conjugated with Alexa flour 488 (Molecular Probes Inc., Eugene, OR) was used to label EPS according to the method developed by Hassan et al. (2002). The working solution of the dye was prepared by diluting the stock solution (1 mg of the dye in 1 ml of phosphate buffer at pH 6.8) to 1: 5 with fermented milk whey. Some drops of the dye were added to an undisturbed fermented milk sample and left for 1 h at 5°C to allow diffusion. Another sample was gently stirred after adding the dye. An excitation wavelength of 488 nm was used.

Rheological Measurements

Fermented milk samples were gently stirred 10 times by spoon prior to rheological analysis. Rheological measurements were done in triplicate on all samples.

Flow curves were obtained using a Bohlin VOR Rheometer (Bohlin Ltd., Cirencester, UK) fitted with a Couette measuring geometry (25 mm diameter). The shear rate was varied from 0.00185 to 116 s⁻¹, and the shear stress was recorded at increasing shear rates (upward flow curve) followed by decreasing shear rates

Table 2. Rheological parameters of yogurt made with different culture combinations. The experiment was replicated three times, and all measurements were done in triplicate.

Parameter	Significance, <i>P</i>	Culture combination			
		A	B	C	D
Yield stress, σ_0^1 (Pa)	<0.0001	15.4 ^{a2}	0.0 ^b	17.4 ^a	0.0 ^b
Consistency coefficient, K^1 (Pa s ⁿ)	<0.0001	0.9 ^a	20.2 ^b	0.6 ^a	16.6 ^b
Flow behavior index, n^1 (–)	<0.0001	0.65 ^a	0.23 ^b	0.65 ^a	0.24 ^b
A_{up} (Pa s ^{–1}) ³	0.0007	3100 ^a	5600 ^b	3000 ^a	4800 ^b
ΔA (%) ⁴	0.0006	18 ^a	23 ^b	18 ^a	34 ^c

¹Determined by fitting to the Herschel-Bulkley model.

²Different letters within same row indicate significant differences.

³Area under the upward curve when plotting shear stress vs. shear rate.

⁴Percentage differences in area under the upward part of the shear stress vs. shear rate curve and the corresponding downward curve.

(downward flow curve). The temperature was maintained at 5°C, and continuous shear was applied with a delay time of 5 s between measurements at a given shear rate.

The Herschel-Bulkley model proved to give significantly better fits to the upward flow curves (results not shown) than did the power law, the Casson or the QRS-models (Skriver et al., 1993) and was therefore chosen to model flow behavior:

$$\sigma = \sigma_0 + K \dot{\gamma}^n$$

where σ = shear stress, K = consistency index, n = flow behavior index, $\dot{\gamma}$ = shear rate, and σ_0 = yield stress.

In addition, the area under the upward flow curve (A_{up}) and the percentage difference in area under the upward flow curve and the downward flow curve (ΔA) were determined using an in-house program written in MathCad 2000 (Mathsoft, 1999).

A small strain oscillation frequency sweep (Rao, 1999) was also performed on the samples at frequencies ranging from 0.01 to 9 Hz. The temperature was 5°C and the applied strain 0.00412 (determined by a strain sweep to be within the linear viscoelastic range). The elastic modulus (G'), the viscous modulus (G'') and the phase angle (δ , where $\tan(\delta) = G''/G'$) were recorded as functions of frequency. The slope of log-log plots of moduli vs. frequency was determined using an in-house program written in Mathcad 2000.

Statistical Analysis

Data preprocessing was done using in-house programs written in Mathcad 2000. ANOVA was done with SAS version 8 (2001). Replication was used as a block effect.

RESULTS

Rheological Properties

Figure 1 shows flow curves for yogurt made with the four culture combinations. The EPS[–] cultures (A, C) resulted in yogurts with lower shear stress values than when the EPS⁺ cultures (B, D) were used. When fitted to the Herschel-Bulkley model, yogurt data obtained using EPS⁺ did not exhibit any yield stress and had higher K values and lower n compared to yogurt made with EPS[–], indicating a thicker consistency and more deviation from Newtonian flow behavior (Table 2). In addition, A_{up} was higher in yogurt made with EPS⁺, as was ΔA , indicating that more structural breakdown during shearing took place. The only significant difference found between the two EPS⁺ (B and D) or EPS[–]

Figure 1. Flow curves of yogurts made with culture combinations A through D (see Table 1). Shear rate first increased, then decreased. Measurement temperature was 5°C.

Table 3. Viscoelastic parameters of yogurt made with different culture combinations. The experiment was replicated three times, and all measurements were done in triplicate.

Parameter	Significance, <i>P</i> (–)	Culture combination			
		A	B	C	D
Elastic modulus, <i>G'</i> , at 1 Hz (Pa)	0.0002	284 ^a	201 ^b	260 ^c	184 ^b
Viscous modulus, <i>G''</i> , at 1 Hz (Pa)	0.0003	73 ^a	45 ^b	63 ^c	42 ^b
Phase angle, δ , at 1 Hz (°)	0.0054	14.4 ^a	12.6 ^b	13.6 ^{ab}	12.9 ^b
Slope of log (<i>G'</i>) vs. log frequency (–)	0.0038	0.150 ^a	0.136 ^b	0.146 ^a	0.138 ^b
Slope of log (<i>G''</i>) vs. log frequency (–)	0.0066	0.145 ^a	0.117 ^b	0.152 ^a	0.118 ^b

^{a,b,c}Different letters within same row indicate significant differences.

(A and C) cultures was that ΔA was higher for D than for B.

Table 3 shows the results from the frequency sweep. The yogurt made with EPS⁺ had lower *G'*, *G''*, and δ values than the corresponding yogurt made using EPS[–]. Plotting log (*G'*) or log (*G''*) vs. log (frequency) gave reasonable straight lines for yogurt made with both types of cultures (Figure 2). All yogurts exhibited characteristics typical of a weak viscoelastic gel, with *G'* greater than *G''* at all the frequencies investigated, and both showing some frequency dependence. *G'* and *G''* showed similar frequency dependence, but the moduli of yogurt made with EPS[–] showed more frequency dependence [higher slope of log (*G'*) and log (*G''*) vs. log (frequency)] than yogurt made with EPS⁺. The slope of log (*G'*) vs. log (frequency) ranged from 0.136 to 0.138 for EPS⁺, whereas it was 0.146 to 0.150 for EPS[–], and the slope of log (*G''*) vs. log (frequency) ranged from 0.117 to 0.118 for EPS⁺ and from 0.145 to 0.152 for EPS[–] (Table 3).

Figure 2. Small strain oscillation frequency sweeps of yogurts made with culture combinations A through D (see Table 1). Measurement temperature was 5°C. The elastic modulus, *G'* is shown by thick lines, and the viscous modulus *G''* by thinner lines.

Microstructure

Figure 3 shows the microstructure of yogurt made with the different cultures. The yogurt made using EPS[–] appears homogenous, with rather small and evenly distributed pores, and a network consisting of relatively thin strands. Yogurt made with EPS⁺, however, have rather large pores, which are associated with the presence of EPS, and the protein network appears to be made up from rather thick strands consisting of densely aggregated protein particles.

Stirring of the yogurt caused immediate whey separation in the samples made with EPS[–], resulting in formation of large areas of separated whey and a denser protein network (Figure 3b, f) containing smaller pores than the unstirred yogurt (Figure 3a, e). In yogurt made using EPS⁺, stirring led to formation of channels containing EPS within the protein network (Figure 3d, 3h).

DISCUSSION

In the present study we observe distinct phase-separation in a yogurt made from cultures producing EPS (Figure 3). Had attractive interactions between EPS and CN been present, we would expect association points between the two to show up in the two-dimensional micrographs in Figure 3 in proportion to their presence in three dimensions, since we can assume yogurt structure to be isotropic. Also, we observe notable differences in the rheological behavior between yogurt made from EPS⁺ and EPS[–] (Tables 2 and 3).

Casein micelles have been observed to become mutually attractive when EPS was added to skim milk due to depletion interaction induced by the EPS (Tuinier et al., 1999). Phase-separation caused by a similar depletion-interaction has also been seen when EPS was added to aggregated whey protein particles (Tuinier et al., 2000). These experiments were done near neutral pH, and the EPS used did not interact with the proteins. In these conditions the EPS can, therefore, be assumed to be excluded from the surface of the protein particles, resulting in a depletion layer, where the osmotic pres-

Figure 3. Confocal laser scanning micrographs showing distribution of protein and exopolysaccharide in unstirred (a, c, e, g) and stirred samples (b, d, f, h) of yogurt made using cultures A through D (see Table 1). Culture combinations: A: a, b; B: c, d; C: e, f; D: g, h. Protein network appears red, exopolysaccharide green, and whey dark. Identical magnification used for all images. Scale bar in field h is 10 μ m long.

sure generated by the EPS is smaller than in the bulk. When two protein particles meet as a result of Brownian motion, they share this depleted volume, hence increas-

ing the volume available for the EPS and decreasing the free energy of the system. The system thus has an entropic driving force towards phase separation (de Kruif and Tuinier, 2001).

Although the above experiments were done near neutral pH, a similar type of interaction can be expected at pH 4.3 because EPS is generally not highly charged (Ruas-Madiedo et al., 2002). The protein particles present in the heat-treated milk used in our experiment consist of CN micelles with associated whey protein. If we assume incompatibility between these particles and the EPS produced by the bacteria during the fermentation, denser and larger protein aggregates can be expected to appear prior to the gel point than if EPS was not present, and the EPS can be assumed to be concentrated in the continuous phase as aggregation proceeds. The final protein network in the yogurt can, therefore, be expected to be more densely aggregated in yogurt made with EPS⁺, which indeed was the case (Figure 3c, e). Further, Hassan et al. (2001a) reported that production of EPS caused gelation to occur at higher pH values. This might allow for more rearrangement to take place after the gel point, again favoring the formation of more densely aggregated structures (Walstra, 1993).

When stirring the yogurts, we visually observed that EPS⁺ samples quickly and easily became homogeneous, whereas samples made using EPS⁻ after identical stirring exhibited syneresis, which led to a macroscopically granular appearance. A likely reason why EPS⁺ samples (Figure 3c, g) broke down more easily than EPS⁻ samples (Figure 3a, e) is that there are fewer protein-protein interactions at the critical sites (where the strands are thinnest) in the network to overcome.

When the pores in the original, unsheared network contain EPS, a result of shearing will be to concentrate the EPS in the continuous liquid phase between the protein aggregates (Figure 3). We have observed earlier (Hassan et al., 2002) that the EPS appears to collect in larger strands during stirring. This also seems to be the case in the present study and is consistent with incompatibility between EPS and protein. When sheared, the samples made using EPS⁺ will contain a continuous phase rich in EPS surrounding the protein aggregates and, hence, will tend to prevent syneresis through increased viscosity, as was observed. Due to incompatibility, the EPS in the continuous phase can be expected to decrease interactions between the protein aggregates.

We did not find a yield stress in yogurts made using EPS⁺. Substantially lower yield stress in yogurt made using EPS⁺ was found by Skriver et al. (1993), and Hassan et al. (1996) notes that the use of a ropy strain resulted in yogurt with a lower value for yield stress

than that of yogurt made with unencapsulated nonropy strains. The absence (or decrease) of the yield stress in yogurt made with EPS⁺ can be explained by the decreased possibility of interactions between the protein aggregates during flow, due to the presence of EPS in the continuous phase surrounding the aggregates. This most probably also contributed to the lower values of G' and G'' in yogurts made with EPS⁺, as compared to those in yogurts made with EPS⁻ (Table 3), which was also noted by Skriver (1995). Lucey et al. (1997, 1998) suggested that extensive particle rearrangement during structure formation results in dense clusters of aggregates and lower G' values. Such an effect may also have been at work here.

The presence of EPS channels in the serum will confer a more polymer-like rheological behavior to the continuous phase, thus resulting in increased consistency index, more deviation from Newtonian behavior, and increased viscosity in the yogurt (Table 2). Also the difference in protein network structure between EPS⁺ and EPS⁻ samples will contribute to differences in flow behavior. Presence of EPS reduced G' relatively more than G'' (Figure 2), and therefore, EPS⁺ yogurts appeared more elastic in nature than EPS⁻ yogurts, as indicated by smaller values for δ and the slope of log-log plots of moduli vs. frequency (Table 3).

The increased structural breakdown (as indicated by A_{up} and ΔA , Table 2) in yogurt when EPS is present can be explained by the difficulty for the protein aggregates to reform into a coherent network structure after shearing due to the compartmentalization of the aggregates caused by the presence of EPS in channels in the continuous phase.

Generally the difference in rheological behavior microstructure between the mutant EPS⁺/EPS⁻ pair (B/C) was similar to the difference between the nonmutant pair (D/A), suggesting that, even in the case of the nonmutant pair, the major part of the difference in rheological behavior and in microstructure of the investigated yogurts was caused by the presence (or absence) of EPS. This adds support to the results of prior studies on the effects of EPS, in which mutant pairs identical in all aspects except EPS were not available.

CONCLUSIONS

The effect of EPS on the rheological properties and the microstructure of yogurt can, to a large extent, be explained by incompatibility with the protein aggregates in the product. This incompatibility probably affects the aggregation prior to gelation, as well as the rearrangement of the protein aggregates after the gel point, resulting in a network with a microstructure composed of rather thick, aggregated protein strands in-

terspaced with pores containing EPS in the unstirred product.

Stirring affects yogurt made with EPS⁺ differently than yogurt made with EPS⁻, as it does not induce immediate syneresis. The shear-induced microstructure in a yogurt made with EPS⁺ was shown to consist of compartmentalized protein aggregates between channels containing EPS. These EPS containing channels cause yogurt made with EPS⁺ to have lower moduli (G', G'') and yield stress, and they hindered syneresis and buildup of structure after stirring. The increased consistency index and deviation from Newtonian flow of stirred yogurt may also be influenced by the effects of incompatibility on structure but are probably affected directly by the rheological properties of the EPS as well.

ACKNOWLEDGMENTS

This research was kindly supported by Chr. Hansen A/S, DK-2970 Hørsholm, Denmark and we thank Anne Skriver for valuable discussions and access to the cultures used.

REFERENCES

- Ariga, H., T. Urashima, E. Michihata, M. Ito, N. Morizono, T. Kimura, and S. Takahashi. 1992. Extracellular polysaccharide from encapsulated *Streptococcus salivarius* subsp. *thermophilus* OR 901 isolated from commercial yogurt. *J. Food Sci.* 57:625-628.
- Bouzar, F., J. Cerning, and M. Desmazeaud. 1997. Exopolysaccharide production and texture-promoting abilities of mixed-strain starter cultures in yogurt production. *J. Dairy Sci.* 80:2310-2317.
- De Kruif, C. G., and R. Tuinier. 2001. Polysaccharide protein interactions. *Food Hydrocolloids* 15(4):555-564.
- Duboc, P., and B. Mollet. 2001. Application of exopolysaccharides in the dairy industry. *Int. Dairy J.* 11:759-768.
- Hassan, A. N., J. F. Frank, M. A. Farmer, K. A. Schmidt, and S. I. Shalabi. 1995. Observation of encapsulated lactic acid bacteria using confocal scanning laser microscopy. 78:2624-2628.
- Hassan, A. N., J. F. Frank, K. A. Schmidt, and S. I. Shalabi. 1996. Rheological properties of yogurt made with encapsulated nonropy lactic cultures. *J. Dairy Sci.* 79:2091-2097.
- Hassan, A. N., M. Corredig, and J. F. Frank. 2001a. Capsule formation by nonropy starter cultures affects the viscoelastic properties of yogurt during structure formation. *J. Dairy Sci.* 85:716-720.
- Hassan, A. N., M. Corredig, and J. F. Frank. 2001b. Viscoelastic properties of yogurt made with ropy and nonropy exopolysaccharides producing cultures. *Milchwissenschaft* 56:661-720.
- Hassan, A. N., J. F. Frank, and P. Azadi. 2001c. Composition of attached (capsular) and unattached polysaccharides produced by *Streptococcus thermophilus* as affected by growth medium. First Int. Symp. on Exopolysaccharides from Lactic Acid Bacteria. Brussels, Belgium.
- Hassan, A. N., J. F. Frank, and K. B. Qvist. 2002. Direct observation of bacterial exopolysaccharides in dairy products using confocal scanning laser microscopy. *J. Dairy Sci.* 85:1705-1708.
- Kleerebezem, M., R. van Kranenburg, R. Tuinier, I. Boels, P. Zoon, E. Looijesteijn, J. Hugenholtz, and W. M. de Vos. 1999. Exopolysaccharides produced by *Lactococcus lactis*: From genetic engineering to improved rheological properties? *Antonie van Leeuwenhoek* 76:357-365.
- Lucey, J. A., M. Tamehana, H. Singh, and P. A. Munro. 1998. A comparison of the formation, rheological properties and microstructure of acid skim milk gels made with bacterial cultures or glucono- δ -lactone. *Food Res. Int.* 31:147-155.

- Lucey, J. A., T. van Vliet, K. Grolle, T. Geurts, and P. Walstra. 1997. Properties of acid casein gels made by acidification with glucono- δ -lactone. 2. Syneresis, permeability and microstructural properties. *Int. Dairy J.* 7:389–397.
- Mathsoft 1999: Mathcad 2000 User's Guide. Mathsoft Inc., Cambridge, MA.
- Rao, M. A. 1999. *Rheology of Fluid and Semi Solid Foods. Principles and Applications*. Aspen Publishers Inc. Gaithersburg, MD.
- Ruas-Madiedo, P., J. Hugenholtz, and P. Zoon. 2002. An overview of the functionality of exopolysaccharides produced by lactic acid bacteria. *Int. Dairy J.* 12:163–171.
- Skriver, A. 1995. Characterization of stirred yoghurt by rheology, microscopy and sensory analysis. Ph.D. Diss., Royal Veterinary and Agricultural University, Denmark.
- Skriver, A., H. Roemer, and K. B. Qvist. 1993. Rheological characterization of stirred yoghurt. *Viscometry. J. Texture Stud.* 24:185–198.
- Tuinier, R., J. K. G. Dhont, and C. G. de Kruif. 2000. Depletion-induced phase separation of aggregated whey protein colloids by an exocellular polysaccharide. *Langmuir* 16:1497–1507.
- Tuinier, R., E. ten Grotenhuis, C. Holt, P. A. Timmins, and C. G. de Kruif. 1999. Depletion interaction of casein micelles and an exocellular polysaccharide. *Phys. Rev. E* 60:848–856.
- Van Marle, M. E., and P. Zoon. 1995. Permeability and rheological properties of microbially and chemically acidified skim-milk gels. *Neth. Milk Dairy J.* 49:47–65.
- Walstra, P. 1993. The syneresis of curd. Pages 141–191 in *Cheese: Chemistry, Physics and Microbiology*. 2nd ed. P. F. Fox, ed. Chapman & Hall, London, United Kingdom.